frontiers

RESEARCH TOPICS

OBESITY-INDUCED INFLAMMATION AND INSULIN RESISTANCE

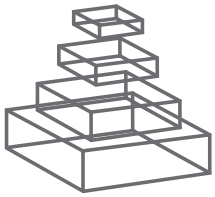
Topic Editor
Tsuguhito Ota

frontiers in ENDOCRINOLOGY

frontiers in PUBLIC HEALTH

frontiers in NEUROSCIENCE

frontiers in NUTRITION



frontiers

FRONTIERS COPYRIGHT STATEMENT

© Copyright 2007-2015
Frontiers Media SA.
All rights reserved.

All content included on this site, such as text, graphics, logos, button icons, images, video/audio clips, downloads, data compilations and software, is the property of or is licensed to Frontiers Media SA ("Frontiers") or its licensees and/or subcontractors. The copyright in the text of individual articles is the property of their respective authors, subject to a license granted to Frontiers.

The compilation of articles constituting this e-book, wherever published, as well as the compilation of all other content on this site, is the exclusive property of Frontiers. For the conditions for downloading and copying of e-books from Frontiers' website, please see the Terms for Website Use. If purchasing Frontiers e-books from other websites or sources, the conditions of the website concerned apply.

Images and graphics not forming part of user-contributed materials may not be downloaded or copied without permission.

Individual articles may be downloaded and reproduced in accordance with the principles of the CC-BY licence subject to any copyright or other notices. They may not be re-sold as an e-book.

As author or other contributor you grant a CC-BY licence to others to reproduce your articles, including any graphics and third-party materials supplied by you, in accordance with the Conditions for Website Use and subject to any copyright notices which you include in connection with your articles and materials.

All copyright, and all rights therein, are protected by national and international copyright laws.

The above represents a summary only. For the full conditions see the Conditions for Authors and the Conditions for Website Use.

ISSN 1664-8714
ISBN 978-2-88919-428-5
DOI 10.3389/978-2-88919-428-5

ABOUT FRONTIERS

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

FRONTIERS JOURNAL SERIES

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing.
All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

DEDICATION TO QUALITY

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view.
By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

WHAT ARE FRONTIERS RESEARCH TOPICS?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area!
Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

OBESITY-INDUCED INFLAMMATION AND INSULIN RESISTANCE

Topic Editor:
Tsuguhito Ota, Kanazawa University, Japan

Immune response and metabolic regulation are highly integrated and this interface maintains a central homeostatic system, dysfunction of which can cause obesity-associated metabolic disorder such as type 2 diabetes, fatty liver disease and cardiovascular disease. Insulin resistance is an underlying basis for the pathogenesis of these metabolic diseases. Overnutrition or obesity activates the innate immune system with subsequent recruitment of immune cells such as macrophages and T cells, which contributes to the development of insulin resistance. In particular, a significant advance in our understanding of obesity-associated inflammation and insulin resistance has been recognition of the critical role of adipose tissue macrophages (ATMs). ATMs are a prominent source of proinflammatory cytokines, such as TNF-α and IL-6, that can block insulin action in adipose tissue, skeletal muscle, and liver autocrine/paracrine signaling and cause systemic insulin resistance via endocrine signaling, providing a potential link between inflammation and insulin resistance.

All articles in this topic highlight the interconnection between obesity, inflammation, and insulin resistance in all its diversity to the mechanisms of obesity-induced inflammation and role of immune system in the pathogenesis of insulin resistance and diabetes.

Table of Contents

04 ***Obesity-induced inflammation and insulin resistance***

Tsuguhito Ota

06 ***Implication of inflammatory signaling pathways in obesity-induced insulin resistance***

Jean-François Tanti, Franck Ceppo, Jennifer Jager and Flavien Berthou

21 ***RAGE-mediated inflammation, type 2 diabetes, and diabetic vascular complication***

Yasuhiko Yamamoto and Hiroshi Yamamoto

24 ***Retinoic acid-related orphan receptors α and γ: key regulators of lipid/glucose metabolism, inflammation, and insulin sensitivity***

Anton M. Jetten, Hong Soon Kang and Yukimasa Takeda

32 ***Transgenic mice overexpressing renin exhibit glucose intolerance and diet-genotype interactions***

Sarah J. Fletcher, Nishan S. Kalupahana, Morvarid Soltani-Bejnood, Jung Han Kim, Arnold M. Saxton, David H. Wasserman, Bart De Taeye, Brynn H. Voy, Annie Quignard-Boulange and Naima Moustaid-Moussa

41 ***Angiotensinogen gene silencing reduces markers of lipid accumulation and inflammation in cultured adipocytes***

Wenting X. Carroll, Nishan S. Kalupahana, Suzanne L. Booker, Nalin Siriwardhana, Monique LeMieux, Arnold M. Saxton and Naima Moustaid-Moussa

53 ***Mechanisms of obesity-induced inflammation and insulin resistance: insights into the emerging role of nutritional strategies***

Maeve A. McArdle, Orla M. Finucane, Ruth M. Connaughton, Aoibheann M. McMorrow and Helen M. Roche

76 ***Recent advances in obesity-induced inflammation and insulin resistance***

Sanshiro Tateya, Francis Kim and Yoshikazu Tamori

90 ***Clinical implications of adipocytokines and newly emerging metabolic factors with relation to insulin resistance and cardiovascular health***

Sung Hee Choi, Eun Shil Hong and Soo Lim

97 ***Sphingolipid metabolism and obesity-induced inflammation***

Se-Chan Kang, Bo-Rahm Kim, Su-Yeon Lee and Tae-Sik Park

108 ***Effects of bariatric surgery on adipokine-induced inflammation and insulin resistance***

Zeynep Goktas, Naima Moustaid-Moussa, Chwan-Li Shen, Mallory Boylan, Huanbiao Mo and Shu Wang

frontiers in
ENDOCRINOLOGY

EDITORIAL
published: 04 December 2014
doi: 10.3389/fendo.2014.00204

Obesity-induced inflammation and insulin resistance

Tsuguhito Ota*

Department of Cell Metabolism and Nutrition, Brain/Liver Interface Medicine Research Center, Kanazawa University, Kanazawa, Japan
**Correspondence: tota@staff.kanazawa-u.ac.jp*

Edited and reviewed by:
Aaron Vinik, Eastern Virginia Medical School, USA

Keywords: obesity, insulin resistance, inflammation, macrophage, innate immunity, adipose tissue, pattern-recognition receptor, bariatric surgery

Immune response and metabolic regulation are highly integrated and this interface maintains a central homeostatic system, dysfunction of which can cause obesity-associated metabolic disorder such as type 2 diabetes, fatty liver disease, and cardiovascular disease. Insulin resistance is an underlying basis for the pathogenesis of these metabolic diseases. Overnutrition or obesity activates the innate immune system with subsequent recruitment of immune cells such as macrophages and T cells, which contributes to the development of insulin resistance. In particular, a significant advance in our understanding of obesity-associated inflammation and insulin resistance has been recognition of the critical role of adipose tissue macrophages (ATMs). ATMs are a prominent source of proinflammatory cytokines, such as TNF-α and IL-6, that can block insulin action in adipose tissue, skeletal muscle, and liver autocrine/paracrine signaling and cause systemic insulin resistance via endocrine signaling, providing a potential link between inflammation and insulin resistance.

All articles in this topic highlight the interconnection between obesity, inflammation, and insulin resistance in all its diversity to the mechanisms of obesity-induced inflammation and role of immune system in the pathogenesis of insulin resistance and diabetes. These articles give some insight into unanswered questions in relation to this topic. First, the relationship between nutrient sensing systems and the interface of metabolic and inflammatory responses is complex. Pattern-recognition receptors (PRRs) such as Toll-like receptors (TLR) and the receptor for advanced glycation end-products (RAGE) are activated in response to dietary nutrients and changes of gut microbiota as described in the review articles by Tanti et al. and Yamamoto et al. (1, 2). The activation of PRRs plays a crucial role as a trigger of this metabolic inflammation. Retinoic acid-related orphan receptors RORα and RORγ, also provide an important link between the circadian clock machinery and its regulation of metabolic genes (3). Two studies by Moustaid-Moussa et al. show a potential role of the renin angiotensin system in the development of insulin resistance and diabetes by using gain and loss of function in mice (4, 5). Second, it is not clear which cell recruits and activates or which tissue inflammation initially occurs upon obesity, which then causes systemic inflammation and subsequent development of insulin resistance. McArdle et al. and Tateya et al. describe how obesity causes alteration of the immune cells, in which TH1 cells, B cells, neutrophils, or mast cells induce M1 activation and polarization of macrophages by the elevated secretion of TNFα and IFNγ (6, 7). In addition to proinflammatory cytokines and adipokines, more metabolic regulators including fibroblast growth factor (FGF) family such as FGF 21 and FGF19 and bioactive lipids, sphingolipids can contribute to the development of systemic inflammation and subsequent development of insulin resistance (8, 9). Interestingly, these novel adipokines can be dramatically changed after bariatric surgery and these changes contribute to improvement of obesity-associated inflammation, insulin resistance, and glucose homeostasis (10).

Overall, all the original articles and review articles covering this topic in all its diversity to contribute somewhat to clarify unanswered questions on the mechanisms of obesity-induced inflammation and insulin resistance.

REFERENCES

1. Tanti JF, Ceppo F, Jager J, Berthou F. Implication of inflammatory signaling pathways in obesity-induced insulin resistance. *Front Endocrinol* (2012) **3**:181. doi:10.3389/fendo.2012.00181
2. Yamamoto Y, Yamamoto H. RAGE-mediated inflammation, type 2 diabetes, and diabetic vascular complication. *Front Endocrinol* (2013) **4**:105. doi:10.3389/fendo.2013.00105
3. Jetten AM, Kang HS, Takeda Y. Retinoic acid-related orphan receptors alpha and gamma: key regulators of lipid/glucose metabolism, inflammation, and insulin sensitivity. *Front Endocrinol* (2013) **4**:1. doi:10.3389/fendo.2013.00001
4. Fletcher SJ, Kalupahana NS, Soltani-Bejnood M, Kim JH, Saxton AM, Wasserman DH, et al. Transgenic mice overexpressing Renin exhibit glucose intolerance and diet-genotype interactions. *Front Endocrinol* (2012) **3**:166. doi:10.3389/fendo.2012.00166
5. Carroll WX, Kalupahana NS, Booker SL, Siriwardhana N, Lemieux M, Saxton AM, et al. Angiotensinogen gene silencing reduces markers of lipid accumulation and inflammation in cultured adipocytes. *Front Endocrinol* (2013) **4**:10. doi:10.3389/fendo.2013.00010
6. McArdle MA, Finucane OM, Connaughton RM, McMorrow AM, Roche HM. Mechanisms of obesity-induced inflammation and insulin resistance: insights into the emerging role of nutritional strategies. *Front Endocrinol* (2013) **4**:52. doi:10.3389/fendo.2013.00052
7. Tateya S, Kim F, Tamori Y. Recent advances in obesity-induced inflammation and insulin resistance. *Front Endocrinol* (2013) **4**:93. doi:10.3389/fendo.2013.00093
8. Choi SH, Hong ES, Lim S. Clinical implications of adipocytokines and newly emerging metabolic factors with relation to insulin resistance and cardiovascular health. *Front Endocrinol* (2013) **4**:97. doi:10.3389/fendo.2013.00097
9. Kang SC, Kim BR, Lee SY, Park TS. Sphingolipid metabolism and obesity-induced inflammation. *Front Endocrinol* (2013) **4**:67. doi:10.3389/fendo.2013.00067
10. Goktas Z, Moustaid-Moussa N, Shen CL, Boylan M, Mo H, Wang S. Effects of bariatric surgery on adipokine-induced inflammation and insulin resistance. *Front Endocrinol* (2013) **4**:69. doi:10.3389/fendo.2013.00069

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 02 October 2014; accepted: 16 November 2014; published online: 04 December 2014.
*Citation: Ota T (2014) Obesity-induced inflammation and insulin resistance. Front. Endocrinol. **5**:204. doi: 10.3389/fendo.2014.00204*
This article was submitted to Diabetes, a section of the journal Frontiers in Endocrinology.

Copyright © 2014 Ota. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in
ENDOCRINOLOGY

REVIEW ARTICLE
published: 08 January 2013
doi: 10.3389/fendo.2012.00181

Implication of inflammatory signaling pathways in obesity-induced insulin resistance

Jean-François Tanti[1,2]*, Franck Ceppo[1,2], Jennifer Jager[1,2†] and Flavien Berthou[1,2]

[1] *INSERM U1065, Mediterranean Center of Molecular Medicine (C3M), Team "Molecular and Cellular Physiopathology of Obesity and Diabetes", Nice, France*
[2] *Faculty of Medicine, University of Nice Sophia-Antipolis, Nice, France*

Edited by:
Tsuguhito Ota, Kanazawa University, Japan

Reviewed by:
Undurti N. Das, UND LIfe Sciences, USA
Marek Bolanowski, Wroclaw Medical University, Poland

****Correspondence:***
Jean-François Tanti, INSERM U1065, Centre Méditerranéen de Médecine Moléculaire, Bâtiment Archimed, 151, route de St. Antoine de Ginestière, BP 2 3194, 06204, Nice Cedex 3, France.
e-mail: tanti@unice.fr

†Present address:
Jennifer Jager, Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, Department of Genetics, and The Institute for Diabetes, Obesity, and Metabolism, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA.

Obesity is characterized by the development of a low-grade chronic inflammatory state in different metabolic tissues including adipose tissue and liver. This inflammation develops in response to an excess of nutrient flux and is now recognized as an important link between obesity and insulin resistance. Several dietary factors like saturated fatty acids and glucose as well as changes in gut microbiota have been proposed as triggers of this metabolic inflammation through the activation of pattern-recognition receptors (PRRs), including Toll-like receptors (TLR), inflammasome, and nucleotide oligomerization domain (NOD). The consequences are the production of pro-inflammatory cytokines and the recruitment of immune cells such as macrophages and T lymphocytes in metabolic tissues. Inflammatory cytokines activate several kinases like IKKβ, mTOR/S6 kinase, and MAP kinases as well as SOCS proteins that interfere with insulin signaling and action in adipocytes and hepatocytes. In this review, we summarize recent studies demonstrating that PRRs and stress kinases are important integrators of metabolic and inflammatory stress signals in metabolic tissues leading to peripheral and central insulin resistance and metabolic dysfunction. We discuss recent data obtained with genetically modified mice and pharmacological approaches suggesting that these inflammatory pathways are potential novel pharmacological targets for the management of obesity-associated insulin resistance.

Keywords: obesity, insulin resistance, inflammation, adipose tissue, pattern-recognition receptors, stress kinases, macrophages

INTRODUCTION

Obesity is characterized by an excessive adipose tissue expansion due to an increase in nutrients intake and insufficient energetic expenditure. Obesity has dramatically increased worldwide and leads to numerous adverse metabolic disorders including cardiovascular diseases, type 2 diabetes, and some forms of cancer. Insulin resistance is associated with obesity and is a central component of type 2 diabetes, leading to altered glucose and lipid metabolism in adipose tissue, liver, and skeletal muscles. Insulin resistance is characterized by a decrease in insulin signaling mainly in the Insulin Receptor Substrate (IRS)/PI-3-kinase/PKB axis that is responsible for most of the metabolic actions of the hormone (Taniguchi et al., 2006). It is now recognized that a chronic low-grade systemic and local inflammation that develops during obesity could connect obesity to the development of insulin resistance (Gregor and Hotamisligil, 2011). This inflammatory state has been reported in different organs involved in the control of metabolic homeostasis including adipose tissue, liver, endocrine pancreas, hypothalamus, and possibly skeletal muscles. The chronic inflammation is caused by an excess of nutrient intake and has been named metabolic inflammation or metainflammation (Gregor and Hotamisligil, 2011). Several dietary factors including saturated fatty acids and glucose as well as a change in gut microbiota have been proposed as triggers of this metabolic inflammation that involves both metabolic cells, such as adipocytes, and a change in the population of immune cells in metabolic tissues (Lolmede et al., 2011; Bertola et al., 2012; Sun et al., 2012). Hypoxia that develops in adipose tissue could also participate in its inflammation and has been recently involved in insulin resistance of adipocytes (Regazzetti et al., 2009; Wood et al., 2009).

In this review, we will first describe the major mediators that link the excess of nutrients to the production of inflammatory cytokines, focusing on pattern-recognition receptors (PRRs). We will then discuss the intracellular signaling pathways activated by inflammatory mediators and involved in the desensitization of insulin signaling. We will also discuss whether and how the blockade of these mechanisms could improve insulin sensitivity.

IMMUNE SENSORS LINKING NUTRITIONAL STRESS TO OBESITY-INDUCED INFLAMMATION AND INSULIN RESISTANCE

TOLL-LIKE RECEPTORS

Toll-like receptors (TLR) belong to the family of PRRs and play a crucial role in innate immunity by their ability to sense pathogens through the pathogen-associated molecular patterns (PAMPs)

and to detect tissue injury through the danger-associated molecular patterns (DAMPs) (Mogensen, 2009). TLRs 1/2/4/5/6/11 are plasma membrane proteins whereas TLRs 3/7/8/9 are present in intracellular compartments. Microbial components induce the activation of the TLR signaling through a MyD88 (myeloid differentiation factor)-dependent pathway, except for TLR3, leading to the activation of the transcription factors NF-κB and AP-1 and the production of inflammatory cytokines. Mitogen-activated protein kinases including extracellular signal-regulated kinases (ERK1/2), JNK, and p38 are also activated by TLRs engagement. TLR3 and TLR4 activation also induces an IFN-β response through a MyD88-independent but TRIF (TIR domain-containing adaptor inducing interferon)-dependent pathway (Mogensen, 2009).

Among the different members of the TLR family, several groups have reported a role for TLR2, TLR4 in inflammation and insulin resistance during obesity (Fresno et al., 2011; Könner and Brüning, 2011). Most of the studies focused on TLR4 which is expressed in macrophages, dendritic cells but also in adipocytes, hepatocytes, muscles, and in the hypothalamus. TLR4 expression is increased in obese mice and obese and diabetic patients and negatively correlates with insulin sensitivity (Könner and Brüning, 2011). Recently it has been proposed that during obesity, metabolic endotoxemia contributes to the development of inflammation and metabolic disorders through the activation of TLR4 in metabolic tissues (**Figure 1**). Metabolic endotoxemia is defined as a moderate increase in circulating lipopolysaccharide (LPS) from Gram-negative bacteria and it develops owing to alterations in the composition of gut microbiota and to an increase in gut permeability (Cani and Delzenne, 2009; Burcelin et al., 2011). Further, high-fat diet could also enhance the translocation of live Gram-negative bacteria from the gut to the adipose

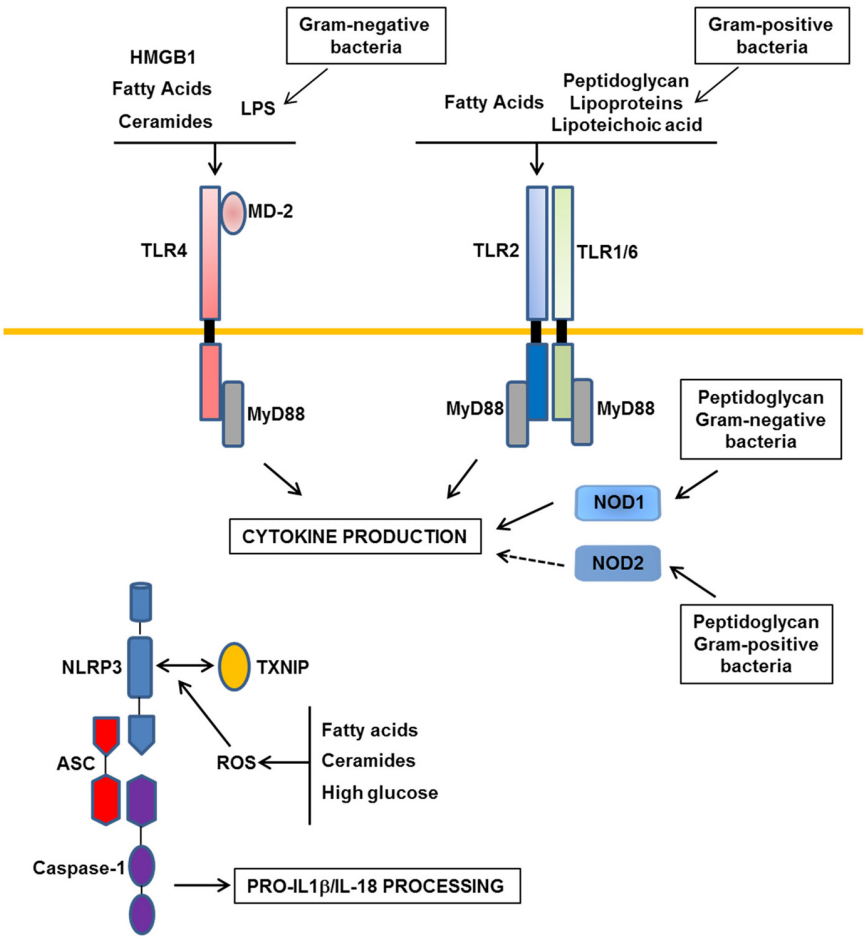


FIGURE 1 | Major Patterns-Recognition Receptors involved in obesity-induced inflammation. In obesity, the moderate increase in LPS derived from gram-negative commensal bacteria activates TLR4 (Toll-like receptors). TLR2 and NOD1/2 (Nucleotide Oligomerization Domain) could be activated by peptidoglycan, lipoproteins, and lipoteichoic acid from gram-negative or -positive commensal bacteria. In addition, nutrients such as saturated fatty acids and their metabolites ceramides could interact with TLR4 or could indirectly activate this receptor through the production of DAMPs such as HMGB1. TLR2 could also be a receptor for saturated fatty acids. Following activation of these receptors, inflammatory cytokines are produced. Among them, IL-1β and IL-18 should be processed in their mature forms by the NLRP3 inflammasome composed of NLRP3, ASC, and caspase-1. ROS production in response to a high level of fatty acids, ceramides, or glucose could trigger the association between TXNIP and NLRP3 leading to the activation of caspase-1 in the inflammasome complex.

tissue, a process that is dependent, at least partly, on CD14 that acts as a co-receptor with TLR4 to sense LPS (Amar et al., 2011).

Saturated fatty acids are other potential ligands of TLR4 both in adipocytes and macrophages leading to the production of inflammatory cytokines and also ceramides (Shi et al., 2006b; Radin et al., 2008; Fresno et al., 2011; Könner and Brüning, 2011) (**Figure 1**). TLR4 also mediates the cross-talk between adipocytes and macrophages induced by fatty acids (Suganami et al., 2007b). However, a direct binding of saturated fatty acids to TLR4 has been recently challenged due to LPS contamination (Erridge and Samani, 2009). It is thus possible that saturated fatty acids indirectly interact with TLR4 through fetuin A (Pal et al., 2012). Alternatively, fatty acids might activate TLR4 signaling through the production of endogenous DAMPs such as HMGB1 (High-Mobility Group Box1) and/or through ceramides production (Fischer et al., 2007; Li et al., 2011) (**Figure 1**).

The *in vivo* pathophysiological importance of TLR4 in obesity-induced inflammation and insulin resistance was investigated by using mice deficient in TLR4 signaling owing to invalidation of TLR4 ($TLR4^{-/-}$ mice) or to a loss-of-function mutation in the *Tlr4* gene (C3H/HeJ and C57BL/10ScN) (**Table 1**). The different studies have reported a mild reduction in inflammation in adipose tissue and liver or in the vasculature (Shi et al., 2006b; Kim et al., 2007; Poggi et al., 2007; Suganami et al., 2007a; Tsukumo et al., 2007; Li et al., 2011; Orr et al., 2012; Ye et al., 2012). The lower inflammation in adipose tissue was linked to a decrease in macrophage infiltration or to a change in macrophage polarization toward a M2 anti-inflammatory phenotype (Shi et al., 2006b; Tsukumo et al., 2007; Davis et al., 2008; Orr et al., 2012). Reduction in inflammation in liver Kupffer cells and in liver parenchymal cells (Li et al., 2011; Ye et al., 2012) was associated with a decrease in hepatic steatosis or with a reduction in the progression from steatosis to non-alcoholic steatohepatitis (Poggi et al., 2007; Tsukumo et al., 2007; Radin et al., 2008; Li et al., 2011; Orr et al., 2012; Ye et al., 2012). In contrast, contradictory results have been obtained concerning the development of obesity. Some studies have reported that C3H/HeJ, 10ScN, or male $TLR4^{-/-}$ mice gained less weight on a high-fat diet than their respective controls (Tsukumo et al., 2007; Davis et al., 2008; Radin et al., 2008; Saberi et al., 2009; Orr et al., 2012). This phenotype could be related to a protection against diet-induced leptin or insulin resistance in the hypothalamus in the absence of a functional TLR4 signaling (Kleinridders et al., 2009; Milanski et al., 2009; Könner and Brüning, 2011). However, other studies have described a higher feeding efficiency of the C3H/HeJ mice with increased adipose tissue mass and adipocyte hypertrophy (Poggi et al., 2007), an increase in body weight gain and

Table 1 | Phenotype of the different TLR4-deficient mice fed with a high-fat diet.

	Genotype	% Fat in diet	Body Weight gain	Whole-Body IS	Food Intake	Adipose Tissue inflammation	ATM	Adipose Tissue IS	Steatosis
Tsukumo et al.	C3H/HeJ	55	↓	↑	=	↓	↓	↑	↓
Poggi et al.	C3H/HeJ	45	=	=	↓	↓	=	↑	↓
Suganami et al.	C3H/HeJ	60	=	↑	ND	↓	=	ND	ND
Davis et al.	10 ScN	60	↓	↑	ND	↓ ±	↓	ND	ND
Radin et al.	10 ScN	45	↓	=	↓	ND	ND	ND	↓
Li et al.	10 ScN	60	↓		=	ND	ND	ND	↓
Shi et al.	$TLR4^{-/-}$ F	60	↑	↑	↑	↓	↓	ND	ND
Shi et al.	$TLR4^{-/-}$M	60	=	=	=	↓	ND	ND	ND
Orr et al.	$TLR4^{-/-}$	45	↓	=	↓	↓ ±	↑ M2	ND	↓
Saberi et al.	$TLR4^{-/-}$	No data	↓	ND	ND	ND	ND	ND	ND
Ding et al.	$TLR4^{-/-}LDLR^{-/-}$	35.5	=	=	=	=	=	ND	ND
Kim et al.	$TLR4^{-/-}$	60	=	ND	ND	ND	ND	ND	ND
Saberi et al.	BMT-10ScN	No data	=	↑	=	↓	↓	↑	↓
Orr et al.	BMT-$TLR4^{-/-}$	45	=	=	ND	↓	↑ M2	ND	=
Coenen et al.	BMT-$TLR4^{-/-}$	41	=	=	ND	=	=	ND	ND

C3H/HeJ: Mice harboring a spontaneous missense mutation in the third exon in the Tlr4 gene leading to a loss-of-function of TLR4.

10ScN: Mice that display a spontaneous mutation resulting in 7 kb deletion in the Tlr4 gene that results in absence of both mRNA and protein expression.

$TLR4^{-/-}$: Mice with a knockout of the Tlr4 gene. F female. M male.

BMT-10ScN: Transplantation of bone marrow from 10ScN mice into C57BL6 mice.

BMT-$TLR4^{-/-}$: Transplantation of bone marrow from $TLR4^{-/-}$ mice into C57BL/6 mice (Orr et al) or into agouty (A^y/a)/LDL-receptor deficient mice (A^y/a;$Ldlr^{-/-}$) (Coenen et al.).

IS: Insulin Sensitivity.

ATM: Adipose tissue macrophages.

M2: macrophages with a M2 anti-inflammatory polarization.

ND: not determined in the study.

adipose tissue mass in female TLR4$^{-/-}$ mice (Shi et al., 2006b) or no protection against obesity in male TLR4$^{-/-}$ mice (Shi et al., 2006b; Kim et al., 2007). The majority of the studies have reported a reduction in the insulin resistance, at least in adipose tissue and liver (Shi et al., 2006b; Suganami et al., 2007a; Tsukumo et al., 2007; Poggi et al., 2007; Davis et al., 2008). However, some of them, mainly concerning male TLR4$^{-/-}$, did not show any improvement in whole-body insulin sensitivity (Shi et al., 2006b; Radin et al., 2008; Ding et al., 2012; Orr et al., 2012).

Different studies have investigated the consequences of TLR4 invalidation specifically in immune cells by transplantation of bone marrow from TLR4-deficient mice into wild-type recipients. In one study, mice developed obesity but with less inflammation and insulin resistance in adipose tissue and liver and no change in muscles (Saberi et al., 2009). In contrast, two other studies using the same experimental strategy but in different genetic backgrounds, failed to observe any improvement in insulin resistance despite a reduction in adipose tissue inflammation and/or a shift in adipose tissue macrophage polarization toward a M2 anti-inflammatory state (Coenen et al., 2009; Orr et al., 2012).

In summary, the consequences on weight gain and insulin resistance are different depending on the genetic background, the sex of the mice, the duration, and the lipid composition of the diet. In this regard, the percentage of saturated lipids in the diet could be important since the protection against insulin resistance seemed to occur selectively when the diet contained a high level of saturated lipids (Davis et al., 2008). It remains to be determined whether the activation of TLR4 signaling by a saturated fat diet is due to a direct action of fatty acids on TLR4 or to an indirect effect through the production of endogenous DAMPs or through the modification of the gut microbiota leading to an increase in circulating LPS. Further, clarification is needed for the respective contribution of TLR4 signaling in hematopoietic vs. non-hematopoietic compartment to the development of insulin resistance. Of note, the analysis of the metabolic phenotype of the TLR4-deficient mice could be complicated due to the potential compensatory increase in TLR2 expression (Ding et al., 2012).

TLR2 detects lipoproteins, lipoteichoic acid, and peptidoglycan from gram-positive bacteria and dimerizes with TLR1 and TLR6 (Mogensen, 2009). TLR2 has a broad pattern of expression and besides its expression in immune cells, is also expressed in insulin sensitive cells and islets (Ehses et al., 2010). A role for TLR2 in the metabolic complications of obesity was suggested by its increased level in different metabolic tissues of obese mice and patients and in circulating monocytes of type 2 diabetic patients (Fresno et al., 2011; Könner and Brüning, 2011). Like TLR4, TLR2 could be a sensor for saturated fatty acids mediating their pro-inflammatory effects in adipose tissue and macrophages and participating in the development of insulin resistance in cultured myotubes or adipocytes (Fresno et al., 2011; Könner and Brüning, 2011). However, as for TLR4, a direct activation of TLR2 by saturated fatty acids has been questioned owing to possible contamination by LPS (Erridge and Samani, 2009). Thus, both TLR4 and TLR2 could participate in the sensing of abnormal levels of nutrients, especially fatty acids and in the detection of gut microflora modification in obesity (**Figure 1**). Cooperation between these two TLRs might be involved since TLR4 activation increases the synthesis of TLR2 in adipocytes. This overlapping function could explain the similar metabolic phenotype of TLR4 and TLR2 deficient mice. Indeed, invalidation of TLR2 improved diet-induced insulin resistance and inflammation of adipose tissue, liver, or muscles (Caricilli et al., 2008; Kuo et al., 2011; Ehses et al., 2010 #3013, Himes and Smith, 2010 #3010). Further, TLR2 signaling is involved in diet-induced pancreatic islet inflammation and beta-cell dysfunction (Ehses et al., 2010). TLR2 knockout mice have also a reduced adiposity (Ehses et al., 2010; Himes and Smith, 2010; Kuo et al., 2011) suggesting that the lack of TLR2 signaling could decrease lipid uptake or increase lipid oxidation in different tissues. Whether the effect of TLR2 activation on lipid metabolism is direct or the consequence of an inflammatory state remains to be clarified. However, the reported interaction between activated TLR2 and CD36, a transporter of fatty acids, supports a role for TLR2 signaling in fatty acids uptake in metabolic tissues (Triantafilou et al., 2006).

Although the findings discussed above support a protective role of TLR2 inactivation in the context of obesity, a recent study reported that TLR2 knockout mice had a phenotype reminiscent of metabolic syndrome even on low-fat diet. In this setting, it was demonstrated that the gut microbiota was responsible for the development of insulin resistance (Caricilli et al., 2011). This finding illustrates the concept that complex interactions between environment, gut microbiota, and the genetic of the host drive the metabolic phenotype (Nicholson et al., 2012). In this regard, a specific environmental condition and the innate immune system may have shaped a harmful gut microbiota that overcomes the protective effect of the genetic deficiency in TLR2. Alternatively the loss of TLR in immune cells may alter gut microbiota leading to the development of inflammation, obesity, and insulin resistance as described for mice lacking TLR5 (Vijay-Kumar et al., 2010), a TLR highly expressed in the intestinal mucosa and involved in the detection of bacterial flagellin (Mogensen, 2009).

INFLAMMASOME AND NOD

Nucleotide oligomerization domain (NOD) 1 and 2 are intracellular proteins that recognize cell wall peptidoglycan moieties from gram-negative or gram-positive bacteria, respectively (Mogensen, 2009). NOD proteins have recently emerged as immune sensors involved in inflammation-induced insulin resistance (**Figure 1**). Peptidoglycan-induced activation of NOD1 in adipocytes or hepatocytes (Schertzer et al., 2011; Zhao et al., 2011) and NOD2 in muscle cells (Tamrakar et al., 2010) trigger insulin resistance through the production of inflammatory mediators and the activation of MAP kinases signaling leading to desensitization of IRS1 function. Injection of specific NOD1 ligand in mice promoted adipose tissue inflammation and induced whole-body insulin resistance with a strong decrease in insulin action in the liver. NOD2 ligand injection caused a milder insulin resistance and preferentially in muscles (Schertzer et al., 2011). NOD1-deficient mice, but not NOD2, were protected against glucose intolerance and diabetes induced by a high-fat diet (Amar et al., 2011). These studies demonstrate the ability of NOD activation to induce insulin resistance and support

the implication of NOD1 in the control of metabolic diseases through the sensing of components from gram-negative bacteria (**Figure 1**).

Inflammasomes are multi-protein complexes composed of three proteins: the nucleotide-binding domain leucine-rich repeat (NLR) protein, the adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD) and the caspase-1. Four different inflammasomes have been identified so far namely NLRP1, NLRP3, NLRC4, and AIM2. Pathogen and danger-associated signals activate inflammasomes leading to the processing of IL-1β and IL-18 by caspase-1 (Mogensen, 2009). It is recognized that IL-1β is one of the main cytokines implicated in the desensitization of insulin signaling (Lagathu et al., 2006; Jager et al., 2007) and its genetic invalidation protects mice against diet-induced insulin resistance (Stienstra et al., 2010; Wen et al., 2011). Pharmacological inhibition of IL-1β signaling by the IL-1 receptor antagonist anakinra, mitigates inflammation, and improves glycemic control in type 2 diabetic patients (Larsen et al., 2007). With this in mind, several groups have investigated the implication of inflammasome activation in insulin resistance.

The expression of NLRP3 and caspase-1 is increased in adipose tissue of obese mice, overweight subjects, or obese individuals with type 2 diabetes (Stienstra et al., 2010; Koenen et al., 2011; Vandanmagsar et al., 2011). The identity of cells within the adipose tissue, in which the NLRP3 inflammasome is activated, remains controversial. Two studies reported an expression and activation mainly in adipose tissue macrophages with a low expression in adipocytes whereas Stienstra et al. found an important contribution of adipocytes (Stienstra et al., 2010; Vandanmagsar et al., 2011; Wen et al., 2011). However, invalidation of different components of the NLRP3 inflammasome (NLRP3, ASC, caspase-1) univocally protected the mice against high-fat diet-induced inflammation and insulin resistance. This phenotype was associated with a reduced expression of IL-1β in adipose tissue and a reduced level of circulating IL-18 (Stienstra et al., 2010; Vandanmagsar et al., 2011; Wen et al., 2011). The lack of the NLRP3 inflammasome had consequences on the subset of immune cells within the adipose tissue. The number of M2 anti-inflammatory macrophages was increased in subcutaneous adipose tissue (SAT) and the activation of pro-inflammatory macrophages was dampened in visceral adipose tissue (VAT). In parallel, the number of naive $CD4^+$ and $CD8^+$ T cells was increased in SAT and the amount of effector memory $CD4^+$ and $CD8^+$ T cells was decreased in VAT (Vandanmagsar et al., 2011). These findings suggest a model whereby the NLRP3 inflammasome-dependent production of IL-1β and IL-18 by adipose tissue macrophages favors macrophage-T cell activation leading to a sustained inflammation of adipose tissue (Vandanmagsar et al., 2011). Besides its role in the control of adipose tissue inflammation, inflammasome activation in adipocytes could limit energy expenditure, fat oxidation, and adipogenesis as revealed by the phenotype of the caspase1$^{-/-}$ mice (Stienstra et al., 2010, 2011). In addition to adipose tissue, activation of NLRP3-inflammasome in other tissues such as pancreatic islets has been reported (Zhou et al., 2010).

These findings strongly support a model whereby danger signals generated in obesity are detected by the NLRP3-inflammasome that in turn promotes inflammation and the dysfunction of different organs involved in the control of glucose and lipid homeostasis (**Figure 1**). The identity of the danger signals that activate the NLRP3 inflammasome in obesity remains ill-defined. However, the observation that the NLRP3 inflammasome is activated by fatty acids and ceramides suggests that the lipotoxic environment in obesity might trigger its activation (Vandanmagsar et al., 2011; Wen et al., 2011). However, the NLRP3 inflammasome can be activated by other molecules such as ATP, glucose, oxidized LDL, uric acid, and crystals of cholesterol. Since all of them are elevated in obesity, their respective contributions to the activation of inflammasome deserve further investigation. The common feature of these danger signals is their ability to increase ROS production that is prerequisite for NLRP3 inflammasome activation (Tschopp and Schroder, 2010). Thus, the activation of NLRP3 inflammasome in obesity might be related to the oxidative stress that develops in the different metabolic tissues. How NLRP3 inflammasome is activated by ROS is not completely understood but the thioredoxin-interacting protein (TXNIP) has recently emerged as a potential link with the demonstration of its binding with NLRP3 in a ROS sensitive manner leading to NLRP3 inflammasome activation (Zhou et al., 2010) (**Figure 1**). This function might explain the similar phenotype between TXNIP- and NLRP3-deficient mice when fed a high-fat diet (Zhou et al., 2010). Further TXNIP might also connect organelle stress, such as reticulum endoplasmic stress, to NLRP3 inflammasome activation (Oslowski et al., 2012).

All together, these findings render the NLRP3 inflammasome as an attractive pharmacological target against the complications of obesity. In this multi-proteins complex, the easiest target is the caspase-1 since inhibitors already exist. The caspase-1 inhibitor Pralnacasan reduced body weight and improved insulin sensitivity of genetically obese *ob/ob* mice (Stienstra et al., 2010). However, one important caveat for the treatment of a chronic disease such as diabetes is that caspase-1 is involved in different inflammasome complexes and its inhibition may reduce the ability to fight infection.

Despite the potential role of the NLRP3 inflammasome in obesity-induced inflammation and insulin resistance, it remains to determine whether its activation is a primary event in the disease that drives the inflammation. Further, the relative contribution of NLRP3 inflammasome to the development of inflammation in the metabolic tissues and in the different subset of cells of these tissues should be clarified. The role of other inflammasome complexes also deserves investigation since ablation of NLRP3 markedly reduced but did not totally abrogate caspase-1 activation in adipose tissue or liver of obese mice (Vandanmagsar et al., 2011). Finally, other caspase-1 substrates besides IL-1β and IL-18 might be involved in the deleterious effect of inflammasome activation on metabolic control. In this regard, the transcription factors SREBPs are activated by caspase-1(Gurcel et al., 2006) and it was recently shown that SREBP-1a regulated the expression of inflammasome components in macrophages (Im et al., 2011). Thus, a feed-forward loop involving caspase-1 activation

and SREBP might link lipid metabolism to the inflammasome activation in obesity.

In conclusion, it is now recognized that the immune sensors described above (TLR, NOD, inflammasome) and others such as the pathogen-sensing kinase (PKR) (Nakamura et al., 2010) participate in the development of the metabolic inflammation. As recently discussed by Gregor and Hotamisligil in an outstanding review (Gregor and Hotamisligil, 2011), it is possible that in obesity the levels of nutrient intake may rise enough to stimulate pathogen- and danger-sensing pathways ultimately leading to the activation of immune cells in the different metabolic tissues. In other words, the organism, in overfeeding situation, recognizes the nutrients as harmful biological molecules and activates pathways that are usually engaged by pathogen or endogenous danger signals. In addition, modification of the gut microbiota and of the intestinal permeability in obesity may fuel the organism with inflammatory molecules such as LPS and other bacterial antigens or favor the translocation of commensal bacteria within metabolic tissues (Cani and Delzenne, 2009; Amar et al., 2011; Nicholson et al., 2012). As a consequence, inflammatory cytokines are overproduced and they activate different signaling pathways in metabolic cells that desensitize insulin signaling and alter the expression of proteins involved in glucose transporter trafficking (Kaddai et al., 2009).

INFLAMMATORY SIGNALING PATHWAYS INVOLVED IN THE DESENSITIZATION OF INSULIN ACTION

THE SOCS PROTEINS

The Suppressor of cytokine signaling (SOCS) protein family also named Janus family kinase-binding (JAB) proteins or SSI (signal transducer and activator of transcription induced Stat inhibitor) includes eight members (SOCS1–7 and CIS), which possess a SH2 domain, and a SOCS-box domain controlling the degradation of interacting proteins. They are induced by several inflammatory cytokines and are involved in a negative feedback loop leading to the termination of cytokines action. At the molecular level, the SOCS proteins interact with the tyrosine kinases Janus-activated kinases (JAK) or directly with the receptor of some cytokines, thus blocking the tyrosine phosphorylation of the transcription factors STAT for review see Lebrun and Van Obberghen (2008). Several cellular studies have demonstrated that SOCS negatively regulate the signaling pathway of hormones including leptin and insulin. In this regard, SOCS3 is induced by leptin and insulin and is involved in a negative feedback loop and in a cross-down regulation (Emanuelli et al., 2000; Lebrun and Van Obberghen, 2008; Benomar et al., 2009). SOCS1, SOCS6, and SOCS7 are also involved in the desensitization of insulin signaling. SOCS3 inhibits insulin signaling by a direct binding through its SH2 domain with the juxtamembrane phosphotyrosine 960 on the insulin receptor, thus preventing the interaction of IRS1 and 2 with the receptor (**Figure 2**). SOCS1 interacts with the catalytic domain of the insulin receptor which contains an interaction motif for IRS2, blocking thus more selectively the tyrosine phosphorylation of IRS2. SOCS1 and SOCS6 also inhibit the tyrosine kinase activity of the insulin receptor. It has also been shown that SOCS proteins interact with the tyrosine phosphorylated IRS1 and IRS2 resulting in their ubiquitination and degradation by the proteasome (Lebrun and Van Obberghen, 2008) (**Figure 2**).

In obesity, inflammation leads to an up-regulation of SOCS proteins in hypothalamus, liver, muscles, and adipose tissue (Rieusset et al., 2004; Lebrun and Van Obberghen, 2008). A causal role for the up-regulation of SOCS proteins in the development of insulin resistance has been investigated in metabolic tissues. Overexpression of SOCS1 or SOCS3 in mouse liver or adipose tissue reduced the expression of IRS1 or IRS2 as well as their tyrosine phosphorylation induced by insulin. As a consequence insulin resistance developed in those tissues, as well as systemic insulin resistance for the overexpression in liver (Ueki et al., 2005; Shi et al., 2006a). Unexpectedly, overexpression of SOCS3 in adipose tissue protected the mice against systemic insulin resistance when fed a high-fat diet owing to a decrease in adipocyte hypertrophy (Shi et al., 2006a). Overexpression of SOCS3 in muscle exacerbated diet-induced obesity and insulin resistance but this effect was not due to a decreased insulin signaling but to an alteration in muscle integrity leading to a reduction in locomotor activity and energy expenditure (Lebrun et al., 2009).

The inhibition of SOCS proteins might thus be useful to prevent the development of obesity-induced insulin resistance. In agreement with such a possibility, heterozygous SOCS3 ($SOCS3^{+/-}$) mice or mice with targeted invalidation of SOCS3 in the central nervous system (CNS) were protected against diet-induced obesity and associated insulin resistance (Howard et al., 2004; Mori et al., 2004; Kievit et al., 2006). This phenotype was explained by increased leptin sensitivity and possibly by an effect on insulin action for the $SOCS3^{+/-}$ mice (Howard et al., 2004). In the same way, targeted invalidation of SOCS3 in adipose tissue or in muscles protected mice against obesity-induced insulin resistance (Jorgensen et al., 2012; Palanivel et al., 2012). SOCS7-deficient mice also displayed improved glucose tolerance and insulin sensitivity (Banks et al., 2005). However, several considerations should be taken into account before inhibiting SOCS proteins for therapeutic purpose. First, some SOCS proteins positively regulate insulin action as demonstrated by the improved insulin sensitivity of SOCS6-overexpressing mice (Li et al., 2004). Second, given that SOCS proteins negatively regulate the signaling of inflammatory cytokines, their chronic inhibition might exacerbate inflammation that could counterbalance its beneficial effect on insulin sensitivity. This dual action of SOCS proteins is well-illustrated by differential effects of short- and long-term invalidation of SOCS1 or SOCS3 in liver. Short-term invalidation by antisense oligonucleotides in obese and diabetic *db/db* mice improved hepatic steatosis with a mild reduction in insulin resistance (Ueki et al., 2005). In contrast, while targeted deletion of SOCS3 in liver-enhanced liver insulin sensitivity on chow diet, it accelerated the onset of high-fat diet- or age-induced insulin resistance with an increased inflammation in liver (Torisu et al., 2007; Sachithanandan et al., 2010). This dual action is not restricted to SOCS3, since SOCS1 in immune cells limited the metabolic inflammation in liver and perhaps adipose tissue (Sachithanandan et al., 2011). Thus, the beneficial effect of SOCS1-deletion on insulin sensitivity

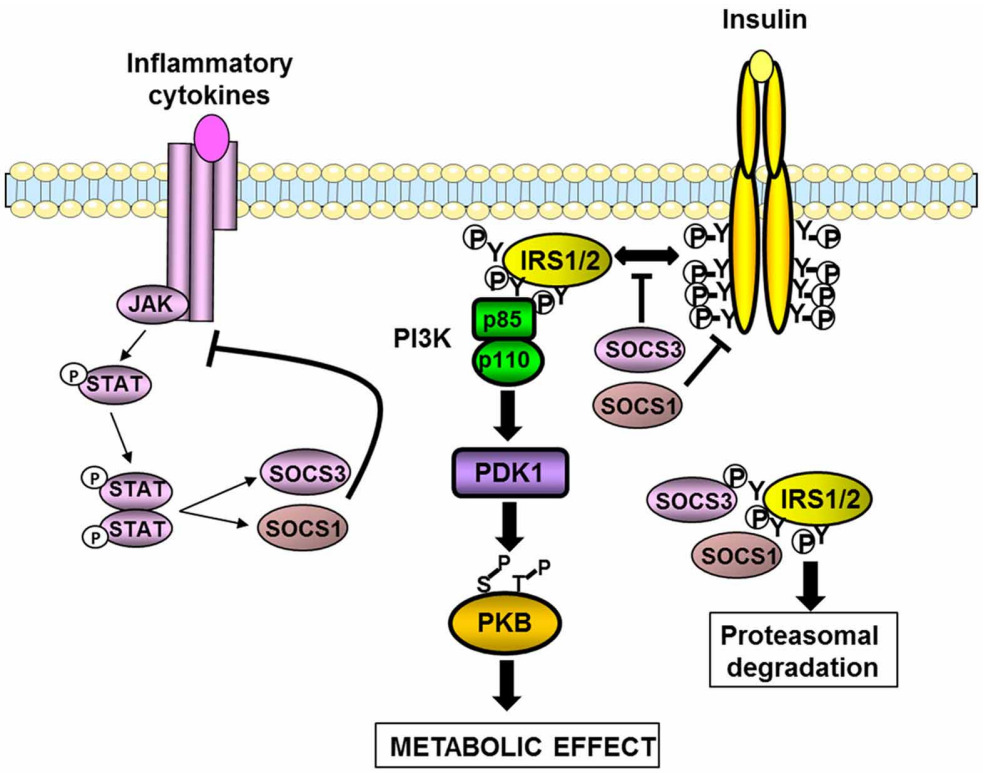


FIGURE 2 | Inhibition of insulin signaling pathway by SOCS1 and SOCS3. SOCS1 and SOCS3 are induced by cytokines and involved in a negative feed-back loop. SOCS1 and SOCS3 also inhibit insulin signaling by different mechanisms. They interfere with the binding between the insulin receptor and IRS1/2 proteins. SOCS1 also inhibits the tyrosine kinase activity of the insulin receptor. Both SOCS1 and SOCS3 can interact with the tyrosine-phosphorylated IRS proteins leading to their degradation by the proteasome. The resulting effect is a decrease in the insulin-induced activation of the IRS1/2-PI3K-PKB axis leading to a reduction in the metabolic effects of insulin.

was only visible when SOCS1-deficient mice were crossed with interferon-gamma- or RAG2-deficient mice to limit inflammation. Of note, this beneficial effect was seen on chow diet (Jamieson et al., 2005; Emanuelli et al., 2008b) but was lost on a high-fat diet when the inflammation is enhanced (Emanuelli et al., 2008b). Further, SOCS2-deficient mice were protected against diet-induced hepatic steatosis probably due to an increase in the lipid mobilizing effect of growth hormone. However, this beneficial effect was overridden by a hyperproduction of inflammatory mediators by the resident macrophages leading to a higher level of inflammation in liver and adipose tissue and the worsening of insulin resistance (Zadjali et al., 2012).

In summary, it appears to date that SOCS3 is mainly involved in the regulation of energy balance through the down-regulation of leptin signaling whereas several SOCS such as SOCS1/3/6/7 are involved in the regulation of the insulin sensitivity. Since SOCS proteins contribute to the development of diet-induced obesity and insulin resistance, their targeting could be open new avenues for the treatment of metabolic disorders. However, careful examination of the balance between pro-inflammatory and insulin sensitizing effects of future inhibitors of SOCS proteins will be needed.

THE IKKβ/NF-κB AND JNK PATHWAYS IN INFLAMMATORY CYTOKINES PRODUCTION AND INSULIN RESISTANCE

The activity of both IκB-kinase β (IKKβ) and JNK is elevated in metabolic tissues in obesity, and these kinases are important nodes in the production of inflammatory mediators and in the desensitization of insulin signaling (Tanti and Jager, 2009; Solinas and Karin, 2010; Donath and Shoelson, 2011). JNK and IKKβ are activated downstream of immune sensors such as TLRs and participate in the production of inflammatory cytokines via the transcription factors AP-1 and NF-κB respectively (**Figure 3**). Many of the produced inflammatory cytokines are able to activate these two kinases leading to a feed-forward amplification loop (Donath and Shoelson, 2011; Gregor and Hotamisligil, 2011). Another important activator of these kinases is the endoplasmic reticulum (ER) stress (**Figure 3**).

ER stress occurs when the synthesis capacity of the ER is exceeded. In this case, the unfolded protein response (UPR) is activated in order to restore ER homeostasis. Three pathways are involved in the UPR, namely PERK (PKR-like eukaryotic initiation factor 2a), IRE-1 (inositol requiring enzyme 1), and ATF6 (activating transcription factor 6) pathways. If these mechanisms fail to restore proper ER homeostasis, cells undergo apoptosis (Xu et al., 2005). In a pioneer study, Hotamisligil and colleagues

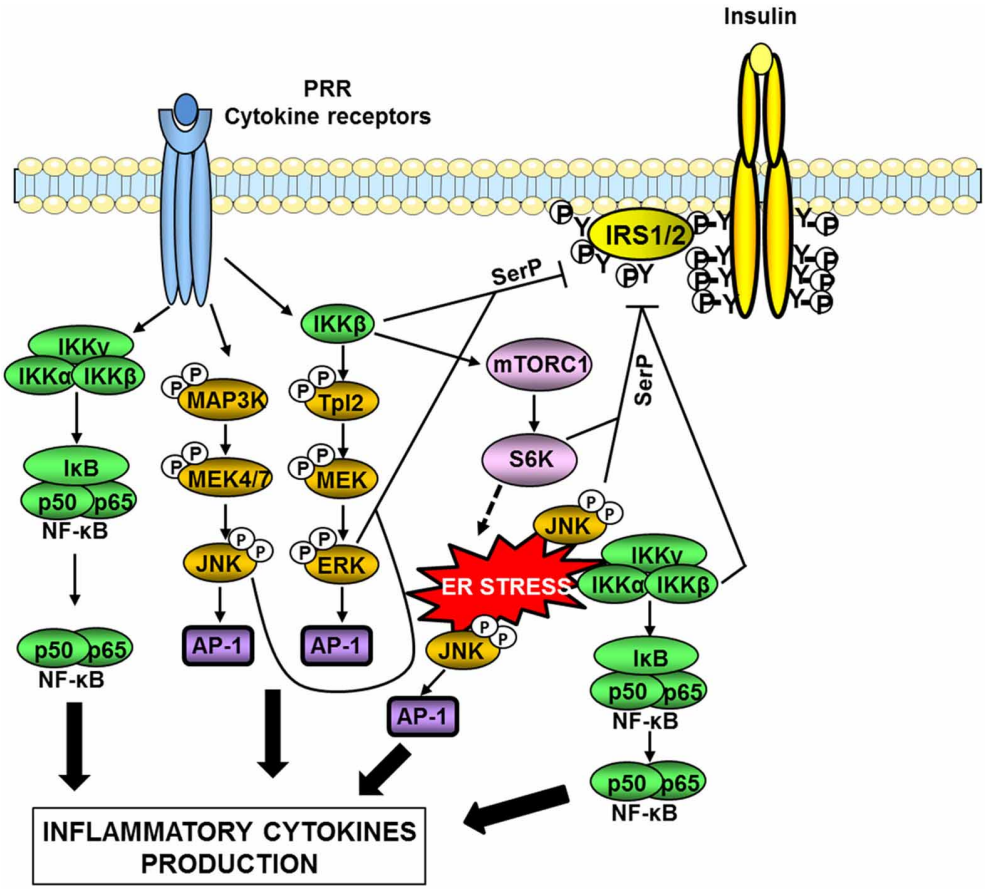


FIGURE 3 | Serine kinases involved in obesity-induced inflammation and insulin resistance. In obesity, a network of serine kinases is activated including IKKβ, JNK, and ERK1/2. JNK and IKKβ are activated downstream of pattern-recognition receptors (PRRs) such as TLRs or by ER stress. IKKβ is also involved in the activation of the Tpl2/ERK pathway. These pathways participate in the production of inflammatory cytokines via the transcription factors AP-1 and NF-κB. Many of the produced inflammatory cytokines are able to activate these kinases leading to a feed-forward amplification loop. JNK and ERK1/2 are involved in the desensitization of insulin signaling through phosphorylation of IRS1/2 on inhibitory serine sites (SerP). IKKβ can directly phosphorylate IRS1/2 on serine sites but can also act indirectly through activation of mTORC1/S6 kinase. Over-activation of the mTORC1/S6K pathway could promote ER stress leading to an amplification loop.

(Ozcan et al., 2004) have demonstrated that ER stress developed in liver and adipose tissue during obesity owing to nutrient overload and participated in the onset of insulin resistance. Several recent reviews have discussed in details the mechanisms linking ER stress to obesity-induced inflammation, insulin resistance, and alterations in tissue metabolism (Cnop et al., 2011; Gregor and Hotamisligil, 2011; Flamment et al., 2012). The activation of IKKβ/NF-κB and JNK by the IRE-1 arm of the UPR is one of these mechanisms (Gregor and Hotamisligil, 2011).

At the molecular level, one important mechanism by which IKKβ and JNK attenuate insulin signaling is the phosphorylation of IRS proteins on inhibitory serine phosphorylation sites (**Figure 3**). The mechanism by which IRS phosphorylation regulates insulin signaling is complex and described in details in recent reviews (Gual et al., 2005; Boura-Halfon and Zick, 2009; Tanti and Jager, 2009; Copps and White, 2012). In physiological condition, insulin induces a time-controlled phosphorylation of both positive and inhibitory serine sites in IRS1 and IRS2 in order to ensure the fine tuning of IRS tyrosine phosphorylation that is necessary for the propagation of insulin action (Tanti and Jager, 2009; Copps and White, 2012). Since our pioneer study (Tanti et al., 1994), it is now admitted that during obesity, activation of inflammatory, and stress kinases such as JNK and IKK is responsible for an uncontrolled phosphorylation of IRS on inhibitory serine sites resulting in a decrease in IRS tyrosine phosphorylation and a desensitization of insulin signaling (Boura-Halfon and Zick, 2009; Tanti and Jager, 2009). It is noteworthy that JNK seems more involved in the direct IRS serine phosphorylation than IKKβ (Tanti and Jager, 2009). IKKβ activation could promote IRS1 serine phosphorylation through activation of TSC1/TSC2/mTORC1/S6 Kinase-1 pathway leading to inhibitory IRS1 serine phosphorylation by S6K1 (Lee et al., 2008). Of note, activation of mTORC1 by IKKβ could be also involved in feed-forward mechanism induced by inflammatory cytokines to promote ER stress since over-activation of mTORC1 has been linked to the development of ER stress (Ozcan et al., 2008)

(**Figure 3**). In addition, activation of the IKKβ/NFκB pathway increases the expression of PTP1B, a tyrosine phosphatase that dephosphorylates IRS1 (Zabolotny et al., 2008).

Several *in vivo* studies in mice have demonstrated the importance of the IKKβ/NF-κB and JNK pathways in the development of insulin resistance (**Figures 4**, **5**). Heterozygous IKKβ and whole-body JNK1-deficient mice were partially protected against diet-induced insulin resistance (Yuan et al., 2001; Hirosumi et al., 2002). JNK2 could also play a role in insulin resistance but to a lesser extent (Tuncman et al., 2006). A cell-autonomous mechanism that involves the negative regulation of IRS1 function by serine phosphorylation was implicated in the regulation of insulin resistance induced by JNK1 and possibly by IKKβ during obesity (Hirosumi et al., 2002; Sabio et al., 2010b). IKKβ haplo-insufficiency or JNK1invalidation could also reduce the pro-inflammatory effect of high-fat diet. In this regard, mice lacking IKKβ or JNK1 in immune cells were partially protected against obesity-induced inflammation (Arkan et al., 2005; Solinas et al., 2007; Vallerie et al., 2008). However, if the protection against systemic insulin resistance was obvious for IKKβ deletion (Arkan et al., 2005), contradictory results were reported for JNK1 invalidation (Solinas et al., 2007; Sabio et al., 2008; Vallerie et al., 2008).

The picture that emerges is that activation of these pathways in non-hematopoietic cells also participates in tissues inflammation and local or systemic insulin resistance. However, the consequences of IKKβ and JNK activation on the development of insulin resistance could totally differ and depend on the site of action, the level of expression and the impact on adiposity. Indeed, the activation of IKKβ/NF-κB in hepatocytes-induced liver inflammation and insulin resistance (Arkan et al., 2005; Cai et al., 2005; Tamura et al., 2007; Wunderlich et al., 2008) and was associated with a reduced ability of insulin to suppress neoglucogenesis (Arkan et al., 2005) and with an increased production of VLDL leading to the development of hypertriglyceridemia (van Diepen et al., 2011). At the opposite of these findings, the development of inflammation induced by a moderate IKKβ activation in adipose tissue and before the onset of obesity has been shown to be protective against diet-induced insulin resistance by limiting adipose tissue expansion and by increasing energy expenditure (Jiao et al., 2012). Furthermore, in contrast to the deleterious effect of IKKβ, the activation of JNK pathway in liver

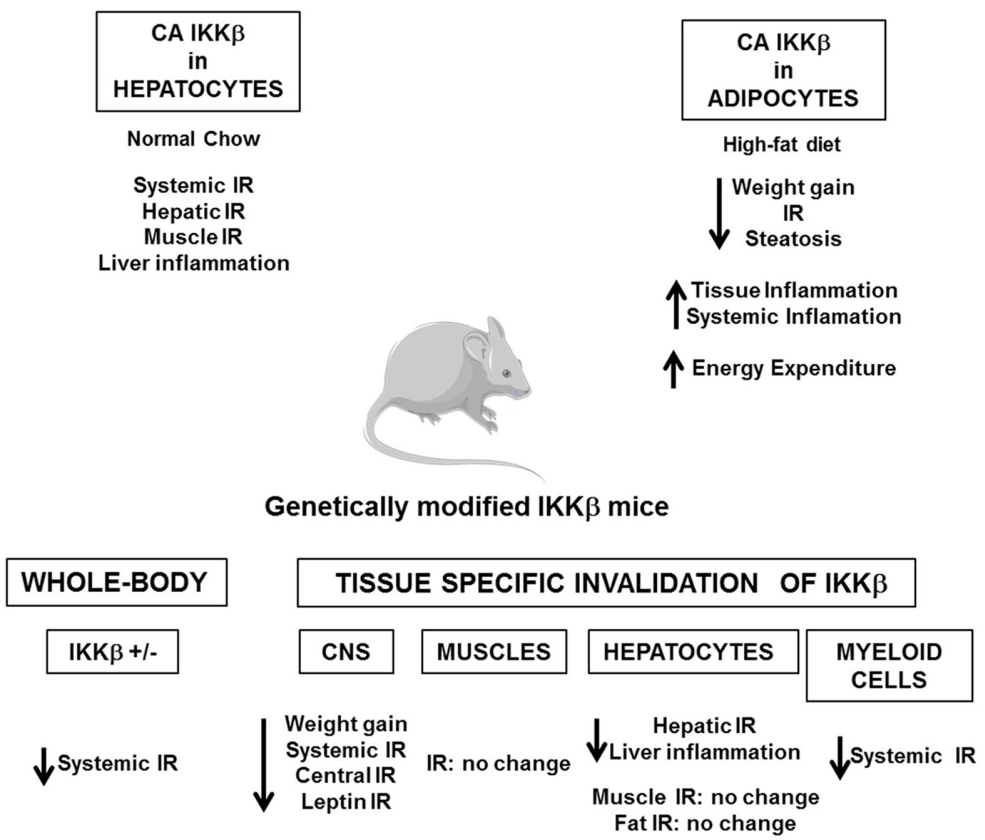


FIGURE 4 | Consequences of the genetic manipulation of IKKβ on the metabolic phenotype of mice. Constitutive activation of IKKβ (CA IKKβ) in hepatocytes results in a deterioration of the insulin sensitivity of lean mice. At the opposite constitutive activation of IKKβ in adipocytes protects the mice against obesity and insulin resistance when fed a high-fat diet. Study of heterozygote IKKβ mice ($IKK\beta^{+/-}$) or of mice with a tissue specific inactivation of IKKβ in the central nervous system (CNS), hepatocytes, myeloid cells, or muscles demonstrates that activation of IKKβ/NF-κB pathway is a core mechanism that connects metabolic inflammation and insulin resistance in peripheral metabolic tissues, except muscles, and in the central nervous system.

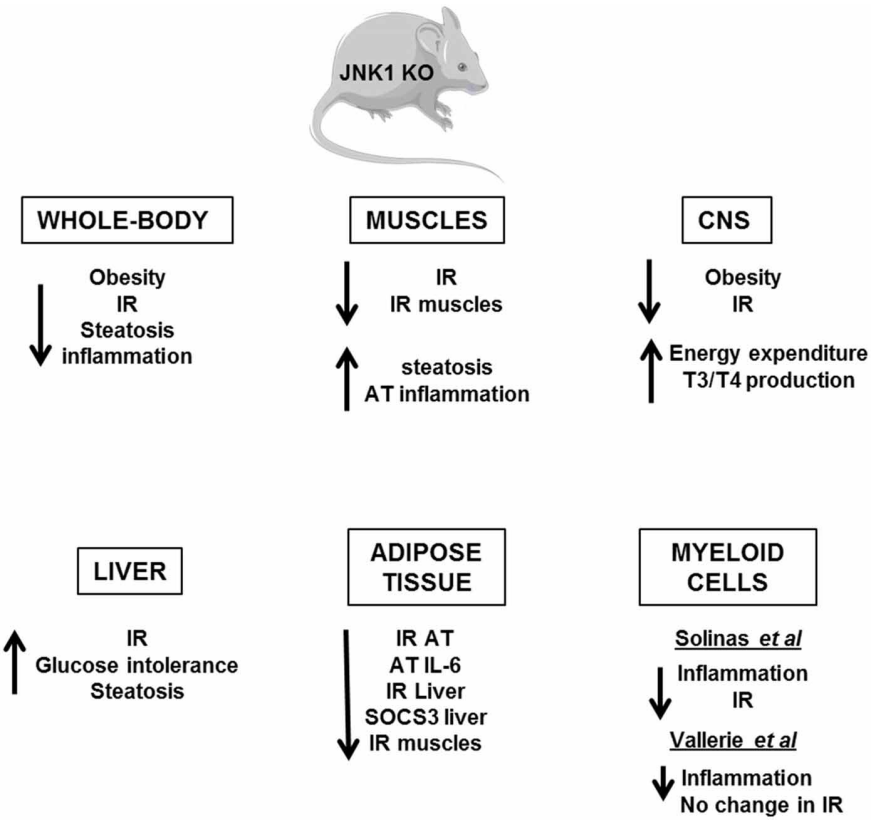


FIGURE 5 | Metabolic phenotype of whole-body JNK1 knockout mice and of conditional JNK1 knockout mice. Whole-body invalidation of JNK1 protects mice against diet-induced obesity and insulin resistance. Tissue-specific invalidation of JNK1 reveals that the lack of JNK1 in adipose tissue, muscles, or CNS protects against the development of obesity and/or insulin resistance. The lack of JNK1 in myeloid cells decreases obesity-induced inflammation but the impact on insulin resistance development is controversial. At the opposite, the lack of JNK1 in the hepatocytes alters liver function suggesting a protective role of JNK1.

seems to have a protective role since hepatocyte-specific invalidation of JNK1 led to the development of glucose intolerance, insulin resistance, and liver steatosis even in lean mice (Sabio et al., 2009). This finding was quite unexpected given that the liver of obese whole-body JNK1-deficient mice was more insulin sensitive with less inflammation. Thus, it is possible that the inhibition of JNK1 in hepatocytes *per se* is detrimental whereas the combined inhibition of JNK1 in hepatocytes and in other liver cells of the liver has a protective effect. Further, organ to organ communication, especially cross-talk between liver and adipose tissue, could be another explanation since JNK1 invalidation in adipose tissue ameliorated liver insulin sensitivity (Sabio et al., 2008; Zhang et al., 2011). One important mediator of this cross-talk could be the adipocytokines, especially IL-6, which altered liver insulin sensitivity through induction of SOCS3 (Sabio and Davis, 2010). The consequences of JNK1 or IKKβ activation in muscles are also totally different. While inactivation of IKKβ in cultured muscle cells markedly reduced cytokine-induced insulin resistance (Austin et al., 2008), the study of mice with conditional knockout of IKKβ in muscles argues against a major role of muscular IKKβ activation in obesity-associated insulin resistance (Rohl et al., 2004). Muscle-specific invalidation of JNK1 improved high-fat diet-induced muscles insulin resistance but also led to an enhanced liver steatosis and to a mild increase in inflammatory mediator expression in adipose tissue. The cross-talk between these different organs was mediated by an increase in circulating triglycerides owing to a reduction in lipoprotein lipase expression in muscles (Sabio et al., 2010b). Systemic insulin sensitivity was slightly improved in those mice suggesting that the improved insulin sensitivity in muscles was sufficient to overcome the enhanced liver steatosis and adipose tissue inflammation. However, it is possible that with age, a worsening in insulin sensitivity develops.

Several evidences suggest that activation of IKKβ and JNK pathways in the hypothalamus by over-nutrition contributes to energy imbalance and weight gain in addition to their role in the development of insulin resistance. At the molecular level, ER stress that develops in hypothalamus owing to an oversupply in nutriments activates the IKKβ/NF-κB pathway leading to local SOCS3 expression that interferes with both insulin and leptin signaling (Zhang et al., 2008). The mechanism by which JNK1 activation in the nervous system regulates body mass is different and complex. Two studies have revealed that invalidation of JNK1 in the nervous system markedly enhanced the

production of thyroid hormones through the hypothalamic-pituitary-thyroid axis leading to an increase in energy expenditure and a protection against obesity (Belgardt et al., 2010; Sabio et al., 2010a).

These studies support the idea that the over-activation of IKKβ/NF-κB and JNK pathways is a core mechanism that connects metabolic inflammation and insulin resistance both in peripheral tissues and in the CNS. This central role highlights IKKβ and JNK as potential pharmacological targets against the development of insulin resistance. In this regard, Shoelson and colleagues have shown that high-doses of salicylate and its derivative salsalate were able to inhibit IKKβ activity and to improve insulin sensitivity in obese mice (Donath and Shoelson, 2011). Importantly, recent proof of concept studies and clinical trials in type 2 diabetic patients suggest that salsalate ameliorates glucose tolerance through better insulin sensitivity, and/or *via* an increased insulin secretion (Koska et al., 2009; Goldfine et al., 2010). The consequences of the pharmacological targeting of JNK have been studied only in mice. Competitive inhibitors of ATP directed against JNK and specific small substrate-competitive inhibitors of JNK displayed protective effects against diet-induced insulin resistance and/or weight gain (Bogoyevitch and Arthur, 2008; Cho et al., 2008; Yang and Trevillyan, 2008). These results suggest that IKKβ or JNK inhibitors could be interesting therapeutic agents against insulin resistance and type 2 diabetes. However, it should be kept in mind that IKKβ/NF-κB pathway is a central regulator of immunity and that JNK pathway regulates numerous physiologic processes. Chronic inhibition of these pathways might thus have side effects and favor the emergence of other pathologies.

THE EXTRACELLULAR SIGNAL-REGULATED KINASES IN OBESITY DEVELOPMENT AND INSULIN RESISTANCE

The Extracellular signal-Regulated Kinases (ERK) 1/2 (also known as p44 and p42 MAP kinase) are activated by several growth factors but also by inflammatory cytokines. The activation of ERK1/2 requires the phosphorylation of both tyrosine and threonine residues located in a TEY sequence. This phosphorylation is mediated by the MAP kinase kinase (MAP2K) MEK. The activation of MEK also requires its phosphorylation by MAP kinase kinase kinase (MAP3K). Depending on the stimuli, different MAP3Ks are engaged to phosphorylate MEK. The activated ERK1/2 phosphorylates numerous substrates with serine or threonine residues close to a proline residue (Keshet and Seger, 2010). The activity of ERK1/2 is increased in adipose tissue, liver, and muscles of obese/diabetic patients or mice for review see Tanti and Jager (2009). Several cellular studies have shown that activation of ERK1/2 by diabetogenic factors-induced IRS1 serine phosphorylation. These phosphorylation events decrease the interaction between IRS1 and the PI3K or inhibit the association between IRS1 and the insulin receptor and would thus diminish the metabolic effects of insulin (Tanti and Jager, 2009). This mechanism has relevant implications in human pathology since basal ERK activity and IRS1 phosphorylation are abnormally increased in primary muscle cells from type 2 diabetic patients (Bouzakri et al., 2003). In addition, activation of the ERK pathway by the inflammatory cytokines, especially IL-1β, in adipocytes also induced a decrease in the transcription of IRS-1 mRNA, leading to a decrease in insulin signaling and glucose transport (Jager et al., 2007). ERK activation by inflammatory cytokines could also indirectly promote insulin resistance by the stimulation of adipocyte lipolysis and the release of free fatty acids (Souza et al., 2003).

The contribution of ERK pathway in the development of obesity and insulin resistance was first demonstrated by our study of ERK1-deficient mice (Bost et al., 2005). Those mice were protected against obesity when fed a high-fat diet, because of a decrease in adipogenesis and an increase in postprandial energy expenditure. The lack of obesity was associated with a better glucose and insulin tolerance compared to wild-type mice (Bost et al., 2005). Conversely, over-activation of the ERK pathway owing to deletion of the signaling adapter p62 resulted in the development of mature-onset obesity and insulin resistance with reduced energy expenditure and increased adipogenesis (Rodriguez et al., 2006). This phenotype was probably due to the over-activation of ERK1 rather than ERK2 since deletion of ERK1 in the $p62^{-/-}$ genetic background reversed the phenotype (Lee et al., 2010). Those studies clearly highlighted the role of ERK1 in the development of obesity but did not allow concluding whether ERK1 could modulate the insulin sensitivity independently of its effect on body weight. However, we have recently shown that invalidation of ERK1 protected obese *ob/ob* mice against insulin resistance and adipose tissue inflammation without any changes in obesity (Jager et al., 2011). Another study also suggested that inhibition of ERK had beneficial effects on insulin resistance independently of an effect on body weight gain (Emanuelli et al., 2008a).

The pharmacological targeting of ERK1/2 against insulin resistance could have a series of drawbacks since they are involved in numerous biological processes. A possible alternative choice would be to target proteins which control ERK activity, specifically in response to inflammatory stresses which develop during obesity. In this regard, interesting candidates could be specific MAP3 kinases that are important in innate immune receptor signaling to MAP kinase activation (Symons et al., 2006).

Among the different MAP3K that control ERK activity, our team recently identified the kinase Tpl2 (Tumor progression locus, MAP3K8) as a potential new player in adipose tissue dysfunction and inflammation (Jager et al., 2010). In immune cells, Tpl2 plays an important role in the production of inflammatory cytokines, especially TNFα (Dumitru et al., 2000), and it is also involved in cytokines signaling downstream of innate immunoreceptors (Gantke et al., 2011). The importance of Tpl2 in inflammation has been demonstrated by the resistance to endotoxin shock and the lack of TNF-α production by macrophages of the Tpl2-deficient mice (Dumitru et al., 2000). Further, Tpl2 is a critical regulator of pancreatic, lung, and bowel inflammation in mice (Gantke et al., 2011). In non-stimulated cells, Tpl2 binds in its inactive form to p105NF-κB and is activated by different inflammatory stimuli through phosphorylation and degradation of p105NF-κB induced by IKKβ (Gantke et al., 2011). Among them, LPS, TNF-α, IL-1β, and CD40 are involved in obesity-induced inflammation and insulin resistance (Poggi et al., 2009; Tanti and Jager, 2009) suggesting a potential role for Tpl2 in this

pathology. Recently, we found Tpl2 up-regulated in adipose tissue of obese mice and patients. Tpl2 was involved in the lipolytic effect the inflammatory cytokines and in the serine phosphorylation of IRS1 in adipocytes (Jager et al., 2010). Based on these findings, one can hypothesize that the targeting of Tpl2 could have beneficial effects in the context of obesity by reducing the production of inflammatory cytokines by adipose tissue immune cells and by blocking their deleterious effects in adipocytes. In agreement, one recent study has shown that Tpl2 inactivation protected the mice against insulin resistance with a reduction in liver and adipose tissue inflammation (Perfield et al., 2011). However, another study failed to confirm this finding (Lancaster et al., 2012). Thus, further studies are needed to conclude whether the targeting of Tpl2 could improve the complications of obesity.

CONCLUSION

The discovery that metabolic diseases are associated with a low-grade inflammatory state has opened a new area of research to understand how inflammation develops and how it impact on metabolic pathways. It appears that a cross-talk between immune cells and metabolic cells plays a central role in the disturbance of metabolic homeostasis. High levels of dietary saturated fatty acids or of their metabolites can be detected by immune sensors such as TLR or inflammasome leading to the synthesis of inflammatory cytokines in different metabolic tissues. Dietary fat can also modify the intestinal microbiota that produces different inflammatory molecules leading to an inappropriate immune reaction. The inflammatory cytokines, saturated fatty acids, and LPS activate a network of signaling pathways that impinges on insulin signaling leading to alterations in metabolic cell functions. Hence, the importance of immune sensors and of different kinases suggests that new strategies targeting these proteins could be conceived to improve the metabolic complications of obesity.

ACKNOWLEDGMENTS

We thank Drs. Mireille Cormont and Yannick Le Marchand-Brustel for helpful suggestions and critical reading of the manuscript. We would like to apologize to colleagues for works which were not cited due to space limitation.

Author's work was supported by INSERM and CNRS and the University of Nice- Sophia Antipolis (France). Jean-François Tanti acknowledges support from ALFEDIAM-Abbott Laboratory, the French National Research Agency (grant ANR-2010-BLAN-1117-01) and the European Commission (Brussels, Belgium) (Contract LSHM-CT-2005–018734, Hepatic and Adipose Tissue and Functions in the Metabolic Syndrome, HEPADIP). Franck Ceppo is supported by a fellowship from the Region Provence Alpes-Cote d'Azur. Flavien Berthou is supported by the ANR grant ANR-2010-BLAN-1117-01.

REFERENCES

Amar, J., Chabo, C., Waget, A., Klopp, P., Vachoux, C., Bermudez-Humaran, L. G., et al. (2011). Intestinal mucosal adherence and translocation of commensal bacteria at the early onset of type 2 diabetes: molecular mechanisms and probiotic treatment. *EMBO Mol. Med.* 3, 559–572.

Arkan, M. C., Hevener, A. L., Greten, F. R., Maeda, S., Li, Z. W., Long, J. M., et al. (2005). IKK-beta links inflammation to obesity-induced insulin resistance. *Nat. Med.* 11, 191–198.

Austin, R. L., Rune, A., Bouzakri, K., Zierath, J. R., and Krook, A. (2008). siRNA-mediated reduction of inhibitor of nuclear factor-kappaB kinase prevents tumor necrosis factor-alpha-induced insulin resistance in human skeletal muscle. *Diabetes* 57, 2066–2073.

Banks, A., Li, J., McKeag, L., Hribal, M., Kashiwada, M., Accili, D., et al. (2005). Deletion of SOCS7 leads to enhanced insulin action and enlarged islets of Langerhans. *J. Clin. Invest.* 115, 2462–2471.

Belgardt, B. F., Mauer, J., Wunderlich, F. T., Ernst, M. B., Pal, M., Spohn, G., et al. (2010). Hypothalamic and pituitary c-Jun N-terminal kinase 1 signaling coordinately regulates glucose metabolism. *Proc. Natl. Acad. Sci. U.S.A.* 107, 6028–6033.

Benomar, Y., Berthou, F., Vacher, C. M., Bailleux, V., Gertler, A., Djiane, J., et al. (2009). Leptin but not ciliary neurotrophic factor (CNTF) induces phosphotyrosine phosphatase-1B expression in human neuronal cells (SH-SY5Y): putative explanation of CNTF efficacy in leptin-resistant state. *Endocrinology* 150, 1182–1191.

Bertola, A., Ciucci, T., Rousseau, D., Bourlier, V., Duffaut, C., Bonnafous, S., et al. (2012). Identification of adipose tissue dendritic cells correlated with obesity-associated insulin-resistance and inducing Th17 responses in mice and patients. *Diabetes* 61, 2238–2247.

Bogoyevitch, M. A., and Arthur, P. G. (2008). Inhibitors of c-Jun N-terminal kinases: JuNK no more? *Biochim. Biophys. Acta* 1784, 76–93.

Bost, F., Aouadi, M., Caron, L., Even, P., Belmonte, N., Prot, M., et al. (2005). The extracellular signal-regulated kinase isoform ERK1 is specifically required for *in vitro* and *in vivo* adipogenesis. *Diabetes* 54, 402–411.

Boura-Halfon, S., and Zick, Y. (2009). Phosphorylation of IRS proteins, insulin action, and insulin resistance. *Am. J. Physiol. Endocrinol. Metab.* 296, E581–E591.

Bouzakri, K., Roques, M., Gual, P., Espinosa, S., Guebre-Egziabher, F., Riou, J. P., et al. (2003). Reduced activation of phosphatidylinositol-3 kinase and increased serine 636 phosphorylation of insulin receptor substrate-1 in primary culture of skeletal muscle cells from patients with type 2 diabetes. *Diabetes* 52, 1319–1325.

Burcelin, R., Serino, M., Chabo, C., Blasco-Baque, V., and Amar, J. (2011). Gut microbiota and diabetes: from pathogenesis to therapeutic perspective. *Acta Diabetol.* 48, 257–273.

Cai, D., Yuan, M., Frantz, D. F., Melendez, P. A., Hansen, L., Lee, J., et al. (2005). Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat. Med.* 11, 183–190.

Cani, P. D., and Delzenne, N. M. (2009). The role of the gut microbiota in energy metabolism and metabolic disease. *Curr. Pharm. Des.* 15, 1546–1558.

Caricilli, A., Picardi, P., de Abreu, L. L., Ueno, M., Prada, P., Ropelle, E., et al. (2011). Gut microbiota is a key modulator of insulin resistance in TLR 2 knockout mice. *PLoS Biol.* 9:e1001212. doi: 10.1371/journal.pbio.1001212

Caricilli, A. M., Nascimento, P. H., Pauli, J. R., Tsukumo, D. M., Velloso, L. A., Carvalheira, J. B., et al. (2008). Inhibition of toll-like receptor 2 expression improves insulin sensitivity and signaling in muscle and white adipose tissue of mice fed a high-fat diet. *J. Endocrinol.* 199, 399–406.

Cho, H., Black, S. C., Looper, D., Shi, M., Kelly-Sullivan, D., Timofeevski, S., et al. (2008). Pharmacological characterization of a small molecule inhibitor of c-Jun kinase. *Am. J. Physiol. Endocrinol. Metab.* 295, E1142–E1151.

Cnop, M., Foufelle, F., and Velloso, L. A. (2011). Endoplasmic reticulum stress, obesity and diabetes. *Trends Mol. Med.* 18, 59–68.

Coenen, K., Gruen, M., Lee-Young, R., Puglisi, M., Wasserman, D., and Hasty, A. (2009). Impact of macrophage toll-like receptor 4 deficiency on macrophage infiltration into adipose tissue and the artery wall in mice. *Diabetologia* 52, 318–328.

Copps, K. D., and White, M. F. (2012). Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2. *Diabetologia* 55, 2565–2582.

Davis, J. E., Gabler, N. K., Walker-Daniels, J., and Spurlock, M. E. (2008). Tlr-4 deficiency selectively protects against obesity induced by diets high in saturated fat. *Obesity (Silver Spring)* 16, 1248–1255.

Ding, Y., Subramanian, S., Montes, V., Goodspeed, L., Wang, S., Han, C., et al. (2012). Toll-like receptor 4 deficiency decreases atherosclerosis but does not protect against inflammation in obese low-density lipoprotein receptor-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* 32, 1596–1604.

Donath, M. Y., and Shoelson, S. E. (2011). Type 2 diabetes as an inflammatory disease. *Nat. Rev. Immunol.* 11, 98–107.

Dumitru, C. D., Ceci, J. D., Tsatsanis, C., Kontoyiannis, D., Stamatakis, K., Lin, J. H., et al. (2000). TNF-alpha induction by LPS is regulated posttranscriptionally via a Tpl2/ERK-dependent pathway. *Cell* 103, 1071–1083.

Ehses, J., Meier, D., Wueest, S., Rytka, J., Boller, S., Wielinga, P., et al. (2010). Toll-like receptor 2-deficient mice are protected from insulin resistance and beta cell dysfunction induced by a high-fat diet. *Diabetologia* 53, 1795–1806.

Emanuelli, B., Eberle, D., Suzuki, R., and Kahn, C. R. (2008a). Overexpression of the dual-specificity phosphatase MKP-4/DUSP-9 protects against stress-induced insulin resistance. *Proc. Natl. Acad. Sci. U.S.A.* 105, 3545–3550.

Emanuelli, B., Macotela, Y., Boucher, J., and Ronald Kahn, C. (2008b). SOCS-1 deficiency does not prevent diet-induced insulin resistance. *Biochem. Biophys. Res. Commun.* 377, 447–452.

Emanuelli, B., Peraldi, P., Filloux, C., Sawka-Verhelle, D., Hilton, D., and Van Obberghen, E. (2000). SOCS-3 is an insulin-induced negative regulator of insulin signaling. *J. Biol. Chem.* 275, 15985–15991.

Erridge, C., and Samani, N. J. (2009). Saturated fatty acids do not directly stimulate Toll-like receptor signaling. *Arterioscler. Thromb. Vasc. Biol.* 29, 1944–1949.

Fischer, H., Ellstrom, P., Ekstrom, K., Gustafsson, L., Gustafsson, M., and Svanborg, C. (2007). Ceramide as a TLR4 agonist; a putative signalling intermediate between sphingolipid receptors for microbial ligands and TLR4. *Cell. Microbiol.* 9, 1239–1251.

Flamment, M., Hajduch, E., Ferre, P., and Foufelle, F. (2012). New insights into ER stress-induced insulin resistance. *Trends Endocrinol. Metab.* 23, 381–390.

Fresno, M., Alvarez, R., and Cuesta, N. (2011). Toll-like receptors, inflammation, metabolism and obesity. *Arch. Physiol. Biochem.* 117, 151–164.

Gantke, T., Sriskantharajah, S., and Ley, S. C. (2011). Regulation and function of TPL-2, an IkB kinase-regulated MAP kinase kinase kinase. *Cell Res.* 21, 131–145.

Goldfine, A. B., Fonseca, V., Jablonski, K. A., Pyle, L., Staten, M. A., and Shoelson, S. E. (2010). The effects of salsalate on glycemic control in patients with type 2 diabetes: a randomized trial. *Ann. Intern. Med.* 152, 346–357.

Gregor, M. F., and Hotamisligil, G. S. (2011). Inflammatory mechanisms in obesity. *Annu. Rev. Immunol.* 29, 415–445.

Gual, P., Le Marchand-Brustel, Y., and Tanti, J. F. (2005). Positive and negative regulation of insulin signaling through IRS-1 phosphorylation. *Biochimie* 87, 99–109.

Gurcel, L., Abrami, L., Girardin, S., Tschopp, J., and van der Goot, F. G. (2006). Caspase-1 activation of lipid metabolic pathways in response to bacterial pore-forming toxins promotes cell survival. *Cell* 126, 1135–1145.

Himes, R., and Smith, C. (2010). Tlr2 is critical for diet-induced metabolic syndrome in a murine model. *FASEB J.* 24, 731–739.

Hirosumi, J., Tuncman, G., Chang, L., Gorgun, C. Z., Uysal, K. T., Maeda, K., et al. (2002). A central role for JNK in obesity and insulin resistance. *Nature* 420, 333–336.

Howard, J. K., Cave, B. J., Oksanen, L. J., Tzameli, I., Bjorbaek, C., and Flier, J. S. (2004). Enhanced leptin sensitivity and attenuation of diet-induced obesity in mice with haploinsufficiency of Socs3. *Nat. Med.* 10, 734–738.

Im, S. S., Yousef, L., Blaschitz, C., Liu, J. Z., Edwards, R. A., Young, S. G., et al. (2011). Linking lipid metabolism to the innate immune response in macrophages through sterol regulatory element binding protein-1a. *Cell Metab.* 13, 540–549.

Jager, J., Corcelle, V., Gremeaux, T., Laurent, K., Waget, A., Pages, G., et al. (2011). Deficiency in the extracellular signal-regulated kinase 1 (ERK1) protects leptin-deficient mice from insulin resistance without affecting obesity. *Diabetologia* 54, 180–189.

Jager, J., Gremeaux, T., Cormont, M., Le Marchand-Brustel, Y., and Tanti, J. F. (2007). Interleukin-1beta-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. *Endocrinology* 148, 241–251.

Jager, J., Gremeaux, T., Gonzalez, T., Bonnafous, S., Debard, C., Laville, M., et al. (2010). The Tpl2 kinase is up-regulated in adipose tissue in obesity and may mediate IL-1(Minamino et al.) and TNF-(alpha) effects on ERK activation and lipolysis. *Diabetes* 59, 61–70.

Jamieson, E., Chong, M., Steinberg, G., Jovanovska, V., Fam, B., Bullen, D., et al. (2005). Socs1 deficiency enhances hepatic insulin signaling. *J. Biol. Chem.* 280, 31516–31521.

Jiao, P., Feng, B., Ma, J., Nie, Y., Paul, E., Li, Y., et al. (2012). Constitutive activation of IKKbeta in adipose tissue prevents diet-induced obesity in mice. *Endocrinology* 153, 154–165.

Jorgensen, S., O'Neill, H., Sylow, L., Honeyman, J., Hewitt, K., Palanivel, R., et al. (2012). Deletion of skeletal muscle SOCS3 prevents insulin resistance in obesity. *Diabetes* 62, 56–64.

Kaddai, V., Jager, J., Gonzalez, T., Najem-Lendom, R., Bonnafous, S., Tran, A., et al. (2009). Involvement of TNF-alpha in abnormal adipocyte and muscle sortilin expression in obese mice and humans. *Diabetologia* 52, 932–940.

Keshet, Y., and Seger, R. (2010). The MAP kinase signaling cascades: a system of hundreds of components regulates a diverse array of physiological functions. *Methods Mol. Biol.* 661, 3–38.

Kievit, P., Howard, J., Badman, M., Balthasar, N., Coppari, R., Mori, H., et al. (2006). Enhanced leptin sensitivity and improved glucose homeostasis in mice lacking suppressor of cytokine signaling-3 in POMC-expressing cells. *Cell Metab.* 4, 123–132.

Kim, F., Pham, M., Luttrell, I., Bannerman, D. D., Tupper, J., Thaler, J., et al. (2007). Toll-like receptor-4 mediates vascular inflammation and insulin resistance in diet-induced obesity. *Circ. Res.* 100, 1589–1596.

Kleinridders, A., Schenten, D., Könner, A., Belgardt, B., Mauer, J., Okamura, T., et al. (2009). MyD88 signaling in the CNS is required for development of fatty acid-induced leptin resistance and diet-induced obesity. *Cell Metab.* 10, 249–259.

Koenen, T. B., Stienstra, R., van Tits, L. J., Joosten, L. A., van Velzen, J. F., Hijmans, A., et al. (2011). The inflammasome and caspase-1 activation: a new mechanism underlying increased inflammatory activity in human visceral adipose tissue. *Endocrinology* 152, 3769–3778.

Könner, A., and Brüning, J. (2011). Toll-like receptors: linking inflammation to metabolism. *Trends Endocrinol. Metab.* 22, 16–23.

Koska, J., Ortega, E., Bunt, J. C., Gasser, A., Impson, J., Hanson, R. L., et al. (2009). The effect of salsalate on insulin action and glucose tolerance in obese non-diabetic patients: results of a randomised double-blind placebo-controlled study. *Diabetologia* 52, 385–393.

Kuo, L. H., Tsai, P. J., Jiang, M. J., Chuang, Y. L., Yu, L., Lai, K. T. A., et al. (2011). Toll-like receptor 2 deficiency improves insulin sensitivity and hepatic insulin signalling in the mouse. *Diabetologia* 54, 168–179.

Lagathu, C., Yvan-Charvet, L., Bastard, J. P., Maachi, M., Quignard-Boulange, A., Capeau, J., et al. (2006). Long-term treatment with interleukin-1beta induces insulin resistance in murine and human adipocytes. *Diabetologia* 49, 2162–2173.

Lancaster, G. I., Kowalski, G. M., Estevez, E., Kraakman, M. J., Grigoriadis, G., Febbraio, M. A., et al. (2012). Tumor progression locus 2 (Tpl2) deficiency does not protect against obesity-induced metabolic disease. *PLoS ONE* 7:e39100. doi: 10.1371/journal.pone.0039100

Larsen, C. M., Faulenbach, M., Vaag, A., Volund, A., Ehses, J. A., Seifert, B., et al. (2007). Interleukin-1-receptor antagonist in type 2 diabetes mellitus. *N. Engl. J. Med.* 356, 1517–1526.

Lebrun, P., Cognard, E., Bellon-Paul, R., Gontard, P., Filloux, C., Jehl-Pietri, C., et al. (2009). Constitutive expression of suppressor of cytokine signalling-3 in skeletal muscle leads to reduced mobility and overweight in mice. *Diabetologia* 52, 2201–2212.

Lebrun, P., and Van Obberghen, E. (2008). SOCS proteins causing trouble in insulin action. *Acta Physiol. (Oxf.)* 192, 29–36.

Lee, D. F., Kuo, H. P., Chen, C. T., Wei, Y., Chou, C. K., Hung, J. Y., et al. (2008). IKKbeta suppression of TSC1 function links the mTOR pathway with insulin resistance. *Int. J. Mol. Med.* 22, 633–638.

Lee, S. J., Pfluger, P. T., Kim, J. Y., Nogueiras, R., Duran, A., Pages, G., et al. (2010). A functional role for the p62-ERK1 axis in the control of energy homeostasis and adipogenesis. *EMBO Rep.* 11, 226–232.

Li, L., Chen, L., Hu, L., Liu, Y., Sun, H.-Y., Tang, J., et al. (2011). Nuclear factor high-mobility group box1 mediating the activation of Toll-like receptor 4 signaling in hepatocytes in the early stage of

nonalcoholic fatty liver disease in mice. *Hepatology* 54, 1620–1630.

Li, L., Grønning, L., Anderson, P., Li, S., Edvardsen, K., Johnston, J., et al. (2004). Insulin induces SOCS-6 expression and its binding to the p85 monomer of phosphoinositide 3-kinase, resulting in improvement in glucose metabolism. *J. Biol. Chem.* 279, 34107–34114.

Lolmede, K., Duffaut, C., Zakaroff-Girard, A., and Bouloumie, A. (2011). Immune cells in adipose tissue: key players in metabolic disorders. *Diabetes Metab.* 37, 283–290.

Milanski, M., Degasperi, G., Coope, A., Morari, J., Denis, R., Cintra, D., et al. (2009). Saturated fatty acids produce an inflammatory response predominantly through the activation of TLR4 signaling in hypothalamus: implications for the pathogenesis of obesity. *J. Neurosci.* 29, 359–370.

Mogensen, T. H. (2009). Pathogen recognition and inflammatory signaling in innate immune defenses. *Clin. Microbiol. Rev.* 22, 240–273.

Mori, H., Hanada, R., Hanada, T., Aki, D., Mashima, R., Nishinakamura, H., et al. (2004). Socs3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet-induced obesity. *Nat. Med.* 10, 739–743.

Nakamura, T., Furuhashi, M., Li, P., Cao, H., Tuncman, G., Sonenberg, N., et al. (2010). Double-stranded RNA-dependent protein kinase links pathogen sensing with stress and metabolic homeostasis. *Cell* 140, 338–348.

Nicholson, J. K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., et al. (2012). Host-gut microbiota metabolic interactions. *Science* 336, 1262–1267.

Orr, J., Puglisi, M., Ellacott, K., Lumeng, C., Wasserman, D., and Hasty, A. (2012). Toll-like receptor 4 deficiency promotes the alternative activation of adipose tissue macrophages. *Diabetes* 61, 2718–2727.

Oslowski, C. M., Hara, T., O'Sullivan-Murphy, B., Kanekura, K., Lu, S., Hara, M., et al. (2012). Thioredoxin-interacting protein mediates ER stress-induced beta cell death through initiation of the inflammasome. *Cell Metab.* 16, 265–273.

Ozcan, U., Cao, Q., Yilmaz, E., Lee, A. H., Iwakoshi, N. N., Ozdelen, E., et al. (2004). Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 306, 457–461.

Ozcan, U., Ozcan, L., Yilmaz, E., Duvel, K., Sahin, M., Manning, B. D., et al. (2008). Loss of the tuberous sclerosis complex tumor suppressors triggers the unfolded protein response to regulate insulin signaling and apoptosis. *Mol. Cell* 29, 541–551.

Pal, D., Dasgupta, S., Kundu, R., Maitra, S., Das, G., Mukhopadhyay, S., et al. (2012). Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. *Nat. Med.* 18, 1279–1285.

Palanivel, R., Fullerton, M., Galic, S., Honeyman, J., Hewitt, K., Jorgensen, S., et al. (2012). Reduced Socs3 expression in adipose tissue protects female mice against obesity-induced insulin resistance. *Diabetologia* 55, 3083–3093.

Perfield, J. W. 2nd., Lee, Y., Shulman, G. I., Samuel, V. T., Jurczak, M. J., Chang, E., et al. (2011). Tumor progression locus 2 (TPL2) regulates obesity-associated inflammation and insulin resistance. *Diabetes* 60, 1168–1176.

Poggi, M., Bastelica, D., Gual, P., Iglesias, M., Gremeaux, T., Knauf, C., et al. (2007). C3H/HeJ mice carrying a toll-like receptor 4 mutation are protected against the development of insulin resistance in white adipose tissue in response to a high-fat diet. *Diabetologia* 50, 1267–1276.

Poggi, M., Jager, J., Paulmyer-Lacroix, O., Peiretti, F., Gremeaux, T., Verdier, M., et al. (2009). The inflammatory receptor CD40 is expressed on human adipocytes: contribution to crosstalk between lymphocytes and adipocytes. *Diabetologia* 52, 1152–1163.

Radin, M., Sinha, S., Bhatt, B., Dedousis, N., and O'Doherty, R. (2008). Inhibition or deletion of the lipopolysaccharide receptor Toll-like receptor-4 confers partial protection against lipid-induced insulin resistance in rodent skeletal muscle. *Diabetologia* 51, 336–346.

Regazzetti, C., Peraldi, P., Gremeaux, T., Najem-Lendom, R., Ben-Sahra, I., Cormont, M., et al. (2009). Hypoxia decreases insulin signaling pathways in adipocytes. *Diabetes* 58, 95–103.

Rieusset, J., Bouzakri, K., Chevillotte, E., Ricard, N., Jacquet, D., Bastard, J. P., et al. (2004). Suppressor of cytokine signaling 3 expression and insulin resistance in skeletal muscle of obese and type 2 diabetic patients. *Diabetes* 53, 2232–2241.

Rodriguez, A., Duran, A., Selloum, M., Champy, M.-F., Diez-Guerra, F. J., Flores, J. M., et al. (2006). Mature-onset obesity and insulin resistance in mice deficient in the signaling adapter p62. *Cell Metab.* 3, 211–222.

Rohl, M., Pasparakis, M., Baudler, S., Baumgartl, J., Gautam, D., Huth, M., et al. (2004). Conditional disruption of IkappaB kinase 2 fails to prevent obesity-induced insulin resistance. *J. Clin. Invest.* 113, 474–481.

Saberi, M., Woods, N. B., de Luca, C., Schenk, S., Lu, J. C., Bandyopadhyay, G., et al. (2009). Hematopoietic cell-specific deletion of toll-like receptor 4 ameliorates hepatic and adipose tissue insulin resistance in high-fat-fed mice. *Cell Metab.* 10, 419–429.

Sabio, G., Cavanagh-Kyros, J., Barrett, T., Jung, D. Y., Ko, H. J., Ong, H., et al. (2010a). Role of the hypothalamic-pituitary-thyroid axis in metabolic regulation by JNK1. *Genes Dev.* 24, 256–264.

Sabio, G., Kennedy, N. J., Cavanagh-Kyros, J., Jung, D. Y., Ko, H. J., Ong, H., et al. (2010b). Role of muscle c-Jun NH2-terminal kinase 1 in obesity-induced insulin resistance. *Mol. Cell. Biol.* 30, 106–115.

Sabio, G., Cavanagh-Kyros, J., Ko, H. J., Jung, D. Y., Gray, S., Jun, J. Y., et al. (2009). Prevention of steatosis by hepatic JNK1. *Cell Metab.* 10, 491–498.

Sabio, G., Das, M., Mora, A., Zhang, Z., Jun, J. Y., Ko, H. J., et al. (2008). A stress signaling pathway in adipose tissue regulates hepatic insulin resistance. *Science* 322, 1539–1543.

Sabio, G., and Davis, R. J. (2010). cJun NH2-terminal kinase 1 (JNK1): roles in metabolic regulation of insulin resistance. *Trends Biochem. Sci.* 35, 490–496.

Sachithanandan, N., Fam, B., Fynch, S., Dzamko, N., Watt, M., Wormald, S., et al. (2010). Liver-specific suppressor of cytokine signaling-3 deletion in mice enhances hepatic insulin sensitivity and lipogenesis resulting in fatty liver and obesity. *Hepatology* 52, 1632–1642.

Sachithanandan, N., Graham, K., Galic, S., Honeyman, J., Fynch, S., Hewitt, K., et al. (2011). Macrophage deletion of SOCS1 increases sensitivity to LPS and palmitic acid and results in systemic inflammation and hepatic insulin resistance. *Diabetes* 60, 2023–2031.

Schertzer, J. D., Tamrakar, A. K., Magalhaes, J. G., Pereira, S., Bilan, P. J., Fullerton, M. D., et al. (2011). NOD1 activators link innate immunity to insulin resistance. *Diabetes* 60, 2206–2215.

Shi, H., Cave, B., Inouye, K., Bjorbaek, C., and Flier, J. S. (2006a). Overexpression of suppressor of cytokine signaling 3 in adipose tissue causes local but not systemic insulin resistance. *Diabetes* 55, 699–707.

Shi, H., Kokoeva, M., Inouye, K., Tzameli, I., Yin, H., and Flier, J. (2006b). TLR4 links innate immunity and fatty acid-induced insulin resistance. *J. Clin. Invest.* 116, 3015–3025.

Solinas, G., and Karin, M. (2010). JNK1 and IKKbeta: molecular links between obesity and metabolic dysfunction. *FASEB J.* 24, 2596–2611.

Solinas, G., Vilcu, C., Neels, J. G., Bandyopadhyay, G. K., Luo, J. L., Naugler, W., et al. (2007). JNK1 in hematopoietically derived cells contributes to diet-induced inflammation and insulin resistance without affecting obesity. *Cell Metab.* 6, 386–397.

Souza, S. C., Palmer, H. J., Kang, Y. H., Yamamoto, M. T., Muliro, K. V., Paulson, K. E., et al. (2003). TNF-alpha induction of lipolysis is mediated through activation of the extracellular signal related kinase pathway in 3T3-L1 adipocytes. *J. Cell. Biochem.* 89, 1077–1086.

Stienstra, R., Joosten, L. A., Koenen, T., van Tits, B., van Diepen, J. A., van den Berg, S. A., et al. (2010). The inflammasome-mediated caspase-1 activation controls adipocyte differentiation and insulin sensitivity. *Cell Metab.* 12, 593–605.

Stienstra, R., van Diepen, J. A., Tack, C. J., Zaki, M. H., van de Veerdonk, F. L., Perera, D., et al. (2011). Inflammasome is a central player in the induction of obesity and insulin resistance. *Proc. Natl. Acad. Sci. U.S.A.* 108, 15324–15329.

Suganami, T., Mieda, T., Itoh, M., Shimoda, Y., Kamei, Y., and Ogawa, Y. (2007a). Attenuation of obesity-induced adipose tissue inflammation in C3H/HeJ mice carrying a Toll-like receptor 4 mutation. *Biochem. Biophys. Res. Commun.* 354, 45–49.

Suganami, T., Tanimoto-Koyama, K., Nishida, J., Itoh, M., Yuan, X., Mizuarai, S., et al. (2007b). Role of the Toll-like receptor 4/NF-kappaB pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages. *Arterioscler. Thromb. Vasc. Biol.* 27, 84–91.

Sun, S., Ji, Y., Kersten, S., and Qi, L. (2012). Mechanisms of inflammatory responses in obese adipose tissue. *Annu. Rev. Nutr.* 32, 261–286.

Symons, A., Beinke, S., and Ley, S. C. (2006). MAP kinase kinase kinases and innate immunity. *Trends Immunol.* 27, 40–48.

Tamrakar, A. K., Schertzer, J. D., Chiu, T. T., Foley, K. P., Bilan, P. J., Philpott, D. J., et al. (2010). NOD2 activation induces muscle cell-autonomous innate immune responses and insulin resistance. *Endocrinology* 151, 5624–5637.

Tamura, Y., Ogihara, T., Uchida, T., Ikeda, F., Kumashiro, N., Nomiyama, T., et al. (2007). Amelioration of glucose tolerance by hepatic inhibition of nuclear factor kappaB in db/db mice. *Diabetologia* 50, 131–141.

Taniguchi, C. M., Emanuelli, B., and Kahn, C. R. (2006). Critical nodes in signalling pathways: insights into insulin action. *Nat. Rev. Mol. Cell Biol.* 7, 85–96.

Tanti, J. F., Gremeaux, T., van Obberghen, E., and Le Marchand-Brustel, Y. (1994). Serine/threonine phosphorylation of insulin receptor substrate 1 modulates insulin receptor signaling. *J. Biol. Chem.* 269, 6051–6057.

Tanti, J. F., and Jager, J. (2009). Cellular mechanisms of insulin resistance: role of stress-regulated serine kinases and insulin receptor substrates (IRS) serine phosphorylation. *Curr. Opin. Pharmacol.* 9, 753–762.

Torisu, T., Sato, N., Yoshiga, D., Kobayashi, T., Yoshioka, T., Mori, H., et al. (2007). The dual function of hepatic SOCS3 in insulin resistance *in vivo*. *Genes Cells* 12, 143–154.

Triantafilou, M., Gamper, F. G., Haston, R. M., Mouratis, M. A., Morath, S., Hartung, T., et al. (2006). Membrane sorting of toll-like receptor (TLR)-2/6 and TLR2/1 heterodimers at the cell surface determines heterotypic associations with CD36 and intracellular targeting. *J. Biol. Chem.* 281, 31002–31011.

Tschopp, J., and Schroder, K. (2010). NLRP3 inflammasome activation: the convergence of multiple signalling pathways on ROS production? *Nat. Rev. Immunol.* 10, 210–215.

Tsukumo, D., Carvalho-Filho, M., Carvalheira, J., Prada, P. C., Hirabara, S., Schenka, A., et al. (2007). Loss-of-function mutation in Toll-like receptor 4 prevents diet-induced obesity and insulin resistance. *Diabetes* 56, 1986–1998.

Tuncman, G., Hirosumi, J., Solinas, G., Chang, L., Karin, M., and Hotamisligil, G. S. (2006). Functional *in vivo* interactions between JNK1 and JNK2 isoforms in obesity and insulin resistance. *Proc. Natl. Acad. Sci. U.S.A.* 103, 10741–10746.

Ueki, K., Kadowaki, T., and Kahn, C. R. (2005). Role of suppressors of cytokine signaling SOCS-1 and SOCS-3 in hepatic steatosis and the metabolic syndrome. *Hepatol. Res.* 33, 185–192.

Vallerie, S. N., Furuhashi, M., Fucho, R., and Hotamisligil, G. S. (2008). A predominant role for parenchymal c-Jun amino terminal kinase (JNK) in the regulation of systemic insulin sensitivity. *PLoS ONE* 3:e3151. doi: 10.1371/journal.pone.0003151

Vandanmagsar, B., Youm, Y. H., Ravussin, A., Galgani, J. E., Stadler, K., Mynatt, R. L., et al. (2011). The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nat. Med.* 17, 179–188.

van Diepen, J. A., Wong, M. C., Guigas, B., Bos, J., Stienstra, R., Hodson, L., et al. (2011). Hepatocyte-specific IKK-beta activation enhances VLDL-triglyceride production in APOE*3-Leiden mice. *J. Lipid Res.* 52, 942–950.

Vijay-Kumar, M., Aitken, J., Carvalho, F., Cullender, T., Mwangi, S., Srinivasan, S., et al. (2010). Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. *Science* 328, 228–231.

Wen, H., Gris, D., Lei, Y., Jha, S., Zhang, L., Huang, M. T., et al. (2011). Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. *Nat. Immunol.* 12, 408–415.

Wood, I. S., de Heredia, F. P., Wang, B., and Trayhurn, P. (2009). Cellular hypoxia and adipose tissue dysfunction in obesity. *Proc. Nutr. Soc.* 68, 370–377.

Wunderlich, F. T., Luedde, T., Singer, S., Schmidt-Supprian, M., Baumgartl, J., Schirmacher, P., et al. (2008). Hepatic NF-kappa B essential modulator deficiency prevents obesity-induced insulin resistance but synergizes with high-fat feeding in tumorigenesis. *Proc. Natl. Acad. Sci. U.S.A.* 105, 1297–1302.

Xu, C., Bailly-Maitre, B., and Reed, J. C. (2005). Endoplasmic reticulum stress: cell life and death decisions. *J. Clin. Invest.* 115, 2656–2664.

Yang, R., and Trevillyan, J. M. (2008). c-Jun N-terminal kinase pathways in diabetes. *Int. J. Biochem. Cell Biol.* 40, 2702–2706.

Ye, D., Li, F., Lam, K., Li, H., Jia, W., Wang, Y., et al. (2012). Toll-like receptor-4 mediates obesity-induced non-alcoholic steatohepatitis through activation of X-box binding protein-1 in mice. *Gut* 61, 1058–1067.

Yuan, M., Konstantopoulos, N., Lee, J., Hansen, L., Li, Z. W., Karin, M., et al. (2001). Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* 293, 1673–1677.

Zabolotny, J. M., Kim, Y. B., Welsh, L. A., Kershaw, E. E., Neel, B. G., and Kahn, B. B. (2008). Protein-tyrosine phosphatase 1B expression is induced by inflammation *in vivo*. *J. Biol. Chem.* 283, 14230–14241.

Zadjali, F., Santana-Farre, R., Vesterlund, M., Carow, B., Mirecki-Garrido, M., Hernandez-Hernandez, I., et al. (2012). SOCS2 deletion protects against hepatic steatosis but worsens insulin resistance in high-fat-diet-fed mice. *FASEB J.* 26, 3282–3291.

Zhang, X., Xu, A., Chung, S. K., Cresser, J. H., Sweeney, G., Wong, R. L., et al. (2011). Selective inactivation of c-Jun NH2-terminal kinase in adipose tissue protects against diet-induced obesity and improves insulin sensitivity in both liver and skeletal muscle in mice. *Diabetes* 60, 486–495.

Zhang, X., Zhang, G., Zhang, H., Karin, M., Bai, H., and Cai, D. (2008). Hypothalamic IKKbeta/NF-kappaB and ER stress link overnutrition to energy imbalance and obesity. *Cell* 135, 61–73.

Zhao, L., Hu, P., Zhou, Y., Purohit, J., and Hwang, D. (2011). NOD1 activation induces proinflammatory gene expression and insulin resistance in 3T3-L1 adipocytes. *Am. J. Physiol. Endocrinol. Metab.* 301, E587–E598.

Zhou, R., Tardivel, A., Thorens, B., Choi, I., and Tschopp, J. (2010). Thioredoxin-interacting protein links oxidative stress to inflammasome activation. *Nat. Immunol.* 11, 136–140.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 29 November 2012; accepted: 19 December 2012; published online: 08 January 2013.

*Citation: Tanti J-F, Ceppo F, Jager J and Berthou F (2013) Implication of inflammatory signaling pathways in obesity-induced insulin resistance. Front. Endocrin. **3**:181. doi: 10.3389/fendo.2012.00181*

This article was submitted to Frontiers in Diabetes, a specialty of Frontiers in Endocrinology.

Copyright © 2013 Tanti, Ceppo, Jager and Berthou. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.

frontiers in
ENDOCRINOLOGY

MINI REVIEW ARTICLE
published: 21 August 2013
doi: 10.3389/fendo.2013.00105

RAGE-mediated inflammation, type 2 diabetes, and diabetic vascular complication

Yasuhiko Yamamoto* and Hiroshi Yamamoto

Department of Biochemistry and Molecular Vascular Biology, Kanazawa University Graduate School of Medical Sciences, Kanazawa, Japan

Edited by:
Tsuguhito Ota, Kanazawa University, Japan

Reviewed by:
Undurti Narasimha Das, UND Life Sciences, USA
Chul-Hee Kim, Soonchunhyang University, South Korea

****Correspondence:***
Yasuhiko Yamamoto, Department of Biochemistry and Molecular Vascular Biology, Kanazawa University Graduate School of Medical Sciences, 13-1 Takara-machi, Kanazawa 920-8640, Japan
e-mail: yasuyama@med.kanazawa-u.ac.jp

Obesity is associated with inflammation and type 2 diabetes. Innate immune system comprised of cellular and molecular components plays an important role in the inflammatory reactions. Immune cells like macrophages and their cell surface pattern-recognition receptors (PRRs) are representative for innate immunity promoting inflammatory reactions. The receptor for advanced glycation end-products (RAGE) is a member of PRRs and a proinflammatory molecular device that mediates danger signals to the body. The expression of RAGE is observed in adipocytes as well as immune cells, endothelial cells, and pancreatic β cells under certain conditions. It has been reported that RAGE is implicated in adipocyte hypertrophy and insulin resistance. RAGE-mediated regulation of adiposity and inflammation may attribute to type 2 diabetes and diabetic vascular complications.

Keywords: rage, obesity, inflammation, toll-like receptors, pattern-recognition receptors

Obesity is associated with an increased risk of developing type 2 diabetes, fatty liver disease, hypertension, and vascular complications (1). Proinflammatory and anti-inflammatory bioactive molecules produced from adipose tissues, known as adipokines, contribute to the burden of obesity-related diseases (2). Adipose tissue consists of heterogeneous populations of adipocytes, stromal preadipocytes, immune cells, and vascular cells, and it can respond rapidly and dynamically to alterations in nutrient excess caused by enhanced food consumption through adipocyte hypertrophy and hyperplasia (3). This results in a local inflammation in adipose tissue that propagates an overall systemic but chronic low-grade inflammation associated with the development of obesity-related comorbidities such as type 2 diabetes and cardiovascular diseases (2).

INNATE IMMUNITY AND RAGE-MEDIATED INFLAMMATORY REACTIONS

The innate immune system can act as a double-edged sword in protecting the host against foreign enemies and destroying tissues via inflammation. It may represent an evolutionary strategy adopted by multicellular organisms to prevent the survival of cells that would otherwise cause more disastrous consequences in the individuals and their descendants. Toll-like receptors (TLR) and receptor for advanced glycation end-products (RAGE) can participate in innate immunity maintaining a delicate balance between clearance of pathogens and induction of exaggerated inflammatory responses.

Receptor for advanced glycation end-products is originally identified for recognizing advanced glycation end-products (AGE) (4). RAGE belongs to the immunoglobulin superfamily, and is now known as a member of pattern-recognition receptors (PRRs) and as a proinflammatory device. RAGE recognizes a variety of endogenous and exogenous ligands, including AGE, advanced oxidation protein products, high-mobility group box protein 1 (HMGB1), calcium-binding S100 proteins, β2-integrin Mac-1/CD11b, amyloid β peptide/fibril, lipopolysaccharide (LPS), phosphatidylserine, C1q, and lysophosphatidic acid (LPA) (5). It has been hypothesized that RAGE engagement of such ligands causes diabetic vascular complications, atherosclerosis, cancer, neurodegeneration, and inflammatory diseases (6). Anti-RAGE antibody treatment is reported to suppress lung metastasis of cancer cells and to offer a survival advantage to septic mice (7, 8). Downstream intracellular signaling molecules of RAGE include NFκB, ERK (extracellular signal-regulated kinase) 1/2, p38MAPK (mitogen-activated protein kinases), JNK (c-Jun N-terminal kinases), PKC (protein kinase C), Rac/Cdc42, and TIRAP and MyD88, adaptor proteins for TLR 2 and 4 (9). A functional link between RAGE and TLR is thus considered to be in a coordinated manner (10).

Among the above ligands, HMGB1 is known to be readily released from necrotic or damaged cells and to be actively secreted by activated endothelial cells and immune cells such as monocytes, macrophages, dendritic cells, and natural killer cells (11). HMGB1 can form a complex with proinflammatory molecules of CpG DNA, LPS, and interleukin 1β, and this further induces the activation of RAGE signaling (12). HMGB1 is also found to be expressed in human adipose tissues with the expression levels associated with the fat mass and obesity-related genes (13). TLR2 and 4 also recognize HMGB1 and can be involved in HMGB1-induced cellular responses (14). S100 proteins are a family of over 20 proteins that show a structural similarity with their two EF-hand Ca^{2+}-binding domains flanked by α-helices. Higher oligomerization states of S100 proteins lead to the activation of RAGE (15).

AGE-modified S100A8/A9 have been reported to strongly activate inflammatory responses via RAGE (16). S100A8/A9 was also shown to interact with TLR4 (17). Our groups have also shown that phosphatidylserine on the surface of apoptotic cells and LPS are also RAGE ligands (18, 19). Rapid removal of apoptotic cells by phagocytes is crucial for tissue development, homeostasis, resolution of inflammation, and prevention of autoimmune responses. RAGE was found to function as one of the PS receptors that recognize and initiate apoptotic cell clearance (18). LPS and the lipid A component responsible for LPS toxicity and known as endotoxin were found to directly interact with RAGE (19). LPS is also a well-known TLR ligand.

RAGE AND ADIPOSITY

Using RAGE and apoE double deficient mice, Ueno et al. demonstrated that absence of RAGE is associated with decreased epididymal fat weight and smaller adipocyte size, which are significantly associated with the decrease in atherosclerotic lesions (20). They also reported that circulating anti-inflammatory adiponectin levels in apoE$^{-/-}$RAGE$^{-/-}$ were higher than apoE$^{-/-}$RAGE$^{+/+}$ mice, and their levels were significantly and inversely associated with aortic atherosclerosis. Very recently, Monden et al. demonstrated that RAGE directly regulated adipogenesis and hypertrophic process of adipocyte differentiation *in vitro* (21). Adenoviral overexpression of RAGE markedly increased generation of hypertrophic adipocytes and RAGE knockdown by using siRNA system significantly suppressed generation of hypertrophic adipocytes. Under high fat diet feeding in mice, RAGE deficiency is associated with less body weight, less epididymal fat weight, less adipocyte size, higher serum adiponectin, higher expressions of Glut4 and adiponectin in epididymal fat, and greater insulin sensitivity. It is now acceptable that direct role of RAGE in adipocyte hypertrophy and insulin resistance (**Figure 1**). However, RAGE ligands are still unknown to be involved in the RAGE-dependent adiposity. Further studies are required to characterize the interplays among a variety of RAGE ligands and inflammatory reactions in obesity and type 2 diabetes.

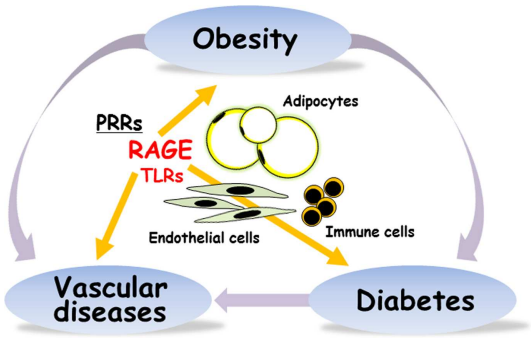


FIGURE 1 | RAGE is implicated in obesity, type 2 diabetes, and vascular diseases. PRRs, pattern-recognition receptors; RAGE, receptor for advanced glycation end-products; TLRs, toll-like receptors.

RAGE POLYMORPHISMS, OBESITY, AND INFLAMMATION

Several functional single nucleotide polymorphisms have been identified in human RAGE gene. The G82S occurs in the ligand-binding V domain of RAGE and affects ligand affinity, resulting in the enhancement of proinflammatory reactions and immune/inflammatory diseases (22, 23). In obese subjects, S/S carriers showed significantly higher concentrations of AGE and C reactive protein than G allele carrier and lower concentration of soluble RAGE, a decoy receptor (24). S allele at RAGE G82S polymorphism may be more closely associated with proinflammatory reactions under obese conditions rather than non-obese status, thus linking to the development of obesity-associated complications.

We very recently reported that the induction of RAGE expression in pancreatic β-cell by insufficient leptin action under obesity conditions could trigger β-cell failure in type 2 diabetes (25). It is thus considered that RAGE could be a potential targeting receptor for the prevention and treatment of the development of obesity, β-cell failure, vascular complications, and inflammation in type 2 diabetes (**Figure 1**).

REFERENCES

1. Romeo GR, Lee J, Shoelson SE. Metabolic syndrome, insulin resistance, and roles of inflammation – mechanisms and therapeutic targets. *Arterioscler Thromb Vasc Biol* (2012) **32**:1771–6. doi:10.1161/ATVBAHA.111.241869
2. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol* (2011) **11**:98–107. doi:10.1038/nri2925
3. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest* (2006) **116**:1793–801. doi:10.1172/JCI29069
4. Neeper M, Schmidt AM, Brett J, Yan SD, Wang F, Pan YC, et al. Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *J Biol Chem* (1992) **267**:14998–5004.
5. Yamamoto Y, Yamamoto H. RAGE-mediated inflammation and diabetic vascular complications. *J Diabetes Invest* (2011) **2**:155–7. doi:10.1111/j.2040-1124.2011.00125.x
6. Yan SF, Yan SD, Ramasamy R, Schmidt AM. Tempering the wrath of RAGE: an emerging therapeutic strategy against diabetic complications, neurodegeneration, and inflammation. *Ann Med* (2009) **25**:1–15. doi:10.1080/07853890902806576
7. Christaki E, Opal SM, Keith JC Jr, Kessimian N, Palardy JE, Parejo NA, et al. A monoclonal antibody against RAGE alters gene expression and is protective in experimental models of sepsis and pneumococcal pneumonia. *Shock* (2011) **35**:492–8. doi:10.1097/SHK.0b013e31820b2e1c
8. Mizumoto S, Takahashi J, Sugahara K. Receptor for advanced glycation end products (RAGE) functions as receptor for specific sulfated glycosaminoglycans, and anti-RAGE antibody or sulfated glycosaminoglycans delivered in vivo inhibit pulmonary metastasis of tumor cells. *J Biol Chem* (2012) **287**:18985–94. doi:10.1074/jbc.M111.313437
9. Yamamoto Y, Yamamoto H. Controlling RAGE to conquer diabetic vascular complications. *J Diabetes Invest* (2012) **3**:107–14. doi:10.1111/j.2040-1124.2011.00191.x
10. Sakaguchi M, Murata H, Yamamoto K, Ono T, Sakaguchi Y, Motoyama A, et al. TIRAP, an adaptor protein for TLR2/4, transduces a signal from RAGE phosphorylated upon ligand binding. *PLoS ONE* (2011) **6**:e23132. doi:10.1371/journal.pone.0023132
11. Tsan MF. Heat shock proteins and high mobility group box 1 protein lack cytokine function. *J Leukoc Biol* (2011) **89**:847–53. doi:10.1189/jlb.0810471
12. Tian J, Avalos AM, Mao SY, Chen B, Senthil K, Wu H, et al. Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. *Nat Immunol* (2007) **8**:487–96. doi:10.1038/ni1457

13. Qin YH, Dai SM, Tang GS, Zhang J, Ren D, Wang ZW, et al. HMGB1 enhances the proinflammatory activity of lipopolysaccharide by promoting the phosphorylation of MAPK p38 through receptor for advanced glycation end products. *J Immunol* (2009) **183**:6244–50. doi:10.4049/jimmunol.0900390
14. Park JS, Svetkauskaite D, He Q, Kim JY, Strassheim D, Ishizaka A, et al. Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. *J Biol Chem* (2004) **279**: 7370–7. doi:10.1074/jbc.M306793200
15. Leclerc E, Fritz G, Vetter SW, Heizmann CW. Binding of S100 proteins to RAGE: an update. *Biochim Biophys Acta* (2009) **1793**: 993–1007. doi:10.1016/j.bbamcr.2008.11.016
16. Turovskaya O, Foell D, Sinha P, Vogl T, Newlin R, Nayak J, et al. RAGE, carboxylated glycans and S100A8/A9 play essential roles in colitis-associated carcinogenesis. *Carcinogenesis* (2008) **29**:2035–43. doi:10.1093/carcin/bgn188
17. Vogl T, Tenbrock K, Ludwig S, Leukert N, Ehrhardt C, van Zoelen MA, et al. Mrp8 and Mrp14 are endogenous activators of Toll-like receptor 4, promoting lethal, endotoxin-induced shock. *Nat Med* (2007) **13**:1042–9. doi:10.1038/nm1638
18. He M, Kubo H, Morimoto K, Fujino N, Suzuki T, Takahasi T, et al. Receptor for advanced glycation end products binds to phosphatidylserine and assists in the clearance of apoptotic cells. *EMBO Rep* (2011) **12**:358–64. doi:10.1038/embor.2011.28
19. Yamamoto Y, Harashima A, Saito H, Tsuneyama K, Munesue S, Motoyoshi S, et al. Septic shock is associated with receptor for advanced glycation end products ligation of LPS. *J Immunol* (2011) **186**:3248–57. doi:10.4049/jimmunol.1002253
20. Ueno H, Koyama H, Shoji T, Monden M, Fukumoto S, Tanaka S, et al. Receptor for advanced glycation end-products (RAGE) regulation of adiposity and adiponectin is associated with atherogenesis in apoE-deficient mouse. *Atherosclerosis* (2000) **211**:431–6. doi:10.1016/j.atherosclerosis.2010.04.006
21. Monden M, Koyama H, Otsuka Y, Morioka T, Mori K, Shoji T, et al. Receptor for advanced glycation end products regulates adipocyte hypertrophy and insulin sensitivity in mice: involvement of Toll-like receptor 2. *Diabetes* (2013) **62**:478–89. doi:10.2337/db11-1116
22. Osawa M, Yamamoto Y, Munesue S, Murakami N, Sakurai S, Watanabe T, et al. De-N-glycosylation or G82S mutation of RAGE sensitizes its interaction with advanced glycation endproducts. *Biochim Biophys Acta* (2007) **1770**: 1468–74. doi:10.1016/j.bbagen.2007.07.003
23. Han Z, Liu Q, Sun C, Li Y. The interaction between obesity and RAGE polymorphisms on the risk of knee osteoarthritis in Chinese population. *Cell Physiol Biochem* (2012) **30**:898–904. doi:10.1159/000341467
24. Kim OY, Jo SH, Jang Y, Chae JS, Kim JY, Hyun YJ, et al. G allele at RAGE SNP82 is associated with proinflammatory markers in obese subjects. *Nutr Res* (2009) **29**:106–13. doi:10.1016/j.nutres.2009.01.006
25. Han D, Yamamoto Y, Munesue S, Motoyoshi S, Saito H, Win MT, et al. Induction of receptor for advanced glycation end products by insufficient leptin action triggers pancreatic β-cell failure in type 2 diabetes. *Genes Cells* (2013) **18**:302–14. doi:10.1111/gtc.12036

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 09 April 2013; accepted: 06 August 2013; published online: 21 August 2013.
*Citation: Yamamoto Y and Yamamoto H (2013) RAGE-mediated inflammation, type 2 diabetes, and diabetic vascular complication. Front. Endocrinol. **4**:105. doi: 10.3389/fendo.2013.00105*
This article was submitted to Diabetes, a section of the journal Frontiers in Endocrinology.
Copyright © 2013 Yamamoto and Yamamoto. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in
ENDOCRINOLOGY

REVIEW ARTICLE
published: 25 January 2013
doi: 10.3389/fendo.2013.00001

Retinoic acid-related orphan receptors α and γ: key regulators of lipid/glucose metabolism, inflammation, and insulin sensitivity

Anton M. Jetten*, Hong Soon Kang and Yukimasa Takeda

Cell Biology Section, Division of Intramural Research, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC, USA

Edited by:
Tsuguhito Ota, Kanazawa University, Japan

Reviewed by:
Krzysztof W. Nowak, Pozan University of Life Sciences, Poland
Venu Lagishetty, University of South Florida, USA

****Correspondence:***
Anton M. Jetten, Cell Biology Section, Division of Intramural Research, National Institute of Environmental Health Sciences, National Institutes of Health, 111 T.W. Alexander Drive, Research Triangle Park, NC 27709, USA.
e-mail: jetten@niehs.nih.gov

Retinoic acid-related orphan receptors RORα and RORγ play a regulatory role in lipid/glucose homeostasis and various immune functions, and have been implicated in metabolic syndrome and several inflammatory diseases. RORα-deficient mice are protected against age- and diet-induced obesity, hepatosteatosis, and insulin resistance. The resistance to hepatosteatosis in RORα-deficient mice is related to the reduced expression of several genes regulating lipid synthesis, transport, and storage. Adipose tissue-associated inflammation, which plays a critical role in the development of insulin resistance, is considerably diminished in RORα-deficient mice as indicated by the reduced infiltration of M1 macrophages and decreased expression of many proinflammatory genes. Deficiency in RORγ also protects against diet-induced insulin resistance by a mechanism that appears different from that in RORα deficiency. Recent studies indicated that RORs provide an important link between the circadian clock machinery and its regulation of metabolic genes and metabolic syndrome. As ligand-dependent transcription factors, RORs may provide novel therapeutic targets in the management of obesity and associated metabolic diseases, including hepatosteatosis, adipose tissue-associated inflammation, and insulin resistance.

Keywords: retinoic acid-related orphan receptor, obesity, inflammation, adipose tissue, hepatosteatosis, diabetes, insulin-resistance, circadian rhythm

INTRODUCTION

In the past 50 years, the occurrence of obesity has greatly increased worldwide in both adults and children and has become a major health-care concern in many countries. In the United States 30% of the population is considered obese, while more than 66% of adults and almost 17% of children and adolescents are overweight (Browning et al., 2004; Ogden et al., 2012). Obesity is associated with an increased risk of several pathologies, including type 2 diabetes, cardiovascular disease, and non-alcoholic fatty liver disease (NAFLD). Accumulating evidence indicates that networks regulating lipid metabolism and inflammation are highly integrated and play a critical role in the development of these pathologies (Hotamisligil, 2006; Donath and Shoelson, 2011; Ouchi et al., 2011; Glass and Olefsky, 2012). Obesity leads to a systemic state of low-grade inflammation, particularly involving adipose tissue, that is causally involved in the development of insulin resistance and other diseases. Blood levels of free fatty acids (FFA) are elevated in obesity and through their interaction with Toll-like receptor 4 (TLR4) FFA induce proinflammatory pathways in macrophages and other cell types that may promote insulin resistance (Samuel and Shulman, 2012). Recent studies demonstrated that retinoic acid-related orphan receptors (RORs) are among many factors that through their modulation of immune responses and lipid/glucose homeostasis regulate the development of inflammation, metabolic syndrome, and insulin resistance (Jetten, 2009; Solt and Burris, 2012).

RORα AND γ PROTEINS

The RORs alpha, beta, and gamma (RORα–γ or NR1F1–3) constitute a subfamily of nuclear receptors that function as ligand-dependent transcription factors (Jetten, 2004, 2009; Solt and Burris, 2012). RORs exhibit a domain structure typical of nuclear receptors and contain an N-terminal domain, the function of which has not yet been clearly defined, a highly conserved DNA-binding domain (DBD) consisting of two zinc finger motifs, a LBD, and a hinge domain spacing the DBD and LBD. By using different promoters and/or alternative splicing each ROR gene produces several isoforms that vary only in their N-terminal region. Some of these isoforms exhibit a distinct tissue-specific pattern of expression and control different genes and biological processes. RORs regulate transcription by binding as monomers to ROR response elements (RORE), which consist of the core sequence "AGGTCA" preceded by an A/T-rich sequence, in the regulatory region of target genes. The activation function (AF-2), localized at the C-terminus within the LBD of RORs, is involved in the recruitment of co-activators or co-repressors that mediate the transcriptional activation or repression by RORs. Recent studies have identified a number of (ant)agonists that interact with the LBD of ROR and either activate or inhibit ROR transcriptional activity (Kallen et al., 2002; Huh and Littman, 2012; Solt and Burris, 2012). Interaction with agonists induces a conformational change in the LBD that allows release of the co-repressor complex and promotes assembly of a co-activator complex that mediates the transcriptional

activation by ROR, while the inverse happens for antagonists. These observations not only indicated that RORs function as ligand-dependent transcription factors, but also suggested that RORs might be potential therapeutic targets to treat disease.

RORs AS REGULATORS OF SEVERAL IMMUNE PROCESSES

RORα and RORγ are important regulators of several diverse immune functions. RORγ-deficient mice lack lymph nodes and Peyer's patches indicating that it is essential for lymph node development (Kurebayashi et al., 2000; Sun et al., 2000). Recent studies demonstrated that RORα and the RORγt isoform play a key role in T cell lineage determination (Ivanov et al., 2006; Yang et al., 2008; Jetten, 2009). The RORγt isoform in particular and to a lesser extent RORα, is required for the differentiation of naïve T cells into interleukin 17 (IL-17) producing T helper 17 (Th17) cells. IL-17A expression is directly regulated by RORs through their interaction with ROREs in the *Il17* promoter (Yang et al., 2008). Proinflammatory Th17 cells and IL-17 have been implicated in several autoimmune diseases and other inflammatory disorders. Deficiency in RORγt or both RORα/γ receptors has been shown to greatly inhibit the generation of Th17 cells and the development of experimental encephalomyelitis in mice. In addition, mice deficient in RORα or RORγ displayed a diminished susceptibility to allergen-induced lung inflammation and collagen-induced arthritis (Jaradat et al., 2006; Tilley et al., 2007) and polymorphisms in RORα have been associated with increased susceptibility to asthma (Ramasamy et al., 2012). A recent study identified a role for RORα in the generation of natural helper (NH) cells (Halim et al., 2012). RORα-deficient, but not RORγ-deficient, mice lack NH cells. NH cell-deficient mice generated by RORα-deficient bone marrow transplantation exhibited normal Th2 cell responses, but failed to develop acute lung inflammation in response to a protease allergen. These findings might at least in part explain the reduced susceptibility to allergen-induced lung inflammation in RORα-deficient mice (Jaradat et al., 2006).

An increased Th17 response has been reported to correlate with white adipose tissue (WAT)-associated inflammation and the development of insulin resistance in obese mice and patients (Ahmed and Gaffen, 2010; Bertola et al., 2012). Whether inhibition of Th17 differentiation plays a role in the protection RORα- and RORγ-deficient mice against diet-induced insulin resistance needs further study. RORα or RORγ have also been implicated in the regulation of thymopoiesis. Loss of RORγt results in accelerated apoptosis of double-positive thymocytes, while RORα deficiency significantly reduces the generation of single positive thymocytes (Kurebayashi et al., 2000; Sun et al., 2000; Dzhagalov et al., 2004).

RORα IN DIET- AND AGE-INDUCED OBESITY

Study of *Staggerer* (*RORα*$^{sg/sg}$) mice, a natural mutant strain containing a deletion in the *RORα* gene that results in loss of RORα expression, indicated that RORα plays a critical role in the control of lipid metabolism and the development of various aspects of metabolic syndrome. These investigations showed that *RORα*$^{sg/sg}$ mice are protected against age- and diet-induced obesity and the development of several obesity-linked pathologies, including adipose tissue-associated inflammation, hepatosteatosis, and insulin resistance (Kang et al., 2011; Lau et al., 2011). *RORα*$^{sg/sg}$ mice fed a high fat diet (HFD) gain relatively less weight and exhibit a significantly lower total body fat index compared to wild-type (WT) littermates on a HFD. Similarly, male *RORα*$^{sg/sg}$ mice were also protected against age-induced obesity. Adipose tissue is the main site of storage of excess energy that is stored in the form of triglycerides in single large lipid droplets. The reduced adiposity in *RORα*$^{sg/sg}$ mice was largely related to smaller adipocyte size due to diminished deposition of triglycerides.

RORα, particularly the RORα4 isoform, has been shown to be highly expressed in WAT and to be induced during differentiation of D1 and 3T3-L1 preadipocytes (Austin et al., 1998). Overexpression of RORα in preadipocytes inhibits adipocyte differentiation (Duez et al., 2009; Ohoka et al., 2009). This appears to be mediated through a direct interaction of RORα with CCAAT/enhancer-binding protein β (C/EBPβ) that results in the inhibition of the recruitment of the co-activator CBP and repression of C/EBPβ transcriptional activity. These studies suggest that RORα has a negative regulatory role in adipocyte differentiation. This function, however, does not explain the reduced adiposity observed in RORα-deficient mice.

Obesity is a consequence of an imbalance between energy intake and expenditure (Glass and Olefsky, 2012; Samuel and Shulman, 2012). However, the decrease in diet-induced adiposity in *RORα*$^{sg/sg}$ mice was found not to be due to reduced food intake or increased fecal lipid excretion. Indirect calorimetric analysis showed that VO_2, VCO_2, and heat generation were significantly enhanced in *RORα*$^{sg/sg}$ mice on a HFD (Kang et al., 2011). This suggested that elevated energy expenditure might at least in part be responsible for the reduced weight gain and resistance to hepatosteatosis and insulin insensitivity in *RORα*$^{sg/sg}$ mice.

RORα AND WAT-ASSOCIATED INFLAMMATION

In addition to functioning as the main site of storage of extra energy in the form of triglycerides derived from food intake, white adipocytes produce a variety of endocrine hormones, including leptin, adiponectin, resistin, and retinol-binding protein 4 (RBP-4) which regulate food intake, lipid metabolism, and inflammation (Hotamisligil, 2006; Guilherme et al., 2008; Glass and Olefsky, 2012). Leptin and adiponectin promote insulin sensitivity, while resistin and RBP4 have the opposite effect and impair insulin sensitivity. It is now well-recognized that obesity is associated with a chronic state of low grade, systemic inflammation and that this is an important contributory factor in the development of insulin resistance (Hotamisligil, 2006; Odegaard and Chawla, 2008; Nishimura et al., 2009; Glass and Olefsky, 2012). Progressive infiltration of various immune cells, including macrophages and $CD8^+$ effector T lymphocytes, in WAT lead to increased release of proinflammatory cyto- and chemokines. In addition to the accumulation of bone marrow-derived macrophages, there is also a shift from anti-inflammatory "alternatively activated" ($CD11c^-CD206^+$) M2 macrophages to proinflammatory "classically activated" ($CD11c^+CD206^-$) M1 macrophages (Sun et al., 2011; Glass and Olefsky, 2012), which in advanced obesity aggregate into crown-like structures (CLS) surrounding necrotic adipocytes. Recent studies indicated that $CD8^+$ T cells are critical in promoting recruitment of macrophages in WAT in obesity (Weisberg et al., 2003; Odegaard and Chawla, 2008; Nishimura

et al., 2009). In addition, a reduction in anti-inflammatory T regulatory (Treg) cells and an increase in proinflammatory Th17 response further stimulate WAT-associated inflammation (**Figure 1**).

Deficiency of RORα greatly inhibits diet-induced adipose tissue-associated inflammation in mice (Kang et al., 2011; Lau et al., 2011). This is indicated by the greatly reduced infiltration of macrophages and $CD8^+$ T lymphocytes in WAT of $RORα^{sg/sg}$ mice fed a HFD. This was further supported by the significant reduction in the formation of CLS and the expression of several macrophage markers, such as F4/80, Mac-2, macrophage expressed 1 (Mpeg1), and macrophage scavenger receptor 1 (Msr1), in WAT of $RORα^{sg/sg}$ mice. Moreover, the relative percentage of proinflammatory M1 macrophages was significantly diminished in $RORα^{sg/sg}$ WAT. This was supported by flow cytometric analysis and the much lower levels of Cd11c expression. The reduced inflammation in $RORα^{sg/sg}$ WAT is further indicated by gene expression profiling showing a greatly reduced expression of a large number of chemokines, including *Ccl2*, *Ccl8*, *Ccl3*, and *Ccl7*, the chemokine receptors *Ccr3*, *Ccr5*, and *Ccr7*, the proinflammatory cytokines *Tnfα* and *IL-6*, the interleukin 1 receptor antagonist (*Il1rn*), osteopontin (*Opn*), *CD44*, serum amyloid 3 (*Saa3*), and several TLRs and metalloproteinases in WAT of $RORα^{sg/sg}$ mice compared to their WT counterparts (Kang et al., 2011). The expression of these genes has been reported to be elevated in obesity and many of these genes have been implicated in obesity-induced inflammation in WAT as well as insulin resistance. For example, both the CCL2/CCR2 and CCL3/CCR5 pathways have been reported to promote recruitment of macrophages in adipose tissue (Kanda et al., 2006; Kitade et al., 2012). CD44, a multifunctional cell membrane protein that acts as a receptor for hyaluronan and Opn, has been shown to regulate migration of macrophages and neutrophils (Johnson and Ruffell, 2009). CD44 and Opn null mice are protected against the development of HFD-induced hepatosteatosis, WAT-associated inflammation, and insulin resistance (Nomiyama et al., 2007; Bertola et al., 2009; Kiefer et al., 2011; Kodama et al., 2012). These observations suggest that suppression of several proinflammatory genes and pathways in $RORα^{sg/sg}$ WAT is causally linked to the reduced inflammation (**Figure 1**). Future studies have to determine what the primary effects are by which RORα regulate the expression of these genes.

RORα AND HEPATOSTEATOSIS

Obesity is associated with increased prevalence of NAFLD, which is characterized by elevated lipid accumulation in hepatocytes (Fabbrini et al., 2010). NAFLD develops when the rate of fatty acid uptake and synthesis and subsequent esterification to triglycerides is greater than the rate of fatty acid oxidation and secretion. Advanced NAFLD progresses into increased inflammation and hepatotoxicity. Several studies showed that compared to WT mice hepatic triglyceride levels are considerably reduced in $RORα^{sg/sg}$ mice fed a HFD or aging male $RORα^{sg/sg}$ mice (Raspe et al., 2001; Lau et al., 2008; Kang et al., 2011). These observations indicated that $RORα^{sg/sg}$ mice are protected against the development of age- and diet-induced hepatosteatosis. Gene expression profiling revealed that the expression of a number of lipogenic genes was

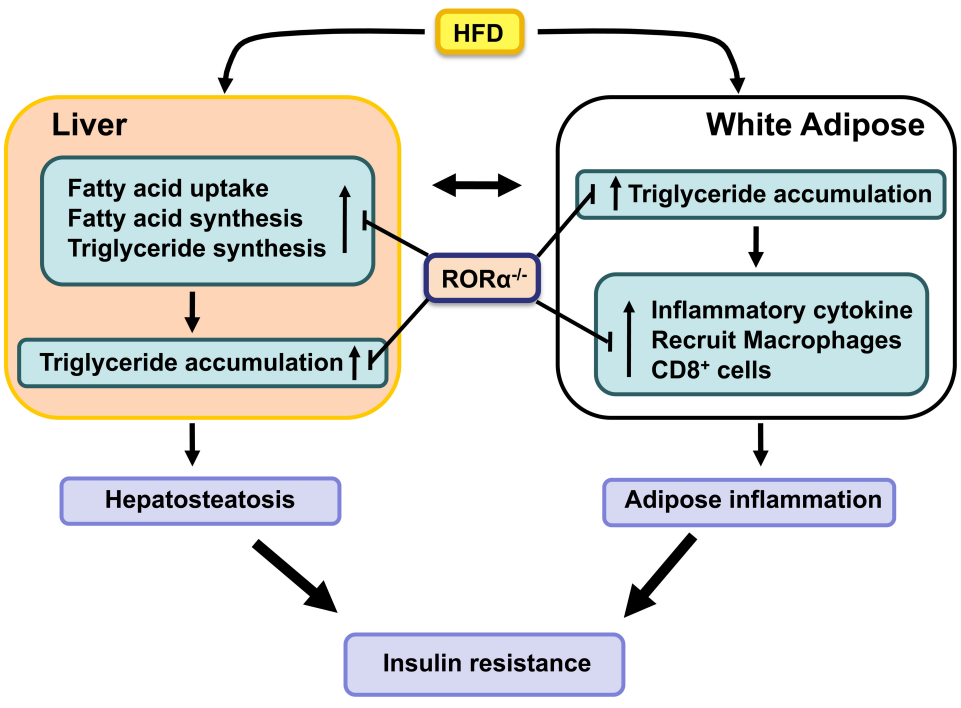


FIGURE 1 | RORα functions as a positive regulator of hepatosteatosis, WAT-associated inflammation, and insulin-resistance in diet- and age-induced obesity. Loss of RORα inhibits the hepatic expression of lipogenic genes and suppresses the expression of proinflammatory genes and the infiltration of macrophages in WAT, and as a result protects against these pathologies.

significantly reduced in the liver of *RORα*$^{sg/sg}$ mice fed a HFD. Expression of *Srebp-1c* and fatty acid synthase (*Fas*), key regulators for lipogenesis, was reduced in liver of *RORα*$^{sg/sg}$ mice. In addition, the expression of several genes involved in the main pathway of triglyceride synthesis, including glycerol-3-phosphate acyltransferase (*Gpam* or *Gpat1*) and acyl-glycerol-3-phosphate acyltransferase 9 (*Agpat9*) and *Mogat1*, which is part of an alternative pathway of triglyceride synthesis, were significantly diminished in *RORα*$^{sg/sg}$ liver (Kang et al., 2011). The hepatic expression of the cell death-inducing DFFA-like effectors a and c (*Cidea* and *Cidec*) and perilipin 2 (*Plin2* or *Adfp*), which play a critical role in the regulation of lipid storage, lipid droplet formation, and lipolysis (Gong et al., 2009; Greenberg et al., 2011), was also suppressed in *RORα*-deficient mice. RORα has been reported to activate *Plin2* transcription directly through interaction with ROREs in the *Plin2* promoter (Kang et al., 2011). Recently, the expression of fibroblast growth factor (*Fgf21*), an important regulator of lipid/glucose metabolism, was found to be directly regulated by RORα in hepatocytes (Wang et al., 2010c). Together these observations suggest that the protection against hepatosteatosis in *RORα*$^{sg/sg}$ mice is related to reduced expression of many genes involved in promoting lipogenesis and triglyceride storage, some of which are directly regulated by RORα (**Figure 1**).

RORα AND INSULIN RESISTANCE

Both adipose-associated inflammation and hepatosteatosis have been linked to the pathogenesis of insulin resistance in obesity (Guilherme et al., 2008; Donath and Shoelson, 2011; Samuel and Shulman, 2012), although a cause-effect relationship not always exists between hepatosteatosis and diabetes (Sun and Lazar, 2013). The phenotypic differences observed between WT and *RORα*$^{sg/sg}$ mice fed a HFD are consistent with this correlation. *RORα*$^{sg/sg}$ mice, which are protected against obesity, hepatosteatosis, and WAT-associated inflammation, exhibited a significantly reduced susceptibility to diet-induced insulin resistance and glucose intolerance compared to obese WT mice (Lau et al., 2008; Kang et al., 2011). In humans, two studies have revealed a connection between RORα, obesity, and type 2 diabetes. A rearrangement resulting in disruption of human RORα1 was found to be associated with severe obesity (Klar et al., 2005), while a recent GWAS study showed an association between a single nucleotide polymorphism in RORα (rs7164773) and increased risk for type 2 diabetes in the Mexico Mestizo population (Gamboa-Melendez et al., 2012).

Many inflammatory and lipogenic genes, including *Plin2*, *Il1rn*, *Opn*, *CD44*, and *Cidec*, that are down-regulated in *RORα*$^{sg/sg}$ mice have been reported to also regulate insulin sensitivity. *Plin2* null mice displayed reduced hepatic lipid accumulation and improved insulin sensitivity and glucose tolerance in an ob/ob background (Chang et al., 2010). *Il1rn*, one of the genes most dramatically repressed in WAT of *RORα*$^{sg/sg}$ mice (Kang et al., 2011), has been reported to be highly up-regulated in WAT of obese humans and to regulate insulin sensitivity (Juge-Aubry et al., 2003; Somm et al., 2006). Similarly, *Opn* expression was found to be elevated in obesity, while Opn deficiency was shown to inhibit obesity-induced inflammation and insulin resistance (Bertola et al., 2009; Kiefer et al., 2011). Deficiency in CD44, a receptor for Opn, also results in improved insulin sensitivity (Kodama et al., 2012) suggesting a role for the Opn/CD44 pathway in the control of insulin sensitivity. Mice deficient in Cidea or Cidec, which play a role in lipid storage, are protected from diet-induced obesity and display improved insulin sensitivity (Gong et al., 2009). Thus, the down-regulation of several genes, including *Plin2*, *Il1rn*, *Opn*, *CD44*, and *Cidec* in *RORα*$^{sg/sg}$ mice may collaboratively be responsible for the improved insulin sensitivity through their interrelated effects on inflammation, adipogenesis, and lipid homeostasis (**Figure 1**).

In addition to adipose tissue and liver, the pancreas and the skeletal muscle also play important roles in energy homeostasis and insulin resistance. The pancreatic islets produce a number of hormones, including insulin and glucagon, that are critical in the regulation of lipid and glucose homeostasis (Saltiel and Kahn, 2001; Cryer, 2012). RORα was shown to be selectively expressed in the glucagon-producing alpha cells; however, its role in these cells and its relationship to the phenotype observed in RORα-deficient mice needs yet to be established (Mühlbauer et al., 2013). In skeletal muscle, RORα has been reported to regulate the expression of a number of genes involved in lipid and carbohydrate metabolism (Lau et al., 2011). Ectopic expression of an RORα mutant in skeletal muscle C2C12 cells reduced the expression of the lipogenic genes, sterol regulatory element-binding transcription factor 1 (Srebp1), Fas, and stearoyl-CoA desaturase 1 (Scd1), and genes involved in cholesterol efflux, such as ATP-binding cassette, subfamily A, member 1 (Abca1). Caveolin-3 (Cav3) and carnitine palmitoyltransferase-1 (Cpt1) were found to be directly regulated by RORα. Changes in the expression of these genes may be in part responsible for the modulation of lipid and glucose homeostasis by RORα.

In muscle, insulin stimulates glucose uptake by stimulating the translocation of Glut4 (Slc2a4) to the plasma membrane (Rose and Richter, 2005). This involves phosphorylation of the insulin receptor substrate 1 (IRS1), which leads to the activation of phosphatidylinositol 3-kinase (PI3K) and subsequently AKT, which then promotes Glut4 translocation. Recently, evidence was provided for a role of RORα in PI3K-Akt signaling (Lau et al., 2011). Akt1/2 expression was up-regulated in skeletal muscle of *RORα*$^{sg/sg}$ mice and this correlated with an increase in the level of insulin-induced Akt phosphorylation, Glut4 expression, and glucose uptake. This stimulation in Akt signaling might at least in part account for the improved insulin sensitivity observed in *RORα*$^{sg/sg}$ mice.

RORγ1 AND INSULIN SENSITIVITY

The RORγ gene generates two different isoforms, RORγ1 and RORγt (RORγ2), that are expressed in a highly tissue-specific manner (Jetten, 2009). Expression of the RORγ1 isoform is restricted to several peripheral tissues, including liver, adipose tissue, kidney, small intestines, pancreas, and skeletal muscle. Recent studies identified RORγ1 as a negative regulator of adipocyte differentiation and a modulator of obesity-associated insulin resistance (Meissburger et al., 2011; Tinahones et al., 2012). In obese *RORγ*$^{-/-}$ mice, the number of adipocytes was increased (hyperplasia), while adipocyte size was reduced. Fasting blood insulin levels were shown to be significantly lower in diet-induced obese

RORγ$^{-/-}$ mice and in *RORγ*$^{-/-}$*ob/ob* double knockout mice and mice displayed improved insulin sensitivity. In addition, *RORγ*$^{-/-}$ adipocytes were highly insulin sensitive leading to improved control of circulating FFA. These observations are consistent with a recent study showing that, opposed to adipose hypertrophy, obese patients with adipose tissue hyperplasia (many small adipocytes) exhibit better glucose and lipid profiles and might be less susceptible to developing insulin resistance (Hoffstedt et al., 2010) and with data showing that in human patients the level of *RORγ 1* expression positively correlated with adipocyte size and insulin resistance (Meissburger et al., 2011; Tinahones et al., 2012). Up to now, no association has been established between *RORγ* polymorphisms and susceptibility to insulin resistance in humans. However, in cattle, a single polynucleotide polymorphism in RORγ has been linked to increased adiposity (Barendse et al., 2007). These observations suggest that the loss or potentially the inhibition of RORγ1 might protect against insulin resistance and type 2 diabetes.

In addition to adipose tissue, regulation of lipid and glucose metabolism in other tissues, including liver, pancreas, and skeletal muscle might be part of the mechanism by which by RORγ modulates insulin sensitivity. In skeletal muscle, RORγ has been reported to regulate the expression of genes associated with lipid and carbohydrate metabolism as well as the production of reactive oxygen species (Raichur et al., 2007). A recent study revealed that RORγ was selectively expressed in insulin-producing pancreatic β cells; however, its role in β cells and how this relates to the modulation of insulin sensitivity by RORγ has yet to be established (Mühlbauer et al., 2013). Further study is required to understand the modulation of lipid/glucose homeostasis and insulin sensitivity by RORγ.

CONNECTION BETWEEN RORs, CIRCADIAN RHYTHM, AND METABOLIC SYNDROME

It has been well established that many behavioral and physiological activities display circadian rhythms that are regulated by endogenous clocks (Asher and Schibler, 2011; Bass, 2012; Mohawk et al., 2012). At the molecular level the clockwork consists of an integral network of several interlocking positive and negative transcriptional and translational feedback loops that include the transcriptional regulators brain and muscle ARNT-like 1 (Bmal1), neuronal PAS domain protein 2 (Npas2), circadian locomotor output cycles kaput (Clock), two cryptochrome proteins (Cry1, 2), the nuclear receptors Rev-erbα and -β, E4 promoter-binding protein 4 (E4bp4), and three period proteins (Per1-3).

Accumulating evidence suggests that disruption of circadian rhythm is closely associated with several pathologies, including sleep disorders, cancer and metabolic syndrome (Maury et al., 2010). Recent studies have established a strong link between the circadian clock machinery and the regulation of a number of metabolic pathways (Asher and Schibler, 2011; Bass, 2012). Bmal1, Clock, and Cry1 have been implicated in the regulation of glucose homeostasis and dysfunction in these proteins lead to impaired glucose tolerance (Rudic et al., 2004; Zhang et al., 2010). Hepatic overexpression of Cry1 has been reported to improve insulin-sensitivity in insulin-resistant *db/db* mice (Zhang et al., 2010). In addition, circadian oscillator components, such as Cry1, have been implicated in the regulation of immune responses (Castanon-Cervantes et al., 2010; Logan and Sarkar, 2012; Narasimamurthy et al., 2012). In *Cry1*$^{-/-}$*Cry2*$^{-/-}$ cells, NF-κB and protein kinase A (PKA) signaling pathways are constitutively activated resulting in elevated levels of circulating TNFα, Il-1β, and Il-6 (Narasimamurthy et al., 2012).

A number of studies demonstrated that RORs play a role in the modulation of circadian behavior and clock gene expression (Sato et al., 2004; Ueda et al., 2005; Duez and Staels, 2010; **Figure 2**). *Bmal1*, *Npas2*, *E4bp4*, and *Cry1* transcription are directly regulated by RORγ and RORα in several peripheral tissues through their interaction with ROREs in their regulatory regions (Crumbley et al., 2010; Takeda et al., 2011, 2012). RORγ1 appears to be the major ROR isotype modulating the circadian expression of clock genes in peripheral tissues. RORγ1 itself exhibits a strong oscillatory pattern of expression in several peripheral tissues, including kidney, liver, pancreas, and adipose tissue, while RORα exhibits only a weak circadian expression pattern (Mongrain et al., 2008; Takeda et al., 2012; Mühlbauer et al., 2013). The RORγ1 gene is directly regulated by Bmal1/Clock heterodimers which interact with two successive E-boxes in the RORγ1 promoter (Mongrain et al., 2008; Takeda et al., 2012). Recent studies have suggested that RORγ1 and RORα might provide a link between the clock machinery and their regulation of metabolic genes (Takeda et al., 2012; **Figure 2**). Data demonstrating that the circadian pattern of expression of a number of metabolic genes are regulated by clock proteins and RORs and observations showing that circadian expression of RORγ1 is controlled by the clock machinery suggested that RORs might function as downstream mediators in the mechanism by which clock proteins regulate the circadian expression of

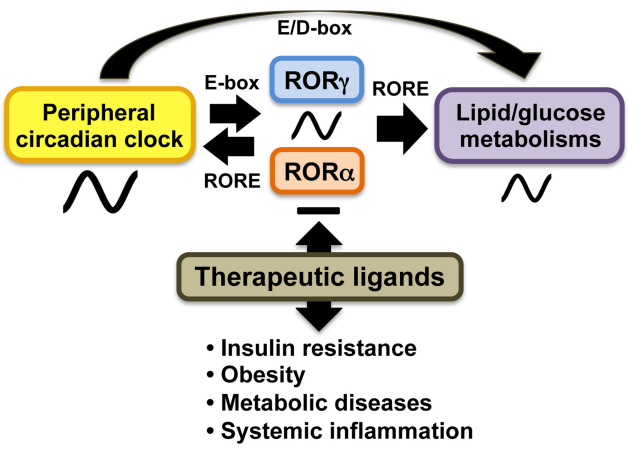


FIGURE 2 | In peripheral tissues, RORs function as intermediate regulators providing a link between clock proteins and their regulation of lipid and glucose metabolism. The oscillatory pattern of expression of *RORγ* is regulated by circadian clock proteins Bmal1 and Clock or Npas2 through E-box, whereas RORα does not exhibit a strong oscillatory pattern of expression. In turn, RORγ activates expression of several clock genes through ROREs together with RORα. Both RORα and RORγ regulate the circadian pattern of expression of target genes involved in lipid/glucose metabolism. ROR (ant)agonists may modulate the amplitude of peripheral clock oscillation of these metabolic outputs and might be useful in the treatment of insulin resistance, obesity, and tissue inflammation.

metabolic genes (Sato et al., 2004; Akashi and Takumi, 2005; Guillaumond et al., 2005; Ueda et al., 2005; Crumbley et al., 2010; Duez and Staels, 2010; Takeda et al., 2011, 2012). This is supported by observations showing that RORs regulate the circadian pattern of expression of a number of genes involved in the lipid/glucose homeostasis, including *Plin2*, sulfotransferase *Sul1E1*, the vasopressin receptor *Avpr1a*, and citrate synthase (*CS*), which exhibit roles in lipogenesis, glycogenolysis, and/or cholesterogenesis (Kang et al., 2007; Crumbley et al., 2012; Takeda et al., 2012). Thus, RORs appear to be part of the mechanism that links the circadian clock to its regulation of lipid/glucose homeostasis, inflammation, and insulin resistance (**Figure 2**).

RORs AS THERAPEUTIC TARGETS FOR METABOLIC SYNDROME AND INSULIN RESISTANCE

X-ray crystallography studies of the LBD of RORα identified the presence of cholesterol in the ligand-binding pocket of RORα (Kallen et al., 2002). Subsequent studies identified cholesterol sulfate, 7-dehydrocholesterol, and 25-hydroxycholesterol as RORα agonists (Kallen et al., 2004). All-*trans* retinoic acid and the synthetic retinoid, ALRT 1550 were reported to bind and function as antagonists for RORβ and RORγ, but not RORα (Stehlin-Gaon et al., 2003). Recently, ursolic acid and several oxygenated sterols, including 7α-hydroxycholesterol (7α-OHC), 7β-hydroxycholesterol, 7-ketocholesterol, and 24S-hydroxycholesterol, were shown to function as inverse agonists to both RORα and RORγ (Wang et al., 2010a; Xu et al., 2011), while 20α-hydroxycholesterol and 22R-hydroxycholesterol acted as agonists (Jin et al., 2010). The LXR agonist T0901317 and several other synthetic derivatives, including SR1001, were identified as RORα and RORγ inverse agonists. Digoxin and several derivatives were identified as specific inhibitors for RORγ transcriptional activity (Fujita-Sato et al., 2011; Huh et al., 2011). The ROR (inverse) antagonists have been reported to repress the expression of ROR target genes and the activation of their promoter regulatory region by inhibiting the recruitment of co-activators. Moreover, ROR antagonists have been shown to inhibit Th17 cell differentiation and IL-17 production both *in vitro* and *in vivo* and to suppress the development of experimental autoimmune encephalomyelitis (Huh et al., 2011; Jetten, 2011; Solt et al., 2011). Therefore, antagonists for RORγ might be potential drugs for pharmacological intervention in the treatment and suppression of several autoimmune diseases, including multiple sclerosis, collagen-induced arthritis, rheumatoid arthritis, and asthma (Solt et al., 2010; Huh and Littman, 2012). Because of their role in regulating various features of metabolic syndrome, RORα and γ antagonists might also have beneficial effects in the management of obesity and insulin resistance.

SUMMARY

The study of ROR-deficient mice has clearly demonstrated that RORα and RORγ are important in several physiological processes, including the regulation of several immune responses, lipid/glucose homeostasis, and circadian rhythm. These studies revealed that loss of RORα protects against the development of diet- and age-induced obesity, hepatosteatosis, glucose intolerance, and insulin resistance, while loss of RORγ protects against insulin resistance. These protective effects have been linked to suppression of the expression of multiple proinflammatory and metabolic genes. RORs regulate expression of some of these genes directly by binding ROREs in their regulatory region and in certain cases involves changes in their circadian pattern of expression. Although much progress has been made, what event or which ROR target genes are the primary driving force by which RORs influences WAT-associated inflammation, hepatosteatosis, and insulin resistance needs further study. With the increasing evidence for an interrelationship between the controls of lipid/glucose metabolism, inflammation and circadian rhythm, RORs might functions as intermediaries between the controls. With the discovery of ROR antagonists, RORs may provide a novel therapeutic target in the management of various aspect of metabolic syndrome.

ACKNOWLEDGMENTS

The authors would like to thank Drs Kristin Lichti-Kaiser and Gary ZeRuth for their comments on the manuscript. This research was supported by the Intramural Research Program of the National Institute of Environmental Health Sciences, the National Institutes of Health [Z01-ES-101586].

REFERENCES

Ahmed, M., and Gaffen, S. L. (2010). IL-17 in obesity and adipogenesis. *Cytokine Growth Factor Rev.* 21, 449–453.

Akashi, M., and Takumi, T. (2005). The orphan nuclear receptor RORα regulates circadian transcription of the mammalian core-clock Bmal1. *Nat. Struct. Mol. Biol.* 12, 441–448.

Asher, G., and Schibler, U. (2011). Crosstalk between components of circadian and metabolic cycles in mammals. *Cell Metab.* 13, 125–137.

Austin, S., Medvedev, A., Yan, Z. H., Adachi, H., Hirose, T., and Jetten, A. M. (1998). Induction of the nuclear orphan receptor RORγ during adipocyte differentiation of D1 and 3T3-L1 cells. *Cell Growth Differ.* 9, 267–276.

Barendse, W., Bunch, R. J., Kijas, J. W., and Thomas, M. B. (2007). The effect of genetic variation of the retinoic acid receptor-related orphan receptor C gene on fatness in cattle. *Genetics* 175, 843–853.

Bass, J. (2012). Circadian topology of metabolism. *Nature* 491, 348–356.

Bertola, A., Ciucci, T., Rousseau, D., Bourlier, V., Duffaut, C., Bonnafous, S., et al. (2012). Identification of adipose tissue dendritic cells correlated with obesity-associated insulin-resistance and inducing Th17 responses in mice and patients. *Diabetes* 61, 2238–2247.

Bertola, A., Deveaux, V., Bonnafous, S., Rousseau, D., Anty, R., Wakkach, A., et al. (2009). Elevated expression of osteopontin may be related to adipose tissue macrophage accumulation and liver steatosis in morbid obesity. *Diabetes* 58, 125–133.

Browning, J. D., Szczepaniak, L. S., Dobbins, R., Nuremberg, P., Horton, J. D., Cohen, J. C., et al. (2004). Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 40, 1387–1395.

Castanon-Cervantes, O., Wu, M., Ehlen, J. C., Paul, K., Gamble, K. L., Johnson, R. L., et al. (2010). Dysregulation of inflammatory responses by chronic circadian disruption. *J. Immunol.* 185, 5796–5805.

Chang, B. H., Li, L., Saha, P., and Chan, L. (2010). Absence of adipose differentiation related protein upregulates hepatic VLDL secretion, relieves hepatosteatosis, and improves whole body insulin resistance in leptin-deficient mice. *J. Lipid Res.* 51, 2132–2142.

Crumbley, C., Wang, Y., Banerjee, S., and Burris, T. P. (2012). Regulation of expression of citrate synthase by the retinoic acid receptor-related orphan receptor α (RORα). *PLoS ONE* 7:e33804. doi: 10.1371/journal.pone.0033804

Crumbley, C., Wang, Y., Kojetin, D. J., and Burris, T. P. (2010). Characterization of the core mammalian clock component, NPAS2, as a

REV-ERBα/RORα target gene. *J. Biol. Chem.* 285, 35386–35392.

Cryer, P. E. (2012). Minireview: glucagon in the pathogenesis of hypoglycemia and hyperglycemia in diabetes. *Endocrinology* 153, 1039–1048.

Donath, M. Y., and Shoelson, S. E. (2011). Type 2 diabetes as an inflammatory disease. *Nat. Rev. Immunol.* 11, 98–107.

Duez, H., Duhem, C., Laitinen, S., Patole, P. S., Abdelkarim, M., Bois-Joyeux, B., et al. (2009). Inhibition of adipocyte differentiation by RORα. *FEBS Lett.* 583, 2031–2036.

Duez, H., and Staels, B. (2010). Nuclear receptors linking circadian rhythms and cardiometabolic control. *Arterioscler. Thromb. Vasc. Biol.* 30, 1529–1534.

Dzhagalov, I., Giguere, V., and He, Y. W. (2004). Lymphocyte development and function in the absence of retinoic acid-related orphan receptor α. *J. Immunol.* 173, 2952–2959.

Fabbrini, E., Sullivan, S., and Klein, S. (2010). Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology* 51, 679–689.

Fujita-Sato, S., Ito, S., Isobe, T., Ohyama, T., Wakabayashi, K., Morishita, K., et al. (2011). Structural basis of digoxin that antagonizes RORγt receptor activity and suppresses Th17 cell differentiation and interleukin (IL)-17 production. *J. Biol. Chem.* 286, 31409–31417.

Gamboa-Melendez, M. A., Huerta-Chagoya, A., Moreno-Macias, H., Vazquez-Cardenas, P., Ordonez-Sanchez, M. L., Rodriguez-Guillen, R., et al. (2012). Contribution of common genetic variation to the risk of type 2 diabetes in the mexican mestizo population. *Diabetes* 61, 3314–3321.

Glass, C. K., and Olefsky, J. M. (2012). Inflammation and lipid signaling in the etiology of insulin resistance. *Cell Metab.* 15, 635–645.

Gong, J., Sun, Z., and Li, P. (2009). CIDE proteins and metabolic disorders. *Curr. Opin. Lipidol.* 20, 121–126.

Greenberg, A. S., Coleman, R. A., Kraemer, F. B., McManaman, J. L., Obin, M. S., Puri, V., et al. (2011). The role of lipid droplets in metabolic disease in rodents and humans. *J. Clin. Invest.* 121, 2102–2110.

Guilherme, A., Virbasius, J. V., Puri, V., and Czech, M. P. (2008). Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat. Rev. Mol. Cell Biol.* 9, 367–377.

Guillaumond, F., Dardente, H., Giguere, V., and Cermakian, N. (2005). Differential control of Bmal1 circadian transcription by REV-ERB and ROR nuclear receptors. *J. Biol. Rhythms* 20, 391–403.

Halim, T. Y., MacLaren, A., Romanish, M. T., Gold, M. J., McNagny, K. M., and Takei, F. (2012). Retinoic-acid-receptor-related orphan nuclear receptor α is required for natural helper cell development and allergic inflammation. *Immunity* 37, 463–474.

Hoffstedt, J., Arner, E., Wahrenberg, H., Andersson, D. P., Qvisth, V., Lofgren, P., et al. (2010). Regional impact of adipose tissue morphology on the metabolic profile in morbid obesity. *Diabetologia* 53, 2496–2503.

Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. *Nature* 444, 860–867.

Huh, J. R., Leung, M. W., Huang, P., Ryan, D. A., Krout, M. R., Malapaka, R. R., et al. (2011). Digoxin and its derivatives suppress TH17 cell differentiation by antagonizing RORγ activity. *Nature* 472, 486–490.

Huh, J. R., and Littman, D. R. (2012). Small molecule inhibitors of RORγt: targeting Th17 cells and other applications. *Eur. J. Immunol.* 42, 2232–2237.

Ivanov, I. I., McKenzie, B. S., Zhou, L., Tadokoro, C. E., Lepelley, A., Lafaille, J. J., et al. (2006). The orphan nuclear receptor RORγ directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* 126, 1121–1133.

Jaradat, M., Stapleton, C., Tilley, S. L., Dixon, D., Erikson, C. J., McCaskill, J. G., et al. (2006). Modulatory role for retinoid-related orphan receptor α in allergen-induced lung inflammation. *Am. J. Respir. Crit. Care Med.* 174, 1299–1309.

Jetten, A. M. (2004). Recent advances in the mechanisms of action and physiological functions of the retinoid-related orphan receptors (RORs). *Curr. Drug Targets Inflamm. Allergy* 3, 395–412.

Jetten, A. M. (2009). Retinoid-related orphan receptors (RORs): critical roles in development, immunity, circadian rhythm, and cellular metabolism. *Nucl. Recept. Signal.* 7, e003.

Jetten, A. M. (2011). Immunology: a helping hand against autoimmunity. *Nature* 472, 421–422.

Jin, L., Martynowski, D., Zheng, S., Wada, T., Xie, W., and Li, Y. (2010). Structural basis for hydroxycholesterols as natural ligands of orphan nuclear receptor RORγ. *Mol. Endocrinol.* 24, 923–929.

Johnson, P., and Ruffell, B. (2009). CD44 and its role in inflammation and inflammatory diseases. *Inflamm. Allergy Drug Targets* 8, 208–220.

Juge-Aubry, C. E., Somm, E., Giusti, V., Pernin, A., Chicheportiche, R., Verdumo, C., et al. (2003). Adipose tissue is a major source of interleukin-1 receptor antagonist: upregulation in obesity and inflammation. *Diabetes* 52, 1104–1110.

Kallen, J., Schlaeppi, J. M., Bitsch, F., Delhon, I., and Fournier, B. (2004). Crystal structure of the human RORα ligand binding domain in complex with cholesterol sulfate at 2.2 A. *J. Biol. Chem.* 279, 14033–14038.

Kallen, J. A., Schlaeppi, J. M., Bitsch, F., Geisse, S., Geiser, M., Delhon, I., et al. (2002). X-ray structure of the hRORα LBD at 1.63 A: structural and functional data that cholesterol or a cholesterol derivative is the natural ligand of RORα. *Structure* 10, 1697–1707.

Kanda, H., Tateya, S., Tamori, Y., Kotani, K., Hiasa, K., Kitazawa, R., et al. (2006). MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J. Clin. Invest.* 116, 1494–1505.

Kang, H. S., Angers, M., Beak, J. Y., Wu, X., Gimble, J. M., Wada, T., et al. (2007). Gene expression profiling reveals a regulatory role for RORα and RORγ in phase I and phase II metabolism. *Physiol. Genomics* 31, 281–294.

Kang, H. S., Okamoto, K., Takeda, Y., Beak, J. Y., Gerrish, K., Bortner, C. D., et al. (2011). Transcriptional profiling reveals a role for RORα in regulating gene expression in obesity-associated inflammation and hepatic steatosis. *Physiol. Genomics* 43, 818–828.

Kiefer, F. W., Neschen, S., Pfau, B., Legerer, B., Neuhofer, A., Kahle, M., et al. (2011). Osteopontin deficiency protects against obesity-induced hepatic steatosis and attenuates glucose production in mice. *Diabetologia* 54, 2132–2142.

Kitade, H., Sawamoto, K., Nagashimada, M., Inoue, H., Yamamoto, Y., Sai, Y., et al. (2012). CCR5 plays a critical role in obesity-induced adipose tissue inflammation and insulin resistance by regulating both macrophage recruitment and M1/M2 status. *Diabetes* 61, 1680–1690.

Klar, J., Asling, B., Carlsson, B., Ulvsback, M., Dellsen, A., Strom, C., et al. (2005). RAR-related orphan receptor A isoform 1 (RORα1) is disrupted by a balanced translocation t(4;15)(q22.3;q21.3) associated with severe obesity. *Eur. J. Hum. Genet.* 13, 928–934.

Kodama, K., Horikoshi, M., Toda, K., Yamada, S., Hara, K., Irie, J., et al. (2012). Expression-based genome-wide association study links the receptor CD44 in adipose tissue with type 2 diabetes. *Proc. Natl. Acad. Sci. U.S.A.* 109, 7049–7054.

Kurebayashi, S., Ueda, E., Sakaue, M., Patel, D. D., Medvedev, A., Zhang, F., et al. (2000). Retinoid-related orphan receptor γ (RORγ) is essential for lymphoid organogenesis and controls apoptosis during thymopoiesis. *Proc. Natl. Acad. Sci. U.S.A.* 97, 10132–10137.

Lau, P., Fitzsimmons, R. L., Pearen, M. A., Watt, M. J., and Muscat, G. E. (2011). Homozygous staggerer (sg/sg) mice display improved insulin sensitivity and enhanced glucose uptake in skeletal muscle. *Diabetologia* 54, 1169–1180.

Lau, P., Fitzsimmons, R. L., Raichur, S., Wang, S. C., Lechtken, A., and Muscat, G. E. (2008). The orphan nuclear receptor, RORα, regulates gene expression that controls lipid metabolism: staggerer (SG/SG) mice are resistant to diet-induced obesity. *J. Biol. Chem.* 283, 18411–18421.

Logan, R. W., and Sarkar, D. K. (2012). Circadian nature of immune function. *Mol. Cell. Endocrinol.* 349, 82–90.

Maury, E., Ramsey, K. M., and Bass, J. (2010). Circadian rhythms and metabolic syndrome: from experimental genetics to human disease. *Circ. Res.* 106, 447–462.

Meissburger, B., Ukropec, J., Roeder, E., Beaton, N., Geiger, M., Teupser, D., et al. (2011). Adipogenesis and insulin sensitivity in obesity are regulated by retinoid-related orphan receptor γ. *EMBO Mol. Med.* 3, 637–651.

Mohawk, J. A., Green, C. B., and Takahashi, J. S. (2012). Central and peripheral circadian clocks in mammals. *Annu. Rev. Neurosci.* 35, 445–462.

Mongrain, V., Ruan, X., Dardente, H., Fortier, E. E., and Cermakian, N. (2008). Clock-dependent and independent transcriptional control of the two isoforms from the mouse RORγ gene. *Genes Cells* 13, 1197–1210.

Mühlbauer, E., Bazwinsky-Wutschke, I., Wolgast, S., Labucay, K., and Peschke,

E. (2013). Differential and daytime dependent expression of nuclear receptors RORα, RORβ, RORγ and RXRα in the rodent pancreas and islet. *Mol. Cell. Endocrinol.* 365, 129–138.

Narasimamurthy, R., Hatori, M., Nayak, S. K., Liu, F., Panda, S., and Verma, I. M. (2012). Circadian clock protein cryptochrome regulates the expression of proinflammatory cytokines. *Proc. Natl. Acad. Sci. U.S.A.* 109, 12662–12667.

Nishimura, S., Manabe, I., Nagasaki, M., Eto, K., Yamashita, H., Ohsugi, M., et al. (2009). CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat. Med.* 15, 914–920.

Nomiyama, T., Perez-Tilve, D., Ogawa, D., Gizard, F., Zhao, Y., Heywood, E. B., et al. (2007). Osteopontin mediates obesity-induced adipose tissue macrophage infiltration and insulin resistance in mice. *J. Clin. Invest.* 117, 2877–2888.

Odegaard, J. I., and Chawla, A. (2008). Mechanisms of macrophage activation in obesity-induced insulin resistance. *Nat. Clin. Pract. Endocrinol. Metab.* 4, 619–626.

Ogden, C. L., Carroll, M. D., Kit, B. K., and Flegal, K. M. (2012). Prevalence of obesity and trends in body mass index among US children and adolescents, 1999–2010. *JAMA* 307, 483–490.

Ohoka, N., Kato, S., Takahashi, Y., Hayashi, H., and Sato, R. (2009). The orphan nuclear receptor RORα restrains adipocyte differentiation through a reduction of C/EBPβ activity and perilipin gene expression. *Mol. Endocrinol.* 23, 759–771.

Ouchi, N., Parker, J. L., Lugus, J. J., and Walsh, K. (2011). Adipokines in inflammation and metabolic disease. *Nat. Rev. Immunol.* 11, 85–97.

Raichur, S., Lau, P., Staels, B., and Muscat, G. E. (2007). Retinoid-related orphan receptor γ regulates several genes that control metabolism in skeletal muscle cells: links to modulation of reactive oxygen species production. *J. Mol. Endocrinol.* 39, 29–44.

Ramasamy, A., Kuokkanen, M., Vedantam, S., Gajdos, Z. K., Couto Alves, A., Lyon, H. N., et al. (2012). Genome-wide association studies of asthma in population-based cohorts confirm known and suggested loci and identify an additional association near HLA. *PLoS ONE* 7:e44008. doi: 10.1371/journal.pone.0044008

Raspe, E., Duez, H., Gervois, P., Fievet, C., Fruchart, J. C., Besnard, S., et al. (2001). Transcriptional regulation of apolipoprotein C-III gene expression by the orphan nuclear receptor RORα. *J. Biol. Chem.* 276, 2865–2871.

Rose, A. J., and Richter, E. A. (2005). Skeletal muscle glucose uptake during exercise: how is it regulated? *Physiology (Bethesda)* 20, 260–270.

Rudic, R. D., McNamara, P., Curtis, A. M., Boston, R. C., Panda, S., Hogenesch, J. B., et al. (2004). BMAL1 and CLOCK, two essential components of the circadian clock, are involved in glucose homeostasis. *PLoS Biol.* 2:e377. doi: 10.1371/journal.pbio.0020377

Saltiel, A. R., and Kahn, C. R. (2001). Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 414, 799–806.

Samuel, V. T., and Shulman, G. I. (2012). Mechanisms for insulin resistance: common threads and missing links. *Cell* 148, 852–871.

Sato, T. K., Panda, S., Miraglia, L. J., Reyes, T. M., Rudic, R. D., McNamara, P., et al. (2004). A functional genomics strategy reveals Rora as a component of the mammalian circadian clock. *Neuron* 43, 527–537.

Solt, L. A., and Burris, T. P. (2012). Action of RORs and their ligands in (patho)physiology. *Trends Endocrinol. Metab.* 23, 619–627.

Solt, L. A., Griffin, P. R., and Burris, T. P. (2010). Ligand regulation of retinoic acid receptor-related orphan receptors: implications for development of novel therapeutics. *Curr. Opin. Lipidol.* 21, 204–211.

Solt, L. A., Kumar, N., Nuhant, P., Wang, Y., Lauer, J. L., Liu, J., et al. (2011). Suppression of TH17 differentiation and autoimmunity by a synthetic ROR ligand. *Nature* 472, 491–494.

Somm, E., Cettour-Rose, P., Asensio, C., Charollais, A., Klein, M., Theander-Carrillo, C., et al. (2006). Interleukin-1 receptor antagonist is upregulated during diet-induced obesity and regulates insulin sensitivity in rodents. *Diabetologia* 49, 387–393.

Stehlin-Gaon, C., Willmann, D., Zeyer, D., Sanglier, S., Van Dorsselaer, A., Renaud, J. P., et al. (2003). All-trans retinoic acid is a ligand for the orphan nuclear receptor RORβ. *Nat. Struct. Biol.* 10, 820–825.

Sun, K., Kusminski, C. M., and Scherer, P. E. (2011). Adipose tissue remodeling and obesity. *J. Clin. Invest.* 121, 2094–2101.

Sun, Z., and Lazar, M. A. (2013). Dissociating fatty liver and diabetes. *Trends Endocrinol. Metab.* 24, 4–12.

Sun, Z., Unutmaz, D., Zou, Y. R., Sunshine, M. J., Pierani, A., Brenner-Morton, S., et al. (2000). Requirement for RORγ in thymocyte survival and lymphoid organ development. *Science* 288, 2369–2373.

Takeda, Y., Jothi, R., Birault, V., and Jetten, A. M. (2012). RORγ directly regulates the circadian expression of clock genes and downstream targets *in vivo*. *Nucleic Acids Res.* 40, 8519–8539.

Takeda, Y., Kang, H. S., Angers, M., and Jetten, A. M. (2011). Retinoic acid-related orphan receptor γ directly regulates neuronal PAS domain protein 2 transcription *in vivo*. *Nucleic Acids Res.* 39, 4769–4782.

Tilley, S. L., Jaradat, M., Stapleton, C., Dixon, D., Hua, X., Erikson, C. J., et al. (2007). Retinoid-related orphan receptor γ controls immunoglobulin production and Th1/Th2 cytokine balance in the adaptive immune response to allergen. *J. Immunol.* 178, 3208–3218.

Tinahones, F. J., Moreno-Santos, I., Vendrell, J., Chacon, M. R., Garrido-Sanchez, L., Garcia-Fuentes, E., et al. (2012). The retinoic acid receptor-related orphan nuclear receptor γ1 (RORγ1): a novel player determinant of insulin sensitivity in morbid obesity. *Obesity* 20, 488–497.

Ueda, H. R., Hayashi, S., Chen, W., Sano, M., Machida, M., Shigeyoshi, Y., et al. (2005). System-level identification of transcriptional circuits underlying mammalian circadian clocks. *Nat. Genet.* 37, 187–192.

Wang, Y., Kumar, N., Crumbley, C., Griffin, P. R., and Burris, T. P. (2010a). A second class of nuclear receptors for oxysterols: regulation of RORα and RORγ activity by 24S-hydroxycholesterol (cerebrosterol). *Biochim. Biophys. Acta* 1801, 917–923.

Wang, Y., Kumar, N., Solt, L. A., Richardson, T. I., Helvering, L. M., Crumbley, C., et al. (2010b). Modulation of retinoic acid receptor-related orphan receptor α and γ activity by 7-oxygenated sterol ligands. *J. Biol. Chem.* 285, 5013–5025.

Wang, Y., Solt, L. A., and Burris, T. P. (2010c). Regulation of FGF21 expression and secretion by retinoic acid receptor-related orphan receptor α. *J. Biol. Chem.* 285, 15668–15673.

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., and Ferrante, A. W. Jr. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* 112, 1796–1808.

Xu, T., Wang, X., Zhong, B., Nurieva, R. I., Ding, S., and Dong, C. (2011). Ursolic acid suppresses interleukin-17 (IL-17) production by selectively antagonizing the function of RORγt protein. *J. Biol. Chem.* 286, 22707–22710.

Yang, X. O., Pappu, B. P., Nurieva, R., Akimzhanov, A., Kang, H. S., Chung, Y., et al. (2008). T helper 17 lineage differentiation is programmed by orphan nuclear receptors RORα and RORγ. *Immunity* 28, 29–39.

Zhang, E.E., Liu, Y., Dentin, R., Pongsawakul, P.Y., Liu, A. C., Hirota, T., et al. (2010). Cryptochrome mediates circadian regulation of cAMP signaling and hepatic gluconeogenesis. *Nat. Med.* 16, 1152–1156.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 30 November 2012; paper pending published: 21 December 2012; accepted: 05 January 2013; published online: 25 January 2013.

*Citation: Jetten AM, Kang HS and Takeda Y (2013) Retinoic acid-related orphan receptors α and γ: key regulators of lipid/glucose metabolism, inflammation, and insulin sensitivity. Front. Endocrin. **4**:1. doi: 10.3389/fendo.2013.00001*

This article was submitted to Frontiers in Diabetes, a specialty of Frontiers in Endocrinology.

Copyright © 2013 Jetten, Kang and Takeda. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.

frontiers in
ENDOCRINOLOGY

ORIGINAL RESEARCH ARTICLE
published: 07 January 2013
doi: 10.3389/fendo.2012.00166

Transgenic mice overexpressing renin exhibit glucose intolerance and diet-genotype interactions

Sarah J. Fletcher[1], Nishan S. Kalupahana[2], Morvarid Soltani-Bejnood[3], Jung Han Kim[4], Arnold M. Saxton[5], David H. Wasserman[6], Bart De Taeye[6], Brynn H. Voy[5], Annie Quignard-Boulange[7] and Naima Moustaid-Moussa[8]*

[1] *Genome Science and Technology Program, University of Tennessee, Knoxville, TN, USA*
[2] *Department of Physiology, Faculty of Medicine, University of Peradeniya, Peradeniya, Sri Lanka*
[3] *Pellissippi State, Knoxville, TN, USA*
[4] *Department of Pharmacology, Physiology and Toxicology, School of Medicine, Marshall University, Huntington, WV, USA*
[5] *Department of Animal Science, University of Tennessee, Knoxville, TN, USA*
[6] *Department of Molecular Physiology and Biophysics, School of Medicine and Mouse Metabolic Phenotyping Center, Vanderbilt University, Nashville, TN, USA*
[7] *INRA-AgroParisTech UMR 914, Paris, France*
[8] *Nutritional Sciences, Texas Tech University, Lubbock, TX, USA*

Edited by:
Tsuguhito Ota, Kanazawa University, Japan

Reviewed by:
Undurti Narasimha Das, UND Life Sciences, USA
Toru Aizawa, Aizawa Hospital, Japan

****Correspondence:***
Naima Moustaid-Moussa, Nutritional Sciences, Texas Tech University, 1301, Akron Street, Lubbock, TX 79423, USA.
e-mail: naima.moustaid-moussa@ttu.edu

Numerous animal and clinical investigations have pointed to a potential role of the renin-angiotensin system (RAS) in the development of insulin resistance and diabetes in conditions of expanded fat mass. However, the mechanisms underlying this association remain unclear. We used a transgenic mouse model overexpressing renin in the liver (RenTgMK) to examine the effects of chronic activation of RAS on adiposity and insulin sensitivity. Hepatic overexpression of renin resulted in constitutively elevated plasma angiotensin II (four- to six-fold increase vs. wild-type, WT). Surprisingly, RenTgMK mice developed glucose intolerance despite low levels of adiposity and insulinemia. The transgenics also had lower plasma triglyceride levels. Glucose intolerance in transgenic mice fed a low-fat diet was comparable to that observed in high-fat fed WT mice. These studies demonstrate that overexpression of renin and associated hyperangiotensinemia impair glucose tolerance in a diet-dependent manner and further support a consistent role of RAS in the pathogenesis of diabetes and insulin resistance, independent of changes in fat mass.

Keywords: adipose tissue, renin-angiotensin system, insulin resistance, angiotensin II

INTRODUCTION

The renin-angiotensin system (RAS) plays an important role in the regulation of blood pressure, fluid, and electrolyte balance (Schmieder et al., 2007). Angiotensinogen (AGT), the precursor peptide of this system, undergoes successive enzymatic cleavages by renin and angiotensin converting enzyme (ACE) to yield angiotensin I (Ang I) and angiotensin II (Ang II) respectively. The latter is the main bioactive peptide of this system, which acts via two G-protein coupled receptors, namely angiotensin Type-1 (AT1) and Type-2 (AT2) receptors, to exert its physiological effects. Because AT1 activation by Ang II leads to elevation of blood pressure, ACE inhibitors (ACEI) and AT1 blockers (ARB) are pharmacologically used as anti-hypertensive agents (Schmieder et al., 2007).

Interestingly, several clinical studies have shown that patients on RAS blockers have a lower risk of developing Type-2 diabetes when compared to patients on other anti-hypertensive medications (Vermes et al., 2003; Bosch et al., 2006). Moreover, RAS blockade prevents and reverses insulin resistance induced by high-fat feeding in rodents (Lee et al., 2008). Given that plasma and tissue levels of several RAS components positively correlate with body mass index (Schorr et al., 1998; Van Harmelen et al., 2000), it is possible that overactivation of the RAS is linked to the pathogenesis of insulin resistance in obesity. Indeed, genetic deletion of AGT, ACE, renin, AT1, or AT2 protects rodents from diet-induced obesity and insulin resistance (Massiera et al., 2001b; Yvan-Charvet et al., 2005; Takahashi et al., 2007; Jayasooriya et al., 2008). Conversely, chronic RAS overactivation via Ang II infusion (Ogihara et al., 2002) leads to glucose intolerance and insulin resistance in rodents, further supporting a role of RAS overactivation in the pathogenesis of insulin resistance.

Although obesity and increased adiposity are associated with RAS overactivation, it is not clear whether systemic RAS overactivation can lead to both obesity and insulin resistance. It is important to test this because studies have documented differences in RAS activity in humans, which have been attributed to polymorphisms in RAS coding (Jeunemaitre et al., 1999; Jeunemaitre, 2008) or promoter regions (Xiao et al., 2006). Therefore, understanding the implications of chronic elevation of RAS may help provide insight into metabolic consequences of chronically elevated RAS in humans.

While overexpression of RAS is consistently associated with insulin resistance and glucose intolerance, the effect of chronic RAS overactivation on adiposity is not clear. This is further complicated by existence of local RAS in several tissues with the local effects complicating the understanding of systemic effects of RAS (Kalupahana and Moustaid-Moussa, 2012b). For example, overexpression of AGT in adipose tissue increases adiposity and blood

pressure and leads to insulin resistance (Massiera et al., 2001a; Kalupahana et al., 2012). However, acute or chronic systemic RAS overactivation leads to decreased fat mass despite the development of insulin resistance (Brink et al., 1996). This suggests that increased fat mass in the case of adipose RAS overexpression may be due to local effects of Ang II production within adipose tissue.

To further dissect effects of elevated systemic Ang II on insulin sensitivity and adiposity, we used a unique mouse model in which Ang II is chronically elevated throughout life time through genetic manipulation. This mouse model is a unique genetic minipump model in which renin is overexpressed in the liver. Given that renin release is the rate-limiting step in the systemic RAS, this model offers the advantage of constant renin overexpression independent of homeostatic control and a lifelong elevated level of Ang II. As expected, these transgenic mice (RenTgMK; Caron et al., 2002) exhibit elevated levels of circulating renin and Ang I and develop chronic hypertension along with other pathological manifestations (Caron et al., 2002, 2004). The RenTgMK mice thus allow us also to study the effects of systemic chronic elevations of Ang II on adiposity and glucose homeostasis, so that we can dissect the effects of systemic vs. adipose RAS by comparing these results with the ones previously reported for local adipose overexpression of RAS (Massiera et al., 2001a).

We report here that elevated circulating Ang II due to renin overexpression leads to glucose intolerance, which is further exacerbated by high-fat feeding. Unexpectedly, these mice exhibit otherwise normal glucose metabolism and a transgene dose-dependent decrease in fat mass and insulinemia.

MATERIALS AND METHODS

ANIMALS

RenTgMK transgenic mice were kindly provided by Dr. Oliver Smithies, University of North Carolina, Chapel Hill, NC, USA (Hatada et al., 1999). Briefly, a renin transgene consisting of portions of the Ren2 and Ren-1d genes (Ren2/1d) was inserted into the genome at the ApoA1/ApoC3 locus via homologous recombination and placed under control of an albumin promoter/enhancer (AlbP/E) to achieve liver-specific expression.

Male heterozygous RenTgMK (RenTgMK$^{-/+}$) mice on an isogenic SvEv 129/6 background were crossed with wild-type (WT) SvEv females. Subsequent heterozygous F1 progeny were mated to generate the F2 offspring that were used in this study. Mice used in this study were bred and maintained at the University of Tennessee accredited animal facility, on a 12h:12h light-dark cycle at 22°C and fed a standard rodent chow and water *ad libitum*. All experiments were approved by the Institutional Animal Care and Use Committee at the University of Tennessee.

GENOTYPING

DNA was extracted from tail tips as previously described (Truett et al., 2000). PCR-based genotyping was performed using three primers: p1, 5′-TGGGATTCTAACCCTGAGGACC-3′; p2, 5′-CACAGATTGTAACTGCAAATCTGTCG-3′; p3, 5′-GTTCTTCTGAGGGGATC-GGC-3′ (Sigma Genosys, The Woodlands, TX, USA) as previously described (Caron et al., 2002).

GLUCOSE TOLERANCE TEST

Mice were fasted overnight prior to the glucose tolerance test (GTT). Blood was collected in heparinized capillary tubes from the orbital sinus prior to intra-peritoneal injection with glucose (1 g/kg body weight), and then 15, 30, 60, 90, and 120 min after injection. Plasma glucose concentrations were calculated using a One Touch ultra-monitoring system (Johnson & Johnson, Co., New Brunswick, NJ, USA). The GTT was performed on mice 10 weeks old and repeated when the mice reached 20 weeks of age and the area under the curve (AUC) for glucose and insulin were calculated.

PLASMA MEASUREMENTS

Serum was separated from blood samples collected during the GTT by centrifugation at 3000 rpm for 15 min at 4°C and then stored in aliquots at −80°C until assayed. Serum insulin, leptin, and adiponectin levels were measured in duplicate using commercially available ELISA kits following the manufacturer's protocol (insulin cat# 90060 and leptin cat# 90030, Crystal Chem, Inc., Downers Grove, IL, USA; adiponectin cat# EZMADP-60, Linco Research, Billerica, MA, USA). Absorbance was read at 450 nm on a Packard SpectraCount microplate reader (Packard Instrument, Co., Meriden, CT, USA).

DIET STUDY

Male heterozygous (RenTgMK$^{+/-}$) mice and their WT littermates were randomly assigned to either a high-fat diet (60% kcal from fat cat# D12492, Research Diets, Inc., New Brunswick, NJ, USA) or a low-fat diet (10% kcal from fat cat# D12450B, Research Diets, Inc., New Brunswick, NJ, USA) for 18 weeks. Each diet group ($n = 6$/group) was comprised of three male RenTgMK$^{+/-}$ mice and three male WT mice. Body weight measurements were acquired weekly for the duration of the study. At the conclusion of the 18-week diet study, a GTT was performed and plasma insulin, leptin, and adiponectin concentrations were measured, as described above. Mice were sacrificed 1 week after the GTT.

METABOLIC STUDIES

Metabolic studies of the RenTgMK mice were performed at the Mouse Metabolic Phenotyping Center (MMPC) at Vanderbilt University, Nashville, TN, USA. Glucose infiltration rate, glucose turnover rate, endogenous glucose turnover rate, and clearance were measured. Whole-body insulin activity *in vivo* was examined via euglycemic hyperinsulinemic clamp. Detailed procedure has been previously reported (Ayala et al., 2006). Briefly, to assess insulin sensitivity and glucose metabolism, insulin was continuously administered via euglycemic hyperinsulinemic clamp. Catheters were chronically implanted in the jugular vein and carotid artery. Arterial glucose levels were measured every 5–10 min during 120 min and glucose infusion rates were determined based on the arterial glucose measurements. Plasma glucose turnover was measured in RenTgMK$^{+/-}$ and WT males ($n = 8$–12/group). Mice were continuously infused with [3-^{3}H]glucose at a rate of 0.4 μCi/min. Glucose appearance (Ra) and disappearance (Rd) rates were estimated as the ratio of the rate of infusion of [3-^{3}H]glucose and the steady-state plasma [^{3}H]glucose specific activity (dpm/mg), and the glucose disappearance was assumed

to be equal to the steady-state Ra rate. Glucose clearance was calculated by dividing the Rd by the arterial glucose concentration. To measure tissue-specific glucose uptake, mice were injected with 12 μCi of [^{3}H]-labeled 2-deoxyglucose ([2-^{3}H] DG). Arterial plasma samples were collected in intervals for 40 min before mice were anesthetized and tissues were extracted and frozen in liquid nitrogen until further analysis.

PANCREAS HISTOLOGY AND IMMUNOSTAINING

The pancreas was collected from WT and transgenic mice. Tissues for immunohistochemistry were fixed in 10% neutral, phosphate-buffered formalin for 24 h and paraffin-embedded. Subsequently, the paraffin-embedded tissues were processed in 4-μm sections. Sections were stained using rabbit anti-glucagon polyclonal antibody and guinea pig anti-insulin serum (both from Millipore, Billerica, MA, USA). For fluorescence detection, goat anti-guinea pig IgG coupled to Texas Red and donkey anti-rabbit IgG coupled to Cy3 were used (both from Jackson ImmunoResearch, West Grove, PA, USA) followed by Vectashield Mounting Medium with 4,6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA, USA) for nuclear staining.

STATISTICAL ANALYSIS

Data were analyzed in SAS (SAS Institute, Inc., Cary, NC, USA) using a mixed model analysis of variance (http://dawg.utk.edu). Fisher's test followed by Tukey's *post hoc* test was used for mean separation. $P < 0.05$ was considered statistically significant. Data are reported as the means ± SE.

RESULTS

BODY WEIGHT, FAT PAD WEIGHT, AND METABOLIC PARAMETERS

Body weights were comparable between mice with either one or two copies of the renin transgene and WT control mice (**Figure 1A**). Gonadal fat pad weight (**Figure 1B**) and adiposity index (gonadal fat pad weight divided by body weight; **Figure 1C**) were significantly lower in homozygous mice compared to WT littermates ($P < 0.05$). Fasting serum glucose, leptin, and adiponectin levels were comparable between all genotypes

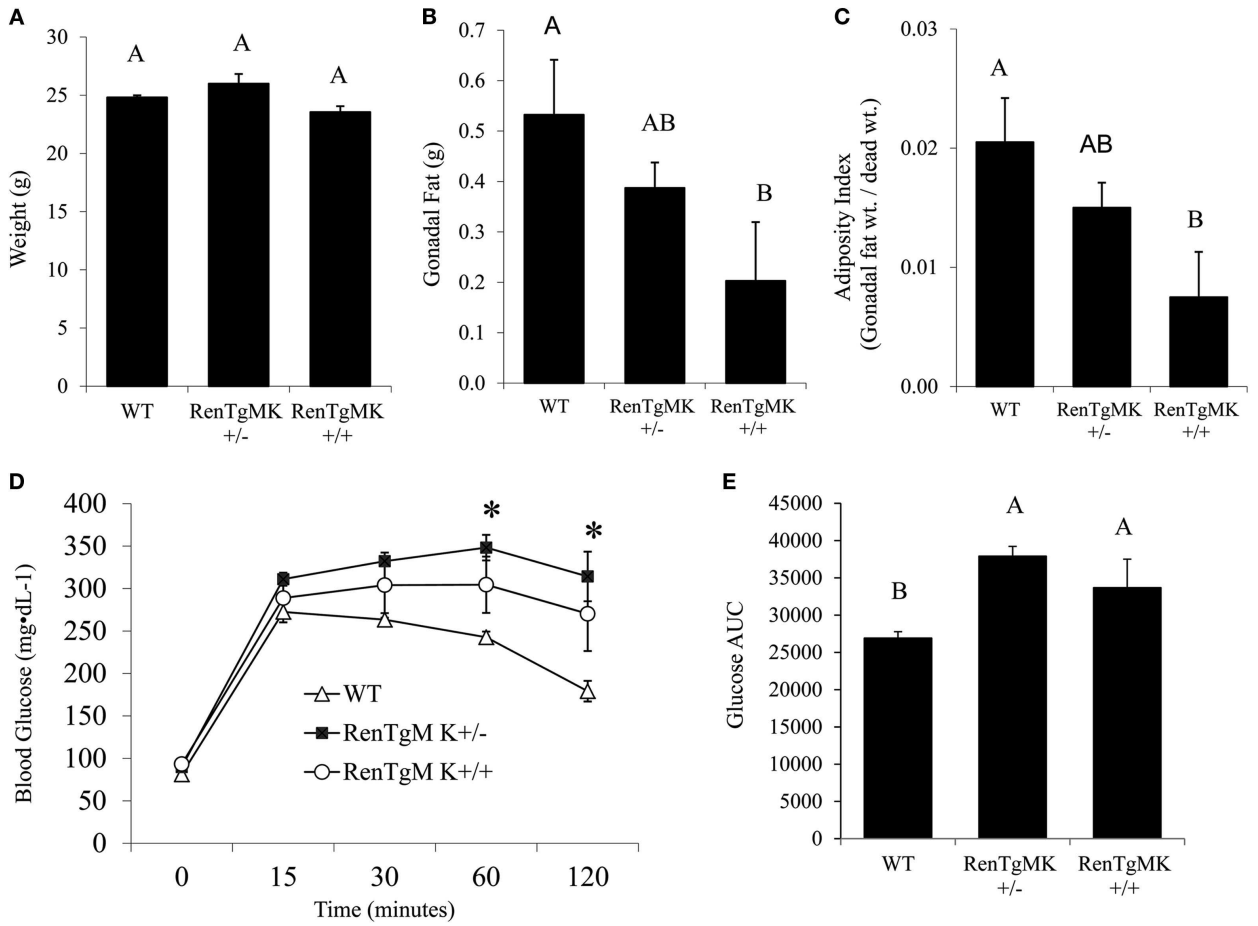


FIGURE 1 | Body and fat pad weight and glucose tolerance in male RenTgMK mice. (A) Body weight at the age of 20 weeks. **(B)** Mice were sacrificed at the end of week 20 and gonadal fat pads were collected and weighed. **(C)** The adiposity index was determined by dividing gonadal fat pad weight by final body weight. A glucose tolerance test (GTT) was administered after overnight fasting. **(D)** Blood glucose levels were measured at 0, 15, 30, 90, and 120 min and plotted on a graph. **(E)** Area Under the Curve (AUC) was calculated as described in the experimental procedures. Values are means ± SE. $n = 6$ For WT; $n = 5$ for RenTgMK$^{+/-}$; $n = 4$ for RenTgMK$^{+/+}$. Different letters indicate a significant difference ($P < 0.05$). *Significantly different ($P < 0.05$) from WT.

Table 1 | Serum metabolic markers in male wild-type and RenTgMK mice.

	WT	RenTgMK$^{+/-}$	RenTgMK$^{+/+}$	*P* value
Glucose, mg/dl	81.2 ± 6.4	89.8 ± 3.2	93.3 ± 5.1	0.292
Insulin, ng/ml	0.62 ± 0.07^{a}	0.42 ± 0.05^{b}	0.36 ± 0.07^{b}	**0.033**
Leptin, ng/ml	2.1 ± 0.7	2.3 ± 0.6	1.3 ± 0.5	0.535
Adiponectin, μg/ml	8.7 ± 1.4	10.8 ± 2.5	7.6 ± 0.9	0.479
C-peptide, ng/ml	1.4 ± 0.1^{b}	1.9 ± 0.2^{a}	2.0 ± 0.1^{a}	**0.007**
FFA, mM	0.84 ± 0.10	0.93 ± 0.06	0.91 ± 0.11	0.791
Triglycerides, mg/dL	60.1 ± 6.4^{a}	44.5 ± 8.6^{a,b}	23.7 ± 8.7^{b}	**0.018**

Values are means ± SE. Animals were 21 weeks old. Initial body weight measurements were taken at 10 weeks. Blood was collected after fasting overnight and metabolic parameters were measured from serum. n = 6 For WT; n = 5 for RenTgMK$^{+/-}$; n = 4 for RenTgMK$^{+/+}$. C-peptide, connecting peptide; FFA, free fatty acid.

Means in a row with superscripts without a common letter differ, P < 0.05. Numbers in bold indicate a significance of P < 0.05.

(**Table 1**). Fasting serum insulin, however, was significantly lower in the transgenic mice (both homozygous and heterozygous) compared to WT littermates ($P < 0.05$). Serum C-peptide levels, on the other hand, were higher in the transgenics than in the WT mice (**Table 1**). Interestingly, serum triglycerides were significantly lower in the homozygous mice compared to WT littermates. On this low-fat chow diet condition, the overall metabolic phenotype was less pronounced in female transgenic mice compared to WT female littermates (data not shown).

GLUCOSE INTOLERANCE IN RenTg MICE

To assess glucose tolerance in the RenTg mice, an intra-peritoneal GTT was administered. Baseline fasting glucose levels were comparable between WT, RenTgMK$^{+/-}$ and RenTgMK$^{+/+}$ mice (81.17 ± 15.68, 89.80 ± 7.16, and 93.25 ± 10.28 mg/dl, respectively). Heterozygous mice maintained significantly higher levels of glycemia compared to WT within 60 min and remained elevated throughout the GTT (**Figure 1D**). These differences were observed as early as 10 weeks of age (data not shown) and became more pronounced with age by 20 weeks. Glucose intolerance in male RenTgMK mice was also evident from a comparison of the glucose AUC (**Figure 1E**). The AUC values for both heterozygous and homozygous mice were higher ($P < 0.05$) than that of WT mice implying greater glucose intolerance in the transgenics. In females, no significant differences in GTT were observed between the three genotypes at 20 weeks of age in these low-fat feeding conditions (data not shown).

METABOLIC PHENOTYPING OF RenTg MICE

Insulin resistance is commonly associated with high adiposity. The paradoxical glucose intolerance despite low adiposity and low insulinemia in the renin transgenic male mice vs. control littermates led us to further investigate whether these differences were due to altered insulin sensitivity and/or glucose production or utilization in this model. Accordingly, metabolic studies at the NIH MMPC at Vanderbilt University were conducted. Male heterozygous mice were compared to WT mice because males exhibited glucose intolerance and sufficient numbers could be obtained from a few litters. Steady-state glucose infusion rate (**Figure 2**), overall tissue-specific glucose uptake, glucose metabolism, and endogenous glucose production (**Table 2**) did not significantly differ between RenTgMK and WT mice, indicating normal insulin sensitivity in the transgenics.

EFFECT OF HIGH-FAT DIET ON BODY WEIGHT, ADIPOSITY, CIRCULATING ADIPOKINES, AND GLUCOSE TOLERANCE

As described above, renin transgene overexpression led to impaired glucose tolerance compared to WT mice when mice were fed a low-fat chow diet. To test whether the genetic differences would be exacerbated by high-fat feeding, we fed male heterozygous and WT mice a low- or high-fat diet to investigate diet-gene interactions.

Body weights were not significantly different between groups at the start of the randomized diet study (**Table 3**). High-fat feeding increased body weight only in the wild-type mice (**Figure 3A**). Mice of both genotypes showed a trend for increased fat pad weight and adiposity with high-fat feeding, although the difference was only significant for adiposity in the RenTgMK$^{+/-}$ mice (**Figures 3B,C**).

Changes in adiposity are known to alter hormonal and metabolite levels. As expected, high-fat feeding increased serum glucose and leptin levels in both male WT and transgenic mice ($P < 0.05$ for diet effect – **Table 3**). Interestingly, high-fat feeding also increased serum resistin levels in WT, but not in transgenic males (**Table 3**). In the WT males, serum triglyceride concentration was higher in the low-fat fed mice when compared to high-fat fed ones (**Table 3**). This effect was minimal in the transgenics.

Low-fat fed male heterozygous mice exhibited a higher glucose excursion and area under the glucose curve compared to their WT counterparts, indicating glucose intolerance (**Figures 3D,E**). High-fat feeding did not exacerbate glucose intolerance in RenTgMK mice.

PANCREAS HISTOLOGY AND IMMUNOSTAINING OF RenTg MICE

Because of the consistently lower insulin levels in heterozygous mice compared to WT mice, we performed immunohistological studies in the pancreas to assess islet morphology and hormone content. In both genotypes, islets appeared normal and exhibited comparable staining for glucagon and insulin (**Figure 4**).

DISCUSSION

Many lines of evidence have linked activation of the RAS to the development of obesity and insulin resistance (Schorr et al., 1998; Van Harmelen et al., 2000), but the effects of increased circulating levels of angiotensins on adiposity remain controversial. We hypothesized that chronic systemic RAS activation via transgenic renin overexpression in the liver would lead to glucose intolerance and systemic insulin resistance. We also predicted that increased systemic Ang II would increase adiposity, based on previous work by us and others showing that Ang II increases adipocyte lipogenesis and triglyceride storage. Our results demonstrate that elevated circulating Ang II due to renin overexpression

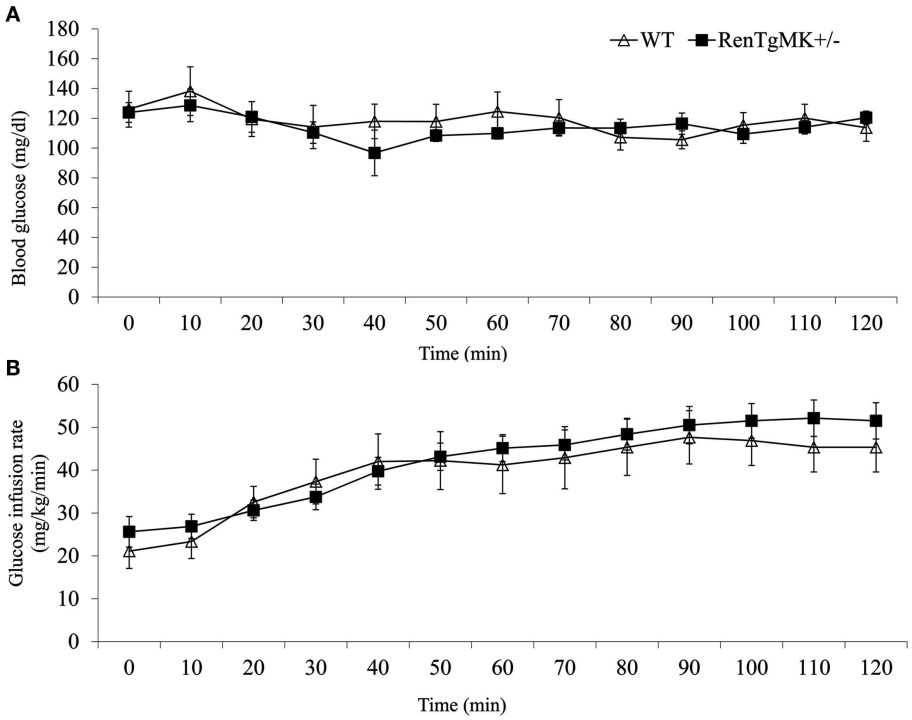


FIGURE 2 | Assessment of insulin sensitivity in male wild-type and RenTgMK$^{+/-}$ mice using hyperinsulinemic euglycemic clamp. Changes in blood glucose concentration **(A)** and glucose infusion rate **(B)** over time are shown. Values are means ± SE. Animals were approximately 9 months old. $n = 8$–9 For WT; $n = 7$ for RenTgMK$^{+/-}$.

Table 2 | Metabolic characteristics and accumulation of [2-^{3}H]DG during the hyperinsulinemic-euglycemic clamp experiments in male wild-type and RenTgMK$^{+/-}$ mice.

	WT	RenTgMK$^{+/-}$	*P* value
GLUCOSE KINETICS			
Blood glucose, mg/dl	114.7 ± 5.8	114.6 ± 5.0	0.996
GTR, mg/kg/min	47.4 ± 4.0	49.4 ± 6.7	0.794
endoGTR, mg/kg/min	4.96 ± 4.62	−2.29 ± 7.60	0.416
Glucose clearance, mg/kg/min	42.0 ± 3.1	43.7 ± 5.7	0.790
GIR, mg/kg/min	42.4 ± 4.3	51.7 ± 4.0	0.142
ACCUMULATION OF [2-^{3}H]DG			
Soleus, μg/min/mg tissue	0.035 ± 0.008	0.036 ± 0.005	0.923
Gastro, μg/min/mg tissue	0.025 ± 0.005	0.033 ± 0.003	0.229
Vastus l., μg/min/mg tissue	0.041 ± 0.007	0.047 ± 0.005	0.550
WAT, μg/min/mg tissue	0.004 ± 0.001	0.006 ± 0.001	0.067
Diaphragm, μg/min/mg tissue	0.131 ± 0.019	0.091 ± 0.007	0.103
Heart, μg/min/mg tissue	0.431 ± 0.057	0.320 ± 0.043	0.161
Brain, μg/min/mg tissue	0.048 ± 0.005	0.049 ± 0.003	0.888

Values are means ± SE. Animals were approximately 9 months old. n = 8–9 For WT; n = 7 for RenTgMK$^{+/-}$. GTR, glucose turnover rate; endoGTR, endogenous glucose turnover rate; GIR, glucose infusion rate; [2-^{3}H]DG, 2-deoxy-[^{3}H]glucose; Gastro, gastrocnemius; Vastus l., vastus lateralis; WAT, white adipose tissue.

leads to glucose intolerance, but with consistently lower levels of plasma insulin. Further, chronic elevation of systemic Ang II by hepatic overexpression of the renin gene led to a reduction rather than an increase in adiposity in male mice. However, these mice exhibit otherwise normal glucose metabolism and a transgene dose-dependent decrease in insulinemia.

GLUCOSE INTOLERANCE IN RenTgMK MICE

Consistent with previous studies of Ang II infusion and transgenic renin expression (Lee et al., 1996), male RenTgMK transgenic mice exhibited glucose intolerance, even on a low-fat diet. However, despite this glucose intolerance, the RenTgMK mice maintained low fasting insulinemia and normal insulin sensitivity, as indicated by normal steady-state glucose infusion during the hyperinsulinemic, euglycemic clamp studies. RenTgMK mice maintained low insulinemia even under high-fat feeding. The glucose intolerance in RenTgMK mice in the presence of normal fasting glucose levels and low insulinemia, a feature that is a rather typical hallmark of increased insulin sensitivity, could be due to decreased insulin production/secretion and/or increased insulin clearance. Serum C-peptide level was higher in heterozygous compared to WT mice arguing against decreased insulin secretion accounting for low insulinemia in the RenTgMK mice. Immunohistochemistry of the pancreas indicated normal islet morphology and hormone content, possibly indicating normal pancreatic function. However, such studies are only qualitative and do not allow to detect clear quantitative differences. Thus, it is probably insulin clearance, rather than insulin secretion that may be altered in this model.

Liver is the primary site of insulin clearance (Duckworth et al., 1998), which can be affected by both nutritional and hormonal signals. Insulin clearance rate is heritable (Goodarzi et al.,

Table 3 | Effects of high-fat diet on body weight and metabolic characteristics in male wild-type and RenTgMK$^{+/-}$ mice.

	WT		RenTgMK$^{+/-}$		*P* value		
	LF	HF	LF	HF	Geno	Diet	Geno X diet
Initial body weight, g	27.2 ± 2.2	29.2 ± 1.4	26.2 ± 0.6	25.7 ± 0.9	NS	NS	NS
Final body weight, g	37.6 ± 3.2^{a,b}	46.9 ± 5.6^{a}	35.4 ± 1.8^{b}	37.8 ± 2.1^{a,b}	NS	NS	NS
Glucose, mg/dL	92.3 ± 3.2^{b}	123.7 ± 7.7^{a}	98.3 ± 5.2^{b}	127.3 ± 2.9^{a}	NS	**0.001**	NS
Insulin, ng/ml	1.29 ± 0.31	2.07 ± 0.60	0.91 ± 0.04	1.14 ± 0.25	NS	NS	NS
Leptin, pg/ml	9.5 ± 1.6	16.2 ± 2.9	7.6 ± 1.1	12.7 ± 2.3	NS	**0.019**	NS
Adiponectin, μg/ml	12.6 ± 0.3	14.1 ± 2.0	12.9 ± 0.4	12.4 ± 0.2	NS	NS	NS
Resistin, pg/ml	475.8 ± 37.3^{b}	702.2 ± 19.0^{a}	545.8 ± 48.7^{a,b}	493.3 ± 83.9^{b}	NS	NS	**0.030**
MCP-1, pg/ml	29.9 ± 8.0	53.0 ± 18.9	30.5 ± 1.6	34.0 ± 12.9	NS	NS	NS
PAI-1, pg/ml	3903.4 ± 666.2	5903.4 ± 834.8	4733.5 ± 734.0	4526.8 ± 961.3	NS	NS	NS
C-peptide, ng/ml	1.9 ± 0.3	3.3 ± 1.2	2.4 ± 0.4	2.3 ± 0.2	NS	NS	NS
FFA, mM	1.00 ± 0.18	0.80 ± 0.04	1.23 ± 0.36	1.16 ± 0.39	NS	NS	NS
Triglycerides, mg/dL	132.4 ± 16.5^{a}	66.9 ± 4.1^{b}	66.8 ± 4.4^{b}	39.2 ± 5.4^{b}	**0.001**	**0.001**	**0.074**

Values are means ± SE. Animals were fed a high-fat or low-fat diet for 19 weeks. Initial body weight measurements were taken at the beginning of the study. Mice were 3–5 months old. Blood was collected after fasting overnight and metabolic parameters were measured from serum. n = 3 For each group. LF, low-fat; HF, high-fat; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor-1; C-peptide, connecting peptide; FFA, free fatty acid.

Means in a row with superscripts without a common letter differ, P < 0.05.

Numbers in bold indicate a significance of P < 0.05.

2005) and is reduced in obesity and Type-2 diabetes (Duckworth et al., 1998). Therefore, it could be an important factor in the pathogenesis of Type-2 diabetes. Conversely, there are mouse models which exhibit increased insulin clearance such as the mouse overexpressing carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) in the liver (Najjar, 2002). Additional studies beyond the scope of this work will be required to address whether the RAS is involved in regulating insulin clearance.

The finding that the glucose intolerance in male transgenic mice did not worsen with high-fat feeding could possibly indicate that RAS overactivation could at least in part play a role in high-fat diet-induced obesity. Along the same lines, mice overexpressing AGT in adipose tissue also develop glucose intolerance on a low-fat diet, which is not further exacerbated by high-fat feeding (Kalupahana et al., 2012). Female transgenic mice exhibited normal glucose tolerance on a low-fat diet while males became glucose intolerant on the same diet when compared to WT littermates. Further, female transgenics became glucose intolerant when fed a high-fat diet (data not shown).

It is likely that the metabolic phenotype of the RenTgMK mice is due to Ang II effects, rather than the effects of renin acting on the renin/prorenin receptor. We argue this because in renin knockout mice, the metabolic phenotype of increased insulin sensitivity and resistance to high-fat diet-induced glucose intolerance and insulin resistance was reversed by Ang II infusion (Takahashi et al., 2007). It is also likely that these effects are mediated via angiotensin receptors, as previous studies on the RenTgMK mice demonstrated that AT1 receptor blockade reversed renal pathology and normalized blood pressure in the RenTgMK mice (Caron et al., 2002). Alternative mechanisms may involve direct effects of renin mediated by the renin/prorenin receptor on the vasculature or adipose tissue. Indeed, renin receptors are expressed in adipose tissue (Achard et al., 2007) and therefore may mediate the observed adipose tissue phenotype.

RAS OVERACTIVATION AND INSULIN RESISTANCE

Renin-angiotensin system overactivation via chronic Ang II infusion leads to the development of systemic insulin resistance in rodents. This is, in most part, due to the Ang II-mediated impairment of skeletal muscle glucose transport and utilization (Kalupahana and Moustaid-Moussa, 2012a). Ang II impedes the insulin-mediated tyrosine phosphorylation of the insulin receptor substrate (IRS)-1, activation of Akt, and translocation of glucose transporter (Glut)-4 in the skeletal muscle in an NADPH oxidase, AT1, and NF-kB-dependent manner. Ang II also increases hepatic glucose production, which also potentially contributes to altered systemic insulin sensitivity. In contrast, the RenTgMK mice in this study exhibited normal systemic insulin sensitivity. While the exact underlying mechanisms for this discrepancy of insulin sensitivity between different models of RAS overactivation are unknown, it is possible that the low insulinemia present in the RenTgMK mice could protect these mice from the development of insulin resistance. Previous studies have shown that an increase in plasma insulin by itself can induce insulin resistance. In the study by Shanik et al. (2008), mice transfected with extra copies of the insulin gene had a two- to four-fold increase in plasma insulin and exhibited normal body weight, insulin resistance and hypertriglyceridemia.

Unlike models of chronic Ang II infusion (Ran et al., 2004), RenTgMK mice exhibited lower plasma triglyceride levels. Thus, the hypoinsulinemia in the RenTgMK could also potentially explain the low serum triglyceride levels seen in these mice. Given this metabolic phenotype of RenTgMK mice, it would be interesting to explore whether the insulin resistance seen in several models of chronic RAS overactivation is insulin-dependent and further

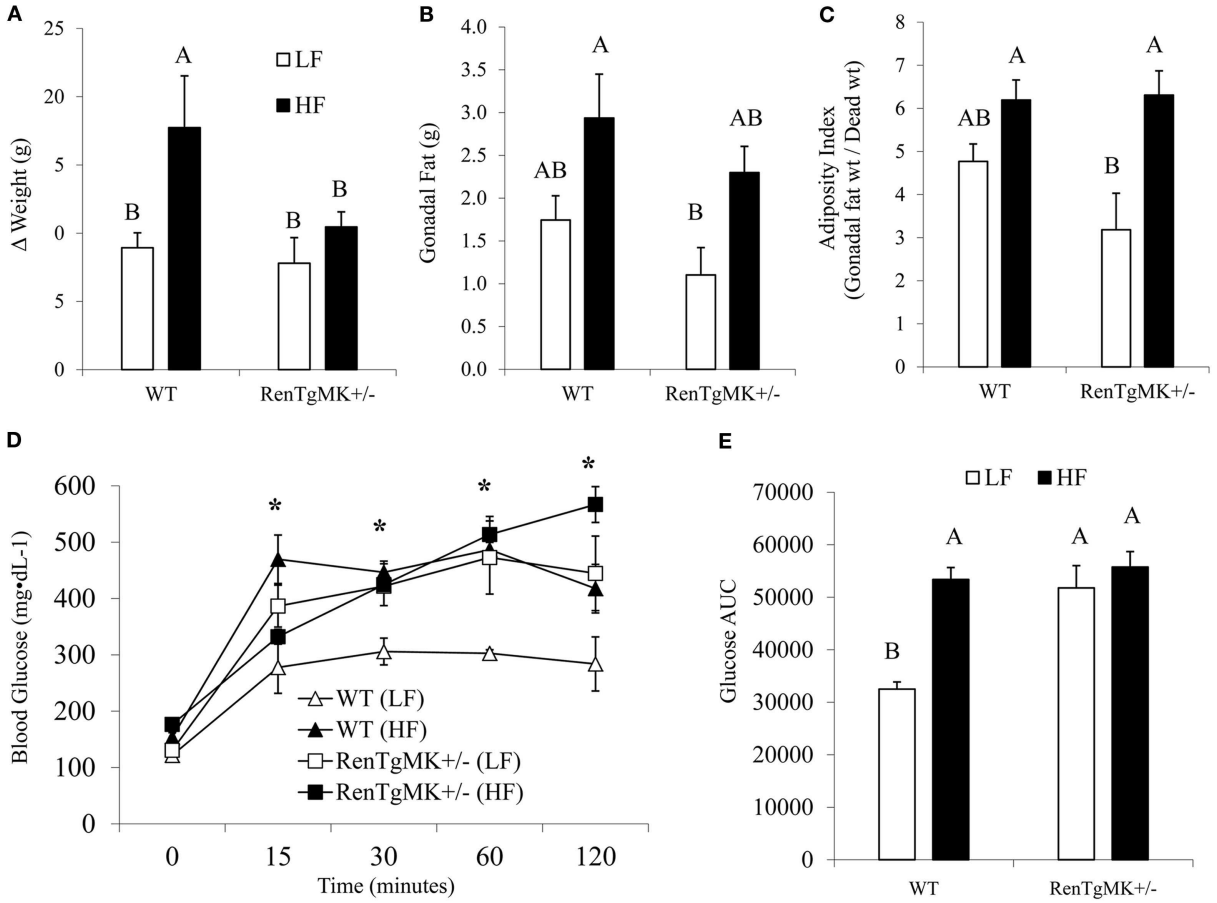


FIGURE 3 | Effect of high-fat diet on body and fat pad weight and glucose tolerance of male wild-type and RenTgMK$^{+/-}$ mice. (A) Weight gain was calculated as the difference between the initial body weight measured at week 1 and the final weight measured after 18 weeks. **(B)** Mice were sacrificed at the end of week 19 and gonadal fat pads were collected and weighed. **(C)** The adiposity index was determined by dividing gonadal fat pad weight by final body weight X 100. **(D)** A glucose tolerance test (GTT) was administered after overnight fasting. Blood glucose levels were measured at 0, 15, 30, 90, and 120 min and plotted on a graph. **(E)** Area Under the Curve (AUC) was calculated as described in the experimental procedures. Values are means ± SE. $n = 3$ For each group. *Significantly different ($P < 0.05$) from WT-LF. Different letters indicate a significant difference ($P < 0.05$).

studies are warranted. The issue of whether the renin receptor may also in part modulate insulin sensitivity merits further investigation as well.

RAS OVERACTIVATION AND ADIPOSITY

Both human and rodent studies have shown that obesity and increased adiposity are associated with both systemic and adipose RAS overactivation (Kalupahana and Moustaid-Moussa, 2012a). However, it is not known whether primary RAS overactivation leads to obesity. Transgenic mouse models clearly demonstrate that manipulating components of the RAS alters adiposity: mice overexpressing AGT in adipose tissue have increased adiposity, while deletion of either the AGT or Ang II receptor genes reduces fatness. Paradoxically, previous studies of chronic Ang II infusion in rodents have shown that chronic systemic RAS overactivation leads to weight loss, rather than weight gain (Griffin et al., 1991; Cassis et al., 1998). The transgenic TGR(mREN2)27 rat overexpressing the mouse Ren2 renin gene also has a lean phenotype (Mullins et al., 1990; Langheinrich et al., 1996; Lee et al., 1996). Similar to these findings, the RenTgMK mice also exhibited lower fat mass compared to WT littermates. The adipose mass was significantly decreased by the renin transgene in a gene dosage-dependent manner. In contrast, mice with primary AGT overproduction in adipose tissue exhibit higher adiposity (Massiera et al., 2001a). Further, deletion of AGT and other RAS genes leads to lower fat mass and resistance to diet-induced obesity (Massiera et al., 2001b; Takahashi et al., 2007). Thus, it appears that while systemic RAS overactivation leads to reductions in body weight, local increases in RAS activity in adipose tissue leads to increased adiposity.

The low-fat mass observed following Ang II infusion is attributed to both increased energy expenditure and reduced energy intake (Brink et al., 1996; Cassis et al., 1998). In the RenTgMK mouse model, we did not detect any significant differences in food intake (data not shown). Activation of the sympathetic nervous system may also account for changes in weight via modulation of lipid metabolism and energy expenditure by catecholamines (Cassis, 2000). The differential effect of systemic vs. adipose

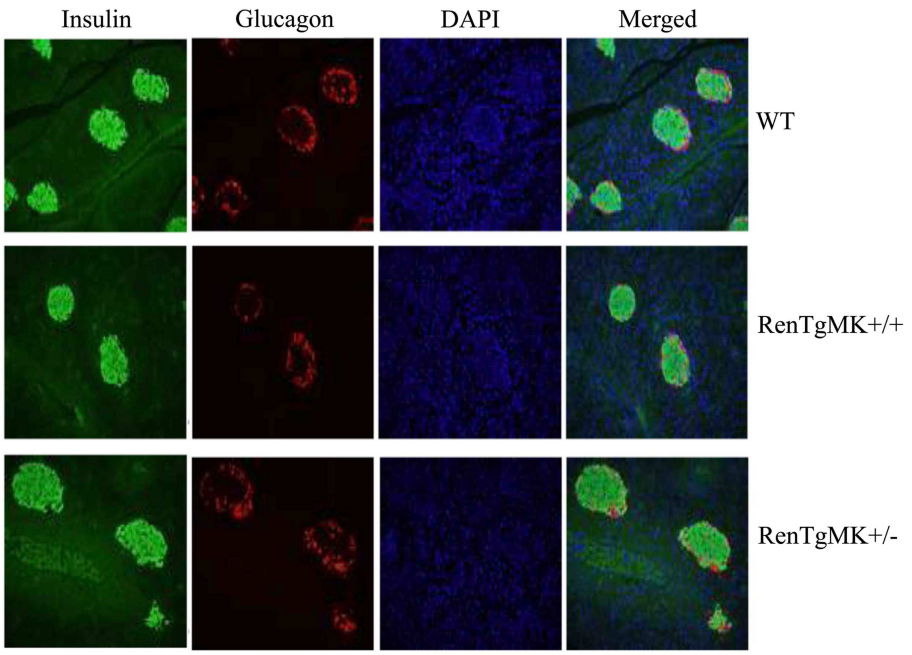


FIGURE 4 | Islet pathology. Pancreas histology and immunostaining were conducted to assess islet morphology and hormone content in male wild-type and RenTgMK mice, 20 weeks of age.

specific RAS overactivation on adiposity indicates that specific local overproduction of AGT in adipose tissue *per se*, may be required for increasing adiposity. Indeed, Ang II exerts local anabolic effects in the adipose tissue (Massiera et al., 2001a). Ang II also increases lipogenic gene expression and enzyme activity in 3T3-L1 murine adipocytes and human adipocytes *in vitro* (Jones et al., 1997). This is also in agreement with studies showing differentiation-dependent increase in AGT gene expression and secretion in preadipocytes (Kim and Moustaid-Moussa, 2000). Ubiquitous inactivation of AGT, on the other hand, results in significant loss of fat mass. However, it is unclear whether targeted inactivation of AGT in adipose tissue would specifically alter fat mass and such studies would convincingly confirm the role of adipose AGT in modulating insulin resistance or fat mass.

In summary, our data demonstrate that transgenic hepatic overexpression of renin leads to glucose intolerance, decreased fat mass, hypoinsulinemia, and hypotriglyceridemia, with normal systemic insulin sensitivity. The hypoinsulinemia in these mice is possibly due to increased insulin clearance, as indicated by elevated C-peptide levels and normal pancreatic insulin levels indicating normal pancreatic function. Whether the unexpected low adiposity and normal insulin sensitivity despite the presence of glucose intolerance in the RenTgMK mice is secondary to hypoinsulinemia merits further investigation.

ACKNOWLEDGMENTS

The authors thank Dr. Oliver Smithies for generously providing us with the RenTg mice we used to perform this study and Dr. K. Carron and J. Hagaman for providing help with animal breeding and genotyping. This work was supported by a USDA NIFA-NRI Grant 2005-35200-15224 and an AHA Grant in Aid (Greater Southeast affiliate 0755626B). The authors would like to thank Jeffrey Morris and Taryn Stewart for their technical assistance.

REFERENCES

Achard, V., Boullu-Ciocca, S., Desbriere, R., Nguyen, G., and Grino, M. (2007). Renin receptor expression in human adipose tissue. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 292, R274–R282.

Ayala, J. E., Bracy, D. P., McGuinness, O. P., and Wasserman, D. H. (2006). Considerations in the design of hyperinsulinemic-euglycemic clamps in the conscious mouse. *Diabetes* 55, 390–397.

Bosch, J., Yusuf, S., Gerstein, H. C., Pogue, J., Sheridan, P., Dagenais, G., et al. (2006). Effect of ramipril on the incidence of diabetes. *N. Engl. J. Med.* 355, 1551–1562.

Brink, M., Wellen, J., and Delafontaine, P. (1996). Angiotensin II causes weight loss and decreases circulating insulin-like growth factor I in rats through a pressor-independent mechanism. *J. Clin. Invest.* 97, 2509–2516.

Caron, K. M., James, L. R., Kim, H. S., Knowles, J., Uhlir, R., Mao, L., et al. (2004). Cardiac hypertrophy and sudden death in mice with a genetically clamped renin transgene. *Proc. Natl. Acad. Sci. U.S.A.* 101, 3106–3111.

Caron, K. M., James, L. R., Kim, H. S., Morham, S. G., Sequeira Lopez, M. L., Gomez, R. A., et al. (2002). A genetically clamped renin transgene for the induction of hypertension. *Proc. Natl. Acad. Sci. U.S.A.* 99, 8248–8252.

Cassis, L. A. (2000). Fat cell metabolism: insulin, fatty acids, and renin. *Curr. Hypertens. Rep.* 2, 132–138.

Cassis, L. A., Marshall, D. E., Fettinger, M. J., Rosenbluth, B., and Lodder, R. A. (1998). Mechanisms contributing to angiotensin II regulation of body weight. *Am. J. Physiol.* 274(Pt 1), E867–E876.

Duckworth, W. C., Bennett, R. G., and Hamel, F. G. (1998). Insulin degradation: progress and potential. *Endocr. Rev.* 19, 608–624.

Goodarzi, M. O., Taylor, K. D., Guo, X., Quinones, M. J., Cui, J., Li, X.,

et al. (2005). Variation in the gene for muscle-specific AMP deaminase is associated with insulin clearance, a highly heritable trait. *Diabetes* 54, 1222–1227.

Griffin, S. A., Brown, W. C., MacPherson, F., McGrath, J. C., Wilson, V. G., Korsgaard, N., et al. (1991). Angiotensin II causes vascular hypertrophy in part by a non-pressor mechanism. *Hypertension* 17, 626–635.

Hatada, S., Kuziel, W., Smithies, O., and Maeda, N. (1999). The influence of chromosomal location on the expression of two transgenes in mice. *J. Biol. Chem.* 274, 948–955.

Jayasooriya, A. P., Mathai, M. L., Walker, L. L., Begg, D. P., Denton, D. A., Cameron-Smith, D., et al. (2008). Mice lacking angiotensin-converting enzyme have increased energy expenditure, with reduced fat mass and improved glucose clearance. *Proc. Natl. Acad. Sci. U.S.A.* 105, 6531–6536.

Jeunemaitre, X. (2008). Genetics of the human renin angiotensin system. *J Mol. Med. (Berl.)* 86, 637–641.

Jeunemaitre, X., Gimenez-Roqueplo, A. P., Celerier, J., and Corvol, P. (1999). Angiotensinogen variants and human hypertension. *Curr. Hypertens. Rep.* 1, 31–41.

Jones, B. H., Standridge, M. K., and Moustaid, N. (1997). Angiotensin II increases lipogenesis in 3T3-L1 and human adipose cells. *Endocrinology* 138, 1512–1519.

Kalupahana, N. S., Massiera, F., Quignard-Boulange, A., Ailhaud, G., Voy, B. H., Wasserman, D. H., et al. (2012). Overproduction of angiotensinogen from adipose tissue induces adipose inflammation, glucose intolerance, and insulin resistance. *Obesity (Silver Spring)* 20, 48–56.

Kalupahana, N. S., and Moustaid-Moussa, N. (2012a). The renin-angiotensin system: a link between obesity, inflammation and insulin resistance. *Obes. Rev.* 13, 136–149.

Kalupahana, Nishan S., and Moustaid-Moussa, N. (2012b). The adipose tissue renin-angiotensin system and metabolic disorders: a review of molecular mechanisms. *Crit. Rev. Biochem. Mol. Biol.* 47, 379–390.

Kim, S., and Moustaid-Moussa, N. (2000). Secretory, endocrine and autocrine/paracrine function of the adipocyte. *J. Nutr.* 130, 3110S–3115S.

Langheinrich, M., Lee, M. A., Bohm, M., Pinto, Y. M., Ganten, D., and Paul, M. (1996). The hypertensive Ren-2 transgenic rat TGR (mREN2)27 in hypertension research. Characteristics and functional aspects. *Am. J. Hypertens.* 9, 506–512.

Lee, M. A., Bohm, M., Paul, M., Bader, M., Ganten, U., and Ganten, D. (1996). Physiological characterization of the hypertensive transgenic rat TGR(mREN2)27. *Am. J. Physiol.* 270(Pt 1), E919–E929.

Lee, M. H., Song, H. K., Ko, G. J., Kang, Y. S., Han, S. Y., Han, K. H., et al. (2008). Angiotensin receptor blockers improve insulin resistance in type 2 diabetic rats by modulating adipose tissue. *Kidney Int.* 74, 890–900.

Massiera, F., Bloch-Faure, M., Ceiler, D., Murakami, K., Fukamizu, A., Gasc, J. M., et al. (2001a). Adipose angiotensinogen is involved in adipose tissue growth and blood pressure regulation. *FASEB J.* 15, 2727–2729.

Massiera, F., Seydoux, J., Geloen, A., Quignard-Boulange, A., Turban, S., Saint-Marc, P., et al. (2001b). Angiotensinogen-deficient mice exhibit impairment of diet-induced weight gain with alteration in adipose tissue development and increased locomotor activity. *Endocrinology* 142, 5220–5225.

Mullins, J. J., Peters, J., and Ganten, D. (1990). Fulminant hypertension in transgenic rats harbouring the mouse Ren-2 gene. *Nature* 344, 541–544.

Najjar, S. M. (2002). Regulation of insulin action by CEACAM1. *Trends Endocrinol. Metab.* 13, 240–245.

Ogihara, T., Asano, T., Ando, K., Chiba, Y., Sakoda, H., Anai, M., et al. (2002). Angiotensin II-induced insulin resistance is associated with enhanced insulin signaling. *Hypertension* 40, 872–879.

Ran, J., Hirano, T., and Adachi, M. (2004). Chronic ANG II infusion increases plasma triglyceride level by stimulating hepatic triglyceride production in rats. *Am. J. Physiol. Endocrinol. Metab.* 287, E955–E961.

Schmieder, R. E., Hilgers, K. F., Schlaich, M. P., and Schmidt, B. M. (2007). Renin-angiotensin system and cardiovascular risk. *Lancet* 369, 1208–1219.

Schorr, U., Blaschke, K., Turan, S., Distler, A., and Sharma, A. M. (1998). Relationship between angiotensinogen, leptin and blood pressure levels in young normotensive men. *J. Hypertens.* 16, 1475–1480.

Shanik, M. H., Xu, Y., Skrha, J., Dankner, R., Zick, Y., and Roth, J. (2008). Insulin resistance and hyperinsulinemia: is hyperinsulinemia the cart or the horse? *Diabetes Care* 31(Suppl. 2), S262–S268.

Takahashi, N., Li, F., Hua, K., Deng, J., Wang, C. H., Bowers, R. R., et al. (2007). Increased energy expenditure, dietary fat wasting, and resistance to diet-induced obesity in mice lacking renin. *Cell Metab.* 6, 506–512.

Truett, G. E., Heeger, P., Mynatt, R. L., Truett, A. A., Walker, J. A., and Warman, M. L. (2000). Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *Biotechniques* 29, 52, 54.

Van Harmelen, V., Ariapart, P., Hoffstedt, J., Lundkvist, I., Bringman, S., and Arner, P. (2000). Increased adipose angiotensinogen gene expression in human obesity. *Obes. Res.* 8, 337–341.

Vermes, E., Ducharme, A., Bourassa, M. G., Lessard, M., White, M., and Tardif, J. C. (2003). Enalapril reduces the incidence of diabetes in patients with chronic heart failure: insight from the Studies Of Left Ventricular Dysfunction (SOLVD). *Circulation* 107, 1291–1296.

Xiao, F., Wei, H., Song, S., Li, G., and Song, C. (2006). Polymorphisms in the promoter region of the angiotensinogen gene are associated with liver cirrhosis in patients with chronic hepatitis B. *J. Gastroenterol. Hepatol.* 21, 1488–1491.

Yvan-Charvet, L., Even, P., Bloch-Faure, M., Guerre-Millo, M., Moustaid-Moussa, N., Ferre, P., et al. (2005). Deletion of the angiotensin type 2 receptor (AT2R) reduces adipose cell size and protects from diet-induced obesity and insulin resistance. *Diabetes* 54, 991–999.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 04 November 2012; accepted: 02 December 2012; published online: 07 January 2013.

*Citation: Fletcher SJ, Kalupahana NS, Soltani-Bejnood M, Kim JH, Saxton AM, Wasserman DH, De Taeye B, Voy BH, Quignard-Boulange A and Moustaid-Moussa N (2013) Transgenic mice overexpressing renin exhibit glucose intolerance and diet-genotype interactions. Front. Endocrin. **3**:166. doi: 10.3389/fendo.2012.00166*

This article was submitted to Frontiers in Diabetes, a specialty of Frontiers in Endocrinology.

Copyright © 2013 Fletcher, Kalupahana, Soltani-Bejnood, Kim, Saxton, Wasserman, De Taeye, Voy, Quignard-Boulange and Moustaid-Moussa. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.

frontiers in
ENDOCRINOLOGY

ORIGINAL RESEARCH ARTICLE
published: 11 March 2013
doi: 10.3389/fendo.2013.00010

Angiotensinogen gene silencing reduces markers of lipid accumulation and inflammation in cultured adipocytes

Wenting X. Carroll[1,2], Nishan S. Kalupahana[3], Suzanne L. Booker[1,2], Nalin Siriwardhana[4], Monique LeMieux[4], Arnold M. Saxton[1,2] and Naima Moustaid-Moussa[4]*

[1] *Department of Animal Science, University of Tennessee, Knoxville, TN, USA*
[2] *Obesity Research Center, University of Tennessee, Knoxville, TN, USA*
[3] *Department of Physiology, Faculty of Medicine, University of Peradeniya, Peradeniya, Sri Lanka*
[4] *Nutritional Sciences Program, College of Human Sciences, Texas Tech University, Lubbock, TX, USA*

Edited by:
Tsuguhito Ota, Kanazawa University, Japan

Reviewed by:
Subrata Chakrabarti, The University of Western Ontario, Canada
Po Sing Leung, The Chinese University of Hong Kong, China

***Correspondence:**
Naima Moustaid-Moussa, Nutritional Sciences Program, College of Human Sciences, Texas Tech University, 1301 Akron Street, Lubbock, TX 79409, USA.
e-mail: naima.moustaid-moussa@ttu.edu

Inflammatory adipokines secreted from adipose tissue are major contributors to obesity-associated inflammation and other metabolic dysfunctions. We and others have recently documented the contribution of adipose tissue renin-angiotensin system to the pathogenesis of obesity, inflammation, and insulin resistance. We hypothesized that adipocyte-derived angiotensinogen (Agt) plays a critical role in adipogenesis and/or lipogenesis as well as inflammation. This was tested using 3T3-L1 adipocytes, stably transfected with Agt-shRNA or scrambled Sc-shRNA as a control. Transfected preadipocytes were differentiated and used to investigate the role of adipose Agt through microarray and PCR analyses and adipokine profiling. As expected, Agt gene silencing significantly reduced the expression of Agt and its hormone product angiotensin II (Ang II), as well as lipid accumulation in 3T3-L1 adipocytes. Microarray studies identified several genes involved in lipid metabolism and inflammatory pathways which were down-regulated by Agt gene inactivation, such as glycerol-3-phosphate dehydrogenase 1 (Gpd1), serum amyloid A 3 (Saa3), nucleotide-binding oligomerization domain containing 1 (Nod1), and signal transducer and activator of transcription 1 (Stat1). Mouse adipogenesis PCR arrays revealed lower expression levels of adipogenic/lipogenic genes such as peroxisome proliferator activated receptor gamma (PPARγ), sterol regulatory element binding transcription factor 1 (Srebf1), adipogenin (Adig), and fatty acid binding protein 4 (Fabp4). Further, silencing of Agt gene significantly lowered expression of pro-inflammatory adipokines including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and monocyte chemotactic protein-1 (MCP-1). In conclusion, this study directly demonstrates critical effects of Agt in adipocyte metabolism and inflammation and further support a potential role for adipose Agt in the pathogenesis of obesity-associated metabolic alterations.

Keywords: angiotensinogen, gene silencing, inflammation, adipocytes, adipokines, adipogenesis

INTRODUCTION

Obesity is a major risk factor for life-threatening diseases in the United States and worldwide. Its comorbidities include hypertension, cardiovascular disease, stroke, type-2 diabetes mellitus (T2DM), and some types of cancer (Ouchi et al., 2011). In obese individuals, imbalanced secretion of pro- vs. anti-inflammatory adipokines from abnormally expanded adipose tissue and a shift in immune cell populations leads to the development of metabolic complications (Ouchi et al., 2011; Kalupahana and Moustaid-Moussa, 2012a). In the past decades, multiple studies (*in vitro*, *in vivo*, and clinical) indicated the critical role of the renin-angiotensin system (RAS) in the pathogenesis of obesity-associated health disorders. RAS is conventionally known as a group of hormones and enzymes (renin and angiotensin-converting enzyme: ACE) which regulate blood pressure and fluid balance. Angiotensinogen (Agt) is the only known precursor from which the hypertensive hormone angiotensin (Ang) II and other bioactive Ang peptides are generated. Ang II mainly functions through two G-protein coupled receptors, type 1 (AT1) and type 2 (AT2) receptors. In lean individuals, the liver is the major site for Agt production but RAS components are also expressed in white and brown adipose tissue, making them relevant to this work (Karlsson et al., 1998; Engeli et al., 2000; Galvez-Prieto et al., 2008). Angiotensinogen expression in general has been shown to be hormonally and nutritionally regulated as well as differentiation-dependent (Jones et al., 1997a; Siriwardhana et al., 2012). Specifically Agt is thought to be a late marker for adipocyte differentiation (Ailhaud et al., 2002), making it relevant to the current study.

Clinical studies showed that RAS blockade using ACE inhibitors or AT1 blockers improved the insulin sensitivity and lowered the incidence of T2DM in patients with hypertension or other cardiovascular diseases (Pollare et al., 1989). RAS blockade also increased the insulin sensitivity and improved

adiposity in obese and diabetic rodent models (reviewed in Kalupahana and Moustaid-Moussa, 2012a). The metabolic effects of RAS were further confirmed in genetic animal models with systemic deletion of certain RAS components (Agt, renin, ACE, AT1a, or AT2 receptor) (reviewed in Kalupahana and Moustaid-Moussa, 2012a). *In vitro* studies showed that Ang II stimulated lipogenesis and secretion of pro-inflammatory adipokines in adipocytes (Jones et al., 1997b; Kalupahana et al., 2012).

In obese humans and rodents, adipose tissue is the major site for Agt production, which significantly increases Agt level in circulation (Van Harmelen et al., 2000; Boustany et al., 2004; Engeli et al., 2005). Our lab and others demonstrated that mice with Agt over-expression in adipose tissue developed obesity with adipocyte hypertrophy, concurrent with insulin resistance and increased expression of lipogenic and pro-inflammatory makers (Massiera et al., 2001a; Kalupahana et al., 2012). Most of these effects were rescued by deletion of AT2 receptor (Yvan-Charvet et al., 2009). The genetic mouse model with adipose-specific Agt gene knock-out exhibited lower systolic blood pressure as they age, however no change was observed in body weight or fat mass when fed a low-fat diet (Yiannikouris et al., 2012). Systemic AGT knock-out mouse models have also been generated in which body weight, adiposity, leptin, and insulin levels were significantly lowered on a high-fat diet compared to wild-type mice. These effects were then reversed when AGT was re-expressed in adipose tissue (Massiera et al., 2001b; Kim et al., 2002).

Studies reviewed above link the elevated secretion of Agt from adipose tissue to obesity-associated local and systemic inflammation as well as insulin resistance, and possibly exacerbated adiposity. Therefore, we hypothesized that inactivation of Agt in adipocytes will limit lipid accumulation, and improve the inflammatory profile.

In the present study, we silenced Agt gene in 3T3-L1 adipocytes using shRNA, and demonstrated that lower Agt expression leads to decreased triglyceride accumulation, which is accompanied by improved expression patterns of adipogenic/lipogenic and inflammatory genes and proteins in adipocytes.

MATERIALS AND METHODS

CELL CULTURE, shRNA TRANSFECTION, AND PREADIPOCYTE DIFFERENTIATION

Initially, cell lines were generated as described below using two different shRNA sequences and prepared as both isolated or pooled clones of stably transfected cells. They were then compared to cells stably transfected with scrambled sequences. Both shRNA sequences reduced inflammatory markers and led to significant inactivation of AGT (>70%). Due to the similarities between the two sequences, only one was chosen and used for further detailed experiments as discussed below.

3T3-L1 preadipocytes (American Type Culture Collection; ATCC, Manassas, VA, USA) were seeded in two 6-well cell culture plates. Each well had 2 ml Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Cells were incubated at 37°C in a humidified CO_2 incubator. The vector-based shRNA targeting Agt gene (Agt-shRNA, GGATC CCGTTTCTACCTTGGATCCTAGATTGATATCCGTCTAAGGA TCCAAGGTAGAAATTTTTTCCAAAAGCTT) was ordered from GenScript (Piscataway, NJ, USA). A scrambled sequence (Sc-shRNA, GGATCCCGTCGCTTACCGATTCAGAATGGTTGAT ATCCGCCATTCTGAATCGGTAAGCGACGAAGCTTAAGTTTA AACCGCTGATCAGCCTCGACTGTGCCTTCTAGT) with no homology to any mouse or rat mRNA sequence in NCBI database was used as experimental control. These vectors carried a neomycin resistance gene.

Cells were stably transfected at ∼50–60% confluence. The transfection was performed using Lipofectamine™ 2000 Transfection Reagent (Life Technologies, Grand Island, NY, USA) method. 3T3-L1 preadipocytes transfected by Agt-shRNA or Sc-shRNA were maintained in regular growth medium (DMEM containing 10% FBS, 1% penicillin/streptomycin) till 90% confluence. To differentiate the preadipocytes to mature adipocytes, cells were maintained in regular growth medium supplemented by isobutylmethylxanthine (0.5 mM), dexamethasone (0.25 μM), and insulin (1 μg/ml) for 3 days, followed by regular growth media for another 3 days. Several cell lines were generated from these stable transfections and referred to as Agt-ShRNA1, 2, etc. Overall, Agt was silenced by more than 70% in these cell lines.

AGT AND ANG II MEASUREMENT

Total protein was extracted using tissue lysis buffer (Life Technologies, Grand Island, NY, USA) containing protease inhibitors (Roche, USA). The concentration of extracted protein samples was determined by Bradford assay (Thermo Scientific/Pierce, Rockford, IL, USA).

The expression level of Agt protein was measured by western blotting. Each sample of 25 μg total protein from 3T3-L1 adipocytes transfected by Agt-shRNA or Sc-shRNA (3 vs. 3) was first separated by electrophoresis using 12% Mini-PROTEAN TGX Precast Gel (Bio-Rad, Hercules, CA, USA), and then transferred to a PVDF membrane. After overnight blocking (3% bovine serum albumin in tris-buffered-saline supplemented by 0.1% Tween 20), the membrane was incubated with polyclonal antibodies targeting Agt protein (Santa Cruz Biotechnology, Santa Cruz, CA, USA). After a wash (tris-buffered-saline with 0.1% Tween 20), the membrane was incubated with the secondary antibodies containing horseradish peroxidase. The expression of Agt was detected with the ECL Plus substrate (Thermo Scientific/Pierce, Rockford, IL, USA), and quantified using the NIH ImageJ software. The expression level of Agt protein was normalized by the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

The intracellular level of Ang II was measured by Angiotensin II EIA Kit (Cayman Chemical, Ann Arbor, MI, USA). To perform the assay, 10 μL of each total protein sample from 3T3-L1 adipocytes transfected by Agt-shRNA or Sc-shRNA ($n = 5$ each) was used. The analyte value was normalized by total protein concentration.

LIPID ACCUMULATION ASSAY

3T3-L1 adipocytes transfected by Agt-shRNA or Sc-shRNA ($n = 5$ each) were washed with PBS, and then harvested in PBS supplemented by 10% IGEPAL CA-630 (detergent, Sigma-Aldrich, St. Louis, MO, USA). Cells were then transferred to a 2 ml autoclaved centrifuge tube and homogenized for 15 s. The triglyceride content in cell lysate was measured using Serum Triglyceride

Determination Kit (Sigma-Aldrich, St. Louis, MO, USA). The analyte value was normalized by total protein concentration.

PRO-INFLAMMATORY ADIPOKINE PROFILING

The intracellular level of pro-inflammatory adipokines (IL-1, TNF-α, MCP-1, PAI-1, and resistin) was measured by the Mouse Serum Adipokine Milliplex Kit (Millipore, Billerica, MA, USA). To perform the assay, total protein sample from 3T3-L1 adipocytes transfected by Agt-shRNA or Sc-shRNA ($n = 5$ each) was used. The analyte value was normalized by protein concentration.

MICROARRAY

Total RNA was extracted from 3T3-L1 adipocytes transfected with Agt-shRNA or Sc-shRNA using RNeasy lipid tissue kit (Qiagen, Valencia, CA, USA). Four sets of RNA samples each were used for microarray analyses. The concentration of these RNA samples was measured using NanoDrop 2000 (Thermo Scientific, USA), and the quality was determined by the Experion RNA StdSens Chips (Bio-Rad, Hercules, CA, USA). Samples were sent to the McGill University's Génome Québec Innovation Center (Montréal, Canada) and Illumina MouseRef-8 V2.0 Expression Beadchips (Illumina, San Diego, CA, USA) were used to measure 25,697 RefSeq transcripts, over 19,100 genes. David Bioinformatics Database 6.7[1] (NIAID/NIH) was used for the ontology and pathway analysis on genes with differential expression.

QUANTITATIVE REAL-TIME PCR

The expression pattern of selected genes with differential expression in the microarray analyses was verified by quantitative Real-Time PCR (qRT-PCR). Total RNA samples extracted from 3T3-L1 adipocytes transfected with Agt-shRNA or Sc-shRNA ($n = 4$ each) were used for cDNA synthesis using the High Capacity cDNA RT kit (Applied Biosystems, Carlsbad, CA, USA). All primers used in this study were purchased from Qiagen (QuantiTect Primer Assay). The real-time PCR was performed on Applied Biosystems 7900HT Fast Real-Time PCR System using the Fast SYBR Master Mix (Applied Biosystems, Carlsbad, CA, USA). The expression level of housekeeping gene β-actin (Actb) was measured as reference to normalize the expression level of genes under investigation.

PCR ARRAY

The expression of genes associated with adipocyte metabolism was profiled by Mouse Adipogenesis RT^2 Profiler™ PCR Array (SABiosciences, Qiagen, Valencia, CA, USA), which targets 84 core genes in mouse adipogenesis pathway. The array kit also contains five housekeeping genes, which include Actb. Total RNA samples extracted from 3T3-L1 adipocytes transfected with Agt-shRNA or Sc-shRNA ($n = 4$ each) were used for the synthesis of cDNA using RT^2 First Strand Kit (SABiosciences, Qiagen, Valencia, CA, USA). RT-PCR was performed on the same equipment as described previously using the RT^2 SYBR Green qPCR Mastermix (SABiosciences, Qiagen, Valencia, CA, USA). David Bioinformatics Database 6.7 (see text footnote 1, NIAID/NIH) was used for the gene ontology analysis.

[1]http://david.abcc.ncifcrf.gov/tools.jsp

STATISTICAL ANALYSES

In this study, microarray data were analyzed by R software (version 2.9.0) using "lumi" package in bioconductor[2]. Significant differential expression in 3T3-L1 adipocytes with Agt gene silencing compared to the adipocytes transfected by Sc-shRNA was defined as an FDR adjusted P value < 0.05.

Data from qRT-PCR and PCR array studies were analyzed by the web-based software[3] (SABiosciences) using $2^{-\Delta\Delta CT}$ method. The expression level for genes of interest was normalized by the expression of Actb housekeeping gene.

All other analyses were performed using SAS software (version 9.2, Cary, NC, USA). Results were reported as means ± SE. One way ANOVA followed by Tukey *post hoc* test was used to compare the group means at $P < 0.05$.

RESULTS

ANGIOTENSINOGEN GENE SILENCING REDUCED AGT PROTEIN EXPRESSION AND INTRACELLULAR LEVEL OF ANG II IN 3T3-L1 ADIPOCYTES

Successful transfection of Agt-shRNA into 3T3-L1 preadipocytes was demonstrated by the significantly lower expression of Agt protein in 3T3-L1 adipocytes expressing Agt-shRNA vs. Sc-shRNA (**Figures 1A,B**; $P < 0.005$). As Agt is the precursor for the hypertensive hormone Ang II, we further measured the intracellular level of Ang II, which was also decreased in 3T3-L1 adipocytes expressing Agt-shRNA vs. Sc-shRNA (**Figure 1C**; $P < 0.0001$).

AGT GENE SILENCING DECREASED LIPID ACCUMULATION AND INTRACELLULAR LEVEL OF PRO-INFLAMMATORY ADIPOKINES IN 3T3-L1 ADIPOCYTES

Previous studies have reported that Ang II increases the triglyceride content in 3T3-L1 adipocytes (Jones et al., 1997b). Here, we determined the role of RAS in mediating lipogenesis by directly knocking down the expression of Ang II precursor – Agt gene. Agt gene silencing significantly reduced the triglyceride content in 3T3-L1 adipocytes expressing Agt-shRNA vs. Sc-shRNA (**Figure 2A**; $P < 0.005$). Studies have also shown that Ang II stimulates the secretion of pro-inflammatory adipokines from both human and 3T3-L1 adipocytes. In this study, we measured the intracellular level of major pro-inflammatory adipokines (IL-6, TNF-α, MCP-1, resistin, and PAI-1). The results showed lower IL-6, TNF-α, and MCP-1 protein levels in 3T3-L1 adipocytes transfected with Agt-shRNA vs. Sc-shRNA (**Figure 2B**). This indicated Agt as an important mediator in adipose inflammation.

AGT GENE SILENCING INDUCED CHANGES IN THE EXPRESSION OF GENES INVOLVED IN LIPID METABOLISM AND IMMUNE RESPONSE PATHWAYS

To dissect the underlying mechanism which mediates the metabolic effects of Agt in adipocytes, a microarray study was performed on 3T3-L1 adipocytes transfected by Agt-shRNA or Sc-shRNA. After filtering out 16,561 transcripts with insignificant expression, we conducted statistical analysis on the remaining

[2]http://www.bioconductor.org/

[3]http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php

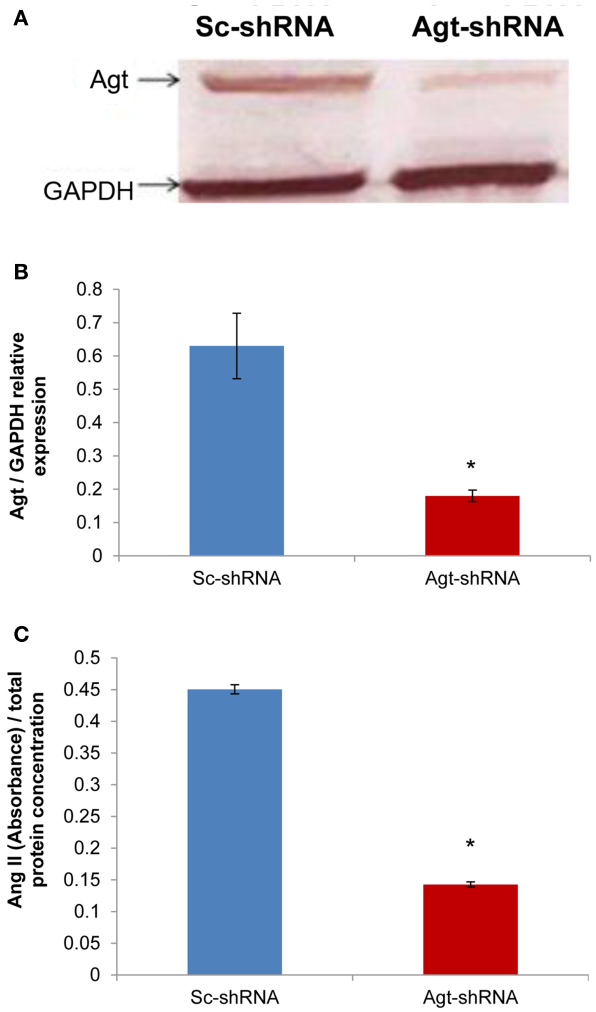


FIGURE 1 | Agt gene silencing induces lower Agt protein expression and intracellular level of Ang II. 3T3-L1 preadipocytes were transfected by shRNA targeting Agt gene (Agt-shRNA) or scrambled shRNA (Sc-shRNA), and then differentiated to mature adipocytes. **(A,B)** Agt protein expression, and **(C)** intracellular level of Ang II are shown. Data are presented as mean ± SE [$n = 3$ for each group in **(A)**, $n = 5$ for each group in **(C)**]. The expression of Agt is normalized by the expression of GAPDH. The intracellular level of Ang II is normalized by total protein concentration. $^*P < 0.005$ vs. adipocytes transfected by Sc-shRNA.

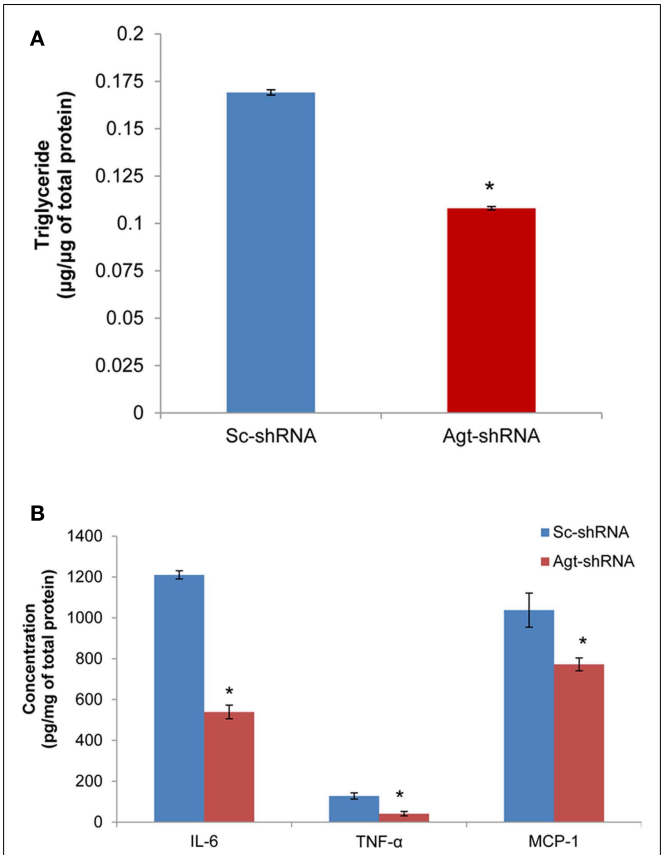


FIGURE 2 | Agt gene silencing leads to lower triglyceride accumulation and intracellular level of pro-inflammatory adipokines in 3T3-L1 adipocytes. 3T3-L1 preadipocytes were transfected by shRNA targeting Agt gene (Agt-shRNA) or scrambled shRNA (Sc-shRNA), and then differentiated to mature adipocytes. Triglyceride content **(A)** and adipokine concentration **(B)** is normalized by total protein concentration. Data are presented as mean ± SE ($n = 5$ for each group). $^*P < 0.05$ vs. adipocytes transfected by Sc-shRNA.

9,136 transcripts. Compared to cells transfected with Sc-shRNA, the expression of 89 genes was significantly down-regulated and the expression of 23 genes was up-regulated in 3T3-L1 adipocytes with Agt gene silencing (**Figure 3A**). A heat map was plotted by cluster analysis on genes with differential expression in 3T3-L1 adipocytes transfected with Agt-shRNA vs. Sc-shRNA. The expression patterns of these genes were highly consistent within each adipocyte cell line, as shown in **Figure 3B**.

Gene ontology and pathway analysis showed that the expression of several critical genes involved in lipid metabolism and inflammatory response was down-regulated in 3T3-L1 adipocytes with Agt gene silencing; selected genes are listed in **Tables 1** and **2**. Other genes with decreased expression in 3T3-L1 adipocytes with Agt gene silencing were genes involved in energy metabolism, immune response, oxidative stress, cell differentiation, and apoptosis. Genes with up-regulated expression in 3T3-L1 adipocytes with Agt gene silencing were ones involved in DNA replication, transcription, translation, repair, chromosomal structure, intracellular protein trafficking, protein phosphorylation, and proteolysis. These genes are listed in **Table A1** in Appendix.

To determine whether the profile of global gene expression from the microarray study were consistent with the relative amounts of mRNA present in parallel samples, qRT-PCR was performed on selected genes (Cxcl12, Stat1, Saa3, Nod1, Gpd1, and Acad10). Consistent with microarray results, the expression level of all these genes were down-regulated in 3T3-L1 adipocytes with Agt gene silencing compared to those transfected with Sc-shRNA (**Figure 4**).

Since knock-down of Agt gene decreased lipid accumulation and the expression of genes associated with lipid metabolism in 3T3-L1 adipocytes, we further profiled the expression pattern of

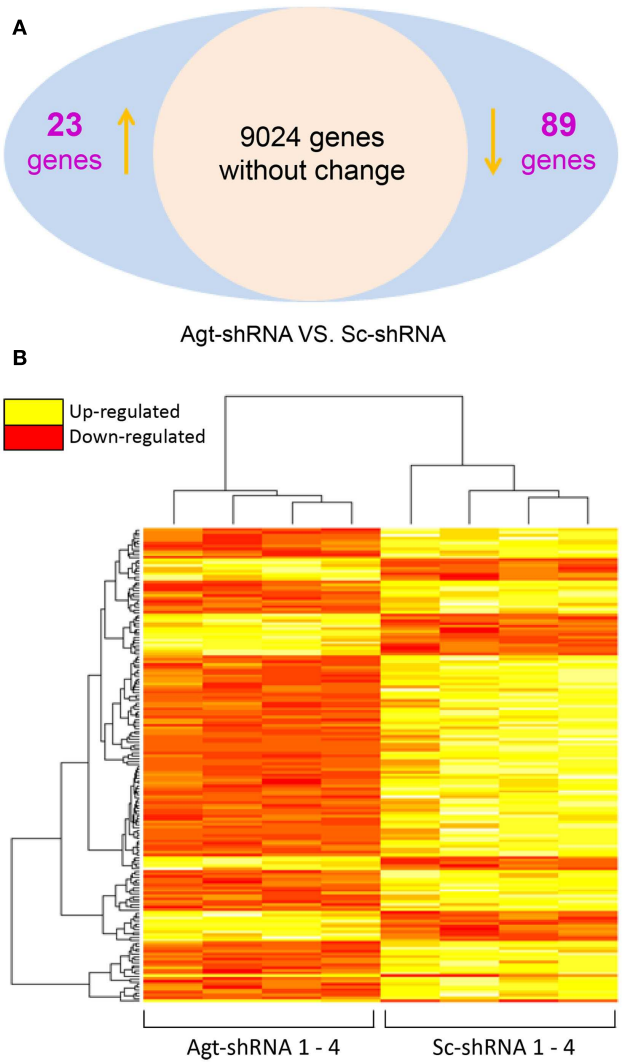


FIGURE 3 | Agt gene silencing induces changes in global gene expression patterns in 3T3-L1 adipocytes. 3T3-L1 preadipocytes were transfected by shRNA targeting Agt gene (Agt-shRNA) or scrambled shRNA (Sc-shRNA), and then differentiated into mature adipocytes. Changes in global gene expression patterns were profiled by microarray study. Genes with significant differential expression in 3T3-L1 adipocytes transfected with Agt-shRNA vs. Sc-shRNA were determined by the false discovery rate (FDR) adjusted P value < 0.05 **(A)**. Heat map with genes of differential expression is shown in **(B)**. The rows represent the patterns of gene expression, and columns represent samples. Red region corresponds to genes with down-regulated expression, and yellow region corresponds to genes with up-regulated expression, in 3T3-L1 adipocytes transfected with Agt-shRNA vs. Sc-shRNA ($n = 4$ for each cell line).

genes in the adipogenesis pathways using a PCR array. The results confirmed the lower expression of Agt gene at transcription level, and showed decreased expression of several adipogenic genes, including PPAR-γ, Cebpb, Srebf1 and Adig, and lipid metabolic markers Ppargc1b and Fabp4 in 3T3-L1 adipocytes with Agt gene silencing. Conversely, the expression of genes with anti-adipogenic effects, Gata3, and Vdr, were up-regulated. The expression pattern of these genes is shown in **Figure 5**, and the functions of these genes are described in **Table 3**.

DISCUSSION

Recent studies indicated the potential role of adipose tissue-derived Agt in the pathogenesis of metabolic disorders. However, the underlying mechanisms mediating these effects are not well clarified. In the present study, we successfully established Agt gene silencing in 3T3-L1 adipocytes using shRNA, which effectively decreased the intracellular level of Agt and Ang II, and enabled us to directly investigate the metabolic effects of adipose Agt. Using this *in vitro* model, we confirmed the direct contribution of adipocyte-derived Agt and Ang II to preadipocyte differentiation, lipogenesis, and adipocyte inflammation, as evidenced by decreased triglyceride accumulation, and pro-inflammatory adipokine production, as well as down-regulated expression of several adipogenic and inflammatory genes.

RAS AND ADIPOSITY

The present study confirmed the effects of Agt/Ang II in promoting lipogenesis. Our lab previously reported that Ang II increases lipogenesis in 3T3-L1 and human adipocytes (Jones et al., 1997b). In line with these findings, we demonstrated in this study that inactivation of Agt gene significantly reduced the lipid accumulation during 3T3-L1 preadipocyte differentiation. This is also in agreement with a recent study where shRNA-mediated Agt gene silencing inhibited triglyceride storage during human visceral preadipocyte differentiation (Ye et al., 2010). Indeed, in these studies, the expression of Gpd1 gene which encodes glycerol-3-phosphate dehydrogenase 1 was increased in Ang II treated 3T3-L1 adipocytes (Jones et al., 1997b) but decreased by knock-down of the Agt gene in human preadipocytes. Consistently, we identified here that the expression of Gpd1 gene was decreased in 3T3-L1 adipocytes with Agt gene silencing. In this study, no change was detected in the gene expression of acetyl-coenzyme A carboxylase or fatty acid synthase, the major enzymes mediating *de novo* lipogenesis. Therefore, the lower lipid accumulation in 3T3-L1 adipocytes with Agt gene silencing is potentially due to the decreased synthesis of glycerol by Gpd1 in the process of lipogenesis. It is also possible that Agt gene silencing down-regulates the activity of lipogenic enzymes, and inhibits the *de novo* lipogenesis in 3T3-L1 adipocytes. Ang II was previously shown to inhibit preadipocyte differentiation, which was potentially mediated by the AT1 receptor. Similarly, certain AT1 blockers enhanced preadipocyte differentiation in both 3T3-L1 and human preadipocytes, and mice with systemic knock-out of AT1a receptor showed adipocyte hypotrophy (Janke et al., 2002, 2006; Schupp et al., 2004). However, in this study, we identified that Agt gene silencing inhibited 3T3-L1 preadipocyte differentiation through down-regulating the expression of core adipogenic genes (C/EBP-β and PPAR-γ) and other adipogenic markers (Srebf1 and Adig) which mediate the initiation of adipogenesis. Agt gene silencing also decreased the expression of several lipid metabolic genes (Fabp4 and Ppargc1b). Genes with anti-adipogenic effects (Gata3 and Vdr) were also up-regulated in Agt-shRNA transfected cells. These indicated Agt as a critical factor with stimulatory effects on preadipocyte differentiation. We propose that the effect of

Table 1 | Lipid metabolic genes differentially expressed in 3T3-L1 adipocytes transfected by Agt-shRNA vs. Sc-shRNA.

Gene symbol	Gene name	Fold change	*P* value (FDR adjusted)	Function
Gpd1	Glycerol-3-phosphate dehydrogenase 1	0.34	0.003919	A lipogenic marker, catalyzes the reversible conversion of dihydroxyacetone phosphate to sn-glycerol-3-phosphate
Ces3	Carboxylesterase 3	0.24	0.000087	Adipocyte lipase, mediates lipolysis
Retsat	Retinol saturase	0.63	0.025349	Saturates13-14 double bond of all-trans-retinol to produce all-trans-13, 14-dihydroretinol
Acad10	Acyl-Coenzyme A dehydrogenase family, member 10	0.78	0.050300	Catalyzes the initial step of mitochondria fatty acid β-oxidation
Apoc1	Apolipoprotein C-I	0.48	0.001845	Modulates the interaction of APOE with beta-migrating VLDL, inhibits the binding of beta-VLDL to LDL receptor-related protein
Hsd3b7	Hydroxy-delta-5-steroid dehydrogenase, 3 beta-, and steroid delta-isomerase 7	0.80	0.048719	Plays a crucial role in the biosynthesis of hormonal steroids
Suclg1	Succinate-CoA ligase, GDP-forming, alpha subunit, mitochondrial	0.72	0.037140	An enzyme in tricarboxylic acid cycle, catalyzes ATP- or GTP-dependent ligation of succinate and CoA to form succinyl-CoA in mitochondrial matrix

FDR, false discovery rate.

Table 2 | Inflammatory genes differentially expressed in 3T3-L1 adipocytes transfected by Agt-shRNA vs. Sc-shRNA.

Gene symbol	Gene name	Fold change	*P* value (FDR adjusted)	Function
Saa3	Serum amyloid A 3	0.57	0.025314	Major acute phase protein, in response to cytokine stimulation
Nod1	Nucleotide-binding oligomerization domain containing 1	0.75	0.029593	Induces NF-κ B activation via RIPK2 and IKK-gamma, enhances caspase-9-mediated apoptosis
Cxcl12	Chemokine (C-X-C motif) ligand 12	0.38	0.046402	Chemoattractant for T-lymphocytes and monocytes
Ptx3	Pentraxin related gene	0.71	0.033282	Activates classical complement pathway, facilitates pathogen recognition by immune cells
Stat1	Signal transducer and activator of transcription 1	0.66	0.029154	A member of signal transducers and activators of transcription (STATs) family, mediates cytokine signaling
Irf9	Interferon regulatory factor 9	0.64	0.029593	Plays a critical role in stimulating IFN-alpha responsive genes

FDR, false discovery rate.

Ang II on adipogenesis is receptor specific, and dose dependent. Possibly, low to regular levels of Ang II stimulate adipogenesis through the AT2 receptor, which overcomes the anti-adipogenic effect mediated by the AT1 receptor. However, high levels of Ang II could activate the AT1 receptor and inhibit preadipocyte differentiation.

Our microarray study detected multiple genes associated with adipocyte metabolism which were down-regulated by Agt gene silencing. Retsat (encodes retinol saturase) is induced during 3T3-L1 preadipocyte differentiation and promotes adipogenesis through increased PPAR-γ transcriptional activity (Schupp et al., 2009).

Ang II is an active mediator of lipolysis in a time-dependent manner. Short term treatment of Ang II inhibits lipolysis in human adipose tissue and adipocytes through the AT1 receptor (Boschmann et al., 2001; Goossens et al., 2004, 2007). However, long term infusion of Ang II markedly increased lipolysis in rats (Cabassi et al., 2005). One explanation for this could be the Ces3 gene which encodes for carboxylesterase 3 (triacylglycerol hydrolase, TGH) – an adipocyte lipase – whose expression was decreased in 3T3-L1 adipocytes with Agt gene silencing.

Lastly, our microarray and RT-PCR data indicated that Agt gene widely affects the whole process of lipid metabolism in adipocytes. This was seen in the expression of other genes down-regulated by

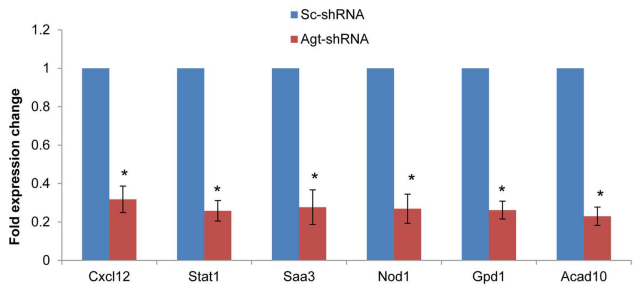


FIGURE 4 | Agt gene silencing induces down-regulated expression of Cxcl12, Stat1, Saa3, Nod1, Gpd1, and Acad10 genes in 3T3-L1 adipocytes. 3T3-L1 preadipocytes were transfected by shRNA targeting Agt gene (Agt-shRNA) or scrambled shRNA (Sc-shRNA), and then differentiated to mature adipocytes. Data are presented as mean ± SE, and calculated according to the $2^{-\Delta\Delta CT}$ method ($n = 4$ for each group). *$P < 0.0001$ vs. adipocytes transfected by Sc-shRNA.

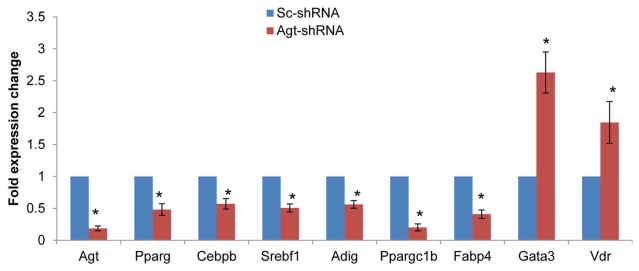


FIGURE 5 | Agt gene silencing induces changes in the expression of genes associated with adipogenesis pathway in 3T3-L1 adipocytes. 3T3-L1 preadipocytes were transfected by shRNA targeting Agt gene (Agt-shRNA) or scrambled shRNA (Sc-shRNA), and then differentiated to mature adipocytes. Data are presented as mean ± SE, and calculated according to the $2^{-\Delta\Delta CT}$ method ($n = 4$ for each group). *$P < 0.05$ vs. adipocytes transfected by Sc-shRNA.

Agt gene silencing, such as Acad10, Apoc1, Suclg1, and Hsd3b7, which are involved in lipid transport, β oxidation, TCA cycle, and biosynthesis of steroid hormones from lipids.

RAS AND INFLAMMATION

While it has been established that obesity leads to chronic low-grade inflammation in adipose tissue (Kalupahana and Moustaid-Moussa, 2012a), the exact trigger for this process is unknown. Adipose tissue-secreted Agt has been shown to induce obesity-associated inflammation, a major factor for the pathogenesis of metabolic disorders, and therefore is a potential target for obesity research as described below (Kalupahana and Moustaid-Moussa, 2012b). In obesity, adipose tissue-derived Agt significantly increases the level of Agt in circulation (Van Harmelen et al., 2000; Boustany et al., 2004; Engeli et al., 2005). Moreover, our lab and collaborators further showed that adipose-specific Agt over-expression induced local and systemic inflammation, as well as insulin resistance in mice (Kalupahana et al., 2012).

Previous studies reported that Ang II stimulated the secretion of several pro-inflammatory adipokines from both 3T3-L1 and human adipocytes (Skurk et al., 2001, 2004). Consistent with those studies, we demonstrated here that Agt gene silencing in 3T3-L1 adipocytes significantly reduced the intracellular level of pro-inflammatory adipokines, including MCP-1, IL-6, and TNFα.

In the current research, the microarray study identified that Agt gene silencing decreased the expression of several genes involved in adipose inflammation including Saa3, Nod1, Stat1, and Cxcl12. Saa3, which encodes for the acute phase protein – serum amyloid A3, was recently identified as a critical pro-inflammatory adipokine involved in obesity-associated metabolic disorders (Yang et al., 2006). A previous study showed that blocking the AT1 receptor suppressed the mRNA expression of Saa3, together with TNF-a, PAI-1, and MCP-1 in mice adipose tissue (Kurata et al., 2006). The present study further confirmed that Saa3 as a critical factor involved in Ang II-induced inflammatory disorders, as silencing of Agt in cultured adipocytes reduced Saa3 expression.

Nod1 gene encodes an intracellular pattern recognition receptor mediating immune response. The activation of this receptor induces peripheral and hepatic insulin resistance, which is prevented when this gene in inactivated (Schertzer et al., 2011). Activation of NOD1 also increases pro-inflammatory adipokine secretion and impairs the insulin-stimulated glucose uptake in 3T3-L1 and primary murine adipocytes (Schertzer et al., 2011; Zhao et al., 2011). Since Agt silencing reduced NOD1, this suggests that Nod1 is a potential mediator for Ang II-induced adipocyte inflammation.

Stat1 is a member in the Stats family – the major component of Jak-Stat pathway which plays a critical role in mediating cytokine signaling. Jak-Stat pathway mediates the action of hormones which impact adipocyte development and functions (Richard and Stephens, 2011). Stats mediate the activation of multiple inflammatory pathways, such as NF-κB and c-Jun (O'Shea et al., 2002). The lowered expression of the Stat1 gene, in part, indicates that Agt gene silencing down-regulates the cytokine signaling in 3T3-L1 adipocytes. Interferon regulatory factors (Irfs), which mainly respond to interferon in the JAK-STAT signaling pathway (O'Shea et al., 2002; Eguchi et al., 2008), could be one avenue of this down-regulation. Previous studies reported that IFN-α/β induced a complex formation of STAT1, STAT2, and IRF9, one of the Irfs which are expressed in adipocytes (Eguchi et al., 2008). Together these indicate that Agt gene silencing induced a consistent change pattern in cytokine signaling pathway (O'Shea et al., 2002; Eguchi et al., 2008).

Several other chemokines were altered by Agt inactivation. These include Cxcl12 and Ptx3. Cxcl12 is mainly expressed in stromal cells, but is also detected in 3T3-L1 adipocytes (Choi et al., 2010). This indicates the potential role of this gene in inducing the immune cells infiltration in adipose tissue. The expression of Ptx3 gene is up-regulated during preadipocyte differentiation, and although almost non-detectable in mature adipocytes, it can be re-activated in 3T3-F442A adipocytes upon TNF-α stimulation (Abderrahim-Ferkoune et al., 2003). The decreased expression of Ptx3 gene in 3T3-L1 adipocytes with Agt gene silencing is potentially mediated by decreased adipogenesis and TNF-α (Abderrahim-Ferkoune et al., 2003).

Table 3 | Genes with differential expression associated with adipogenesis pathway in 3T3-L1 adipocytes transfected by Agt-shRNA vs. Sc-shRNA.

Gene symbol	Gene name	Fold change	*P* value	Function
Pparg	Peroxisome proliferator activated receptor gamma	0.4808	0.030656	Key regulator of glucose homeostasis and preadipocyte differentiation
Cebpb	CCAAT/enhancer binding protein (C/EBP), beta	0.5718	0.038756	Plays a catalytic role in preadipocyte differentiation
Srebf1	Sterol regulatory element binding transcription factor 1	0.5070	0.008977	Regulates glucose metabolism, fatty acid, and lipid (mainly cholesterol) synthesis
Adig	Adipogenin	0.5619	0.005956	An adipocyte-specific protein, promotes adipocyte differentiation
Ppargc1b	Peroxisome proliferative activated receptor, gamma, coactivator 1 beta	0.2017	0.045250	Stimulates the activation of transcription factors. Involved in fat oxidation, and non-oxidative glucose metabolism
Fabp4	Fatty acid binding protein 4	0.4094	0.002358	Adipocytes specific lipid transport protein
Gata3	GATA binding protein 3	2.6291	0.003210	Suppresses preadipocytes differentiation through PPAR-γ
Vdr	Vitamin D receptor	1.8460	0.003185	Suppresses preadipocytes differentiation through C/EBP and PPAR-γ

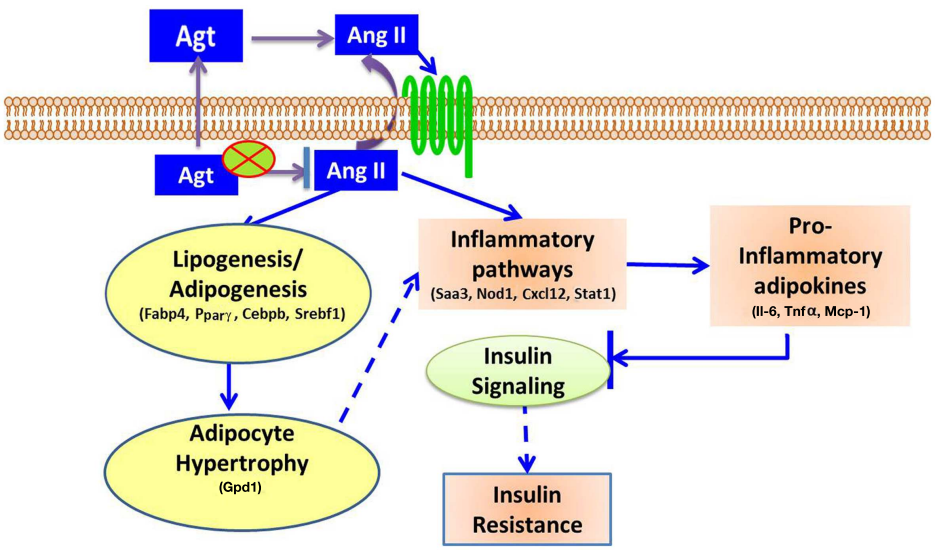


FIGURE 6 | Proposed mechanisms for Ang II-induced metabolic disorders. Adipocyte-derived Agt is converted to Ang II which affects adipocytes in autocrine or paracrine manner through angiotensin receptors. Ang II activates inflammatory pathways, and increases the secretion of pro-inflammatory adipokines, which inhibit insulin signaling and induce insulin resistance. Ang II also stimulates lipogenesis in adipocytes, leading to adipocyte hypertrophy which potentially activates inflammatory pathways and exacerbates these inflammatory conditions.

Elevated level of RAS components was detected in adipose tissue and circulation in obese human subjects and animals (Van Harmelen et al., 2000; Boustany et al., 2004; Engeli et al., 2005). Animal models with systemic deletion of RAS components (Agt, renin, ACE, AT1a, and AT2) were protected from diet-induced obesity and metabolic disorders (Massiera et al., 2001b). Transgenic mice with Agt over-expression in adipose tissue exhibit adiposity, adipocyte hypertrophy, adipose and systemic inflammation, and insulin resistance (Kalupahana et al., 2012). However, mice with Agt gene specifically knock-out from adipose tissue had no change in fat mass or body weight (Yiannikouris et al., 2012). These indicate that elevated secretion of Agt from adipose tissue may in part mediate obesity-associated metabolic disorders.

In summary, this study showed that gene silencing induced lower adipocyte-derived-Agt, which led to decreased lipid accumulation and pro-inflammatory adipokines production, as

well as down-regulated expression of adipogenic and lipid metabolic markers and genes in inflammatory pathways. The underlying mechanism for the metabolic effect of Ang II is summarized in **Figure 6**.

ACKNOWLEDGMENTS

This work was supported by the Physician Medical Education and Research Foundation, The University of Tennessee AgResearch, and The College of Human Sciences/Texas Tech University.

REFERENCES

Abderrahim-Ferkoune, A., Bezy, O., Chiellini, C., Maffei, M., Grimaldi, P., Bonino, F., et al. (2003). Characterization of the long pentraxin PTX3 as a TNFα-induced secreted protein of adipose cells. *J. Lipid Res.* 44, 994–1000.

Ailhaud, G., Teboul, M., and Massiera, F. (2002). Angiotensinogen, adipocyte differentiation and fat mass enlargement. *Curr. Opin. Clin. Nutr. Metab. Care* 5, 385–389.

Boschmann, M., Ringel, J., Klaus, S., and Sharma, A. M. (2001). Metabolic and hemodynamic response of adipose tissue to angiotensin II. *Obes. Res.* 9, 486–491.

Boustany, C. M., Bharadwaj, K., Daugherty, A., Brown, D. R., Randall, D. C., and Cassis, L. A. (2004). Activation of the systemic and adipose renin-angiotensin system in rats with diet-induced obesity and hypertension. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 287, R943–R949.

Cabassi, A., Coghi, P., Govoni, P., Barouhiel, E., Speroni, E., Cavazzini, S., et al. (2005). Sympathetic modulation by carvedilol and losartan reduces angiotensin II-mediated lipolysis in subcutaneous and visceral fat. *J. Clin. Endocrinol. Metab.* 90, 2888–2897.

Choi, S., Kim, J., Yea, K., Suh, P. G., Kim, J., and Ryu, S. H. (2010). Targeted label-free quantitative analysis of secretory proteins from adipocytes in response to oxidative stress. *Anal. Biochem.* 401, 196–202.

Eguchi, J., Yan, Q. W., Schones, D. E., Kamal, M., Hsu, C. H., Zhang, M. Q., et al. (2008). Interferon regulatory factors are transcriptional regulators of adipogenesis. *Cell Metab.* 7, 86–94.

Engeli, S., Bohnke, J., Gorzelniak, K., Janke, J., Schling, P., Bader, M., et al. (2005). Weight loss and the renin-angiotensin-aldosterone system. *Hypertension* 45, 356–362.

Engeli, S., Negrel, R., and Sharma, A. M. (2000). Physiology and pathophysiology of the adipose tissue renin-angiotensin system. *Hypertension* 35, 1270–1277.

Galvez-Prieto, B., Bolbrinker, J., Stucchi, P., de Las Heras, A. I., Merino, B., Arribas, S., et al. (2008). Comparative expression analysis of the renin-angiotensin system components between white and brown perivascular adipose tissue. *J. Endocrinol.* 197, 55–64.

Goossens, G. H., Blaak, E. E., Arner, P., Saris, W. H., and van Baak, M. A. (2007). Angiotensin II: a hormone that affects lipid metabolism in adipose tissue. *Int. J. Obes. (Lond.)* 31, 382–384.

Goossens, G. H., Blaak, E. E., Saris, W. H. M., and van Baak, M. A. (2004). Angiotensin II-induced effects on adipose and skeletal muscle tissue blood flow and lipolysis in normal-weight and obese subjects. *J. Clin. Endocrinol. Metab.* 89, 2690–2696.

Janke, J., Engeli, S., Gorzelniak, K., Luft, F. C., and Sharma, A. M. (2002). Mature adipocytes inhibit in vitro differentiation of human preadipocytes via angiotensin type 1 receptors. *Diabetes* 51, 1699–1707.

Janke, J., Schupp, M., Engeli, S., Gorzelniak, K., Boschmann, M., Sauma, L., et al. (2006). Angiotensin type 1 receptor antagonists induce human in-vitro adipogenesis through peroxisome proliferator-activated receptor-gamma activation. *J. Hypertens.* 24, 1809–1816.

Jones, B. H., Standridge, M. K., Taylor, J. W., Moustaïd, N. (1997a). Angiotensinogen gene expression in adipose tissue: analysis of obese models and hormonal and nutritional control. *Am. J. Physiol.* 273(1 Pt 2): R236–R242.

Jones, B. H., Standridge, M. K., and Moustaid, N. (1997b). Angiotensin II increases lipogenesis in 3T3-L1 and human adipose cells. *Endocrinology* 138, 1512–1519.

Kalupahana, N. S., Massiera, F., Quignard-Boulange, A., Ailhaud, G., Voy, B. H., Wasserman, D. H., et al. (2012). Overproduction of angiotensinogen from adipose tissue induces adipose inflammation, glucose intolerance, and insulin resistance. *Obesity (Silver Spring)* 20, 48–56.

Kalupahana, N. S., and Moustaid-Moussa, N. (2012a). The renin-angiotensin system: a link between obesity, inflammation and insulin resistance. *Obes. Rev.* 13, 136–149.

Kalupahana, N. S., and Moustaid-Moussa, N. (2012b). The adipose tissue renin-angiotensin system and metabolic disorders: a review of molecular mechanisms. *Crit. Rev. Biochem. Mol. Biol.* 47, 379–390.

Karlsson, C., Lindell, K., Ottosson, M., Sjostrom, L., Carlsson, B., and Carlsson, L. M. (1998). Human adipose tissue expresses angiotensinogen and enzymes required for its conversion to angiotensin II. *J. Clin. Endocrinol. Metab.* 83, 3925–3929.

Kim, S., Urs, S., Massiera, F., Wortman, P., Joshi, R., Heo, Y. R., et al. (2002). Effects of high-fat diet, angiotensinogen (agt) gene inactivation, and targeted expression to adipose tissue on lipid metabolism and renal gene expression. *Horm. Metab. Res.* 34, 721–725.

Kurata, A., Nishizawa, H., Kihara, S., Maeda, N., Sonoda, M., Okada, T., et al. (2006). Blockade of Angiotensin II type-1 receptor reduces oxidative stress in adipose tissue and ameliorates adipocytokine dysregulation. *Kidney Int.* 70, 1717–1724.

Massiera, F., Bloch-Faure, M., Ceiler, D., Murakami, K., Fukamizu, A., Gasc, J. M., et al. (2001a). Adipose angiotensinogen is involved in adipose tissue growth and blood pressure regulation. *FASEB J.* 15, 2727–2729.

Massiera, F., Seydoux, J., Geloen, A., Quignard-Boulange, A., Turban, S., Saint-Marc, P., et al. (2001b). Angiotensinogen-deficient mice exhibit impairment of diet-induced weight gain with alteration in adipose tissue development and increased locomotor activity. *Endocrinology* 142, 5220–5225.

O'Shea, J. J., Gadina, M., and Schreiber, R. D. (2002). Cytokine signaling in 2002: new surprises in the jak/stat pathway. *Cell* 109 (Suppl. 1), S121–S131.

Ouchi, N., Parker, J. L., Lugus, J. J., and Walsh, K. (2011). Adipokines in inflammation and metabolic disease. *Nat. Rev. Immunol.* 11, 85–97.

Pollare, T., Lithell, H., and Berne, C. (1989). A comparison of the effects of hydrochlorothiazide and captopril on glucose and lipid metabolism in patients with hypertension. *N. Engl. J. Med.* 321, 868–873.

Richard, A. J., and Stephens, J. M. (2011). Emerging roles of JAK–STAT signaling pathways in adipocytes. *Trends Endocrinol. Metab.* 22, 325–332.

Schertzer, J. D., Tamrakar, A. K., Magalhaes, J. G., Pereira, S., Bilan, P. J., Fullerton, M. D., et al. (2011). NOD1 activators link innate immunity to insulin resistance. *Diabetes* 60, 2206–2215.

Schupp, M., Janke, J., Clasen, R., Unger, T., and Kintscher, U. (2004). Angiotensin type 1 receptor blockers induce peroxisome proliferator-activated receptor-gamma activity. *Circulation* 109, 2054–2057.

Schupp, M., Lefterova, M. I., Janke, J., Leitner, K., Cristancho, A. G., Mullican, S. E., et al. (2009). Retinol saturase promotes adipogenesis and is downregulated in obesity. *Proc. Natl. Acad. Sci. U.S.A.* 106, 1105–1110.

Siriwardhana, N., Kalupahana, N. S., Fletcher, S., Xin, W., Claycombe, K. J., Quignard-Boulange, A., et al. (2012). n-3 and n-6 polyunsaturated fatty acids differentially regulate adipose angiotensinogen and other inflammatory adipokines in part via NF-κB-dependent mechanisms. *J. Nutr. Biochem.* 23, 1661–1667.

Skurk, T., Lee, Y. M., and Hauner, H. (2001). Angiotensin II and its metabolites stimulate PAI-1 protein release from human adipocytes in primary culture. *Hypertension* 37, 1336–1340.

Skurk, T., van Harmelen, V., and Hauner, H. (2004). Angiotensin II stimulates the release of interleukin-6 and interleukin-8 from cultured human adipocytes by activation of NF-kappaB. *Arterioscler. Thromb. Vasc. Biol.* 24, 1199–1203.

Van Harmelen, V., Ariapart, P., Hoffstedt, J., Lundkvist, I., Bringman, S., and Arner, P. (2000). Increased adipose angiotensinogen gene expression in human obesity. *Obes. Res.* 8, 337–341.

Yang, R. Z., Lee, M. J., Hu, H., Pollin, T. I., Ryan, A. S., Nicklas, B. J., et al. (2006). Acute-phase serum amyloid a: an inflammatory adipokine and potential link between obesity and its metabolic complications. *PLoS Med.* 3:e287. doi:10.1371/journal.pmed.0030287

Ye, Z. W., Wu, X. M., and Jiang, J. G. (2010). Knockdown of angiotensinogen by shRNA-mediated RNA interference inhibits human visceral preadipocytes differentiation. *Int. J. Obes. (Lond.)* 34, 157–164.

Yiannikouris, F., Karounos, M., Charnigo, R., English, V. L., Rateri, D. L., Daugherty, A., et al. (2012). Adipocyte-specific deficiency of angiotensinogen decreases plasma angiotensinogen concentration and systolic blood pressure in mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 302, R244–R251.

Yvan-Charvet, L., Massiera, F., Lamande, N., Ailhaud, G., Teboul, M., Moustaid-Moussa, N., et al. (2009). Deficiency of angiotensin type 2 receptor rescues obesity but not hypertension induced by overexpression of angiotensinogen in adipose tissue. *Endocrinology* 150, 1421–1428.

Zhao, L., Hu, P., Zhou, Y., Purohit, J. S., and Hwang, D. H. (2011). NOD1 activation induces proinflammatory gene expression and insulin resistance in 3T3-L1 adipocytes. *Am. J. Physiol. Endocrinol. Metab.* 301, E587–E598.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 17 November 2012; accepted: 28 January 2013; published online: 11 March 2013.

Citation: Carroll WX, Kalupahana NS, Booker SL, Siriwardhana N, LeMieux M, Saxton AM and Moustaid-Moussa N (2013) Angiotensinogen gene silencing reduces markers of lipid accumulation and inflammation in cultured adipocytes. Front. Endocrinol. ***4****:10. doi:10.3389/fendo.2013.00010*

This article was submitted to Frontiers in Diabetes, a specialty of Frontiers in Endocrinology.

Copyright © 2013 Carroll, Kalupahana, Booker, Siriwardhana, LeMieux, Saxton and Moustaid-Moussa. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.

APPENDIX

Table A1 | Genes differentially expressed in 3T3-L1 adipocytes transfected with Agt-shRNA vs. Sc-shRNA.

Gene symbol	Gene name	Fold change	*P* value (FDR adjusted)
ENERGY METABOLIC PROCESS			
Por	P450 (cytochrome) oxidoreductase	0.613868	0.004184
Aldh2	Aldehyde dehydrogenase 2, mitochondrial	0.656561	0.022585
Cdo1	Cysteine dioxygenase 1, cytosolic	1.806253	0.041216
Cyb561	Cytochrome b-561	0.661127	0.029593
Idh3g	Isocitrate dehydrogenase 3 (NAD+), gamma	0.65022	0.021874
Hibadh	3-hydroxyisobutyrate dehydrogenase	0.729005	0.033282
Phgdh	Phosphoglycerate dehydrogenase	0.688725	0.035963
gm2a	GM2 ganglioside activator protein	0.511569	0.029154
AU018778	Expressed sequence AU018778 (type-B carboxylesterase/lipase)	0.439825	0.000356
Atp6ap1	ATPase, H+ transporting, lysosomal accessory protein 1	0.666187	0.031342
Acaa1b	Acetyl-Coenzyme A acyltransferase 1B	0.627201	0.031219
Adhfe1	Alcohol dehydrogenase, iron containing, 1	0.669428	0.029465
Gpt	Glutamic pyruvic transaminase, soluble	0.482968	0.014529
Gsta4	Glutathione S-transferase, alpha 4	0.541488	0.025158
Gstm2	Glutathione S-transferase, mu 2	0.71847	0.008982
Cox8b	Cytochrome c oxidase, subunit VIIIb	0.32511	0.003044
Cox6c	Cytochrome c oxidase subunit Vic	2.011121	0.022585
IMMUNE RESPONSE TO EXTRACELLULAR STIMULUS			
C4b	Complement component 4B	0.6	0.041924
B2m	Beta-2 microglobulin	0.717475	0.0322
Tap2	Transporter 2, ATP-binding cassette, sub-family B	0.634635	0.043325
Igtp	Interferon gamma induced GTPase	0.533293	0.031471
Ifit3	Interferon-induced protein with tetratricopeptide repeats 3	0.198333	0.031219
Cd99l2	CD99 antigen-like 2	0.647073	0.043325
RESPONSE TO HORMONE STIMULUS			
Serpina3c	Serine peptidase inhibitor, clade A, member 3C	0.712025	0.045332
Serpina3g	Serine peptidase inhibitor, clade A, member 3G	0.388773	0.001845
Serpina3h	Serine peptidase inhibitor, clade A, member 3H	0.494143	0.013389
Serpina3n	Serine peptidase inhibitor, clade A, member 3N	0.505226	0.000321
SMALL GTPASE MEDIATED SIGNAL TRANSDUCTION			
Rasl11a	RAS-like, family 11, member A	0.674084	0.019568
Rasl11b	RAS-like, family 11, member B	0.573156	0.001066
Rab3d	RAB3D, member RAS oncogene family	0.72951	0.034662
Rhob	Ras homolog gene family, member B	1.666706	0.040915
Rhoj	Ras homolog gene family, member J	0.691116	0.031471
OTHER GENES OF INTEREST			
Aatk	Apoptosis-associated tyrosine kinase	0.564091	0.022585
Renbp	Renin binding protein	0.643495	0.031471
Aqp7	Aquaporin 7	0.565265	0.003405
Bmp1	Bone morphogenetic protein 1	0.634635	0.034934
Bst2	Bone marrow stromal cell antigen 2	0.334250	0.013389
Cenpa	Centromere protein A	1.455989	0.022585
Dbp	D site of albumin promoter binding protein	0.645728	0.043573
Enpp5	Ectonucleotide pyrophosphatase/phosphodiesterase 5	0.732550	0.0322
Fhod1	Formin homology 2 domain containing 1	1.371733	0.041924
Fkbp5	FK506 binding protein 5	1.215879	0.036674
Gas6	Growth arrest-specific 6	0.468785	0.037595

(Continued)

Table A1 | Continued

Gene symbol	Gene name	Fold change	*P* value (FDR adjusted)
Gatad1	GATA zinc finger domain containing 1	1.747145	0.003405
Gtf3c5	General transcription factor IIIC, polypeptide 5	1.264003	0.0322
Hist1h2ah	Histone cluster 1, H2ah	1.634670	0.040326
Hp	Haptoglobin	0.657015	0.030981
Hspb8	Heat shock 22kDa protein 8	0.668963	0.022585
Ifi27	Interferon, alpha-inducible protein 27	0.309497	0.03069
Ift20	Intraflagellar transport 20 homolog	1.485552	0.031471
Itih4	Inter-alpha (globulin) inhibitor H4	0.243332	0.000025
Klc1	Kinesin light chain 1	1.278985	0.035963
Matn4	Matrilin 4	0.641712	0.031471
Mettl7B	Methyltransferase like 7B	0.704660	0.048333
Mid1ip1	MID1 interacting protein 1	0.720464	0.045125
Mpp1	Membrane protein, palmitoylated 1, 55kDa	0.744322	0.041924
Nasp	Nuclear autoantigenic sperm protein (histone-binding)	1.336074	0.048719
Nup107	Nucleoporin 107kDa	0.657927	0.029465
Pde1b	Phosphodiesterase 1B, calmodulin-dependent	1.522033	0.013389
Pde4dip	Phosphodiesterase 4D interacting protein	0.654289	0.038052
Prelp	Proline/arginine-rich end leucine-rich repeat protein	0.677362	0.034934
Psmc5	Proteasome (prosome, macropain) 26S subunit, ATPase, 5	1.387030	0.029465
Pxmp2	Peroxisomal membrane protein 2, 22 kDa	0.660669	0.033282
Rdbp	RD RNA binding protein	1.263127	0.045125
Rps24	Ribosomal protein S24	1.791291	0.022585
S100A13	S100 calcium binding protein A13	1.580082	0.022585
Aimp1	AminoacylItRNAsynthetase complex-interacting multifunctional protein 1	1.354724	0.025849
Slc5a6	Solute carrier family 5 (sodium-dependent vitamin transporter), member 6	0.756283	0.048936
Thrsp	Thyroid hormone responsive	0.264621	0.001714
Tk1	Thymidine kinase 1, soluble	1.629015	0.022786
Tmed4	Transmembrane emp24 protein transport domain containing 4	0.777007	0.045125
Tmem43	Transmembrane protein 43	0.637722	0.022585
Tmem45B	Transmembrane protein 45B	0.718470	0.041924
Tpm2	Tropomyosin 2 (beta)	1.418140	0.041924
Trafd1	Traf-type zinc finger domain containing 1	0.692074	0.004184
Uba7	Ubiquitin-like modifier activating enzyme 7	0.668037	0.048719
Uck2	Uridine-cytidine kinase 2	1.286097	0.019568
Uhrf1	Ubiquitin-like with PHD and ring finger domains 1	1.420107	0.043072
Usp18	Ubiquitin specific peptidase 18	0.238489	0.029154
Yeats4	YEATS domain containing 4	0.503128	0.019128

frontiers in
ENDOCRINOLOGY

REVIEW ARTICLE
published: 10 May 2013
doi: 10.3389/fendo.2013.00052

Mechanisms of obesity-induced inflammation and insulin resistance: insights into the emerging role of nutritional strategies

Maeve A. McArdle*, Orla M. Finucane, Ruth M. Connaughton, Aoibheann M. McMorrow and Helen M. Roche

Nutrigenomics Research Group, UCD Conway Institute, School of Public Health, Physiotherapy and Population Science, University College Dublin, Dublin, Republic of Ireland

Edited by:
Tsuguhito Ota, Kanazawa University, Japan

Reviewed by:
Andrzej Bartke, Southern Illinois University, USA
Shinichi Oikawa, Nippon Medical School, Japan

****Correspondence:***
Maeve A. McArdle, Nutrigenomics Research Group, UCD Conway Institute, School of Public Health, Physiotherapy and Population Science, University College Dublin, Dublin 4, Republic of Ireland.
e-mail: maeve.mc-ardle@ucdconnect.ie

Obesity and associated chronic inflammation initiate a state of insulin resistance (IR). The secretion of chemoattractants such as MCP-1 and MIF and of cytokines IL-6, TNF-α, and IL-1β, draw immune cells including dendritic cells, T cells, and macrophages into adipose tissue (AT). Dysfunctional AT lipid metabolism leads to increased circulating free fatty acids, initiating inflammatory signaling cascades in the population of infiltrating cells. A feedback loop of pro-inflammatory cytokines exacerbates this pathological state, driving further immune cell infiltration and cytokine secretion and disrupts the insulin signaling cascade. Disruption of normal AT function is causative of defects in hepatic and skeletal muscle glucose homeostasis, resulting in systemic IR and ultimately the development of type 2 diabetes. Pharmaceutical strategies that target the inflammatory milieu may have some potential; however there are a number of safety concerns surrounding such pharmaceutical approaches. Nutritional anti-inflammatory interventions could offer a more suitable long-term alternative; whilst they may be less potent than some pharmaceutical anti-inflammatory agents, this may be advantageous for long-term therapy. This review will investigate obese AT biology, initiation of the inflammatory, and insulin resistant environment; and the mechanisms through which dietary anti-inflammatory components/functional nutrients may be beneficial.

Keywords: obesity, inflammation, immune cell infiltration, insulin resistance, nutrient sensing, PUFA

INTRODUCTION

The global increase in body mass is an escalating societal concern. Concomitant environmental factors such as poor dietary habits, sedentary lifestyle, socioeconomic influences; and less frequently, genetic disorders that impact on hormone secretion and metabolism, result in weight gain. The world health organization (WHO) projects that by 2015, 2.3 billion adults will be overweight, body mass index (BMI) >25 (kg/m^2) and more than 700 million will be obese BMI >30 (kg/m^2). Consequently obesity-related co-morbidities including type 2 diabetes (T2D), cardiovascular disease (CVD), and non-alcoholic fatty liver disease (NAFLD) will continue to escalate (Kopelman, 2000; Mokdad et al., 2003).

Abbreviations: AA, arachidonic acid; ACC, acetyl-CoA carboxylase; AKT, protein kinase B; AMPK, adenosine monophosphate activated protein kinase; AP-1, activator protein-1; APC, antigen presenting cell; ASC, adipocyte stem cells; ASC, apoptotic speck-like protein containing a CARD; ASO, antisense oligonucleotide; AT, adipose tissue; ATM, adipose tissue macrophage; ATP, adenosine triphosphate; BAT, brown adipose tissue; bFGF, basic fibroblast growth factor; BMDC, bone marrow derived dendritic cell; BMI, body mass index; C/EBP, CCAAT/enhancer binding protein; C3, complement component 3; CLA, conjugated linoleic acid; CLS, crown-like structures; COX, cyclooxygenase; CVD, cardiovascular disease; DAG, diacylglycerol; DC, dendritic cell; DHA, docosahexaenoic acid; DPP, dipeptidyl peptidase; EGCG, epigallocatechin gallate; EPA, eicosapentaenoic acid; ERK, extracellular signal-regulated kinase; FA, fatty acid; FFA, free fatty acid; G6P, glucose 6-phosphatase; GLP, glucagon-like peptide; GLUT, glucose transporter; GPCR, G protein-coupled receptor; HDL, high-density lipoprotein; HFD, high-fat diet; HIF, hypoxia-inducible factor; HMW, high-molecular weight; HSD1, hydroxysteroid dehydrogenase type 1; IFN, interferon; IGT, impaired glucose tolerance; IκB, inhibitor of NF-κB; IKK, IκB kinase; IL, interleukin; iNKT, invariant natural killer cell; iNOS, inducible nitric oxide synthase; IR, insulin resistance; IRAK, interleukin-1 receptor-associated kinase-1; IRS, insulin receptor substrate; IκB, inhibitor of NF-κB kinase complex; JAK, janus kinase; JNK, Jun N-terminal kinase; KC, Kupffer cell; LC, long chain; LDL, low-density lipoprotein; LMW, low molecularweight; LPS, lipopolysaccharide; MAPK, mitogen activated protein kinase; MCP, macrophage chemoattractant protein; MDMs monocyte derived macrophages; MetS, metabolic syndrome; MHO, metabolically healthy obese; MIF, macrophage migration inhibitory factor; MMW, middle-molecular weight; MnSOD, mitochondrial dismutase; MONW, metabolically obese normal weight; mTOR, mammalian target of rapamycin; MYD88, myeloid differentiation primary response gene 88; NAFLD, non-alcoholic fatty liver disease; NK, natural killer cell; NLRP3, NOD, like receptor 3; NO, nitric oxide; $O_2^{\bullet-}$ superoxide; PI, phosphatidylinositol; PI3K, phosphatidylinositide 3-kinase; PKC, protein kinase C; PLC, phospholipase; PPAR, peroxisome proliferator-activated receptor; Pref-1, Pre-adipocyte factor-1; PRR, pattern recognition receptor; PUFA, polyunsaturated fatty acid; RA, receptor antagonist; ROS, reactive oxygen species; SAT, subcutaneous adipose tissue; SFA, saturated fatty acid; SGBS, Simpson–Gibson–Behmel syndrome; SHP2, protein tyrosine phosphatase, non-receptor type 11; SIRT1, sirtuin1; SNP, single nucleotide polymorphism; SOCS, suppressor of cytokine signaling; SREBP, sterol receptor binding protein; STAT, signal-transducer and activator of transcription protein; SVF, stroma-vascular fraction; T2D, type 2 diabetes; TAG, triacylglyceride; TAK, transforming growth factor β activated kinase; TH, T helper cell; TLR, toll like receptor; TNF, tumor-necrosis factor; TRADD, TNF receptor death domain; TRAF, TNF receptor-associated factor; Treg, regulatory T cell; VAT, visceral adipose tissue; WAT, white adipose tissue; WHO, world health organization; WT, wild type.

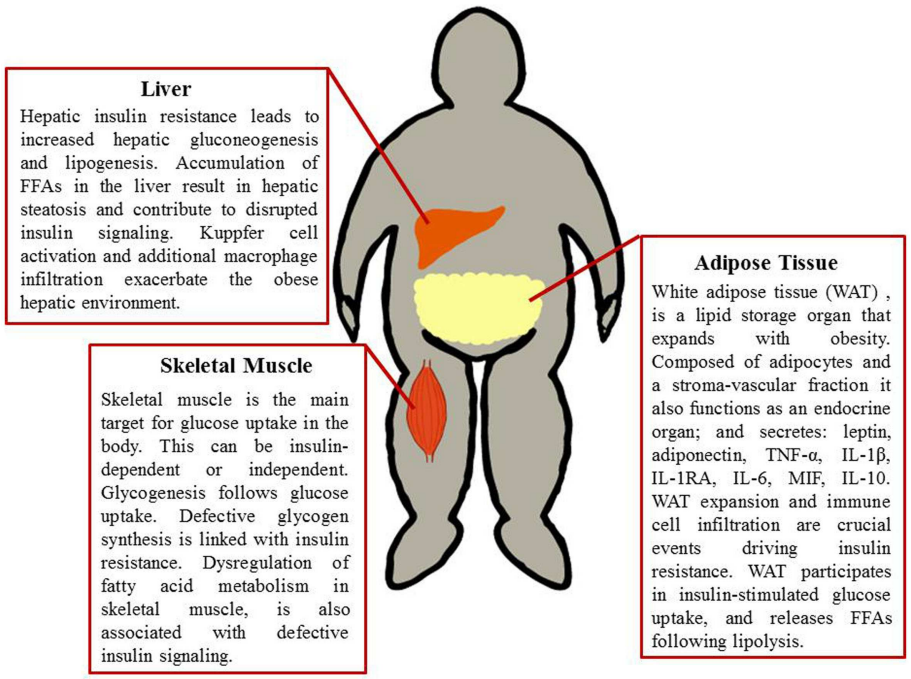


FIGURE 1 | Metabolic tissues implicated in obesity-induced insulin resistance. Adipose tissue, liver, and skeletal muscle are involved in glucose uptake, glucose production, and glucose processing. These tissues therefore are paramount in obesity and the progression of insulin resistance. A combination of defective fatty acid storage and metabolism together with immune cell infiltration and a pro-inflammatory tissue milieu result in dysregulation of insulin signaling.

Substantial evidence indicates that obesity is linked to a state of chronic low-grade inflammation. Initially Hotamisligil et al. (1993) determined a link between obesity and inflammation; demonstrating that the pro-inflammatory cytokine tumor-necrosis factor (TNF)-α was expressed in adipose tissue (AT) of obese mice and linked to insulin resistance (IR). Significant advances in understanding the highly complex role of immuno-metabolism in health have since been accomplished. Consequently obesity is linked to pro-inflammatory cytokine secretion, immune cell infiltration, and disrupted function of tissues involved in glucose homeostasis, summarized in **Figure 1**. Dysfunctional lipid metabolism accompanies obesity and can impair insulin signaling; circulating free fatty acids (FFAs) have a negative effect on insulin target tissues, through the activation of inflammatory pathways, via cell surface pattern recognition receptors (PRRs) (Shi et al., 2006). Furthermore, accumulation of lipid derivatives, such as diacylglycerol (DAG) and ceramides can negatively regulate insulin action (Schenk et al., 2008).

The purpose of this review is to evaluate the current evidence in relation to obesity-induced IR, whereby the inflammatory axis plays a critical role in the progression and severity of IR. It is possible to attenuate the pro-inflammatory, insulin de-sensitizing milieu within the obesogenic environment. Weight management programs can attenuate T2D risk by reducing obesity and improving insulin sensitivity; however the long-term success of this approach is not optimal. Thus, are there effective nutritional strategies to attenuate the impact of inflammation mediated IR beyond weight loss?

ADIPOSE TISSUE, OBESITY-INDUCED INFLAMMATION, AND INSULIN RESISTANCE

There are two distinct forms of AT, brown adipose tissue (BAT) and white adipose tissue (WAT). BAT is typically associated with thermogenesis, although initially thought to disappear soon after birth in humans, evidence now suggests that BAT is present in adult humans in the supraclavical and paraspinal regions (Yoneshiro et al., 2011). Recent studies support the role of BAT functionality in adult humans relating to the control of body temperature and adiposity, which is of direct relevance to the metabolic syndrome (MetS) (Jacene et al., 2011; Ouellet et al., 2011; Nishio et al., 2012). BAT levels and activity are inversely correlated with BMI and conversely loss of BAT activity may be associated with the accumulation of WAT (Saito et al., 2009; Van Marken Lichtenbelt et al., 2009; Vijgen et al., 2011).

The depot most important to obesity and IR is WAT. WAT traditionally functions in lipid storage, storing triacylglycerides (TAG) following energy excess, and mobilizing these stores during periods of nutrient deprivation (Gregoire et al., 1998). With obesity and IR there is increased lipolysis, consequently there is an inappropriate spill over of TAG derived FFAs (Sethi and Vidal-Puig, 2007) and these FFAs can activate inflammatory pathways and impair insulin signaling. WAT also acts as an endocrine organ, releasing bioactive substances that have been coined adipokines, these include interleukin (IL)-6, IL-1β, TNF-α, leptin, and adiponectin. WAT is a complex multi-cellular organ, composed primarily of adipocytes. The stroma-vascular fraction (SVF) of AT contains several highly potent cells including adipocyte progenitor cells, or

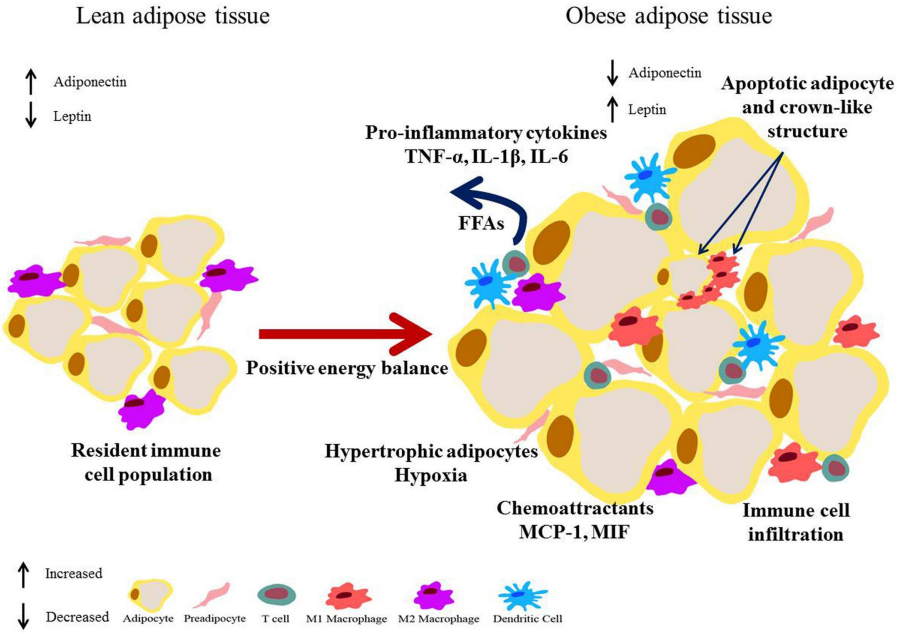


FIGURE 2 | Obese adipose tissue expansion – resultant inflammation and metabolic dysregulation. Excess energy leads to adipose expansion with hypertrophic adipocytes that secrete chemoattractants such as MCP-1, drawing immune cells into the tissue. Secretion of pro-inflammatory mediators such as TNF-α, IL-1β, and IL-6 by adipocytes, pre-adipocytes, and infiltrating immune cells results in polarization of macrophages to a pro-inflammatory M1 phenotype, and drive an inflammatory T cell population. Augmented lipolysis leads to increased levels of FFAs. This environment negatively impacts on the insulin signaling pathway and a state of insulin resistance results. Additionally hypertrophic adipocytes are also linked with hypoxia.

pre-adipocytes, and resident immune cell populations. In the IR state, pro-inflammatory cytokines activate several serine kinases, including IκB kinase (IKK) and JNK (Gual et al., 2005). These kinases have been shown to inhibit insulin action by promoting the phosphorylation of serine residues of the insulin signaling pathway, including serine phosphorylation of insulin receptor substrate-1 (IRS-1). In contrast with tyrosine phosphorylation of IRS-1 in the insulin sensitive state, serine phosphorylation impairs normal insulin signaling (Schenk et al., 2008).

ADIPOCYTES

White adipose tissue is unique in its plasticity, it can adapt quickly to nutrient deprivation and hyper-nutrition alike. The flexibility of WAT is largely due to the hypertrophic and hyperplastic changes in adipocytes. WAT plasticity has an important role in determining metabolic health (Virtue and Vidal-Puig, 2008). The expansion of WAT that occurs with weight gain is accompanied by changes in cytokine and chemokine secretion, hypoxia, cell death, immune cell infiltration, and dysregulation of fatty acid (FA) metabolism and storage, see **Figure 2**. Adipocyte hypertrophy modulates the adipose secretome (Osborn and Olefsky, 2012). Mature adipocytes secrete IL-6, monocyte chemoattractant protein (MCP)-1, leptin, and adiponectin; which can act in an autocrine, paracrine, or endocrine manner to regulate lipid and glucose homeostasis (Curat et al., 2004). Expression of inducible nitric oxide synthase (iNOS), MCP-1 and IL-6 are concomitantly increased in the mature adipocyte fraction of obese mice, in parallel with increased 11β-hydroxysteroid dehydrogenase type I (HSD1) levels (Ishii-Yonemoto et al., 2010). In *in vitro* cultures TNF-α, IL-1β, and IL-6 up-regulate 11β-HSD1 (Tomlinson et al., 2001). Increased MCP-1 expression promotes monocyte infiltration of the WAT, these then differentiate into adipose tissue macrophages (ATM). Adipocytes also induce the expression of the adhesion molecules ICAM-1 and PECAM-1 on endothelial cells (Curat et al., 2004) which further attract monocytes to the region. ATM secrete additional chemokines and cytokines, further exacerbating the pro-inflammatory environment (Osborn and Olefsky, 2012).

STROMA-VASCULAR FRACTION

The SVF of WAT is composed of several metabolically active and inflammatory cells, summarized in **Figure 3**; including pre-adipocytes, fibroblasts, endothelial cells, dendritic cells (DCs), T cells, mast cells, granulocytes, and macrophages (Calder et al., 2011) embedded in an extra cellular matrix. This fraction plays a critical role in healthy fat pad expansion (Sun et al., 2011). In response to a high-fat diet (HFD) and obesity, there is an increase in SVF cell number, the phenotype of which adversely affects metabolism (Strissel et al., 2010).

PRE-ADIPOCYTES

Two distinct adipocyte cell types were identified in human omental fat (Julien et al., 1989). Mature adipocytes with lipid droplets were observed in close proximity to much smaller nucleated cells

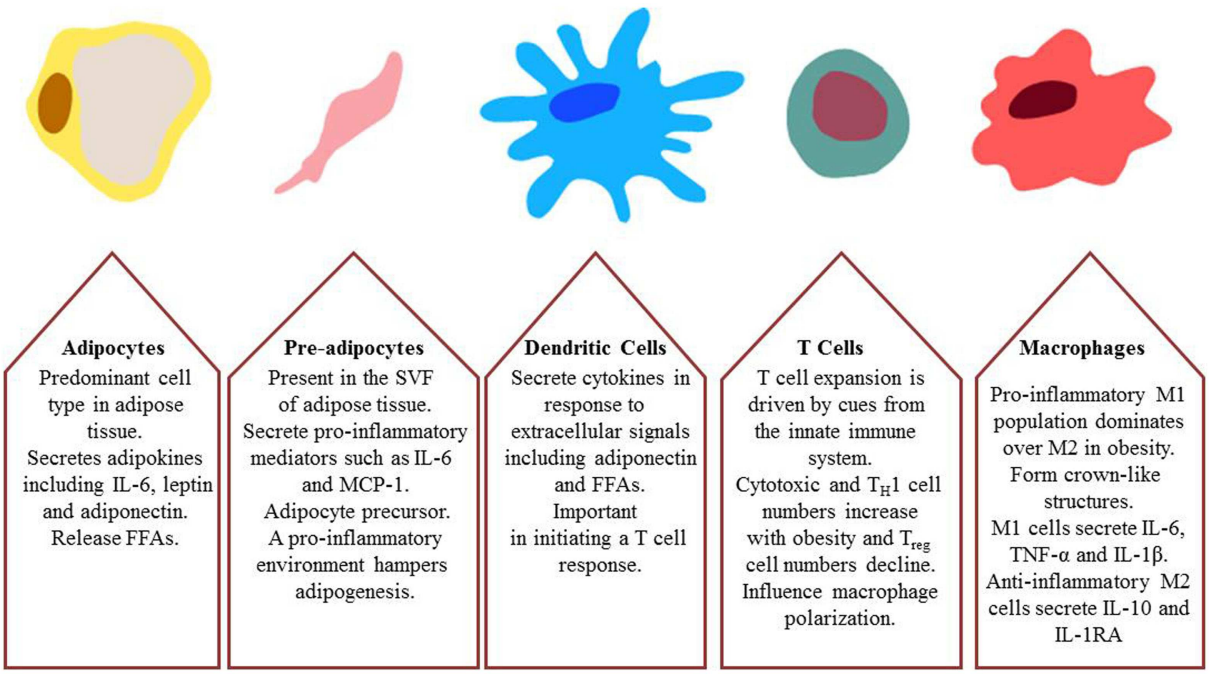


FIGURE 3 | Major cell types involved in obesity-induced inflammation and insulin resistance; adipocytes, pre-adipocytes, dendritic cells, T cells, and macrophages.

containing less lipid, referred to as pre-adipocytes. Increasing body weight is associated with increased numbers of pre-adipocytes and it has been suggested that the expansion of this cellular population is associated with IR (McLaughlin et al., 2007). There is much speculation as to the origins of pre-adipocytes (Cawthorn et al., 2012). Hollenberg and Vost (1969) utilized tritiated thymidine incorporation in WAT and identified the SVF as the source of new adipocyte formation. Adipocyte stem cells (ASCs) are likely mesenchymal stem cells that reside in the WAT and give rise to adipocytes; with pre-adipocytes representing an intermediary stage in this process (Zuk et al., 2001; Cawthorn et al., 2012).

Cytokines have potent effects on pre-adipocyte biology. TNF-α, interferon (IFN)-γ, IL-1β, and IL-6 impair adipogenesis and lipid accumulation in 3T3-L1 pre-adipocytes (Gustafson and Smith, 2006; McGillicuddy et al., 2009). Specifically, IL-6 treatment reduced adiponectin, resistin, glucose transporter type (GLUT)-4, and IRS-1 expression; while TNF-α treatment increased secretion of IL-6 and MCP-1 from pre-adipocytes (Chung et al., 2006). Indeed pre-adipocytes secrete greater levels of pro-inflammatory mediators such as IL-6 and MCP-1 than adipocytes (Poulain-Godefroy and Froguel, 2007; Mack et al., 2009). Phospholipase Cδ1 (PLCδ1) has also been shown to regulate 3T3-L1 pre-adipocyte differentiation (Hirata et al., 2011), this enzyme regulates adipogenesis. Pre-adipocytes secrete basic fibroblast growth factor (bFGF/FGF-2), involved in promoting vascular endothelial cell growth, levels of which are increased with obesity (Bell et al., 2006; Sun et al., 2011). Basic fibroblast growth factor is involved in recruiting monocytes and neutrophils under chronic inflammatory conditions, it acts synergistically with TNF-α and IFN-γ and in the absence of these cytokines bFGF could not induce leukocytes recruitment (Zittermann and Issekutz, 2006).

Pre-adipocyte factor-1 (Pref-1) is a transmembrane protein expressed exclusively by pre-adipocytes and inhibits adipogenesis (Chung et al., 2006; O'Connell et al., 2011). Pref-1 levels are increased in metabolically unhealthy WAT (O'Connell et al., 2011) and levels correlate with ATM number. The capacity of pre-adipocytes to undergo adipogenesis is a critical factor in obesity and IR. Adipogenic potential is impeded with age and by various cytokines, via the transcription factors CCAAT/enhancer binding protein (C/EBP)1α (Karagiannides et al., 2001) and peroxisome proliferator-activated receptors (PPAR)-γ (Hotta et al., 1999). Reduced capacity for *de novo* adipogenesis, coupled with increased FFA storage demand in obesity may account for the switch from hyperplastic to hypertrophic WAT, which in turn has negative implications in terms of impeding FFA storage capacity and leakage.

ADIPOSE TISSUE MACROPHAGES

Adipose tissue macrophages can be classified based on their surface marker expression and/or their chemokine and cytokine secretion profile. ATM number and phenotype is altered in genetic and diet induced obesity. In the obese state there are greater proportions of classically activated M1 but less M2 macrophages; which would otherwise play a role in the resolution of inflammation (Lumeng et al., 2007; Fujisaka et al., 2009). M1 macrophages secrete pro-inflammatory cytokines TNF-α, IL-1β, and IL-6; whereas alternatively activated M2 macrophages secrete anti-inflammatory cytokines including IL-10 and IL-1 receptor antagonist (RA)

(Lumeng et al., 2007). The degree of ATM infiltration is associated with the progression of IR (Osborn and Olefsky, 2012). It is likely that M1 ATMs originate from monocytes in circulation rather than originating in the SVF (Calder et al., 2011; Oh et al., 2012).

The immuno-phenotype of macrophages is highly plastic in response to their surrounding milieu (Mosser and Edwards, 2008). As obesity progresses the ATM phenotype switches from anti-inflammatory M2 to the pro-inflammatory M1 type, through a dynamic process spanning a spectrum from M1 to M2 states (Lumeng et al., 2007; Osborn and Olefsky, 2012). ATM cluster around necrotic adipocytes in arrangements coined crown-like structures (CLS). M1 ATM infiltration decreases insulin sensitivity as a result of greater TNF-α, IL-1β, and IL-6 secretion, which induce an insulin resistant environment. FFAs can activate macrophages *in vitro* acting through Toll like receptor (TLR)2 and TLR4 to induce MCP-1, IL-6, and IL-1β expression (Nguyen et al., 2007). FFAs also induce PAI-1 secretion from macrophages *in vitro*; however this induction occurred only in the presence of 3T3-L1 adipocytes in the culture environment, suggesting FFA's and adipocyte secretions act synergistically to influence macrophage secretions (Kishore et al., 2010). ATM infiltration can be detected after only 1 week of HFD feeding (Lynch et al., 2012) and increase progressively with time and in proportion to the degree of obesity. With obesity ATM also take on a foam cell-like role, accumulating excess lipid (Prieur et al., 2011). Weight loss is associated with lower ATM number (Kosteli et al., 2010) however initial rapid weight loss is also accompanied by transient infiltration of macrophages (Granneman et al., 2005; Sun et al., 2011).

There is some uncertainty when distinguishing macrophages from DCs (Geissmann et al., 2010); these cell types overlap in terms of function and molecular characterization. Both are phagocytic antigen presenting cells (APC) and also share certain cell surface markers including CD11c, MHC II, and F4/80. The Immunological Genome Project has generated detailed gene expression profiles and regulatory pathways that enable discrimination between macrophages and DCs and additionally to distinguish between different populations of these cell types (Gautier et al., 2012). This project shows that there was great diversity between different macrophage populations but detected only 39 mRNA transcripts common to all macrophage types but not to DCs.

T CELLS

Adipose tissue macrophages were the first immune cells associated with IR, however in terms of WAT infiltration they are preceded by other immune cells including T cells. Different T cell subsets are involved in obesity and WAT infiltration. Regulatory T cells (T_{reg}) normally constitute 5–20% of the $CD4^+$ T cell population and play a crucial role in maintaining immune homeostasis (Feuerer et al., 2009). T_{reg} secrete anti-inflammatory cytokines, inhibiting macrophage migration, and promoting an M2 macrophage phenotype (Osborn and Olefsky, 2012). A decline in T_{regs} accompanies increasing weight gain in both mouse and human (Feuerer et al., 2009; Winer et al., 2009a). The T_{reg} depleted mouse has lower insulin-stimulated insulin receptor tyrosine phosphorylation in both epididymal fat and liver, accompanied by reduced AKT phosphorylation (Feuerer et al., 2009). Anti-inflammatory CD4+ T helper (T_H) 2 cells secrete IL-4 and IL-13 (Winer et al., 2009a). Interleukin-4 induces M2 macrophages that secrete IL-10, which in turn is proposed to have insulin sensitizing potential.

Pro-inflammatory T_H1 cells secrete IFN-γ that drives polarization of pro-inflammatory macrophages, that in turn secrete IL-1, IL-6, and TNF-α (Winer et al., 2009a). T_H1 cells can activate macrophages (Zhu and Paul, 2008). HFD feeding increased T_H1 cell number thus negating the anti-inflammatory secretions from T_{reg} and T_H2 cells (Winer et al., 2009a). Interleukin-6 in conjunction with IL-23, TGF-β, and IL-1, drive the proliferation of $CD4^+$ T_H17 cells (Kikly et al., 2006; Brereton et al., 2009). Activation of ERK in DCs is crucial in driving IL-1β and IL-23 production that further drive the expansion of T_H17 cells (Brereton et al., 2009). T_H17 cells secrete the pro-inflammatory cytokines IL-17A and IL-17F and also TNF-α, IL-6, GM-CSF, CXCL1, and CCL20. Winer et al. (2009b) reported an increase in T_H17 cells in the spleens of HFD-fed mice, but T_H17 cells were absent in HFD-fed IL-$6^{-/-}$ mice. HFD-fed Rag$1^{-/-}$ mice reconstituted with $CD4^+$ T cells had lower weight gain than those reconstituted with $CD8^+$ T cells, both had similar food intake (Winer et al., 2009a). The $CD4^+$ T cell recipient mice also had smaller adipocytes in both subcutaneous and epididymal fat pads. The infiltration of $CD8^+$ T cells to WAT is an early event in obesity, whereby obese WAT can activate $CD8^+$ T cells that subsequently recruit macrophages (Nishimura et al., 2009; Harford et al., 2011). The percentage of $CD8^+CD4^-$ T cells in the SVF fraction of epididymal fat increased in HFD-fed mice after just 2 weeks. $CD8^+CD4^-$ T cell number increased for up to 11 weeks on HFD while both $CD8^-CD4^+$ and T_{reg} numbers decreased over time (Nishimura et al., 2009). Depletion of $CD8^+$ cells resulted in a significant reduction in $CD8^+CD4^-$ T cells numbers in epididymal fat pads, and reduced M1 macrophage infiltration; without altering the M2 fraction in HFD-fed mice. In order to demonstrate the effects of obese versus lean WAT on $CD8^+$ T cells, epididymal fat pads from chow and HFD-fed mice were co-cultured with splenic $CD8^+$ T cells; the obese WAT induced T cell proliferation to a greater extent than the lean WAT (Nishimura et al., 2009).

Invariant natural killer T (iNKT) cells are a population of innate T cells present in WAT; obesity is accompanied by a decline in iNKT cells in WAT (Lynch et al., 2012). This cell type can recognize lipid antigens on MHC-like glycoprotein CD1 (Brigl and Brenner, 2004; Lynch et al., 2009b). DCs express CD1 and *in vivo* may activate iNKT cells (Exley and Koziel, 2004). There is uncertainty about naturally occurring ligands recognized by iNKT cells; endogenous lysosomal glycosphingolipid and isoglobotrihexosylceramide in addition to bacterial glycosphingolipids are proposed iNKT ligands (Zhou et al., 2004; Kinjo et al., 2005; Lynch et al., 2009b). iNKT cells are found at a higher frequency in mouse than in human, however they are particularly present in the human omentum, this region also contains the highest concentration of $CD1d^+$ cells (Lynch et al., 2009b). iNKT cells comprise up to 50% of resident T cells in the liver (Eberl et al., 1999) and in the WAT they produce T_H2 type cytokines. Jα18 deficient mice and $CD1d^{-/-}$ mice lack iNKT cells, with this absence there is an increase in

macrophage number in WAT (Lynch et al., 2012). Both show greater M1 infiltration of WAT following HFD feeding. CD1d$^{-/-}$ mice have decreased adiponectin and increased leptin levels in WAT and plasma (Schipper et al., 2012). Lean mice deficient in iNKT develop an insulin resistant phenotype in the absence of WAT inflammation (Schipper et al., 2012). These lean mice also displayed adipocyte hypertrophy but there was no change in epididymal fat pad weight relative to wild-type mice.

DENDRITIC CELLS

Dendritic cells are considered the principal APC of the immune system and are crucial for initiating a T cell response (Sallusto and Lanzavecchia, 1994). They process phagocytosed particles and use peptide information from these to present to T cells via MHC class I and class II molecules. DCs are the only cell type that can induce naïve T cell differentiation (Sallusto and Lanzavecchia, 1994; Bhattacharya et al., 2011). Mature DCs secrete cytokines that influence different cell responses, e.g., naïve T cells to T_H1, -2, -17, T_{regs} and therefore DCs link innate and adaptive arms of the immune system. T_H1 cell induction follows pro-inflammatory IL-12p70 secretion by DCs (McGuirk et al., 2005); T_H1 cells then produce IFN-γ and can prevent the development of T_{reg} cells (DePaolo et al., 2011). Additionally IFN-γ feeds back to DCs ensuring the continued production of IL-12p70. Inactivation of MEK1/2 in the MAPK pathway leads to a reduction in IL-1β and IL-23 production but does not result in diminished IL-12p70 production.

Adipose and bone marrow DC number increases following HFD feeding in mice (Reynolds et al., 2012). Further studies have shown HFD feeding also results in DC infiltration of the liver in addition to the WAT (Stefanovic-Racic et al., 2012). DCs present in the liver of obese mice have increased CD86 expression, suggesting that the obese environment promotes DC maturation. Additionally Flt3$^{-/-}$ mice, lacking DCs, showed reduced macrophage infiltration of liver and WAT. DCs isolated from the SVF of lean and obese mice contained different population subsets (Bertola et al., 2012); CDllc^{high}/F4/80^{low} DCs from obese mice induced the differentiation of T_H17 cells that produced high levels of IL-17, whereas CDllc^{high}/F4/80^{neg} DCs induced both T_H1 and T_H17 cells that produced low levels of their respective cytokines IFN-γ and IL-17. In lean mice CDllc^{high}/F4/80^{neg} DCs only induced T_H1 cells and CDllc^{high}/F4/80^{low} DCs initiated a weak T_H17 cell expansion.

Zhong et al. (2013) investigated the role of dipeptidyl peptidase-4 (DDP4/CD26) in human DCs in relation to T cell activation. Dipeptidyl peptidase-4 was first identified as an enzyme involved in the degradation of glucagon-like peptide (GLP)-1 (Yazbeck et al., 2009). Dipeptidyl peptidase-4 expression was investigated in human peripheral blood and the SVF fraction from the omentum of healthy lean subjects (Zhong et al., 2013). Expression of DPP4 was greater on DCs and macrophages from the SVF fraction than those from the peripheral blood. Dipeptidyl peptidase-4 expressing macrophages were measured from visceral adipose tissue (VAT) of obese subjects and levels were higher than observed in lean controls; both HFD-fed mice and *ob/ob* mice also showed increase DPP4 expression in WAT, and on DCs and macrophages (Zhong et al., 2013).

NATURAL KILLER CELLS

Natural killer (NK) cells represent the first line of immune defense (Shi et al., 2000). They produce IFN-γ and can drive T_H1 cell expansion (Yadav et al., 2011), they also produce IL-17 (Cua and Tato, 2010). Subjects deemed obese yet metabolically healthy had a greater number of CD8$^+$ cells and NK cells than their "unhealthy" counterparts, but both groups had fewer circulating NK cells than lean subjects (Lynch et al., 2009a). NK cells differed in phenotype between the two groups of obese patients; there was increased expression of inhibitory markers such as NKB1 and CD158b in the unhealthy group. Adipokines can influence NK cells and leptin was shown to have an inhibitory effect on NK cells in lean but not obese subjects while adiponectin enhanced NK activity in obese subjects (O'Shea et al., 2010).

EOSINOPHILS

Eosinophils are classically associated with allergy but are also associated with M2 macrophages. Eosinophils are present in the WAT of mice and produce IL-4 thus act to replenish M2 macrophages; they account for 4–5% of SVF cells in the WAT (Wu et al., 2011). T_H2 cell IL-4 production may complement eosinophil IL-4 secretion, or indeed make it redundant (Maizels and Allen, 2011). A decline in eosinophil numbers has been reported in HFD-fed mice (Wu et al., 2011) and in the absence of eosinophils M2 macrophage numbers become diminished.

THE LIVER AND SKELETAL MUSCLE ARE CRITICAL ORGANS ADVERSELY AFFECTED IN OBESITY-INDUCED INSULIN RESISTANCE

LIVER

Obesity induces morphological and metabolic alterations in other peripheral tissues including the liver and skeletal muscle. NAFLD is a complex metabolic disorder transpiring to be one of the most prevalent liver conditions in the western world (Flegal et al., 2002; Utzschneider and Kahn, 2006). NAFLD is strongly linked with obesity, IR, and T2D (Seppala-Lindroos et al., 2002; Adams et al., 2005). This condition represents multiple pathogenic states from steatosis to steatohepatitis which can progress to cirrhosis and liver failure (Farrell and Larter, 2006). In an obese state the liver exhibits selective IR; the inhibitory effect of insulin on hepatic gluconeogenesis is interrupted while its action on *de novo* lipogenesis is enhanced, resulting in chronic hyperglycemia and hypertriglyceridemia (Schwarz et al., 2003; Brown and Goldstein, 2008). The mechanisms responsible for hepatic IR are controversial and causality has been attributed to several factors.

The "portal hypothesis" suggests enhanced visceral tissue lipolysis, resultant from obesity-induced IR, exacerbates FFA influx into the liver via the portal vein, contributing to hepatic steatosis (Bjorntorp, 1990; Berraondo and Martínez, 2000; Utzschneider and Kahn, 2006). This increase in hepatic FFA may induce hepatic IR by promoting protein kinase C (PKC)δ translocation from the cytosol to the membrane, leading to impaired IRS-associated phosphatidylinositol (PI)3-kinase activity (Lam et al., 2002). Additionally elevated WAT derived pro-inflammatory cytokine secretion including TNF-α and reduced adiponectin secretion have also been implicated in hepatic IR (Berg et al., 2001). Alternatively excessive TAG accumulation within the liver has been implicated in

hepatic IR (Utzschneider and Kahn, 2006). Patients with NAFLD display elevated *de novo* lipogenesis, which reflects the inability for insulin to impede lipogenesis (Donnelly et al., 2005). NAFLD associated hyperinsulinemia can stimulate lipogenic transcription factor sterol receptor binding protein (SREBP)-1c, and inhibit fork head transcription factor Foxa2 activity, promoting enhanced FA synthesis and reduced β-oxidation (Wolfrum et al., 2004; Tamura and Shimomura, 2005; Gonzalez-Baro et al., 2007). Furthermore, HFD-fed mice have elevated hepatocyte ceramide secretion (Boon et al., 2013), this secretion profile is linked to plasma ceramide levels suggesting that the liver is the principal producer of circulating ceramide.

The liver has a large population of resident macrophages or Kupffer cells (KC) (Neyrinck et al., 2009). NAFLD is linked with the inflammatory activation of KCs (Odegaard et al., 2008). Increased adiposity activates KC, without altering total KC number (Cai et al., 2005). Activation of KC is associated with hepatic IR (Neyrinck et al., 2009; Lanthier et al., 2010) and the production of inflammatory mediators including TNF-α and reactive oxygen species (ROS). Similar to ATM, KC present immense plasticity and can alter between a pro-inflammatory M1 and anti-inflammatory M2 phenotype, in response to their specific environment (Gordon, 2003). Ablation of PPAR-δ, an important FA sensor in hematopoietic cells, polarized KC to an M1 activation state augmenting HFD-induced hepatic steatosis through a reduction in hepatic β-oxidation (Odegaard et al., 2008). HFD fed mice depleted of KCs, have decreased lipogenic gene expression and suppression of hepatic glucose 6-phosphatase (G6Pase), a crucial gene involved in gluconeogenesis (Neyrinck et al., 2009). The G protein-coupled receptor (GPCR) GPR105 is involved in additional macrophage infiltration of the liver and the ensuing inflammatory and insulin resistant state (Xu et al., 2012).

SKELETAL MUSCLE

Skeletal muscle is the main target organ for glucose uptake in the body, responsible for 80% of glucose disposal in man (Lorenzo et al., 2008; DeFronzo and Tripathy, 2009). Glycogen synthesis is the principal pathway for glucose disposal in both normal and T2D subjects (Shulman et al., 1990). Defective glycogen synthesis has a causative role in IR and T2D (Shulman, 2000). Insulin stimulation in T2D does not increase glucose-6-phosphate, an intermediary metabolite of glucose transport and glycogen synthesis. This suggests that T2D is associated with either decreased glucose transport activity or decreased hexokinase II activity. Further investigation elucidated that glucose transport is the rate-controlling step in insulin-stimulated glycogen synthesis (Cline et al., 1999).

Increased circulating FFAs and dysregulation of intramyocellular FA metabolism can result in a ∼50% decrease in insulin-stimulated glycogen synthesis in muscle (Roden et al., 1996). This is a consequence of reduced glucose-transport activity and defective PI3K activity (Dresner et al., 1999; Petersen and Shulman, 2002). *In vivo* infusion of a lipid emulsion in rats resulted in an increase in intracellular C18:2 CoA and DAG in skeletal muscle (Yu et al., 2002). Additionally reduced tyrosine phosphorylation of IRS-1 and reduced IRS-1 associated PI3K activity followed lipid infusion; this coincided with increased PKCθ activation. Combined these changes result in decreased insulin-stimulated glucose transport activity. Circulating ceramides are increased with T2D, in particular elevated LDL-ceramide is associated with IR (Boon et al., 2013). Infusion of LDL-ceramide into lean mice resulted in reduced glucose disposal in skeletal muscle and decreased phosphorylation of AKT (Boon et al., 2013). In skeletal muscle, IKKβ signaling increased coincident with increased expression of NF-κB target genes TNF-α, IL-6, and IL-1β. LDL-ceramide also decreased insulin-stimulated glucose uptake through decreased GLUT4 translocation.

Interleukin-6 levels are elevated in obesity, likely due to greater WAT IL-6 secretion; and are associated with IR and T2D risk (Spranger et al., 2003; Pedersen and Febbraio, 2008). The functional role and consequence of IL-6 in skeletal muscle is highly complex and potentially counterintuitive. In its role as a myokine, IL-6 expression is enhanced in contracting skeletal muscle and released after exercise, when insulin sensitivity is enhanced (Ostrowski et al., 1998; Steensberg et al., 2000). Skeletal muscle metabolism is enhanced by IL-6, increasing AMP-activated protein kinase (AMPK) α2 activity, FA oxidation, and glucose uptake (Kelly et al., 2009). Interleukin-6 signaling is abnormal in obese and T2D subjects muscle precursor cells. There is reduced skeletal muscle IL-6 receptor expression in obesity and abnormal STAT3/suppressor of cytokine signaling 3 (SOCS3) signaling, and attenuated IL-6 induced AMPKα2 activation with T2D (Scheele et al., 2012).

Hong et al. (2009) investigated IL-10 action in skeletal muscle glucose homeostasis; using a transgenic mouse model with muscle specific overexpression of IL-10. Following HFD feeding, IL-10 over-expressing mice had improved insulin sensitivity relative to HFD-fed control mice; additionally increased tyrosine phosphorylation of IRS-1 was demonstrated. There was reduced macrophage infiltration of skeletal muscle in the HFD transgenic mice and a corresponding reduction in IL-6 and TNF-α secretion in the muscle. TNF-α expression has been shown to be increased in muscle biopsies from IR subjects (Saghizadeh et al., 1996).

Insulin-independent glucose transport in skeletal muscle occurs following AMPK activation (Fujii et al., 2006). Leptin increases fatty oxidation and decreases fat storage in muscle via AMPK activation. HFD fed mice show decreased leptin-stimulated AMPK activation (Martin et al., 2006), due to abrogated acetyl-CoA carboxylase (ACC) activity downstream of the AMPK pathway. SOCS3, a regulator of leptin, is elevated in the skeletal muscle of HFD-fed mice (Yang et al., 2012). Overexpression of SOCS3 impairs leptin-stimulated AMPK activation, reduced tyrosine phosphorylation of IRS-1, PI-3-kinase activity, and AKT phosphorylation (Yang et al., 2012).

White adipose tissue derivatives, either FA or cytokine/inflammatory derived have a plethora of adverse and synergistic effects on hepatic and skeletal metabolism, that further augment the impact of dysregulated WAT metabolism in obesity.

INFLAMMATORY MEDIATORS INVOLVED IN OBESITY-INDUCED INSULIN RESISTANCE

Metabolic dysregulation has been attributed to numerous pro-inflammatory cytokines secreted by the altered immune cell milieu of obese WAT, including IL-1, TNF-α, MIF, and IL-6, all of which have been documented in disrupting insulin signaling.

INTERLEUKIN-1 FAMILY

The IL-1 superfamily includes IL-1α, IL-1β, IL-1RA, IL-18, IL-33, and IL-37 (Dinarello, 2009; Akdis et al., 2011). Interleukin-1 is a multifunctional pro-inflammatory cytokine, produced by numerous innate immune cells including monocytes/macrophage and DCs (Garcia et al., 2006; Tilg and Moschen, 2008). Interleukin-1α and IL-1β mediate their actions through the IL-1 receptor 1(IL-1R1) and potently induce the production of inflammatory cytokines including IL-6. Conversely IL-1α and IL-1β differ in their maturation and secretion, IL-1α is secreted in a biologically active state while IL-1β activation is dependent on the cleavage of pro-IL-1β to mature IL-1β by caspase-1 (Dinarello et al., 2010). Interleukin-1β disrupts adipogenesis in human and murine cell models (Lagathu et al., 2006). IL-1 signaling cascade involves the activation of NF-κB and JNK MAPK pathways (Stylianou and Saklatvala, 1998). In contrast, anti-inflammatory IL-1RA binds to the IL-1R1 and blocks signaling events due to its lack of an essential IL-1R accessory protein (Tack et al., 2012). In recent years the full importance of IL-1 signaling in obesity, adipocyte dysfunction, and IR has been recognized.

Increased circulating IL-1β concentrations are associated with greater risk of developing T2D (Spranger et al., 2003). Obese VAT expresses more IL-1β and IL-1R1 (Juge-Aubry et al., 2003). Interleukin-1α impairs insulin-stimulated tyrosine phosphorylation of IRS-1 in 3T3-L1-adipocytes (He et al., 2006). Furthermore IL-1β inhibited insulin-stimulated glucose uptake into 3T3-L1-adipocytes, an effect proposed to be mediated via IL-1β induced ERK activation (Jager et al., 2007). Correspondingly lack of IL-1R1 protects mice from HFD-induced glucose intolerance (De Roos et al., 2009; McGillicuddy et al., 2011). Treatment of HFD-fed mice with IL-1RA ameliorated glucose intolerance and protected against obesity-induced pancreatic β-cell destruction (Sauter et al., 2008). However a certain element of controversy still surrounds IL-1 regarding its function *in vivo*. Interleukin-1RA$^{-/-}$ mice exhibit a lean phenotype concomitant with enhanced energy expenditure and improved insulin sensitivity (Matsuki et al., 2003; Isoda et al., 2005). On the other hand IL-1R1$^{-/-}$ mice develop mature onset obesity with reduced glucose homeostasis (Garcia et al., 2006). Together these studies highlight the pathological significance of IL-1 to obesity associated metabolic dysregulation but also highlight the complexity of this signaling molecule.

PROCESSING ACTIVE IL-1β VIA NLRP3 INFLAMMASOME

The processing and activation of IL-1β *in vivo* comprises a multifaceted network of events wherein two individual stress response signals are required to "prime" and "activate" IL-1β. Activation of TLR4 via stress signals such as lipopolysaccharide (LPS) or saturated fatty acids (SFA) delivers the first "hit" necessary for production of pro-IL-1β (Wen et al., 2011). The subsequent processing of pro-IL-1β to mature biologically active IL-1β is dependent on the NOD like Receptor (NLRP)3-caspase 1 inflammasome complex (Mills and Dunne, 2009). The NLRP3 inflammasome is a multimolecular complex recently demonstrated as central to obesity-induced IR. This complex is comprised of NLRP3, the adaptor molecule-apoptosis-associated speck-like protein containing a CARD and pro-caspase-1. Assembly of the NLRP3 inflammasome is mediated through a diverse range of endogenous and exogenous stressors including FFAs, glucose, adenosine triphosphate (ATP), uric acid, and ROS (Netea et al., 2009). Activation of the inflammasome induces caspase-1 activity required for cleaving pro-IL-1β to mature IL-1β. Indeed the importance of the NLRP3 inflammasome and caspase-1 activity in obesity-induced IR has been recently highlighted. Vandanmagsar et al. confirmed that weight loss through calorie restriction or exercise diminished NLRP3 expression in WAT. Moreover NLRP3$^{-/-}$ mice were protected from the adverse metabolic effects elicited by HFD; demonstrated by improved glucose homeostasis, greater insulin-induced pAKT activity in VAT, subcutaneous adipose tissue (SAT), liver and muscle. Interestingly, similar to IL-1R1 deficient mice, lack of NLRP3 did not alter the ATM M1/M2 ratio (Vandanmagsar et al., 2011). Stienstra et al. illustrated that caspase-1 expression was amplified during adipocyte differentiation and this increase was associated with reduced insulin sensitivity. Lack of caspase-1 or NLRP3 increased adipogenesis and lipid accumulation in adipocytes. Furthermore, lack of caspase-1 prevented HFD-induced IR (Stienstra et al., 2010). These studies indicate that the inflammasome which modulates IL-1 activation/mediated inflammation is a critical regulator of inflammation and WAT function.

TUMOR-NECROSIS FACTOR-α

Tumor-necrosis factor-α is a potent pro-inflammatory cytokine, primarily secreted from monocytes and macrophages, via the activation of MAPK and NF-κB signaling pathways, resulting in the release of other inflammatory cytokines such as IL-1β and IL-6 (Chen and Goeddel, 2002; De Luca and Olefsky, 2006). It was the first inflammatory mediator linked with obesity-induced IR (Hotamisligil et al., 1993). Chronic treatment of 3T3-L1 adipocytes with TNF-α activated intracellular IKKβ and reduced tyrosine phosphorylation of IRS-1, ultimately leading to impaired insulin action. Obese rodents administered TNF-α neutralizing antibody exhibited reduced hyperinsulinemia (Hotamisligil et al., 1994). Whole body deletion of TNF-α or its corresponding receptor TNF receptor 1 (TNFR1) gene partially protects mice from obesity-induced IR (Uysal et al., 1997). Several studies have shown TNF-α can also indirectly alter insulin sensitivity. The treatment of 3T3-L1 adipocytes with TNF-α increased lipolysis but also down-regulated adipogenic genes; PPAR-γ and C/EBP. Furthermore TNF-α activated NF-κB suppressed genes involved in lipid uptake and storage (Ruan et al., 2002). Obese human subjects exhibit elevated circulating levels of TNF-α (Hotamisligil et al., 1995; Kern et al., 1995). However the benefits of anti-TNF therapy in T2D are limited.

INTERLEUKIN-10

Interleukin-10 is an anti-inflammatory cytokine produced by monocytes, M2 ATMs, DCs, T cells, and B cells. It was initially discovered through its role in preventing the production of T_H1 cytokines in mice (Moore et al., 2001). It signals via the IL-10 receptor (IL-10R) to activate the JAK/STAT pathway and exerts immuno-suppressive effects by blocking IκK activity (Schottelius et al., 1999) or by inducing tyrosine phosphorylation of STAT-3 (Lumeng et al., 2007). Interleukin-10 may play a protective role in obesity-induced metabolic dysregulation and IR. Interleukin-10 levels are attenuated in T2D (Van Exel et al., 2002)

and weight loss enhances WAT IL-10 expression coincident with reduced pro-inflammatory gene expression (Cancello et al., 2005). Lumeng et al. have demonstrated elevated IL-10 expression in ATM and within the SVF of lean WAT compared with obese WAT. Interleukin-10 reduced MCP-1 secretion from 3T3-L1-adiopcytes. Moreover pre-treatment of 3T3-L1-adiopcytes with IL-10 blocked the insulin-desensitizing effects of TNF-α and enhanced insulin-stimulated glucose transport (Lumeng et al., 2007). Interleukin-10 is associated with many immune cells and production by T_H cells initiates a feedback loop that can limit the effector functions of macrophages and DCs. It can also drive differentiation of IL-10 secreting T_{reg} cells (Saraiva and O'Garra, 2010). Interleukin-10 can inhibit the production of MHC class II and co-stimulatory molecule expression in DCs and macrophages, it can also prevent the production of cytokines from $CD4^+$ T cells (Joss et al., 2000; Couper et al., 2008).

Other studies have challenged the protective effects of IL-10 in obesity-induced IR. Deletion of hematopoietic cell derived IL-10 did not exacerbate WAT or liver inflammation in response to HFD. However IL-10 expression was markedly up-regulated in WAT and liver in these mice compared to wild type (WT) mice, suggesting induction of a compensatory mechanism (Kowalski et al., 2011). Therefore further *in vivo* studies are required to evaluate the significance of IL-10 in obesity-induced IR.

INTERLEUKIN-6

Interleukin-6 is secreted by WAT, skeletal muscle, and the liver (Fasshauer et al., 2003; Weisberg et al., 2003; Wieckowska et al., 2008). Interleukin-6 signals via the JAK/STAT and MAPK pathways (Heinrich et al., 2003). WAT and plasma IL-6 expression is correlated with BMI (Vozarova et al., 2001) and up-regulated by insulin and TNF-α; it negatively impacts on insulin signaling promoting serine phosphorylation of IRS-1 (Fasshauer et al., 2003; Ruge et al., 2009). Interleukin-6 may promote dysregulation of FA metabolism in WAT as it enhances mesenchymal stem cell proliferation, maintaining the cells in an undifferentiated state and inhibiting adipogenesis (Pricola et al., 2009). Increased hepatic IL-6 levels are also associated with steatohepatitis and plasma IL-6 levels (Wieckowska et al., 2008). Additionally IL-6 was recently shown to stimulate insulin secretion via enhanced GLP-1 expression in pancreatic cells (Ellingsgaard et al., 2011). This suggests that obesity-induced IL-6 secretion may reflect a mechanism to increase insulin production in the obese IR state. However, while elevated IL-6 secretion from WAT and the liver is unfavorable, the opposite is true for skeletal muscle. Physical inactivity decreases insulin sensitivity, it is also associated with reduced skeletal muscle IL-6 expression and secretion (Pedersen and Febbraio, 2012). Furthermore the increase in plasma IL-6 levels that result from exercise are followed by increased IL-1RA and IL-10 levels, and exercise induced IL-6 is thought to result from glycogen/MAPK activation rather than through NF-κB (Pedersen, 2011); thus highlighting the pleiotropic role of IL-6.

MACROPHAGE MIGRATION INHIBITORY FACTOR

Several studies suggest an association between MIF and obesity-induced IR (Vozarova et al., 2002; Dandona et al., 2004; Ghanim et al., 2004; Church et al., 2005). Macrophage migration inhibitory factor has a prominent role in macrophage biology, it promotes secretion of TNF-α, IL-6, IL-1β, and inhibits IL-10, propagating a pro-inflammatory response (Calandra et al., 1994; Baugh and Bucala, 2002; Calandra and Roger, 2003; Lue et al., 2007). Moreover MIF can sustain activated macrophage life span by thwarting p53-dependent apoptosis (Mitchell et al., 2002) and induce chemotaxis via CXCR2 and CXCR4 in macrophages and T cells respectively (Bernhagen et al., 2007). Plasma MIF concentrations and PBMC MIF mRNA are positively associated with BMI, FFA concentration, impaired glucose tolerance (IGT), and IR (Vozarova et al., 2002; Skurk et al., 2005). Obese SAT explants demonstrate increased MIF secretion, and expression is increased with adipocyte size IR (Skurk et al., 2005; Koska et al., 2009). Weight loss and treatment with the anti-diabetic drug metformin reduces plasma MIF concentrations coincident with improved pancreatic β-cell function (Dandona et al., 2004; Church et al., 2005). More recently a causal role for MIF in IR was emphasized using an atherosclerotic mouse model which lacked functional MIF ($LDLR^{-/-}MIF^{-/-}$). This model displayed less local WAT inflammation, reduced $MAC3^+$ macrophage infiltration, with enhanced WAT and systemic insulin sensitivity (Verschuren et al., 2009). In addition deletion of MIF reduced monocyte adhesion, macrophage lesion content, and atherosclerotic lesion size (Verschuren et al., 2009). Unpublished work within our group implicates MIF as a critical inflammatory regulator in the pathogenesis of HFD-induced IR with an essential role in HFD-associated ATM recruitment. It has since emerged that lack of MIF signaling results in an age-dependent impairment of glucose homeostasis in mice fed a chow diet (Serre-Beinier et al., 2010), highlighting the intricacy of this molecule and lack of true clarity in terms of its functional role in obesity-induced IR.

ADIPONECTIN

Adiponectin is a 30-kDa secretory hormone produced predominantly by adipocytes, expression, and secretion are elevated during adipocyte differentiation (Carbone et al., 2012). Reduced adiponectin levels such that accompany obesity, result in IGT due to reduced insulin sensitivity (Arita et al., 1999; Fantuzzi, 2005). There are three major isoforms of adiponectin; low molecular weight (LMW), formed from three adiponectin monomers, middle-molecular weight (MMW) which is an octomer, and high-molecular weight (HMW) consisting of 12 or more monomers (Magkos and Sidossis, 2007). HMW adiponectin is the most biologically active form and best reflective of the reduction in total adiponectin levels associated with obesity (Almeda-Valdes et al., 2010). Indeed HMW-adiponectin levels have been identified as an independent risk factor for IR (Aso et al., 2006; Hara et al., 2006; Almeda-Valdes et al., 2010).

Adiponectin can enhance FA oxidation and improve insulin sensitivity (Carbone et al., 2012); suppressing gluconeogenesis in the liver thus reducing circulating glucose levels, also increasing glucose uptake in the muscle by enhanced GLUT4 expression. In the liver, adiponectin stimulates glucose and FA oxidation through activation of the AMPK pathway and reduces lipogenesis by minimizing SREBP-1c expression (Utzschneider and Kahn, 2006).

In addition to its insulin sensitizing role, adiponectin is typically anti-inflammatory. Adiponectin can suppress the production of TNF-α and IFN-γ and it is speculated to be a negative regulator of T cells (Scherer et al., 1995; Carbone et al., 2012). Tsang et al. (2011) demonstrated that chronic adiponectin treatment in DCs decreased the expression of the co-stimulatory molecules CD80 and CD86, MHC II, and also IL-12. Additionally adiponectin treated DCs had altered T cell interactions with reduced proliferation of T_H1 cells and T_{reg} cell induction. Thus targeting adiponectin signaling pathways may be promising in the treatment of metabolic diseases.

CYTOKINE SYNERGY TO AUGMENT OBESITY-INDUCED INSULIN RESISTANCE

Given the complexity of pro-inflammatory signaling, it is highly probable that a combination of stimuli rather than a single entity would elicit greater accountability for the inflammation-IR paradigm. Certainly it is known that IL-1, TNF-α, and TLR4 converge at the inhibitor of NF-κB kinase complex (IκB kinase) (Verstrepen et al., 2008). McGillicuddy et al. (2011) emphasized the potent synergistic capabilities IL-1β and TNF-α in macrophages and WAT. Intriguingly this study showed that lack of IL-1 signaling altered the immunogenic phenotype of ATM, with reduced IL-6 and TNF-α secretion, but without altered ATM number. Correspondingly AT inflammation was reduced concomitant with enhanced insulin-stimulated pAKT expression. This study also showed that adipocytes exposed to IL-1β and TNF-α induced greater activation of NF-κB, with significant amplification of IL-6 secretion compared with either cytokine alone. Furthermore abrogation of IL-1R1 in bone marrow macrophages ablates these synergistic abilities. To investigate this phenomenon in a more physiological relevant setting, WAT explants from WT and IL-$1R1^{-/-}$ mice were cultured in the presence of IL-1β and TNF-α *ex vivo,* loss of synergy was again observed in IL-$1R1^{-/-}$ WAT. Another important inflammatory regulator that offers potential involvement in IL-1, TNF-α, and TLR4 signaling is MIF (Finucane et al., 2012). Endogenous MIF regulates innate immunity via upregulation of TLR4, IL-1R1, and p55 tumor-necrosis factor receptor (TNFR) expression (Roger et al., 2001; Toh et al., 2006). $MIF^{-/-}$ macrophages were shown to be hypo-responsive to LPS and failed to secrete TNF-α and IL-6 due to profound suppression of IKKβ/NF-κB activity (Roger et al., 2001). Macrophage migration inhibitory factor deficiency suppressed IL-1 and TNF-α induced MAPK activity and cell proliferation in mouse fibroblasts. Reconstitution of an upstream MAPK or MIF reversed this suppression (Toh et al., 2006). Furthermore, treatment of 3T3-L1 adipocytes with TNF-α induced MIF secretion, suggesting that TNF-α may regulate MIF production during obesity (Hirokawa et al., 1997, 1998). *In vivo* MIF deficient mice were hypo-responsive to TNF-α and exhibited euglycemia, indicating MIF is required for successful TNF-α action. Additionally 3T3-L1 adipocytes exposed to exogenous MIF demonstrated impaired insulin-stimulated glucose uptake and insulin receptor signal transduction. Similarly in response to inflammatory stress $MIF^{-/-}$ mice exhibit a marked improvement in WAT glucose uptake compared to control mice (Atsumi et al., 2007).

ACTIVATION AND PROPAGATION OF INFLAMMATION AND INSULIN RESISTANCE

The NF-κB and JNK MAPK pathways link obesity, inflammation, and IR, summarized in **Figure 4** (Hirosumi et al., 2002; Shoelson et al., 2003). But despite intense scrutiny, an intricate question persistently evades resolution, what instigates and consequentially propagates this inflammation? See **Figure 5**. Reduced ability of WAT to adequately store excess fat and an increased hypoxic environment have been proposed as two potential contributors. Hypoxia represents a metabolic stressor relevant to obese WAT, this local hypoxia may be partially attributed to an insufficient blood supply reaching the expanding organ (Kabon et al., 2004; Hosogai et al., 2007). Hypoxia-inducible factor-1 (HIF)-1 is a transcription factor with an oxygen regulated α subunit (HIF-1 α) (Bruning et al., 2011; Shin et al., 2012); tissue inflammation is associated with regions of hypoxia, and HIF signaling is also linked to NF-κB activation (Ye et al., 2007; Bruning et al., 2012). WAT hypoxia correlated with reduced expression of adiponectin in a HFD-fed mouse model. Moreover, hypoxic conditions augment WAT derived cytokines including leptin and IL-6 (Sun et al., 2011). Conversely, weight loss improves adipose oxygenation and increases adiponectin expression. HIF-1α is a critical regulator of cellular hypoxic responses and is rapidly degraded under normoxic conditions. HIF-1α activity increases early in obesity. Overexpression of HIF-1α initiates WAT fibrosis, accompanied by increased local inflammation. Halberg et al. (2009) suggest that hypoxia induced WAT fibrosis may be responsible for initiating WAT inflammation during obesity. A HIF-1α antisense oligonucleotide (ASO) suppressed *Hif-1α* gene expression in the liver and WAT of HFD-fed mice (Shin et al., 2012), and resulted in weight loss without a change in food intake or activity levels. *In vitro* analysis of hypoxia in tumor cell lines showed that resveratrol (Zhang et al., 2005; Park et al., 2007) and two isoforms of conjugated linoleic acid (CLA) *c*9,*t*11- and *t*10,*c*12-CLA (Yamasaki et al., 2012) can promote HIF-1α protein degradation; suggesting that the hypoxia related inflammatory response in WAT is nutrient sensitive.

Decreased WAT expandability may result in lipid spillover, elevating circulating FFAs resulting in enhanced ectopic lipid accumulation in peripheral tissues (Khan et al., 2009; Prieur et al., 2011). Long-chain (LC) SFA can serve as a ligand to the innate immune receptor TLR4 (Shi et al., 2006). Indeed, exposing macrophages and adipocytes to varying concentrations of palmitate, the most abundant circulating SFA in obesity, elicits a TLR4 dependent pro-inflammatory response characterized by increased NF-κB and JNK activation with resultant enhanced TNF-α cytokine secretion *in vitro.* Furthermore $TLR4^{-/-}$ mice administered an acute lipid infusion maintained glucose homeostasis (Shi et al., 2006). HFD-fed mice lacking TLR4 had attenuated IR, increased energy expenditure and improved WAT inflammation (Shi et al., 2006; Tsukumo et al., 2007). While bone-marrow transplant studies indicate that TLR4 in the hematopoietic system opposed to the non-hematopoietic system may be responsible for TLR4 mediated IR during HFD wherein TLR4 chimeric mice exhibit reduced hyperinsulinemia, hepatic, and WAT IR (Saberi et al., 2009). Despite abundant reports signifying that TLR4 ameliorates WAT inflammation, the effect on ATM accumulation has

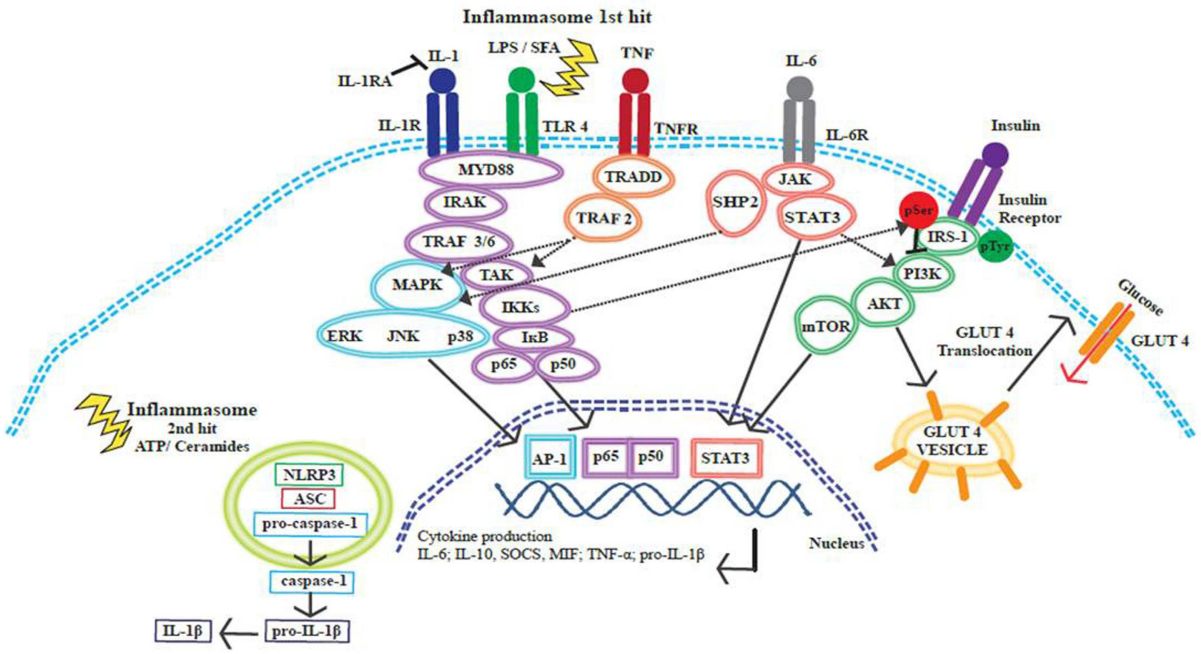


FIGURE 4 | Cross-talk between insulin and inflammatory signaling pathways. Inflammatory signaling pathways activated by SFA or by pro-inflammatory cytokines IL-1β, IL-6, and TNF-α initiate a cascade of events that promote the release of further inflammatory mediators. These signaling events converge at the NF-κB and MAPK pathways, resulting in the translocation of transcription factors to the nucleus, transcriptional activation, and cytokine production. The inflammasome is activated through a two-hit process; with obesity the first hit occurs when TLR4 is activated by SFAs and this results in pro-IL-1β production, ATP or ceramides then provide the second hit. The NLRP3 inflammasome acts on pro-caspase-1 causing the release of caspase-1; caspase-1 then acts upon pro-IL-1β cleaving this precursor to the active IL-1β form. Insulin signaling promotes glucose uptake by promoting the translocation of GLUT4 to the cell surface plasma membrane. Inflammatory signaling pathways can alter the phosphorylation status of IRS-1. IRS-1 is crucial in the insulin signaling pathway, tyrosine phosphorylation is associated with an insulin sensitive state. IKKβ and JNK can promote serine phosphorylation of IRS-1 and this phosphorylation state is linked to insulin resistance and reduced glucose uptake.

remained unclear. In this regards Orr et al. (2012) demonstrated TLR4 deficiency did not alter ATM number but promoted ATM toward an M2 anti-inflammatory phenotype.

Dietary FA have been shown to interact with DCs and alter cell function (Loscher et al., 2005; Weatherill et al., 2005; Zeyda et al., 2005). Saturated fatty acid drives DC maturation through interaction with TLR4. Bone marrow derived dendritic cells (BMDCs) isolated from HFD-fed mice are skewed toward a pro-inflammatory phenotype with increased secretion of IL-1β, IL-12p70, and TNF-α in response to LPS (Reynolds et al., 2012). Interleukin-1R1, TLR4, caspase-1, and NLRP3 mRNA expression also increases in BMDCs from HFD-fed mice; additionally cell surface TLR4 expression was also significantly increased. Polyunsaturated fatty acids (PUFA) have an opposing effect on DC function and can suppress IL-12p70 production while boosting IL-10 release, thus driving a T_{reg} rather than a T_H1 response (Loscher et al., 2005; Weatherill et al., 2005). PUFA largely exert an effect via changes in gene expression causing activation of transcription factors (Bouwens et al., 2010).

Specific FAs as well as some eicosanoids have been shown to bind to receptors of the PPAR family of transcription factors, including the PPAR-α, PPAR-β/δ, and PPAR-γ isoforms (Forman et al., 1997; Delerive et al., 1999). PPAR act as a hub between inflammation and insulin sensitivity, via physical interactions with NF-κB and IκBα (Ricote et al., 1998; Delerive et al., 2002); antagonizing inflammation and promoting insulin signaling. Activation of PPAR-γ is associated with the upregulation of IRS proteins (Hammarstedt and Smith, 2003; Seto-Young et al., 2007); additionally the thiazolidinedione class of drugs which act as PPAR-agonists, promote tyrosine phosphorylation of IRS-1 in both *in vivo* and *in vitro* models (Jiang et al., 2002, 2004). Nevertheless it is impossible to adequately address PPAR functionality in obesity within the current review and this topic was reviewed very comprehensively elsewhere (Stienstra et al., 2007; Varga et al., 2011).

Ectopic accumulation of excessive palmitate-derived lipid plays an antagonistic role in insulin signaling (Gill and Sattar, 2009). Ceramides in particular are postulated to be a critical factor connecting disproportionate lipid accretion, inflammation, and impaired insulin sensitivity (Summers, 2006). Indeed activation of a TLR4 inflammatory response via SFA is essential for SFA induced ceramide biosynthesis (Holland et al., 2011). Ceramides comprise of sphingosine bound to a FA and serve as both structural and signaling molecules (Gill and Sattar, 2009). Ceramides are generated by condensation of palmitoyl-CoA and serine via the rate-limiting enzymatic reaction mediated by serine palmitoyltransferase (Mathias et al., 1998; Summers, 2006). Importantly pro-inflammatory cytokine such as IL-1β amplify ceramide

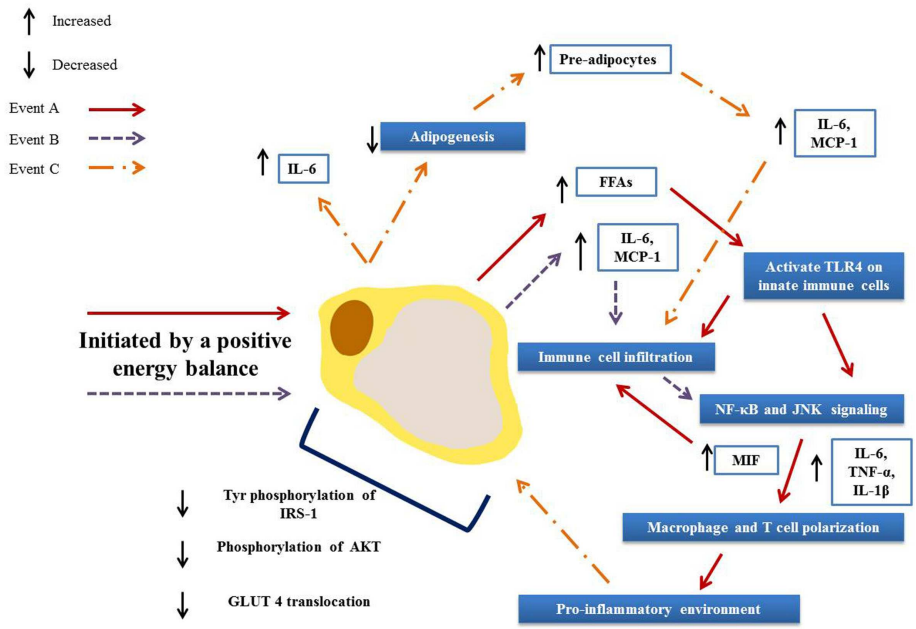


FIGURE 5 | Activation and propagation of inflammation and insulin resistance in obese adipose tissue. Adipocyte hypertrophy results in elevated circulation of FFAs **(A)** and increased secretion of adipokines **(B)**. These in turn result in immune cell infiltration and the activation of pro-inflammatory signaling pathways, driving further infiltration and the polarization of adipose tissue macrophages and T cells toward a pro-inflammatory phenotype. This environment then drives further adipokine secretions **(C)** and hampers adipogenesis, resulting in greater numbers of pre-adipocytes that in turn secrete pro-inflammatory mediators. Together these events lead to defective insulin signaling and ultimately to an insulin resistant state.

biosynthesis by upregulating serine palmitoyltransferase (Summers, 2006). Haus et al. (2009) ascribed a pathogenic association between total plasma ceramide and its subspecies C18:0, C20:0, C24:1, C24:0 concentrations and the degree of IR in obese T2D patients. Elevated circulating TNF-α levels were positively correlated with increased C18:1 and C18:0 ceramide subspecies.

Culturing C2C12 myoblasts in the presence of palmitate induced *de novo* ceramide accumulation, disrupting insulin-stimulated pAKT activation. Inhibiting ceramide accumulation negated the detrimental effects on insulin signaling. However, whether ceramides induced an inflammatory response in myoblasts was not assessed in this study (Chavez et al., 2003). Further, Holland et al. demonstrated enhanced ceramide accumulation in the soleus muscle in response to lard oil infusion but not soy oil infusion, a source of PUFAs. Inhibiting ceramide infusion using serine palmitoyltransferase inhibitors prevented ceramide induced IR. Additionally lard oil evoked a significant increase in circulating pro-inflammatory IL-6 and TNF-α (Holland et al., 2011). Most recently ceramides were identified as an effective second "hit" required for NLRP3 inflammasome activation in macrophages. Furthermore, ceramides evoked NLRP3 dependent caspase-1 activation in obese WAT explants, an event that was lost in NLRP3$^{-/-}$ WAT (Vandanmagsar et al., 2011). Despite significant evidence highlighting the importance of ceramides in inflammation and IR the clinical importance of these findings is yet to be fully deciphered. A subsequent study reported that palmitate was a potential second signal required for NLRP3 activation, inducing IL-1β production from macrophages. Both NLRP3 and the inflammasome adaptor ASC (apoptotic speck-like protein containing a CARD, known also as Pycard) deficiency ameliorated obesity-induced IR in mice (Wen et al., 2011).

INSULIN RESISTANCE AS A PROTECTIVE MECHANISM

It has been hypothesized that the induction of IR is perhaps a protective response. Insulin resistance could potentially act to protect cells from stress and damage through the exclusion of glucose from cells that are heavily lipid loaded; thus reducing the chance of lipotoxic damage (Unger, 2003). *In vivo* and *in vitro* rat studies indicate that glucose toxicity occurs when cells are exposed to a chronic hyperglycemic state (Rossetti et al., 1990), and this may induce or aggravate IR (Baron et al., 1995). Impaired GLUT 4 translocation in response to the metabolite glucosamine has been implicated in the induction of IR (Baron et al., 1995). Roden et al. (1996) determined through lipid infusion studies in healthy volunteers, that FFA's inhibit glucose transport. It has since been demonstrated that prolonged FFA exposure but not short-term exposure, interrupts glucose uptake (Hawkins et al., 1997). Alternatively IR may be induced partly in response to oxidative stress. Oxidative stress describes a biological state that develops following increased ROS production or reduced ROS clearance (Azzi, 2007). ROS production increases in response to increased macronutrient consumption (Codoñer-Franch et al., 2011). Excessive nutrient intake is linked to an increase in superoxide ($O_2^{\bullet}-$) generation by mitochondria (Tiganis, 2011). Mitochondria produce most

of the cell's energy, and in this capacity take up the majority of intracellular oxygen (Finkel and Holbrook, 2000). Cell signaling pathways are activated in response to increasing oxidative stress; these pathways include MAPK, NF-κB, and the PI3K-AKT pathway (Finkel and Holbrook, 2000). Hoehn et al. (2009) propose that IR is linked to mitochondrial $O_2^{\bullet}-$ and that $O_2^{\bullet}-$ acts as a nutrient sensor, which regulates nutrient intake under conditions of overnutrition. This group reports that mitochondrial $O_2^{\bullet}-$ is upstream of IR in skeletal muscle and AT; overexpression of mitochondrial superoxide dismutase (MnSOD) enzymes, with anti-oxidant action, in *in vitro* and *in vivo* rodent models significantly improved insulin sensitivity. Additionally, findings from caloric restriction (CR) studies show reduced oxidative damage (Sohal et al., 1994; Sohal and Weindruch, 1996; Masoro, 2000; Zainal et al., 2000). Sirtuin 1 (SIRT1) a member of the sirtuin family of proteins, may mediate the effect of CR by influencing PPAR-γ, PGC1-α, and FOXO (Han et al., 2010). SIRT1 has previously been shown to improve insulin sensitivity in *in vitro* and *in vivo* mouse studies (Moynihan et al., 2005; Sun et al., 2007).

METABOLICALLY HEALTHY OBESE

Interestingly 20–30% of obese adults do not express the adverse metabolic phenotype typically associated with obesity (Alam et al., 2012). Individuals considered metabolically healthy but obese (MHO) have high levels of insulin sensitivity but may not display symptoms of hypertension, dyslipidemia, or chronic inflammation (Karelis et al., 2005; Stefan et al., 2008; Succurro et al., 2008). MHO individuals have significantly smaller omental adipocytes than metabolically unhealthy individuals (O'Connell et al., 2010). This finding correlates with the degree of IR and hepatic steatosis within the obese groups. Interestingly this study demonstrated that BMI was not associated with adipocyte size nor did it predict general metabolic health or fatty liver disease, in a group of adults with a median BMI of 48 kg/m^2. Brochu et al. (2001) demonstrated in a group of obese postmenopausal women, that MHO individuals had lower levels of VAT; and that these women had been obese for longer than the metabolically unhealthy group. Additionally the MHO group was shown to have lower plasma TAG levels and higher high-density lipoprotein (HDL) cholesterol concentrations. In a study of obese males and females, fitness levels were higher in the MHO group (Ortega et al., 2013). Conversely an individual may be considered metabolically obese but normal weight (MONW) (Ruderman et al., 1981). Adult female subjects determined to be MONW tend to have a similar BMI to their metabolically healthy counterparts but have a higher percentage body fat, lower fat-free mass, and lower physical activity energy expenditure, and are considered to be less aerobically fit (Conus et al., 2004). Female MONW subjects were shown to have increased plasma cholesterol and reduced insulin sensitivity but showed no change in ghrelin, leptin, or adiponectin levels (Conus et al., 2004). MONW subjects also have increased TAG and FFA levels. Low-HDL cholesterol levels have been noted in male MONW subjects when compared with healthy non-obese subjects (Succurro et al., 2008; Lee et al., 2011).

TARGETING NUTRIENT-SENSITIVE INFLAMMATORY PATHWAYS TO TREAT INSULIN RESISTANCE

There is no doubt that weight loss can improve the inflammatory phenotype and insulin sensitivity (Kopp et al., 2005; Kováciková et al., 2011). However, maintenance of weight loss is difficult to achieve and weight regain frequently occurs (Gage, 2012). Thus there is a vital need to identify alternative nutritional interventions that may antagonize inflammation, independent of weight loss. As proof of concept from a pharmaceutical perspective, drugs that (1) interfere with the TLR4/IKK/NF-kB axis, (2) target PPAR-γ, or (3) target pro-inflammatory cytokines, have demonstrated promise with respect to treating IR despite obesity. Salicylate, an inhibitor of IKK has been shown to reverse hyperglycemia, hyperinsulinemia, and dyslipidemia in obese rodents by sensitizing insulin signaling (Yuan et al., 2001). Anakinra, an IL-1 RA, was shown to improve glycemia and beta-cell secretory function in patients with T2D (Malozowski et al., 2007). Small molecule MIF antagonist CPSI-1306 treatment in a mouse model with streptozotocin (STZ)-induced T2D, resulted in reduced circulating IL-6 and TNF-α levels and reduced blood glucose levels (Sanchez-Zamora et al., 2010). Nevertheless, long-term pharmacological immuno-suppressive interventions that attenuate IR, may not be ideal in terms of immunosurveillance and long-term health (Kung and Henry, 2012; Bortolini et al., 2013). Alternatively, anti-inflammatory nutritional interventions may have potential. Albeit producing a more subtle effect, several nutrients have now emerged as potentially insulin sensitizing, affecting the same molecular targets as established pharmaceutical approaches.

Perhaps the most extensively researched in relation to their immunomodulating effects are dietary FA which are known to interact with several inflammatory pathways (Hotamisligil and Erbay, 2008). The effect of FAs on these pathways depends on the degree of FA saturation (Bradley et al., 2008). Within this context, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), two LC $n-3$ PUFAs have emerged as anti-inflammatory nutrients that exert their effects through a number of biological mechanisms. Opposingly, SFA activate TLR4 and increase NF-κB transcriptional activity. EPA and DHA may mitigate this response (Bradley et al., 2008; Reynolds et al., 2012) by reducing nuclear p65 expression and increasing cytoplasmic IκBα expression, and DHA may act as a more potent NF-κB inhibitor than EPA (Weldon et al., 2007). Xue et al. (2012) suggested that this effect is partially mediated via AMPK/SIRT1 activation as DHA does not fully deacetylate p65 in SIRT1 knockdown macrophages. Furthermore pre-treatment of macrophages with DHA promotes an anti-inflammatory phenotype, with reduced IL-6 and increased IL-10 expression; which when co-cultured with adipocytes attenuates the characteristic IR phenotype (Oliver et al., 2012). Therefore the anti-inflammatory effects observed due to DHA pre-treatment were translated into improved insulin sensitivity in adipocytes (Oliver et al., 2012).

The GPCR GPR120 that is highly expressed in both adipocytes and macrophages, plays a pivotal role in LC $n-3$ PUFA mediated inhibition of inflammation and IR (Oh et al., 2010). Both EPA and DHA bind to the GPR120 receptor to inhibit NF-κB and

JNK via reduced TAK1 phosphorylation (Oh et al., 2010). Furthermore it was demonstrated that LC $n-3$ PUFA supplementation increased insulin sensitivity in WT but not in GPR120$^{-/-}$ mice (Oh et al., 2010). Additionally LC $n-3$ PUFA may exert their anti-inflammatory effect by enhancing adiponectin secretion from human adipocytes, an effect that is elicited at least partially, via PPAR-γ (Tishinsky et al., 2011). These results are consistent with *in vivo* work that demonstrated feeding mice a fish oil enriched diet increases plasma adiponectin, and this was completely blocked by the PPAR-γ antagonist BADGE (Neschen et al., 2006).

Long-chain $n-3$ PUFA may increase β-oxidation in WAT and cultured adipocytes (Guo et al., 2005; Flachs et al., 2011), potentially via activation of the AMPK regulatory pathway (Lorente-Cebrián et al., 2009; Figueras et al., 2011). This metabolic switch could increase the mitochondrial content of adipocytes, resulting in reduced accumulation of toxic FA derivatives and improved insulin sensitivity (Kopecky et al., 2009). Furthermore, LC $n-3$ PUFA competitively inhibit the conversion of arachidonic acid (AA) to pro-inflammatory eicosanoids such as prostaglandin E2 and leukotriene B4. Increased intake of LC $n-3$ PUFA promote the incorporation of EPA into membrane phospholipids at the expense of AA, thereby increasing production of EPA-derived anti-inflammatory eicosanoids, such as prostaglandin E3 and leukotriene B5 (Lottenberg et al., 2012). Whilst the potential benefits of LC $n-3$ PUFA supplementation on inflammatory pathways is clear *in vitro*, it is difficult to ascertain a consistent effect in man (Kabir et al., 2007; Tierney et al., 2011). Inconsistencies in human data are reflective of dose variability between studies, duration of supplementation, and the population studied (Calder et al., 2011). Genetic variability between individuals likely influences responsiveness to an intervention (Calder et al., 2011). Single nucleotide polymorphisms (SNPs) can influence responsiveness, MetS patients that are minor allele carriers of an adiponectin SNP have reduced IR following reduced SFA intake (Ferguson et al., 2010). A second study showed that common genetic variants of the complement component 3 (C3) locus conferred an increased risk of MetS, and that PUFA intake may modulate these genetic influences (Phillips et al., 2009a). Therefore gene-nutrient interactions may play an important role in regards to responsiveness to interventions, a personalized nutrition approach may be considered in order to determine an ideal dietary intervention.

There is growing evidence, albeit highly controversial, in relation to the immunomodulating potential of vitamin D. Following *in vitro* induced inflammation, 1,25-dihydroxyvitamin D up-regulates IκBα in macrophages through increased mRNA stability and decreased IκBα phosphorylation, thus reducing NF-κB activity (Cohen-Lahav et al., 2006). Furthermore, 1,25-dihydroxyvitamin D suppresses TLR2 and TLR4 expression in human monocytes (Sadeghi et al., 2006). Du et al. (2009) demonstrated vitamin D_3 pre-treatment of monocytes from T2D patients and controls resulted in similar TLR2 and TLR4 expression, NF-κB p65 phosphorylation state, and IL-1β and TNF-α expression in the two groups. Nevertheless human data is inconsistent, intervention studies have shown little or no effect of vitamin D supplementation on inflammatory or metabolic markers related to insulin sensitivity (Gulseth et al., 2010; O'Sullivan et al., 2011). Furthermore cross-sectional data showed that serum vitamin D status bore no relationship with insulin action or secretion in subjects with the MetS (Gulseth et al., 2010).

Vitamin C and vitamin E have been proposed to improve insulin sensitivity through anti-oxidant and anti-inflammatory mechanisms. As an anti-oxidant, ascorbic acid down-regulates ROS that otherwise would lead to activation of NF-κB (Nathan, 2003). Moreover, after oxidation to dehydroascorbic acid, vitamin C has been shown to directly inhibit IKKα, IKKβ, and p38 MAPK activity (Cárcamo et al., 2004). Vitamin C has also been shown to inhibit nitric oxide (NO) production and decrease insulin-induced MCP-1 and apelin secretion in an adipocyte-macrophage co-culture (Garcia-Diaz et al., 2011). Similarly, the insulin sensitizing effect of lycopene, a carotenoid pigment found in tomatoes involves inhibition of NF-κB, NO, and IL-6, and suppresses the activation of a number of MAP kinases (Feng et al., 2010). Phosphorylation of the MAP kinases ERK, p-38, and JNK, were shown to be ameliorated by lycopene (Kim et al., 2004). In WAT, pro-inflammatory cytokine and chemokine expression were reduced by lycopene treatment (Gouranton et al., 2011). A recent study conducted in young overweight adults showed that daily supplementation with one glass of tomato juice reduced TNF-α, IL-6, and IL-8 after 20 days (Ghavipour et al., 2012). Another study in overweight men demonstrated a decrease in systemic levels of serum amyloid A after 12 weeks of lycopene supplementation (McEneny et al., 2012). However Thies et al. (2012) demonstrated that following a 12-week treatment on a tomato-rich diet, inflammatory markers such as highly sensitive CRP (hsCRP) and IL-6, and HOMA-IR, remained unchanged.

Polyphenols, particularly flavonoids are gaining increasing attention for their anti-inflammatory effect. Resveratrol, a polyphenol that naturally occurs in grapes (Dong, 2003) was shown to inhibit pre-adipocyte proliferation, adipogenic differentiation, and *de novo* lipogenesis in a SIRT1-dependent manner in SGBS (Simpson–Golabi–Behmel syndrome) adipocytes (Fischer-Posovszky et al., 2010). Interestingly in humans it has been demonstrated that resveratrol treatment can improve the metabolic phenotype of healthy obese men, by reducing blood glucose and insulin levels and reducing plasma inflammatory markers (Timmers et al., 2011). Nevertheless it must be acknowledged that there have been other resveratrol interventions which have had little effect (Poulsen et al., 2012; Yoshino et al., 2012). Poulsen et al. demonstrated that resveratrol failed to improve endogenous glucose production and turnover or improve inflammatory and metabolic biomarkers in obese but otherwise healthy men. Additionally plasma lipids and insulin sensitivity did not improve; neither was there a change in molecular targets such as AMPK or SIRT1 in non-obese women treated with resveratrol (Yoshino et al., 2012).

Epigallocatechin gallate (EGCG) present in green tea, has been implicated in inhibiting resistin gene expression in adipocytes (Liu et al., 2006) and was shown to decrease ERK phosphorylation. Yang et al. (2001) demonstrated EGCG also inhibits IKK. A recent investigation of quercetin, demonstrated that this flavonoid, found predominantly in capers, apples, and grapes, attenuated TNF-α-induced NF-κB activity and subsequent expression of inflammatory genes in primary human adipocytes (Chuang et al., 2010).

Curcumin which is the yellow pigment found in the spice turmeric inhibits LPS-induced secretion of TNF-α and IL-1β *in vitro* (Chan, 1995). Curcumin also completely inhibits TNF-α-induced activation of NF-κB and other inflammatory agents such as phorbol esters (Singh and Aggarwal, 1995). Salicylic acid, the basic component of aspirin is present in fruits, vegetables, herbs, and spices (Duthie and Wood, 2011). Relatively low concentrations of salicylic acid have been shown to inhibit cyclooxygenase (COX)-2 *in vitro* (Wu et al., 1991; Xu et al., 1999; Hare et al., 2003) and suppress the transcriptional activation of pro-inflammatory genes such as iNOS (Duthie and Wood, 2011). However, salicylate concentrations sufficient to inhibit NF-κB activation are unlikely to be achieved through diet, limiting the therapeutic potential of salicylic acid in its natural form (Duthie and Wood, 2011; Wood et al., 2011).

Much of the research to date has examined the effect of individual nutrients on inflammation however recent evidence suggests that a combination of nutrients may offer an enhanced immunomodulating effect. Bakker et al. (2010) demonstrated that supplementing overweight men with a combination of nutrients, with known anti-inflammatory properties, increased plasma adiponectin by 7% over a 5-week period, independent of weight loss. Additionally, large-scale profiling of genes, proteins, and metabolites showed that the intervention could influence inflammation, oxidative stress, and metabolism. Anti-inflammatory IL-10Rα and SOCS3 expression were up-regulated in WAT in response to the intervention (Bakker et al., 2010). This study highlights the potential efficacy of nutritional interventions that target multiple signaling pathways to treat IR in obese individuals.

Despite convincing evidence from *in vitro* and animal studies to support the therapeutic potential of several nutrients within the context of obesity-induced inflammation and IR, results from clinical studies are not entirely consistent. Even high dose nutritional supplementation often fails to elicit an anti-inflammatory effect (Blok et al., 1997; Jellema et al., 2004; Pot et al., 2009). Much of the discrepancy observed may stem from the examination of supra-physiological doses in *in vitro* studies (Calder et al., 2011). Taken together a cocktail of nutrients may be more beneficial in ameliorating the inflammatory phenotype observed in a number of pathologies. There is no doubt that there is potential for nutritional anti-inflammatory agents to improve IR within the obese phenotype. Nevertheless demonstrating efficacy is confounded by defining an effective dose of individual dietary elements and/or defining potential synergies between anti-inflammatory nutrients/food components. Determining effective doses may be facilitated by the development of specific functional foods, enriched with the active agents. There is strong evidence that some individuals with the MetS who have a pro-inflammatory status due to their genotype and/or inflammatory phenotype may be more susceptible to the pro-inflammatory effect of dietary SFA (Phillips et al., 2009b, 2013). Furthermore their pro-inflammatory genotype/phenotype may determine potential responsiveness to an intervention. Therefore there may be a difference in efficacy between sub-cohorts of the obese population to anti-inflammatory interventions; and a dual approach in terms of removing SFA and augmenting nutritional anti-inflammatory agents may be required to achieve the desired effect to attenuate IR.

CONCLUSION

Inflammation is a critical mediator in obesity-induced IR. WAT expansion and the influx of immune cells initiate a cascade of inflammatory events that directly contribute to defective insulin signaling and glucose uptake, resulting in systemic IR. The mechanisms driving the pathogenic environment of obese WAT are complex and not fully elucidated; evidence implicates that a combination of events converge, and escalate the pro-inflammatory state. Research suggests that nutritional anti-inflammatory interventions may attenuate IR, independent of weight loss. However, results from human studies so far remain inconsistent. Moving forward, greater consideration should be given to designing nutritional interventions that (1) target multiple signaling pathways and (2) take account of genetic polymorphisms in order to improve efficacy.

ACKNOWLEDGMENTS

Maeve A. McArdle supported by Food for Health Ireland (Enterprise Ireland Grant: CC20080001), Orla M. Finucane supported by Science Foundation Ireland (PI/11/1119), Ruth M. Connaughton and Aoibheann M. McMorrow supported by the National Children's Research Centre (B/11/1).

REFERENCES

Adams, L. A., Angulo, P., and Lindor, K. D. (2005). Nonalcoholic fatty liver disease. *CMAJ* 172, 899–905.

Akdis, M., Burgler, S., Crameri, R., Eiwegger, T., Fujita, H., Gomez, E., et al. (2011). Interleukins, from 1 to 37, and interferon-gamma: receptors, functions, and roles in diseases. *J. Allergy Clin. Immunol.* 127, 701–721.

Alam, I., Ng, T. P., and Larbi, A. (2012). Does inflammation determine whether obesity is metabolically healthy or unhealthy? The aging perspective. *Mediators Inflamm.* 2012, 456–456.

Almeda-Valdes, P., Cuevas-Ramos, D., Mehta, R., Gomez-Perez, F. J., Cruz-Bautista, I., Arellano-Campos, O., et al. (2010). Total and high molecular weight adiponectin have similar utility for the identification of insulin resistance. *Cardiovasc. Diabetol.* 9, 9–26.

Arita, Y., Kihara, S., Ouchi, N., Takahashi, M., Maeda, K., Miyagawa, J., et al. (1999). Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem. Biophys. Res. Commun.* 257, 79–83.

Aso, Y., Yamamoto, R., Wakabayashi, S., Uchida, T., Takayanagi, K., Takebayashi, K., et al. (2006). Comparison of serum high-molecular weight (HMW) adiponectin with total adiponectin concentrations in type 2 diabetic patients with coronary artery disease using a novel enzyme-linked immunosorbent assay to detect HMW adiponectin. *Diabetes* 55, 1954–1960.

Atsumi, T., Cho, Y. R., Leng, L., McDonald, C., Yu, T., Danton, C., et al. (2007). The proinflammatory cytokine macrophage migration inhibitory factor regulates glucose metabolism during systemic inflammation. *J. Immunol.* 179, 5399–5406.

Azzi, A. (2007). Oxidative stress: a dead end or a laboratory hypothesis? *Biochem. Biophys. Res. Commun.* 362, 230–232.

Bakker, G. C., Van Erk, M. J., Pellis, L., Wopereis, S., Rubingh, C. M., Cnubben, N. H., et al. (2010). An antiinflammatory dietary mix modulates inflammation and oxidative and metabolic stress in overweight men: a nutrigenomics approach. *Am. J. Clin. Nutr.* 91, 1044–1059.

Baron, A. D., Zhu, J. S. H., Weldon, H., Maianu, L., and Garvey, W. T. (1995). Glucosamine induces insulin resistance in vivo by affecting GLUT 4 translocation in skeletal muscle. Implications for glucose toxicity. *J. Clin. Invest.* 96, 2792–2801.

Baugh, J. A., and Bucala, R. (2002). Macrophage migration inhibitory factor. *Crit. Care Med.* 30, S27–S35.

Bell, L. N., Ward, J. L., Degawa-Yamauchi, M., Bovenkerk, J. E., Jones, R., Cacucci, B. M., et al. (2006). Adipose tissue production of hepatocyte growth factor contributes to elevated serum HGF in obesity. *Am. J. Physiol. Endocrinol. Metab.* 291, E843–E848.

Berg, A. H., Combs, T. P., Du, X., Brownlee, M., and Scherer, P. E. (2001). The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat. Med.* 7, 947–953.

Bernhagen, J., Krohn, R., Lue, H., Gregory, J. L., Zernecke, A., Koenen, R. R., et al. (2007). MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. *Nat. Med.* 13, 587–596.

Berraondo, B., and Martínez, J. A. (2000). Free fatty acids are involved in the inverse relationship between hormone-sensitive lipase (HSL) activity and expression in adipose tissue after high-fat feeding or beta3-adrenergic stimulation. *Obes. Res.* 8, 255–261.

Bertola, A., Ciucci, T., Rousseau, D., Bourlier, V., Duffaut, C., Bonnafous, S., et al. (2012). Identification of adipose tissue dendritic cells correlated with obesity-associated insulin-resistance and inducing Th17 responses in mice and patients. *Diabetes* 61, 2238–2247.

Bhattacharya, P., Gopisetty, A., Ganesh, B. B., Sheng, J. R., and Prabhakar, B. S. (2011). GM-CSF-induced, bone-marrow-derived dendritic cells can expand natural Tregs and induce adaptive Tregs by different mechanisms. *J. Leukoc. Biol.* 89, 235–249.

Bjorntorp, P. (1990). "Portal" adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. *Arteriosclerosis* 10, 493–496.

Blok, W. L., Deslypere, J. P., Demacker, P. N., Van der Ven-Jongekrijg, J., Hectors, M. P., Van der Meer, J. W., et al. (1997). Pro- and anti-inflammatory cytokines in healthy volunteers fed various doses of fish oil for 1 year. *Eur. J. Clin. Invest.* 27, 1003–1008.

Boon, J., Hoy, A. J., Stark, R., Brown, R. D., Meex, R. C., Henstridge, D. C., et al. (2013). Ceramides contained in LDL are elevated in type 2 diabetes and promote inflammation and skeletal muscle insulin resistance. *Diabetes* 62, 401–410.

Bortolini, M., Wright, M. B., Bopst, M., and Balas, B. (2013). Examining the safety of PPAR agonists – current trends and future prospects. *Expert Opin. Drug Saf.* 12, 65–79.

Bouwens, M., Bromhaar, M. G., Jansen, J., Mu, M., and Afman, L. A. (2010). Postprandial dietary lipid – specific effects on human peripheral blood mononuclear cell gene expression profiles 1–3. *Am. J. Clin. Nutr.* 91, 208–217.

Bradley, R. L., Fisher, F. F. M., and Maratos-Flier, E. (2008). Dietary fatty acids differentially regulate production of TNF-alpha and IL-10 by murine 3T3-L1 adipocytes. *Obesity (Silver Spring)* 16, 938–944.

Brereton, C. F., Sutton, C. E., Lalor, S. J., Lavelle, E. C., and Mills, K. H. G. (2009). Inhibition of ERK MAPK suppresses IL-23- and IL-1-driven IL-17 production and attenuates autoimmune disease. *J. Immunol.* 183, 1715–1723.

Brigl, M., and Brenner, M. B. (2004). CD1: antigen presentation and T cell function. *Annu. Rev. Immunol.* 22, 817–890.

Brochu, M., Tchernof, A., Dionne, I. J., Sites, C. K., Eltabbakh, G. H., Sims, E. A., et al. (2001). What are the physical characteristics associated with a normal metabolic profile despite a high level of obesity in postmenopausal women? *J. Clin. Endocrinol. Metab.* 86, 1020–1025.

Brown, M. S., and Goldstein, J. L. (2008). Selective versus total insulin resistance: a pathogenic paradox. *Cell Metab.* 7, 95–96.

Bruning, U., Cerone, L., Neufeld, Z., Fitzpatrick, S. F., Cheong, A., Scholz, C. C., et al. (2011). MicroRNA-155 promotes resolution of hypoxia-inducible factor 1alpha activity during prolonged hypoxia. *Mol. Cell. Biol.* 31, 4087–4096.

Bruning, U., Fitzpatrick, S. F., Frank, T., Birtwistle, M., Taylor, C. T., and Cheong, A. (2012). NFκB and HIF display synergistic behaviour during hypoxic inflammation. *Cell. Mol. Life Sci.* 69, 1319–1329.

Cai, D., Yuan, M., Frantz, D. F., Melendez, P. A., Hansen, L., Lee, J., et al. (2005). Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat. Immunol.* 11, 183–190.

Calandra, T., Bernhagen, J., Mitchell, R. A., and Bucala, R. (1994). The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor. *J. Exp. Med.* 179, 1895–1902.

Calandra, T., and Roger, T. (2003). Macrophage migration inhibitory factor: a regulator of innate immunity. *Nat. Rev. Immunol.* 3, 791–800.

Calder, P. C., Ahluwalia, N., Brouns, F., Buetler, T., Clement, K., Cunningham, K., et al. (2011). Dietary factors and low-grade inflammation in relation to overweight and obesity. *Br. J. Nutr.* 106(Suppl.), S5–S78.

Cancello, R., Henegar, C., Viguerie, N., Taleb, S., Poitou, C., Rouault, C., et al. (2005). Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. *Diabetes* 54, 2277–2286.

Carbone, F., La Rocca, C., and Matarese, G. (2012). Immunological functions of leptin and adiponectin. *Biochimie* 94, 2082–2088.

Cárcamo, J. M., Pedraza, A., Bórquez-ojeda, O., Zhang, B., Sanchez, R., Golde, D. W., et al. (2004). Vitamin C is a kinase inhibitor: dehydroascorbic acid inhibits IκBα kinase β. *Mol. Cell. Biol.* 24, 6645–6652.

Cawthorn, W. P., Scheller, E. L., and MacDougald, O. A. (2012). Adipose tissue stem cells meet preadipocyte commitment: going back to the future. *J. Lipid Res.* 53, 227–246.

Chan, M. M. (1995). Inhibition of tumour necrosis factor by curcumin, a phytochemical. *Biochem. Pharmacol.* 49, 1551–1556.

Chavez, J. A., Knotts, T. A., Wang, L. P., Li, G., Dobrowsky, R. T., Florant, G. L., et al. (2003). A role for ceramide, but not diacylglycerol, in the antagonism of insulin signal transduction by saturated fatty acids. *J. Biol. Chem.* 278, 10297–10303.

Chen, G., and Goeddel, D. V. (2002). TNF-R1 signaling: a beautiful pathway. *Science* 296, 1634–1635.

Chuang, C., Martinez, K., Xie, G., Kennedy, A., Bumrungpert, A., Overman, A., et al. (2010). Quercetin is equally or more effective than resveratrol in attenuating tumor necrosis factor-alpha – mediated inflammation and insulin resistance in primary human adipocytes 1 – 3. *Am. J. Clin. Nutr.* 92, 1511–1521.

Chung, S., Lapoint, K., Martinez, K., Kennedy, A., Boysen Sandberg, M., and McIntosh, M. K. (2006). Preadipocytes mediate lipopolysaccharide-induced inflammation and insulin resistance in primary cultures of newly differentiated human adipocytes. *Endocrinology* 147, 5340–5351.

Church, T. S., Willis, M. S., Priest, E. L., Lamonte, M. J., Earnest, C. P., Wilkinson, W. J., et al. (2005). Obesity, macrophage migration inhibitory factor, and weight loss. *Int. J. Obes. (Lond.)* 29, 675–681.

Cline, G. W., Petersen, K. F., Krssak, M., Shen, J., Hundal, R. S., Trajanoski, Z., et al. (1999). Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes. *N. Engl. J. Med.* 341, 240–246.

Codoñer-Franch, P., Valls-Bellés, V., Arilla-Codoñer, A., and Alonso-Iglesias, E. (2011). Oxidant mechanisms in childhood obesity: the link between inflammation and oxidative stress. *Transl. Res.* 158, 369–384.

Cohen-Lahav, M., Shany, S., Tobvin, D., Chaimovitz, C., and Douvdevani, A. (2006). Vitamin D decreases NFkappaB activity by increasing Ikappa-Balpha levels. *Nephrol. Dial. Transplant.* 21, 889–897.

Conus, F., Allison, D. B., Rabasa-Lhoret, R., St-Onge, M., St-Pierre, D. H., Tremblay-Lebeau, A., et al. (2004). Metabolic and behavioral characteristics of metabolically obese but normal-weight women. *J. Clin. Endocrinol. Metab.* 89, 5013–5020.

Couper, K. N., Blount, D. G., and Riley, E. M. (2008). IL-10: the master regulator of immunity to infection. *J. Immunol.* 180, 5771–5777.

Cua, D. J., and Tato, C. M. (2010). Innate IL-17-producing cells: the sentinels of the immune system. *Nat. Rev. Immunol.* 10, 479–489.

Curat, C. A., Miranville, A., Sengene, C., Diehl, M., Tonus, C., Busse, R., et al. (2004). From blood monocytes to adipose tissue-resident macrophages: induction of diapedesis by human mature adipocytes. *Diabetes* 53, 1285–1292.

Dandona, P., Aljada, A., Ghanim, H., Mohanty, P., Tripathy, C., Hofmeyer, D., et al. (2004). Increased plasma concentration of macrophage migration inhibitory factor (MIF) and MIF mRNA in mononuclear cells in the obese and the suppressive action of metformin. *J. Clin. Endocrinol. Metab.* 89, 5043–5047.

De Luca, C., and Olefsky, J. M. (2006). Stressed out about obesity and insulin resistance. *Nat. Med.* 12, 41–42.

De Roos, B., Rungapamestry, V., Ross, K., Rucklidge, G., Reid, M., Duncan, G., et al. (2009). Attenuation of inflammation and cellular stress-related pathways maintains insulin sensitivity in obese type I interleukin-1 receptor knockout mice on a high-fat diet. *Proteomics* 9, 3244–3256.

DeFronzo, R. A., and Tripathy, D. (2009). Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care* 32(Suppl. 2), S157–S163.

Delerive, P., De Bosscher, K., Besnard, S., Vanden Berghe, W., Peters, J. M., Gonzalez, F. J., et al. (1999). Peroxisome proliferator-activated receptor

alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-kappaB and AP-1. *J. Biol. Chem.* 274, 32048–32054.

Delerive, P., De Bosscher, K., Vanden, B. W., Fruchart, J., Haegeman, G., and Staels, B. (2002). DNA binding-independent induction of IkBa gene transcription by PPARalpha. *Mol. Endocrinol.* 16, 1029–1039.

DePaolo, R. W., Abadie, V., Tang, F., Fehlner-Peach, H., Hall, J. A., Wang, W., et al. (2011). Co-adjuvant effects of retinoic acid and IL-15 induce inflammatory immunity to dietary antigens. *Nature* 471, 220–224.

Dinarello, C., Arend, W., Sims, J., Smith, D., Blumberg, H., O'Neill, L., et al. (2010). IL-1 family nomenclature. *Nat. Immunol.* 11, 973.

Dinarello, C. A. (2009). Immunological and inflammatory functions of the interleukin-1 family. *Annu. Rev. Immunol.* 27, 519–550.

Dong, Z. (2003). Molecular mechanism of the chemopreventive effect of resveratrol. *Mutat. Res.* 52, 145–150.

Donnelly, K. L., Smith, C. I., Schwarzenberg, S. J., Jessurun, J., Boldt, M. D., and Parks, E. J. (2005). Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J. Clin. Invest.* 115, 1343–1351.

Dresner, A., Laurent, D., Marcucci, M., Griffin, M. E., Dufour, S., Cline, G. W., et al. (1999). Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J. Clin. Invest.* 103, 253–259.

Du, T., Zhou, Z.-G., You, S., Lin, J., Yang, L., Zhou, W.-D., et al. (2009). Regulation by 1, 25-dihydroxy-vitamin D_3 on altered TLRs expression and response to ligands of monocyte from autoimmune diabetes. *Clin. Chim. Acta* 402, 13313–13318.

Duthie, G. G., and Wood, A. D. (2011). Natural salicylates: foods, functions and disease prevention. *Food Funct.* 2, 515–520.

Eberl, G., Lees, R., Smiley, S. T., Taniguchi, M., Grusby, M. J., and MacDonald, H. R. (1999). Tissue-specific segregation of CD1d-dependent and CD1d-independent NK T cells. *J. Immunol.* 162, 6410–6419.

Ellingsgaard, H., Hauselmann, I., Schuler, B., Habib, A. M., Baggio, L. L., Meier, D. T., et al. (2011). Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. *Nat. Med.* 17, 1481–1489.

Exley, M. A., and Koziel, M. J. (2004). To be or not to be NKT: natural killer T cells in the liver. *Hepatology* 40, 1033–1040.

Fantuzzi, G. (2005). Adipose tissue, adipokines, and inflammation. *J. Allergy Clin. Immunol.* 115, 911–919.

Farrell, G. C., and Larter, C. Z. (2006). Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 43, S99–S112.

Fasshauer, M., Klein, J., Lossner, U., and Paschke, R. (2003). Interleukin (IL)-6 mRNA expression is stimulated by insulin, isoproterenol, tumour necrosis factor alpha, growth hormone, and IL-6 in 3T3-L1 adipocytes. *Horm. Metab. Res.* 35, 147–152.

Feng, D., Ling, W.-H., and Duan, R.-D. (2010). Lycopene suppresses LPS-induced NO and IL-6 production by inhibiting the activation of ERK, p38MAPK, and NF-kappaB in macrophages. *Inflamm. Res.* 59, 115–121.

Ferguson, J. F., Phillips, C. M., Tierney, A. C., Perez-Martinez, P., Defoort, C., Helal, O., et al. (2010). Gene-nutrient interactions in the metabolic syndrome: single nucleotide polymorphisms in ADIPOQ and ADIPOR1 interact with plasma saturated fatty acids to modulate insulin resistance. *Am. J. Clin. Nutr.* 91, 794–801.

Feuerer, M., Herrero, L., Cipolletta, D., Naaz, A., Wong, J., Nayer, A., et al. (2009). Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nat. Med.* 15, 930–939.

Figueras, M., Olivan, M., Busquets, S., López-Soriano, F. J., and Argilés, J. M. (2011). Effects of eicosapentaenoic acid (EPA) treatment on insulin sensitivity in an animal model of diabetes: improvement of the inflammatory status. *Obesity (Silver Spring)* 19, 362–369.

Finkel, T., and Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature* 408, 239–247.

Finucane, O. M., Reynolds, C. M., McGillicuddy, F. C., and Roche, H. M. (2012). Insights into the role of macrophage migration inhibitory factor in obesity and insulin resistance. *Proc. Nutr. Soc.* 71, 622–633.

Fischer-Posovszky, P., Kukulus, V., Tews, D., Unterkircher, T., Debatin, K., Fulda, S., et al. (2010). Resveratrol regulates human adipocyte number and function in a Sirt1-dependent manner. *Am. J. Clin. Nutr.* 92, 5–15.

Flachs, P., Rühl, R., Hensler, M., Janovska, P., Zouhar, P., Kus, V., et al. (2011). Synergistic induction of lipid catabolism and anti-inflammatory lipids in white fat of dietary obese mice in response to calorie restriction and n-3 fatty acids. *Diabetologia* 54, 2626–2638.

Flegal, K. M., Carroll, M. D., Ogden, C. L., and Johnson, C. L. (2002). Prevalence and trends in obesity among US adults, 1999-2000. *JAMA* 288, 1723–1727.

Forman, B. M., Chen, J., and Evans, R. M. (1997). Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. *Proc. Natl. Acad. Sci. U.S.A.* 94, 4312–4317.

Fujii, N., Jessen, N., and Goodyear, L. J. (2006). AMP-activated protein kinase and the regulation of glucose transport. *Am. J. Physiol. Endocrinol. Metab.* 291, E867–E877.

Fujisaka, S., Usui, I., Bukhari, A., Ikutani, M., Oya, T., Kanatani, Y., et al. (2009). Regulatory mechanisms for adipose tissue M1 and M2 macrophages in diet-induced obese mice. *Diabetes* 58, 2574–2582.

Gage, D. (2012). Weight loss/maintenance as an effective tool for controlling type 2 diabetes: novel methodology to sustain weight reduction. *Diabetes Metab. Res. Rev.* 28, 214–218.

Garcia, M. C., Wernstedt, I., Berndtsson, A., Enge, M., Bell, M., Hultgren, O., et al. (2006). Mature-onset obesity in interleukin-1 receptor I knockout mice. *Diabetes* 55, 1205–1213.

Garcia-Diaz, D. F., Campion, J., Quintero, P., Milagro, F. I., Moreno-Aliaga, M. J., and Martinez, J. A. (2011). Vitamin C modulates the interaction between adipocytes and macrophages. *Mol. Nutr. Food Res.* 55(Suppl. 2), S257–S263.

Gautier, E. L., Shay, T., Miller, J., Greter, M., Jakubzick, C., Ivanov, S., et al. (2012). Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat. Immunol.* 13, 1118–1128.

Geissmann, F., Manz, M. G., Jung, S., Sieweke, M. H., Merad, M., and Ley, K. (2010). Development of monocytes, macrophages, and dendritic cells. *Science* 327, 656–661.

Ghanim, H., Aljada, A., Hofmeyer, D., Syed, T., Mohanty, P., and Dandona, P. (2004). Circulating mononuclear cells in the obese are in a proinflammatory state. *Circulation* 110, 1564–1571.

Ghavipour, M., Saedisomeolia, A., Djalali, M., Sotoudeh, G., Eshraghyan, M. R., Malekshahi Moghadam, A., et al. (2012). Tomato juice consumption reduces systemic inflammation in overweight and obese females. *Br. J. Nutr.* 15, 1–5.

Gill, J. M., and Sattar, N. (2009). Ceramides: a new player in the inflammation-insulin resistance paradigm? *Diabetologia* 52, 2475–2477.

Gonzalez-Baro, M. R., Lewin, T. M., and Coleman, R. A. (2007). Regulation of triglyceride metabolism. II. Function of mitochondrial GPAT1 in the regulation of triacylglycerol biosynthesis and insulin action. *Am. J. Physiol. Gastrointest. Liver Physiol.* 292, G1195–G1199.

Gordon, S. (2003). Alternative activation of macrophages. *Nat. Rev. Immunol.* 3, 23–35.

Gouranton, E., Thabuis, C., Riollet, C., Malezet-Desmoulins, C., El Yazidi, C., Amiot, M. J., et al. (2011). Lycopene inhibits proinflammatory cytokine and chemokine expression in adipose tissue. *J. Nutr. Biochem.* 22, 642–648.

Granneman, J. G., Li, P., Zhu, Z., and Lu, Y. (2005). Metabolic and cellular plasticity in white adipose tissue I: effects of beta3-adrenergic receptor activation. *Am. J. Physiol. Endocrinol. Metab.* 289, E608–E616.

Gregoire, F. M., Smas, C. M., and Sul, H. S. (1998). Understanding adipocyte differentiation. *Physiol. Rev.* 78, 783–809.

Gual, P., Le Marchand-Brustel, Y., and Tanti, J. F. (2005). Positive and negative regulation of insulin signaling through IRS-1 phosphorylation. *Biochimie* 87, 99–109.

Gulseth, H. L., Gjelstad, I. M. F., Tierney, A. C., Lovegrove, J. A., Defoort, C., Blaak, E. E., et al. (2010). Predict insulin action or secretion in syndrome. *Diabetes Care* 33, 923–925.

Guo, W., Xie, W., Lei, T., and Hamilton, J. A. (2005). Eicosapentaenoic acid, but not oleic acid, stimulates beta-oxidation in adipocytes. *Lipids* 40, 815–821.

Gustafson, B., and Smith, U. (2006). Cytokines promote Wnt signaling and inflammation and impair the normal differentiation and lipid accumulation in 3T3-L1 preadipocytes. *J. Biol. Chem.* 281, 9507–9516.

Halberg, N., Khan, T., Trujillo, M. E., Wernstedt-Asterholm, I., Attie, A. D., Sherwani, S., et al. (2009). Hypoxia-inducible factor 1alpha induces fibrosis and insulin resistance in

white adipose tissue. *Mol. Cell* 29, 4467–4483.

Hammarstedt, A., and Smith, U. (2003). Thiazolidinediones (PPARgamma ligands) increase IRS-1, UCP-2 and C/EBPalpha expression, but not transdifferentiation, in L6 muscle cells. *Diabetologia* 46, 48–52.

Han, L., Zhou, R., Niu, J., McNutt, M. A., Wang, P., and Tong, T. (2010). SIRT1 is regulated by a PPAR{γ}-SIRT1 negative feedback loop associated with senescence. *Nucleic Acids Res.* 38, 7458–7471.

Hara, K., Horikoshi, M., Yamauchi, T., Yago, H., Miyazaki, O., Ebinuma, H., et al. (2006). Measurement of the high-molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. *Diabetes Care* 29, 1357–1362.

Hare, L. G., Woodside, J. V., and Young, I. S. (2003). Dietary salicylates. *J. Clin. Pathol.* 56, 649–650.

Harford, K. A., Reynolds, C. M., McGillicuddy, F. C., and Roche, H. M. (2011). Fats, inflammation and insulin resistance: insights to the role of macrophage and T-cell accumulation in adipose tissue. *Proc. Nutr. Soc.* 70, 408–417.

Haus, J. M., Kashyap, S. R., Kasumov, T., Zhang, R., Kelly, K. R., Defronzo, R. A., et al. (2009). Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. *Diabetes* 58, 337–343.

Hawkins, M., Barzilai, N., Liu, R., Hu, M., Chen, W., and Rossetti, L. (1997). Role of the glucosamine pathway in fat-induced insulin resistance. *J. Clin. Invest.* 99, 2173–2182.

He, J., Usui, I., Ishizuka, K., Kanatani, Y., Hiratani, K., Iwata, M., et al. (2006). Interleukin-1alpha inhibits insulin signaling with phosphorylating insulin receptor substrate-1 on serine residues in 3T3-L1 adipocytes. *Mol. Endocrinol.* 20, 114–124.

Heinrich, P. C., Behrmann, I., Haan, S., Hermanns, H. M., Muller-Newen, G., and Schaper, F. (2003). Principles of interleukin (IL)-6-type cytokine signalling and its regulation. *Biochem. J.* 15, 1–20.

Hirata, M., Suzuki, M., Ishii, R., Satow, R., Uchida, T., Kitazumi, T., et al. (2011). Genetic defect in phospholipase Cδ1 protects mice from obesity by regulating thermogenesis and adipogenesis. *Diabetes* 60, 1926–1937.

Hirokawa, J., Sakaue, S., Furuya, Y., Ishii, J., Hasegawa, A., Tagami, S., et al. (1998). Tumor necrosis factor-alpha regulates the gene expression of macrophage migration inhibitory factor through tyrosine kinase-dependent pathway in 3T3-L1 adipocytes. *J. Biochem.* 123, 733–739.

Hirokawa, J., Sakaue, S., Tagami, S., Kawakami, Y., Sakai, M., Nishi, S., et al. (1997). Identification of macrophage migration inhibitory factor in adipose tissue and its induction by tumor necrosis factor-alpha. *Biochem. Biophys. Res. Commun.* 235, 94–98.

Hirosumi, J., Tuncman, G., Chang, L., Gorgun, C. Z., Uysal, K. T., Maeda, K., et al. (2002). A central role for JNK in obesity and insulin resistance. *Nature* 420, 333–336.

Hoehn, K. L., Salmon, A. B., Hohnen-Behrens, C., Turner, N., Hoy, A. J., Maghzal, G. J., et al. (2009). Insulin resistance is a cellular antioxidant defense mechanism. *Proc. Natl. Acad. Sci. U.S.A.* 106, 17787–17792.

Holland, W. L., Bikman, B. T., Wang, L. P., Yuguang, G., Sargent, K. M., Bulchand, S., et al. (2011). Lipid-induced insulin resistance mediated by the proinflammatory receptor TLR4 requires saturated fatty acid-induced ceramide biosynthesis in mice. *J. Clin. Invest.* 121, 1858–1870.

Hollenberg, C. H., and Vost, A. (1969). Regulation of DNA synthesis in fat cells and stromal elements from rat adipose tissue. *J. Clin. Invest.* 47, 2485–2498.

Hong, E. G., Ko, H. J., Cho, Y. R., Kim, H. J., Ma, Z., Yu, T. Y., et al. (2009). Interleukin-10 prevents diet-induced insulin resistance by attenuating macrophage and cytokine response in skeletal muscle. *Diabetes* 58, 2525–2535.

Hosogai, N., Fukuhara, A., Oshima, K., Miyata, Y., Tanaka, S., Segawa, K., et al. (2007). Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. *Diabetes* 56, 901–911.

Hotamisligil, G. S., Arner, P., Caro, J. F., Atkinson, R. L., and Spiegelman, B. M. (1995). Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J. Clin. Invest.* 95, 2409–2415.

Hotamisligil, G. S., Budavari, A., Murray, D., and Spiegelman, B. M. (1994). Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-alpha. *J. Clin. Invest.* 94, 1543–1549.

Hotamisligil, G. S., and Erbay, E. (2008). Nutrient sensing and inflammation in metabolic diseases. *Nat. Rev. Immunol.* 8, 923–934.

Hotamisligil, G. S., Shargill, N. S., and Spiegelman, B. M. (1993). Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 259, 87–91.

Hotta, K., Bodkin, N. L., Gustafson, T. A., Yoshioka, S., Ortmeyer, H. K., and Hansen, B. C. (1999). Age-related adipose tissue mRNA expression of ADD1/SREBP1, PPARgamma, lipoprotein lipase, and GLUT4 glucose transporter in rhesus monkeys. *J. Gerontol. A Biol. Sci. Med. Sci.* 54, B183–B188.

Ishii-Yonemoto, T., Masuzaki, H., Yasue, S., Okada, S., Kozuka, C., Tanaka, T., et al. (2010). Glucocorticoid reamplification within cells intensifies NF-kappaB and MAPK signaling and reinforces inflammation in activated preadipocytes. *Am. J. Physiol. Endocrinol. Metab.* 298, E930–E940.

Isoda, K., Sawada, S., Ayaori, M., Matsuki, T., Horai, R., Kagata, Y., et al. (2005). Deficiency of interleukin-1 receptor antagonist deteriorates fatty liver and cholesterol metabolism in hypercholesterolemic mice. *J. Biol. Chem.* 280, 7002–7009.

Jacene, H. A., Cohade, C. C., Zhang, Z., and Wahl, R. L. (2011). The relationship between patients' serum glucose levels and metabolically active brown adipose tissue detected by PET/CT. *Mol. Imaging Biol.* 13, 1278–1283.

Jager, J., Gremeaux, T., Cormont, M., Le Marchand-Brustel, Y., and Tanti, J. F. (2007). Interleukin-1beta-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. *Endocrinology* 148, 241–251.

Jellema, A., Plat, J., and Mensink, R. P. (2004). Weight reduction, but not a moderate intake of fish oil, lowers concentrations of inflammatory markers and PAI-1 antigen in obese men during the fasting and postprandial state. *Eur. J. Clin. Invest.* 34, 766–773.

Jiang, G., Dallas-Yang, Q., Biswas, S., Li, Z., and Zhang, B. B. (2004). Rosiglitazone, an agonist of peroxisome-proliferator-activated receptor γ (PPARγ), decreases inhibitory serine phosphorylation of IRS1 in vitro and in vivo. *Biochem. J.* 15, 339–346.

Jiang, G., Dallas-Yang, Q., Li, Z., Szalkowski, D., Liu, F., Shen, X., et al. (2002). Potentiation of insulin signaling in tissues of Zucker obese rats after acute and long-term treatment with PPARgamma agonists. *Diabetes* 51, 2412–2419.

Joss, A., Akdis, M., Faith, A., Blaser, K., and Akdis, C. A. (2000). IL-10 directly acts on T cells by specifically altering the CD28 co-stimulation pathway. *Eur. J. Immunol.* 30, 1683–1690.

Juge-Aubry, C. E., Somm, E., Giusti, V., Pernin, A., Chicheportiche, R., Verdumo, C., et al. (2003). Adipose tissue is a major source of interleukin-1 receptor antagonist: upregulation in obesity and inflammation. *Diabetes* 52, 1104–1110.

Julien, P., Despres, J., and Angel, A. (1989). Scanning electron microscopy of very small fat cells and mature fat cells in human obesity. *J. Lipid Res.* 30, 293–299.

Kabir, M., Skurnik, G., Naour, N., Pechtner, V., Meugnier, E., Rome, S., et al. (2007). Treatment for 2 mo with n 3 polyunsaturated fatty acids reduces adiposity and some atherogenic factors but does not improve insulin sensitivity in women with type 2 diabetes: a randomized controlled study. *Am. J. Clin. Nutr.* 86, 1670–1679.

Kabon, B., Nagele, A., Reddy, D., Eagon, C., Fleshman, J. W., Sessler, D. I., et al. (2004). Obesity decreases perioperative tissue oxygenation. *Anesthesiology* 100, 274–280.

Karagiannides, I., Tchkonia, T., Dobson, D. E., Steppan, C. M., Cummins, P., Chan, G., et al. (2001). Altered expression of C/EBP family members results in decreased adipogenesis with aging. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 280, R1772–R1780.

Karelis, A. D., Faraj, M., Bastard, J.-P., St-Pierre, D. H., Brochu, M., Prud'homme, D., et al. (2005). The metabolically healthy but obese individual presents a favorable inflammation profile. *J. Clin. Endocrinol. Metab.* 90, 4145–4150.

Kelly, M., Gauthier, M.-S., Saha, A. K., and Ruderman, N. B. (2009). Activation of AMP-activated protein kinase by interleukin-6 in rat skeletal muscle: association with changes in cAMP, energy state, and endogenous fuel mobilization. *Diabetes* 58, 1953–1960.

Kern, P. A., Saghizadeh, M., Ong, J. M., Bosch, R. J., Deem, R., and Simsolo, R. B. (1995). The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J. Clin. Invest.* 95, 2111–2119.

Khan, T., Muise, E. S., Iyengar, P., Wang, Z. V., Chandalia, M., Abate, N., et al. (2009). Metabolic dysregulation and adipose tissue fibrosis: role

of collagen VI. *Mol. Cell. Biol.* 29, 1575–1591.

Kikly, K., Liu, L., Na, S., and Sedgwick, J. D. (2006). The IL-23/Th(17) axis: therapeutic targets for autoimmune inflammation. *Curr. Opin. Immunol.* 18, 670–675.

Kim, G., Kim, J., Ahn, S., Lee, H., and Moon, D. (2004). Lycopene suppresses the lipopolysaccharide-induced phenotypic and functional maturation of murine dendritic cells through inhibition of mitogen-activated protein kinases and nuclear factor-kB. *Immunology* 113, 203–211.

Kinjo, Y., Wu, D., Kim, G., Xing, G., Poles, M. A., Ho, D. D., et al. (2005). Recognition of bacterial glycosphingolipids by natural killer T cells. *Nature* 434, 520–525.

Kishore, P., Li, W., Tonelli, J., Lee, D.-E., Koppaka, S., Zhang, K., et al. (2010). Adipocyte-derived factors potentiate nutrient-induced production of plasminogen activator inhibitor-1 by macrophages. *Sci. Transl. Med.* 2, 20ra15.

Kopecky, J., Rossmeisl, M., Flachs, P., Kuda, O., Brauner, P., Jilkova, Z., et al. (2009). n-3 PUFA: bioavailability and modulation of adipose tissue function. *Proc. Nutr. Soc.* 68, 361–369.

Kopelman, P. G. (2000). Obesity as a medical problem. *Nature* 404, 635–643.

Kopp, H.-P., Krzyzanowska, K., Möhlig, M., Spranger, J., Pfeiffer, A. F. H., and Schernthaner, G. (2005). Effects of marked weight loss on plasma levels of adiponectin, markers of chronic subclinical inflammation and insulin resistance in morbidly obese women. *Int. J. Obes. (Lond.)* 29, 766–771.

Koska, J., Stefan, N., Dubois, S., Trinidad, C., Considine, R. V., Funahashi, T., et al. (2009). mRNA concentrations of MIF in subcutaneous abdominal adipose cells are associated with adipocyte size and insulin action. *Int. J. Obes. (Lond.)* 33, 842–850.

Kosteli, A., Sugaru, E., Haemmerle, G., Martin, J. F., Lei, J., Zechner, R., et al. (2010). Weight loss and lipolysis promote a dynamic immune response in murine adipose tissue. *J. Clin. Invest.* 120, 3466–3479.

Kováciková, M., Sengenes, C., Kovácová, Z., Šiklová-Vítková, M., Klimcáková, E., Polák, J., et al. (2011). Dietary intervention-induced weight loss decreases macrophage content in adipose tissue of obese women. *Int. J. Obes. (Lond.)* 35, 91–98.

Kowalski, G. M., Nicholls, H. T., Risis, S., Watson, N. K., Kanellakis, P., Bruce, C. R., et al. (2011). Deficiency of haematopoietic-cell-derived IL-10 does not exacerbate high-fat-diet-induced inflammation or insulin resistance in mice. *Diabetologia* 54, 888–899.

Kung, J., and Henry, R. R. (2012). Thiazolidinedione safety. *Expert Opin. Drug Saf.* 11, 565–579.

Lagathu, C., Yvan-Charvet, L., Bastard, J. P., Maachi, M., Quignard-Boulange, A., Capeau, J., et al. (2006). Long-term treatment with interleukin-1beta induces insulin resistance in murine and human adipocytes. *Diabetologia* 49, 2162–2173.

Lam, T. K., Yoshii, H., Haber, C. A., Bogdanovic, E., Lam, L., Fantus, I. G., et al. (2002). Free fatty acid-induced hepatic insulin resistance: a potential role for protein kinase C-delta. *Am. J. Physiol. Endocrinol. Metab.* 283, E682–E691.

Lanthier, N., Molendi-Coste, O., Horsmans, Y., Van Rooijen, N., Cani, P. D., and Leclercq, I. A. (2010). Kupffer cell activation is a causal factor for hepatic insulin resistance. *Am. J. Physiol. Gastrointest. Liver Physiol.* 298, G107–G116.

Lee, S.-H., Ha, H.-S., Park, Y.-J., Lee, J.-H., Yim, H.-W., Yoon, K.-H., et al. (2011). Identifying metabolically obese but normal-weight (MONW) individuals in a nondiabetic Korean population: the Chungju Metabolic disease Cohort (CMC) study. *Clin. Endocrinol. (Oxf)* 75, 475–481.

Liu, H.-S., Chen, Y.-H., Hung, P.-F., and Kao, Y.-H. (2006). Inhibitory effect of green tea (-)-epigallocatechin gallate on resistin gene expression in 3T3-L1 adipocytes depends on the ERK pathway. *Am. J. Physiol. Endocrinol. Metab.* 290, E273–E281.

Lorente-Cebrián, S., Bustos, M., Marti, A., Martinez, J. A., and Moreno-Aliaga, M. J. (2009). Eicosapentaenoic acid stimulates AMP-activated protein kinase and increases visfatin secretion in cultured murine adipocytes. *Clin. Sci.* 117, 243–249.

Lorenzo, M., Fernández-Veledo, S., Vila-Bedmar, R., Garcia-Guerra, L., De Alvaro, C., and Nieto-Vazquez, I. (2008). Insulin resistance induced by tumor necrosis factor-alpha in myocytes and brown adipocytes. *J. Anim. Sci.* 86, E94–E104.

Loscher, C. E., Draper, E., Leavy, O., Kelleher, D., Mills, K. H. G., and Roche, H. M. (2005). Conjugated linoleic acid suppresses NF-kappa B activation and IL-12 production in dendritic cells through ERK-mediated IL-10 induction. *J. Immunol.* 175, 4990–4998.

Lottenberg, A. M., Afonso, M. D. S., Lavrador, M. S. F., Machado, R. M., and Nakandakare, E. R. (2012). The role of dietary fatty acids in the pathology of metabolic syndrome. *J. Nutr. Biochem.* 23, 1027–1040.

Lue, H., Thiele, M., Franz, J., Dahl, E., Speckgens, S., Leng, L., et al. (2007). Macrophage migration inhibitory factor (MIF) promotes cell survival by activation of the Akt pathway and role for CSN5/JAB1 in the control of autocrine MIF activity. *Oncogene* 26, 5046–5059.

Lumeng, C. N., Bodzin, J. L., and Saltiel, A. R. (2007). Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J. Clin. Invest.* 117, 175–184.

Lynch, L., Nowak, M., Varghese, B., Clark, J., Hogan, A. E., Toxavidis, V., et al. (2012). Adipose tissue invariant NKT cells protect against diet-induced obesity and metabolic disorder through regulatory cytokine production. *Immunity* 37, 574–587.

Lynch, L. A., O'Connell, J. M., Kwasnik, A. K., Cawood, T. J., O'Farrelly, C., and O'Shea, D. B. (2009a). Are natural killer cells protecting the metabolically healthy obese patient? *Obesity (Silver Spring)* 17, 601–615.

Lynch, L., O'Shea, D., Winter, D. C., Geoghegan, J., Doherty, D. G., and O'Farrelly, C. (2009b). Invariant NKT cells and CD1d(+) cells amass in human omentum and are depleted in patients with cancer and obesity. *Eur. J. Immunol.* 39, 1893–1901.

Mack, I., BelAiba, R. S., Djordjevic, T., Görlach, A., Hauner, H., and Bader, B. L. (2009). Functional analyses reveal the greater potency of preadipocytes compared with adipocytes as endothelial cell activator under normoxia, hypoxia, and TNFalpha exposure. *Am. J. Physiol. Endocrinol. Metab.* 297, E735–E748.

Magkos, F., and Sidossis, L. S. (2007). Recent advances in the measurement of adiponectin isoform distribution. *Curr. Opin. Clin. Nutr. Metab. Care* 10, 571–575.

Maizels, R. M., and Allen, J. E. (2011). Immunology. Eosinophils forestall obesity. *Science* 332, 186–187.

Malozowski, S., Sahlroot, J. T., Larsen, C. M., Faulenbach, M., Vaag, A., Vølund, A., et al. (2007). Interleukin-1-receptor antagonist in type 2 diabetes mellitus. *N. Engl. J. Med.* 357, 302–303.

Martin, T. L., Alquier, T., Asakura, K., Furukawa, N., Preitner, F., and Kahn, B. B. (2006). Diet-induced obesity alters AMP kinase activity in hypothalamus and skeletal muscle. *J. Biol. Chem.* 281, 18933–18941.

Masoro, E. J. (2000). Caloric restriction and aging: an update. *Exp. Gerontol.* 35, 299–305.

Mathias, S., Pena, L. A., and Kolesnick, R. N. (1998). Signal transduction of stress via ceramide. *Biochem. J.* 335(Pt 3), 465–480.

Matsuki, T., Horai, R., Sudo, K., and Iwakura, Y. (2003). IL-1 plays an important role in lipid metabolism by regulating insulin levels under physiological conditions. *J. Exp. Med.* 198, 877–888.

McEneny, J., Wade, L., Young, I. S., Masson, L., Duthie, G., McGinty, A., et al. (2012). Lycopene intervention reduces inflammation and improves HDL functionality in moderately overweight middle-aged individuals. *J. Nutr. Biochem.* 1, 163–168.

McGillicuddy, F. C., Chiquoine, E. H., Hinkle, C. C., Kim, R. J., Shah, R., Roche, H. M., et al. (2009). Interferon gamma attenuates insulin signaling, lipid storage, and differentiation in human adipocytes via activation of the JAK/STAT pathway. *J. Biol. Chem.* 284, 31936–31944.

McGillicuddy, F. C., Harford, K. A., Reynolds, C. M., Oliver, E., Claessens, M., Mills, K. H. G., et al. (2011). Lack of interleukin-1 receptor I (IL-1RI) protects mice from high-fat diet-induced adipose tissue inflammation coincident with improved glucose homeostasis. *Diabetes* 60, 1688–1698.

McGuirk, P., Higgins, S. C., and Mills, K. H. G. (2005). Regulatory cells and the control of respiratory infection. *Curr. Allergy Asthma Rep.* 5, 51–55.

McLaughlin, T., Sherman, A., Tsao, P., Gonzalez, O., Yee, G., Lamendola, C., et al. (2007). Enhanced proportion of small adipose cells in insulin-resistant vs insulin-sensitive obese individuals implicates impaired adipogenesis. *Diabetologia* 50, 1707–1715.

Mills, K. H., and Dunne, A. (2009). Immune modulation: IL-1, master mediator or initiator of inflammation. *Nat. Med.* 15, 1363–1364.

Mitchell, R. A., Liao, H., Chesney, J., Fingerle-Rowson, G., Baugh, J., David, J., et al. (2002). Macrophage migration inhibitory factor (MIF) sustains macrophage proinflammatory function by inhibiting p53: regulatory role in the innate immune response. *Proc. Natl. Acad. Sci. U.S.A.* 99, 345–350.

Mokdad, A. H., Ford, E. S., Bowman, B. A., Dietz, W. H., Vinicor, F., Bales, V. S., et al. (2003). Prevalence of

obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA* 289, 76–79.

Moore, K. W., De Waal Malefyt, R., Coffman, R. L., and O'Garra, A. (2001). Interleukin-10 and the interleukin-10 receptor. *Annu. Rev. Immunol.* 19, 683–765.

Mosser, D. M., and Edwards, J. P. (2008). Exploring the full spectrum of macrophage activation. *Nat. Rev. Immunol.* 8, 958–969.

Moynihan, K. A., Grimm, A. A., Plueger, M. M., Bernal-Mizrachi, E., Ford, E., Cras-Méneur, C., et al. (2005). Increased dosage of mammalian Sir2 in pancreatic beta cells enhances glucose-stimulated insulin secretion in mice. *Cell Metab.* 2, 105–117.

Nathan, C. (2003). Specificity of a third kind: reactive oxygen and nitrogen intermediates in cell signaling. *J. Clin. Invest.* 111, 769–778.

Neschen, S., Morino, K., Rossbacher, C., Pongratz, R. L., Cline, G. W., Sono, S., et al. (2006). Fish oil regulates adiponectin secretion by a receptory-dependent mechanism in mice. *Diabetes* 55, 924–928.

Netea, M. G., Nold-Petry, C. A., Nold, M. F., Joosten, L. A., Opitz, B., Van der Meer, J. H., et al. (2009). Differential requirement for the activation of the inflammasome for processing and release of IL-1beta in monocytes and macrophages. *Blood* 113, 2324–2335.

Neyrinck, A. M., Cani, P. D., Dewulf, E. M., De Backer, F., Bindels, L. B., and Delzenne, N. M. (2009). Critical role of Kupffer cells in the management of diet-induced diabetes and obesity. *Biochem. Biophys. Res. Commun.* 385, 351–356.

Nguyen, M. T. A., Favelyukis, S., Nguyen, A.-K. K., Reichart, D., Scott, P. A., Jenn, A., et al. (2007). A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways. *J. Biol. Chem.* 282, 35279–35292.

Nishimura, S., Manabe, I., Nagasaki, M., Eto, K., Yamashita, H., Ohsugi, M., et al. (2009). CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat. Med.* 15, 914–920.

Nishio, M., Yoneshiro, T., Nakahara, M., Suzuki, S., Saeki, K. K., Hasegawa, M., et al. (2012). Production of functional classical brown adipocytes from human pluripotent stem cells using specific hemopoietin cocktail without gene transfer. *Cell Metab.* 16, 394–406.

O'Connell, J., Lynch, L., Cawood, T. J., Kwasnik, A., Nolan, N., Geoghegan, J., et al. (2010). The relationship of omental and subcutaneous adipocyte size to metabolic disease in severe obesity. *PLoS ONE* 5:e9997. doi:10.1371/journal.pone.0009997

O'Connell, J., Lynch, L., Hogan, A., Cawood, T. J., and O'Shea, D. (2011). Preadipocyte factor-1 is associated with metabolic profile in severe obesity. *J. Clin. Endocrinol. Metab.* 96, E680–E684.

Odegaard, J. I., Ricardo-Gonzalez, R. R., Red Eagle, A., Vats, D., Morel, C. R., Goforth, M. H., et al. (2008). Alternative M2 activation of Kupffer cells by PPARdelta ameliorates obesity-induced insulin resistance. *Cell Metab.* 7, 496–507.

Oh, D. Y., Morinaga, H., Talukdar, S., Bae, E. J., and Olefsky, J. M. (2012). Increased macrophage migration into adipose tissue in obese mice. *Diabetes* 61, 346–354.

Oh, D. Y., Talukdar, S., Bae, E. J., Imamura, T., Morinaga, H., Fan, W., et al. (2010). GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell* 142, 687–698.

Oliver, E., McGillicuddy, F. C., Harford, K. A., Reynolds, C. M., Phillips, C. M., Ferguson, J. F., et al. (2012). Docosahexaenoic acid attenuates macrophage-induced inflammation and improves insulin sensitivity in adipocytes-specific differential effects between LC n-3 PUFA. *J. Nutr. Biochem.* 23, 1192–1200.

Orr, J. S., Puglisi, M. J., Ellacott, K. L. J., Lumeng, C. N., Wasserman, D. H., and Hasty, A. H. (2012). Toll-like receptor 4 deficiency promotes the alternative activation of adipose tissue macrophages. *Diabetes* 61, 2718–2727.

Ortega, F. B., Lee, D.-C., Katzmarzyk, P. T., Ruiz, J. R., Sui, X., Church, T. S., et al. (2013). The intriguing metabolically healthy but obese phenotype: cardiovascular prognosis and role of fitness. *Eur. Heart J.* 34, 389–397.

Osborn, O., and Olefsky, J. M. (2012). The cellular and signaling networks linking the immune system and metabolism in disease. *Nat. Med.* 18, 363–374.

O'Shea, D., Cawood, T. J., O'Farrelly, C., and Lynch, L. (2010). Natural killer cells in obesity: impaired function and increased susceptibility to the effects of cigarette smoke. *PLoS ONE* 5:e8660. doi:10.1371/journal.pone.0008660

Ostrowski, K., Rohde, T., Zacho, M., Asp, S., and Pedersen, B. K. (1998). Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. *J. Physiol. (Lond.)* 508, 949–953.

O'Sullivan, A., Balducci, D., Paradisi, F., Cashman, K. D., Gibney, M. J., and Brennan, L. (2011). Effect of supplementation with vitamin D_3 on glucose production pathways in human subjects. *Mol. Nutr. Food Res.* 55, 1018–1025.

Ouellet, V., Routhier-Labadie, A., Bellemare, W., Lakhal-Chaieb, L., Turcotte, E., Carpentier, A. C., et al. (2011). Outdoor temperature, age, sex, body mass index, and diabetic status determine the prevalence, mass, and glucose-uptake activity of 18F-FDG-detected BAT in humans. *J. Clin. Endocrinol. Metab.* 96, 192–199.

Park, S. Y., Jeong, K. J., Lee, J., Yoon, D. S., Choi, W. S., Kim, Y. K., et al. (2007). Hypoxia enhances LPA-induced HIF-1alpha and VEGF expression: their inhibition by resveratrol. *Cancer Lett.* 258, 63–69.

Pedersen, B. K. (2011). Muscles and their myokines. *J. Exp. Biol.* 214, 337–346.

Pedersen, B. K., and Febbraio, M. A. (2008). Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol. Rev.* 88, 1379–1406.

Pedersen, B. K., and Febbraio, M. A. (2012). Muscles, exercise and obesity: skeletal muscle as a secretory organ. *Nat Rev Endocrinol* 8, 457–465.

Petersen, K. F., and Shulman, G. I. (2002). Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. *Am. J. Cardiol.* 90, 11G–18G.

Phillips, C., Tierney, A., Perze-Martinez, P., Defoort, C., Blaak, E., Gjelstad, I., et al. (2013). Obesity and body fat classification in the metabolic syndrome: impact on cardiometabolic risk metabotype. *Obesity (Silver Spring)* 21, E154–E161.

Phillips, C. M., Goumidi, L., Bertrais, S., Ferguson, J. F., Field, M. R., Kelly, E. D., et al. (2009a). Complement component 3 polymorphisms interact with polyunsaturated fatty acids to modulate risk of metabolic syndrome 1 – 3. *Am. J. Clin. Nutr.* 90, 1665–1673.

Phillips, C. M., Goumidi, L., Bertrais, S., Field, M. R., Peloso, G. M., Shen, J., et al. (2009b). Dietary saturated fat modulates the association between STAT3 polymorphisms and abdominal obesity in adults. *J. Nutr.* 139, 2011–2017.

Pot, G. K., Brouwer, I. A., Enneman, A., Rijkers, G. T., Kampman, E., and Geelen, A. (2009). No effect of fish oil supplementation on serum inflammatory markers and their interrelationships: a randomized controlled trial in healthy, middle-aged individuals. *Eur. J. Clin. Nutr.* 63, 1353–1359.

Poulain-Godefroy, O., and Froguel, P. (2007). Preadipocyte response and impairment of differentiation in an inflammatory environment. *Biochem. Biophys. Res. Commun.* 356, 662–667.

Poulsen, M. M., Vestergaard, P. F., Clasen, B. F., Radko, Y., Christensen, L. P., Stødkilde-Jørgensen, H., et al. (2012). High-dose resveratrol supplementation in obese men. *Diabetes* 62, 1186–1195.

Pricola, K. L., Kuhn, N. Z., Haleem-Smith, H., Song, Y., and Tuan, R. S. (2009). Interleukin-6 maintains bone marrow-derived mesenchymal stem cell stemness by an ERK1/2-dependent mechanism. *J. Cell. Biochem.* 108, 577–588.

Prieur, X., Mok, C. Y. L., Velagapudi, V. R., Núñez, V., Fuentes, L., Montaner, D., et al. (2011). Differential lipid partitioning between adipocytes and tissue macrophages modulates macrophage lipotoxicity and M2/M1 polarization in obese mice. *Diabetes* 60, 797–809.

Reynolds, C. M., McGillicuddy, F. C., Harford, K. A., Finucane, O. M., Mills, K. H. G., and Roche, H. M. (2012). Dietary saturated fatty acids prime the NLRP3 inflammasome via TLR4 in dendritic cells-implications for diet-induced insulin resistance. *Mol. Nutr. Food Res.* 56, 1212–1222.

Ricote, M., Li, A. C., Willson, T. M., Kelly, C. J., and Glass, C. K. (1998). The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* 391, 79–82.

Roden, M., Price, T. B., Perseghin, G., Petersen, K. F., Rothman, D. L., Cline, G. W., et al. (1996). Mechanism of free fatty acid-induced insulin resistance in humans. *J. Clin. Invest.* 97, 2859–2865.

Roger, T., David, J., Glauser, M. P., and Calandra, T. (2001). MIF regulates innate immune responses through modulation of Toll-like receptor 4. *Nature* 414, 920–924.

Rossetti, L., Giaccari, A., and DeFronzo, R. A. (1990). Glucose toxicity. *Diabetes Care* 13, 610–630.

Ruan, H., Hacohen, N., Golub, T. R., Van Parijs, L., and Lodish, H. F. (2002). Tumor necrosis factor-alpha suppresses adipocyte-specific genes and activates expression of preadipocyte genes in 3T3-L1 adipocytes: nuclear factor-kappaB activation by TNF-alpha is obligatory. *Diabetes* 51, 1319–1336.

Ruderman, N. B., Schneider, S. H., and Berchtold, P. (1981). The "metabolically-obese," normal-weight individual. *Am. J. Clin. Nutr.* 34, 1617–1621.

Ruge, T., Lockton, J. A., Renstrom, F., Lystig, T., Sukonina, V., Svensson, M. K., et al. (2009). Acute hyperinsulinemia raises plasma interleukin-6 in both nondiabetic and type 2 diabetes mellitus subjects, and this effect is inversely associated with body mass index. *Metab. Clin. Exp.* 58, 860–866.

Saberi, M., Woods, N.-B. B., De Luca, C., Schenk, S., Lu, J. C., Bandyopadhyay, G., et al. (2009). Hematopoietic cell-specific deletion of toll-like receptor 4 ameliorates hepatic and adipose tissue insulin resistance in high-fat-fed mice. *Cell Metab.* 10, 419–429.

Sadeghi, K., Wessner, B., Laggner, U., Ploder, M., Tamandl, D., Friedl, J., et al. (2006). Vitamin D_3 down-regulates monocyte TLR expression and triggers hyporesponsiveness to pathogen-associated molecular patterns. *Eur. J. Immunol.* 36, 361–370.

Saghizadeh, M., Ong, J. M., Garvey, W. T., Henry, R. R., and Kern, P. A. (1996). The expression of TNF-a by human muscle. *J. Clin. Invest.* 97, 1111–1116.

Saito, M., Okamatsu-ogura, Y., Matsushita, M., Watanabe, K., Yoneshiro, T., Nio-kobayashi, J., et al. (2009). High incidence of metabolically active brown adipose effects of cold exposure and adiposity. *Diabetes* 58, 1526–1531.

Sallusto, F., and Lanzavecchia, A. (1994). Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α. *J. Exp. Med.* 179, 1109–1118.

Sanchez-Zamora, Y., Terrazas, L. I., Vilches-Flores, A., Leal, E., Juárez, I., Whitacre, C., et al. (2010). Macrophage migration inhibitory factor is a therapeutic target in treatment of non-insulin-dependent diabetes mellitus. *FASEB J.* 24, 2583–2590.

Saraiva, M., and O'Garra, A. (2010). The regulation of IL-10 production by immune cells. *Nat. Rev. Immunol.* 10, 170–181.

Sauter, N. S., Schulthess, F. T., Galasso, R., Castellani, L. W., and Maedler, K. (2008). The antiinflammatory cytokine interleukin-1 receptor antagonist protects from high-fat diet-induced hyperglycemia. *Endocrinology* 149, 2208–2218.

Scheele, C., Nielsen, S., Kelly, M., Broholm, C., Nielsen, A. R., Taudorf, S., et al. (2012). Satellite cells derived from obese humans with type 2 diabetes and differentiated into myocytes in vitro exhibit abnormal response to IL-6. *PLoS ONE* 7:e39657. doi:10.1371/journal.pone.0039657

Schenk, S., Saberi, M., and Olefsky, J. M. (2008). Insulin sensitivity: modulation by nutrients and inflammation. *J. Clin. Invest.* 118, 2992–3002.

Scherer, P. E., Williams, S., Fogliano, M., Baldini, G., and Lodish, H. F. (1995). A novel serum protein similar to C1q, produced exclusively in adipocytes. *J. Biol. Chem.* 270, 26746–26749.

Schipper, H. S., Rakhshandehroo, M., Graaf, S. F. J., Van De Venken, K., Koppen, A., Stienstra, R., et al. (2012). Natural killer T cells in adipose tissue prevent insulin resistance. *J. Clin. Invest.* 122, 3343–3354.

Schottelius, A. J., Mayo, M. W., Sartor, R. B., and Baldwin, A. S. Jr. (1999). Interleukin-10 signaling blocks inhibitor of kappaB kinase activity and nuclear factor kappaB DNA binding. *J. Biol. Chem.* 274, 31868–31874.

Schwarz, J. M., Linfoot, P., Dare, D., and Aghajanian, K. (2003). Hepatic de novo lipogenesis in normoinsulinemic and hyperinsulinemic subjects consuming high-fat, low-carbohydrate and low-fat, high-carbohydrate isoenergetic diets. *Am. J. Clin. Nutr.* 77, 43–50.

Seppala-Lindroos, A., Vehkavaara, S., Hakkinen, A. M., Goto, T., Westerbacka, J., Sovijarvi, A., et al. (2002). Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J. Clin. Endocrinol. Metab.* 87, 3023–3028.

Serre-Beinier, V., Toso, C., Morel, P., Gonelle-Gispert, C., Veyrat-Durebex, C., Rohner-Jeanrenaud, F., et al. (2010). Macrophage migration inhibitory factor deficiency leads to age-dependent impairment of glucose homeostasis in mice. *J. Endocrinol.* 206, 297–306.

Sethi, J. K., and Vidal-Puig, A. J. (2007). Thematic review series: adipocyte biology. Adipose tissue function and plasticity orchestrate nutritional adaptation. *J. Lipid Res.* 48, 1253–1262.

Seto-Young, D., Avtanski, D., Strizhevsky, M., Parikh, G., Patel, P., Kaplun, J., et al. (2007). Interactions among peroxisome proliferator activated receptor-gamma, insulin signaling pathways, and steroidogenic acute regulatory protein in human ovarian cells. *J. Clin. Endocrinol. Metab.* 92, 2232–2239.

Shi, F. D., Wang, H. B., Li, H., Hong, S., Taniguchi, M., Link, H., et al. (2000). Natural killer cells determine the outcome of B cell-mediated autoimmunity. *Nat. Immunol.* 1, 245–251.

Shi, H., Kokoeva, M. V., Inouye, K., Tzameli, I., Yin, H., and Flier, J. S. (2006). TLR4 links innate immunity and fatty acid-induced insulin resistance. *J. Clin. Invest.* 116, 3015–3025.

Shin, M.-K., Drager, L. F., Yao, Q., Bevans-Fonti, S., Yoo, D.-Y., Jun, J. C., et al. (2012). Metabolic consequences of high-fat diet are attenuated by suppression of HIF-1α. *PLoS ONE* 7:e46562. doi:10.1371/journal.pone.0046562

Shoelson, S. E., Lee, J., and Yuan, M. (2003). Inflammation and the IKK beta/I kappa B/NF-kappa B axis in obesity- and diet-induced insulin resistance. *Int. J. Obes. Relat. Metab. Disord.* 27(Suppl. 3), S49–S52.

Shulman, G. I. (2000). Cellular mechanisms of insulin resistance. *J. Clin. Invest.* 106, 171–176.

Shulman, G. I., Rothman, D. L., Jue, T., Stein, P., DeFronzo, R. A., and Shulman, R. G. (1990). Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. *N. Engl. J. Med.* 322, 223–228.

Singh, S., and Aggarwal, B. B. (1995). Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane) [corrected]. *J. Biol. Chem.* 270, 24995–25000.

Skurk, T., Herder, C., Kraft, I., Muller-Scholze, S., Hauner, H., and Kolb, H. (2005). Production and release of macrophage migration inhibitory factor from human adipocytes. *Endocrinology* 146, 1006–1011.

Sohal, R. S., Agarwal, S., Candas, M., Forster, M. J., and Lal, H. (1994). Effect of age and caloric restriction on DNA oxidative damage in different tissues of C57BL/6 mice. *Mech. Ageing Dev.* 76, 215–224.

Sohal, R. S., and Weindruch, R. (1996). Oxidative stress, caloric restriction, and aging. *Science* 273, 59–63.

Spranger, J., Kroke, A., Mohlig, M., Hoffmann, K., Bergmann, M. M., Ristow, M., et al. (2003). Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Diabetes* 52, 812–817.

Steensberg, A., Van Hall, G., Osada, T., Sacchetti, M., Saltin, B., and Klarlund Pedersen, B. (2000). Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J. Physiol. (Lond.)* 529, 237–242.

Stefan, N., Kantartzis, K., Machann, J., Schick, F., Thamer, C., Rittig, K., et al. (2008). Identification and characterization of metabolically benign obesity in humans. *Arch. Intern. Med.* 168, 1609–1616.

Stefanovic-Racic, M., Yang, X., Turner, M. S., Mantell, B. S., Stolz, D. B., Sumpter, T. L., et al. (2012). Dendritic cells promote macrophage infiltration and comprise a substantial proportion of obesity-associated increases in CD11c+ cells in adipose tissue and liver. *Diabetes* 61, 2330–2339.

Stienstra, R., Duval, C., Müller, M., and Kersten, S. (2007). PPARs, obesity, and inflammation. *PPAR Res.* 2007, 95974.

Stienstra, R., Joosten, L. A., Koenen, T., Van Tits, B., Van Diepen, J. A., Van den Berg, S. A., et al. (2010). The inflammasome-mediated caspase-1 activation controls adipocyte differentiation and insulin sensitivity. *Cell Metab.* 12, 593–605.

Strissel, K. J., DeFuria, J., Shaul, M. E., Bennett, G., Greenberg, A. S., and Obin, M. S. (2010). T-cell recruitment and Th1 polarization in adipose tissue during diet-induced obesity in C57BL/6 mice. *Obesity (Silver Spring)* 18, 1918–1925.

Stylianou, E., and Saklatvala, J. (1998). Interleukin-1. *Int. J. Biochem. Cell Biol.* 30, 1075–1079.

Succurro, E., Marini, M. A., Frontoni, S., Hribal, M. L., Andreozzi, F., Lauro, R., et al. (2008). Insulin secretion in metabolically obese, but normal weight, and in metabolically healthy but obese individuals. *Obesity (Silver Spring)* 16, 1881–1886.

Summers, S. A. (2006). Ceramides in insulin resistance and lipotoxicity. *Prog. Lipid Res.* 45, 42–72.

Sun, C., Zhang, F., Ge, X., Yan, T., Chen, X., Shi, X., et al. (2007). SIRT1 improves insulin sensitivity under insulin-resistant conditions by repressing PTP1B. *Cell Metab.* 6, 307–319.

Sun, K., Kusminski, C. M., and Scherer, P. E. (2011). Adipose tissue remodeling and obesity. *J. Clin. Invest.* 121, 2094–2101.

Tack, C. J., Stienstra, R., Joosten, L. A., and Netea, M. G. (2012). Inflammation links excess fat to insulin resistance: the role of the interleukin-1 family. *Immunol. Rev.* 249, 239–252.

Tamura, S., and Shimomura, I. (2005). Contribution of adipose tissue and de novo lipogenesis to nonalcoholic fatty liver disease. *J. Clin. Invest.* 115, 1139–1142.

Thies, F., Masson, L. F., Rudd, A., Vaughan, N., Tsang, C., Brittenden, J., et al. (2012). Effect of a tomato-rich diet on markers of cardiovascular disease risk in moderately overweight, disease-free, middle-aged adults: a randomized controlled trial. *Am. J. Clin. Nutr.* 95, 1013–1022.

Tierney, A., McMonagle, J., Shaw, D. I., Gulseth, H. L., Helal, O., Saris, W. H. M., et al. (2011). Effects of dietary fat modification on insulin sensitivity and on other risk factors of the metabolic syndrome – LIPGENE: a European randomized dietary intervention study. *Int. J. Obes. (Lond.)* 35, 800–809.

Tiganis, T. (2011). Reactive oxygen species and insulin resistance: the good, the bad and the ugly. *Trends Pharmacol. Sci.* 32, 82–89.

Tilg, H., and Moschen, A. R. (2008). Inflammatory mechanisms in the regulation of insulin resistance. *Mol. Med.* 14, 222–231.

Timmers, S., Konings, E., Bilet, L., Houtkooper, R. H., Van de Weijer, T., Goossens, G. H., et al. (2011). Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. *Cell Metab.* 14, 612–622.

Tishinsky, J. M., Ma, D. W. L., and Robinson, L. E. (2011). Eicosapentaenoic acid and rosiglitazone increase adiponectin in an additive and PPARγ-dependent manner in human adipocytes. *Obesity (Silver Spring)* 19, 262–268.

Toh, M. L., Aeberli, D., Lacey, D., Yang, Y., Santos, L. L., Clarkson, M., et al. (2006). Regulation of IL-1 and TNF receptor expression and function by endogenous macrophage migration inhibitory factor. *J. Immunol.* 177, 4818–4825.

Tomlinson, J. W., Moore, J., Cooper, M. S., Bujalska, I., Shahmanesh, M., Burt, C., et al. (2001). Regulation of expression of 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue: tissue-specific induction by cytokines. *Endocrinology* 142, 1982–1989.

Tsang, J. Y. S., Li, D., Ho, D., Peng, J., Xu, A., Lamb, J., et al. (2011). Novel immunomodulatory effects of adiponectin on dendritic cell functions. *Int. Immunopharmacol.* 11, 604–609.

Tsukumo, D. M. L., Carvalho-Filho, M. A., Carvalheira, J. B. C., Prada, P. O., Hirabara, S. M., Schenka, A. A., et al. (2007). Loss-of-function mutation in Toll-like receptor 4 prevents diet-induced obesity and insulin resistance. *Diabetes* 56, 1986–1998.

Unger, R. (2003). Lipid overload and overflow: metabolic trauma and the metabolic syndrome. *Trends Endocrinol. Metab.* 14, 398–403.

Utzschneider, K. M., and Kahn, S. E. (2006). Review: the role of insulin resistance in nonalcoholic fatty liver disease. *J. Clin. Endocrinol. Metab.* 91, 4753–4761.

Uysal, K. T., Wiesbrock, S. M., Marino, M. W., and Hotamisligil, G. S. (1997). Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature* 389, 610–614.

Van Exel, E., Gussekloo, J., De Craen, A. J. M., Frölich, M., Bootsma-Van Der Wiel, A., Westendorp, R. G. J., et al. (2002). Low production capacity of interleukin-10 associates with the metabolic syndrome and type 2 diabetes: the Leiden 85-Plus Study. *Diabetes* 51, 1088–1092.

Van Marken Lichtenbelt, W. D., Vanhommerig, J. W., Smulders, N. M., Drossaerts, J. M. A. F. L., Kemerink, G. J., Bouvy, N. D., et al. (2009). Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* 360, 1500–1508.

Vandanmagsar, B., Youm, Y.-H. H., Ravussin, A., Galgani, J. E., Stadler, K., Mynatt, R. L., et al. (2011). The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nat. Med.* 17, 179–188.

Varga, T., Czimmerer, Z., and Nagy, L. (2011). PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation. *Biochim. Biophys. Acta* 1812, 1007–1022.

Verschuren, L., Kooistra, T., Bernhagen, J., Voshol, P. J., Ouwens, D. M., Van Erk, M., et al. (2009). MIF deficiency reduces chronic inflammation in white adipose tissue and impairs the development of insulin resistance, glucose intolerance, and associated atherosclerotic disease. *Circ. Res.* 105, 99–107.

Verstrepen, L., Bekaert, T., Chau, T. L., Tavernier, J., Chariot, A., and Beyaert, R. (2008). TLR-4, IL-1R and TNF-R signaling to NF-kappaB: variations on a common theme. *Cell. Mol. Life Sci.* 65, 2964–2978.

Vijgen, G. H. E. J., Bouvy, N. D., Teule, G. J. J., Brans, B., Schrauwen, P., and Van Marken Lichtenbelt, W. D. (2011). Brown adipose tissue in morbidly obese subjects. *PLoS ONE* 6:e17247. doi:10.1371/journal.pone.0017247

Virtue, S., and Vidal-Puig, A. (2008). It's not how fat you are, it's what you do with it that counts. *PLoS Biol.* 6:e237. doi:10.1371/journal.pbio.0060237

Vozarova, B., Stefan, N., Hanson, R., Lindsay, R. S., Bogardus, C., Tataranni, P. A., et al. (2002). Plasma concentrations of macrophage migration inhibitory factor are elevated in Pima Indians compared to Caucasians and are associated with insulin resistance. *Diabetologia* 45, 1739–1741.

Vozarova, B., Weyer, C., Hanson, K., Tataranni, P. A., Bogardus, C., and Pratley, R. E. (2001). Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. *Obes. Res.* 9, 414–417.

Weatherill, A. R., Lee, J. Y., Zhao, L., Lemay, D. G., Youn, H. S., and Hwang, D. H. (2005). Saturated and polyunsaturated fatty acids reciprocally modulate dendritic cell functions mediated through TLR4. *J. Immunol.* 174, 5390–5397.

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., and Ferrante, A. W. Jr. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* 112, 1796–1808.

Weldon, S. M., Mullen, A. C., Loscher, C. E., Hurley, L. A., and Roche, H. M. (2007). Docosahexaenoic acid induces an anti-inflammatory profile in lipopolysaccharide-stimulated human THP-1 macrophages more effectively than eicosapentaenoic acid. *J. Nutr. Biochem.* 18, 250–258.

Wen, H., Gris, D., Lei, Y., Jha, S., Zhang, L., Huang, M. T., et al. (2011). Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. *Nat. Immunol.* 12, 408–415.

Wieckowska, A., Papouchado, B. G., Li, Z., Lopez, R., Zein, N. N., and Feldstein, A. E. (2008). Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am. J. Gastroenterol.* 103, 1372–1379.

Winer, S., Chan, Y., Paltser, G., Truong, D., Tsui, H., Bahrami, J., et al. (2009a). Normalization of obesity-associated insulin resistance through immunotherapy. *Nat. Med.* 15, 921–929.

Winer, S., Paltser, G., Chan, Y., Tsui, H., Engleman, E., Winer, D., et al. (2009b). Obesity predisposes to Th17 bias. *Eur. J. Immunol.* 39, 2629–2635.

Wolfrum, C., Asilmaz, E., Luca, E., Friedman, J. M., and Stoffel, M. (2004). Foxa2 regulates lipid metabolism and ketogenesis in the liver during fasting and in diabetes. *Nature* 432, 1027–1032.

Wood, A., Baxter, G., Thies, F., Kyle, J., and Duthie, G. (2011). A systematic review of salicylates in foods: estimated daily intake of a Scottish population. *Mol. Nutr. Food Res.* 55(Suppl. 1), S7–S14.

Wu, D., Molofsky, A. B., Liang, H.-E., Ricardo-Gonzalez, R. R., Jouihan, H. A., Bando, J. K., et al. (2011). Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. *Science* 332, 243–247.

Wu, K. K., Sanduja, R., Tsai, A. L., Ferhanoglu, B., and Loose-Mitchell, D. S. (1991). Aspirin inhibits interleukin 1-induced prostaglandin H synthase expression in cultured endothelial cells. *Proc. Natl. Acad. Sci. U.S.A.* 88, 2384–2387.

Xu, J., Morinaga, H., Oh, D., Li, P., Chen, A., Talukdar, S., et al. (2012). GPR105 ablation prevents inflammation and improves insulin sensitivity in mice with diet-induced obesity. *J. Immunol.* 189, 1992–1999.

Xu, X. M., Sansores-Garcia, L., Chen, X. M., Matijevic-Aleksic, N., Du, M., and Wu, K. K. (1999). Suppression of inducible cyclooxygenase 2 gene transcription by aspirin and sodium salicylate. *Proc. Natl. Acad. Sci. U.S.A.* 96, 5292–5297.

Xue, B., Yang, Z., Wang, X., and Shi, H. (2012). Omega-3 polyunsaturated fatty acids antagonize macrophage inflammation via activation of AMPK/SIRT1 pathway. *PLoS ONE* 7:e45990. doi:10.1371/journal.pone.0045990

Yadav, P. K., Chen, C., and Liu, Z. (2011). Potential role of NK cells in the pathogenesis of inflammatory bowel

disease. *J. Biomed. Biotechnol.* 2011, 348530.

Yamasaki, M., Nagatomo, T., Matsuyama, T., Ikeho, Y., Kato, E., Nishiyama, K., et al. (2012). Conjugated linoleic acids inhibit hypoxia inducible factor-1α stabilization under hypoxic condition in human hepatocellular carcinoma cells. *J. Oleo Sci.* 496, 491–496.

Yang, F., Oz, H. S., Barve, S., De Villiers, W. J., McClain, C. J., and Varilek, G. W. (2001). The green tea polyphenol (-)-epigallocatechin-3-gallate blocks nuclear factor-kappa B activation by inhibiting I kappa B kinase activity in the intestinal epithelial cell line IEC-6. *Mol. Pharmacol.* 60, 528–533.

Yang, Z., Hulver, M., McMillan, R. P., Cai, L., Kershaw, E. E., Yu, L., et al. (2012). Regulation of insulin and leptin signaling by muscle suppressor of cytokine signaling 3 (SOCS3). *PLoS ONE* 7:e47493. doi:10.1371/journal.pone.0047493

Yazbeck, R., Howarth, G. S., and Abbott, C. A. (2009). Dipeptidyl peptidase inhibitors, an emerging drug class for inflammatory disease? *Trends Pharmacol. Sci.* 30, 600–607.

Ye, J., Gao, Z., Yin, J., and He, Q. (2007). Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice. *Am. J. Physiol. Endocrinol. Metab.* 293, E1118–E1128.

Yoneshiro, T., Aita, S., Matsushita, M., Okamatsu-Ogura, Y., Kameya, T., Kawai, Y., et al. (2011). Age-related decrease in cold-activated brown adipose tissue and accumulation of body fat in healthy humans. *Obesity (Silver Spring)* 19, 1755–1760.

Yoshino, J., Conte, C., Fontana, L., Mittendorfer, B., Imai, S.-I., Schechtman, K. B., et al. (2012). Resveratrol supplementation does not improve metabolic function in nonobese women with normal glucose tolerance. *Cell Metab.* 16, 658–664.

Yu, C., Chen, Y., Cline, G. W., Zhang, D., Zong, H., Wang, Y., et al. (2002). Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. *J. Biol. Chem.* 277, 50230–50236.

Yuan, M., Konstantopoulos, N., Lee, J., Hansen, L., Li, Z. W., Karin, M., et al. (2001). Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* 293, 1673–1677.

Zainal, T. A., Oberley, T. D., Allison, D. B., Szweda, L. I., and Weindruch, R. (2000). Caloric restriction of rhesus monkeys lowers oxidative damage in skeletal muscle. *FASEB J.* 14, 1825–1836.

Zeyda, M., Säemann, M. D., Stuhlmeier, K. M., Mascher, D. G., Nowotny, P. N., Zlabinger, G. J., et al. (2005). Polyunsaturated fatty acids block dendritic cell activation and function independently of NF-kappaB activation. *J. Biol. Chem.* 280, 14293–14301.

Zhang, Q., Tang, X., Lu, Q. Y., Zhang, Z. F., Brown, J., and Le, A. D. (2005). Resveratrol inhibits hypoxia-induced accumulation of hypoxia-inducible factor-1alpha and VEGF expression in human tongue squamous cell carcinoma and hepatoma cells. *Mol. Cancer Ther.* 4, 1465–1474.

Zhong, J., Rao, X., Deiuliis, J., Braunstein, Z., Narula, V., Hazey, J., et al. (2013). A potential role for dendritic cell/macrophage-expressing DPP4 in obesity-induced visceral inflammation. *Diabetes* 62, 149–157.

Zhou, D., Mattner, J., Cantu, C., Schrantz, N., Yin, N., Gao, Y., et al. (2004). Lysosomal glycosphingolipid recognition by NKT cells. *Science* 306, 1786–1789.

Zhu, J., and Paul, W. E. (2008). CD4 T cells: fates, functions, and faults. *Blood* 112, 1557–1569.

Zittermann, S. I., and Issekutz, A. C. (2006). Basic fibroblast growth factor (bFGF, FGF-2) potentiates leukocyte recruitment to inflammation by enhancing endothelial adhesion molecule expression. *Am. J. Pathol.* 168, 835–846.

Zuk, P. A., Zhu, M., Mizuno, H., Huang, J., Futrell, J. W., Katz, A. J., et al. (2001). Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng.* 7, 211–228.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 10 December 2012; accepted: 22 April 2013; published online: 10 May 2013.

Citation: McArdle MA, Finucane OM, Connaughton RM, McMorrow AM and Roche HM (2013) Mechanisms of obesity-induced inflammation and insulin resistance: insights into the emerging role of nutritional strategies. Front. Endocrinol. ***4****:52. doi: 10.3389/fendo.2013.00052*

This article was submitted to Frontiers in Diabetes, a specialty of Frontiers in Endocrinology.

Copyright © 2013 McArdle, Finucane, Connaughton, McMorrow and Roche. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.

frontiers in
ENDOCRINOLOGY

REVIEW ARTICLE
published: 08 August 2013
doi: 10.3389/fendo.2013.00093

Recent advances in obesity-induced inflammation and insulin resistance

Sanshiro Tateya[1], Francis Kim[2,3] and Yoshikazu Tamori[1,4]*

[1] *Department of Internal Medicine, Division of Diabetes, Metabolism, and Endocrinology, Graduate School of Medicine, Kobe University, Kobe, Japan*
[2] *Department of Medicine, University of Washington, Seattle, WA, USA*
[3] *Diabetes and Obesity Center of Excellence, University of Washington, Seattle, WA, USA*
[4] *Department of Internal Medicine, Diabetes Center, Chibune Hospital, Osaka, Japan*

Edited by:
Tsuguhito Ota, Kanazawa University, Japan

Reviewed by:
Undurti Narasimha Das, UND Life Sciences, USA
Venu Lagishetty, University of California Los Angeles, USA

Correspondence:
Yoshikazu Tamori, Department of Internal Medicine, Division of Diabetes, Metabolism, and Endocrinology, Graduate School of Medicine, Kobe University, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan
e-mail: tamori@med.kobe-u.ac.jp

It has been demonstrated in rodents and humans that chronic inflammation characterized by macrophage infiltration occurs mainly in adipose tissue or liver during obesity, in which activation of immune cells is closely associated with insulin sensitivity. Macrophages can be classified as classically activated (M1) macrophages that support microbicidal activity or alternatively activated (M2) macrophages that support allergic and antiparasitic responses. In the context of insulin action, M2 macrophages sustain insulin sensitivity by secreting IL-4 and IL-10, while M1 macrophages induce insulin resistance through the secretion of proinflammatory cytokines, such as TNFα. Polarization of M1/M2 is controlled by various dynamic functions of other immune cells. It has been demonstrated that, in a lean state, T_H2 cells, T_{reg} cells, natural killer T cells, or eosinophils contribute to the M2 activation of macrophages by secreting IL-4 or IL-10. In contrast, obesity causes alteration of the constituent immune cells, in which T_H1 cells, B cells, neutrophils, or mast cells induce M1 activation of macrophages by the elevated secretion of TNFα and IFNγ. Increased secretion of TNFα and free fatty acids from hypertrophied adipocytes also contributes to the M1 activation of macrophages. Since obesity-induced insulin resistance is established by macrophage infiltration and the activation of immune cells inside tissues, identification of the factors that regulate accumulation and the intracellular signaling cascades that define polarization of M1/M2 would be indispensable. Regulation of these factors would lead to the pharmacological inhibition of obesity-induced insulin resistance. In this review, we introduce molecular mechanisms relevant to the pathophysiology and review the most recent studies of clinical applications targeting chronic inflammation.

Keywords: obesity, chronic inflammation, insulin resistance, adipose tissue, TNFα, macrophages

INTRODUCTION

Obesity develops as a consequence of nutritional excess and insufficient exercise; it causes major adverse health outcomes such as type 2 diabetes, cardiovascular diseases, dyslipidemia, chronic kidney diseases, and cancers, which are serious problems worldwide. These pathological states are strongly associated with insulin resistance or hyperinsulinemia. On the basis of efforts over the last two decades, there have been remarkable developments in the investigation of obesity-induced insulin resistance, especially in terms of the mechanisms involved, some of which are expected to lead to treatments of the disease. Among these, low-grade chronic inflammation in obesity is one of the most innovative and newly identified concepts. The metabolic pathway and the immune response pathway, which are strongly evolutionarily conserved among species, have been found to be strongly associated with each other in the development of obesity-induced insulin resistance. In this review, we look back over the initial findings in the research field of inflammation and insulin resistance and discuss recent studies, including those on clinical applications.

OBESITY-INDUCED CHRONIC INFLAMMATION IN ADIPOSE TISSUE AND ADIPOKINE SECRETION

Low-grade chronic inflammation was found to be closely associated with obesity-related metabolic diseases. This association between obesity/type 2 diabetes and inflammation can be traced back to case reports published over a century ago, showing that high-dose sodium salicylate could diminish glycosuria in older diabetic patients (1, 2). Thereafter, several studies also showed that acetylsalicylic acid or sodium salicylate reduced the glucose level and improved glucose tolerance in diabetic patients (3, 4). These reports again drew attention in 1993 with the publication of a report demonstrating in mice that the expression of TNFα in adipose tissue was increased during the development of obesity, while conversely the neutralization of TNFα attenuated insulin resistance (5). Subsequently, the same research group demonstrated that TNFα suppressed insulin signaling by inhibiting insulin receptor tyrosine kinase activity (6) and proposed a model in which inflammation defined as an increased level of TNFα in adipose tissue could be the basis of systemic insulin resistance. Concurrently with these findings, leptin was identified as

a secretory bioactive molecule from adipocytes, which regulates food intake and energy expenditure through the hypothalamus (7). This led to the establishment of an innovative concept in which adipose tissue not only simply stores excess energy as triacylglycerol but is also an organ that secretes the biologically active substances referred to as adipokines. Adipokines could directly regulate the insulin sensitivity of remote insulin-sensitive organs including liver and skeletal muscle through the circulation. Deregulated adipokine secretion from the expanded adipose tissue of obese individuals was shown to contribute to the development of systemic insulin resistance and metabolic diseases. Following the discovery of leptin (7), a number of adipokines have been identified; these include IL-6 (8, 9), resistin (10), retinol-binding protein 4 (RBP-4) (11), omentin (12), chemerin (13–15), progranulin (16), and monocyte chemoattractant protein-1 (MCP-1) (17–19). The proinflammatory cytokine TNFα, produced mainly by macrophages that have infiltrated into adipose tissue, can also be considered as an adipokine (5, 20). Given that TNFα activates proinflammatory signal cascades as well as inhibits insulin receptor signaling, this molecule is thought to be a major player linking adipose tissue inflammation and insulin resistance (21, 22). In contrast, in a lean state, a certain level of "healthy" adipokines contributes to insulin sensitivity and adequate glucose homeostasis. For instance, adiponectin is considered a "healthy" adipokine. Adiponectin-deficient mice exhibited insulin resistance (23, 24) along with increased expression of TNFα in adipose tissue (23). Chronic inflammation, especially in adipose tissue, causes impairment of adipokine secretion, leading to systemic insulin resistance. Thus, adipose tissue inflammation and adipokine secretion are strongly associated with each other and coordinately contribute to insulin resistance in obesity.

MACROPHAGE ACCUMULATION IN ADIPOSE TISSUE

The mechanisms by which TNFα is increased during obesity were unclear until the findings published in 2003 that chronic inflammation observed in rodents and humans was characterized by the accumulation of macrophages into adipose tissue (21, 22). In general, macrophages differentiate in tissue from recruited monocytes and function in innate immunity during host defense. However, these studies demonstrated that macrophages existed even in a lean state, but expanded their populations during the development of obesity in mice and humans (21, 22). It is now considered that macrophages defined as $F4/80^+$ $CD11b^+$ are resident in lean adipose tissue, representing 5% of the stromal vascular fraction (17, 25), but are increased by obesity up to 14–30% (17, 18, 25). In healthy subjects, adipose tissue macrophages show dynamic diversity. Kosteli et al. showed that, although chronic weight loss reduced the macrophage content in adipose tissue, fasting or acute weight loss in turn elicited their accumulation (26). Such conditions seemed to enhance the lipolysis that caused elevation of local free fatty acid (FFA), which induced macrophage accumulation. Infiltrated macrophages incorporate lipids, which act to suppress lipolysis. These findings provide evidence that, not only in a pathological state, but also in physiological circumstances, macrophages in adipose tissue play dynamic roles in the maintenance of homeostasis.

THE ROLE OF CHEMOKINES IN ADIPOSE TISSUE INFLAMMATION AND INSULIN RESISTANCE

Chemokines are a family of low-molecular-weight proteins with an essential role in leukocyte trafficking during both homeostasis and inflammation. On the basis of their molecular structure, chemokines are divided into two major subgroups: CC chemokine ligand (CCL) and CXC chemokine ligand (CXCL), which bind to CC chemokine receptor (CCR) or CXC chemokine receptor (CXCR), respectively (27). Intriguingly, MCP-1 (also known as CCL2), a representative CC chemokine, was found to be remarkably increased in adipose tissue in obesity (21, 22, 28). We and others sought to investigate whether MCP-1 is a factor that enhances the infiltration of macrophages in adipose tissue. Adipose tissue-specific overexpression of MCP-1 in mice indeed increased macrophage infiltration into adipose tissue and insulin resistance (17, 19), whereas disruption of MCP-1 or its receptor, CCR2, impaired high-fat diet (HFD)-induced migration of macrophages into adipose tissue, thereby reducing adipose tissue inflammation and attenuating insulin resistance (17, 18, 29, 30). These findings suggest that MCP-1 secreted from enlarged adipocytes attracted circulating monocytes to adipose tissue, causing inflammatory characteristics of adipose tissue. Infiltrated monocytes differentiate into macrophages and produce additional inflammatory cytokines, leading to further inflammation. Secreted inflammatory cytokines are supposed to induce insulin resistance in liver and skeletal muscle by functioning as adipokines (**Figure 1**). In addition, chronic increase in the circulating level of MCP-1 by the administration of recombinant MCP-1 protein induced insulin resistance, macrophage infiltration into adipose tissue, and an increase in hepatic triacylglycerol content without affecting body weight (18). Acute increase in the circulating MCP-1 concentration also induced insulin resistance but not macrophage infiltration into adipose tissue. These findings indicate that an increase in the concentration of MCP-1 in the circulation is sufficient to induce systemic insulin resistance irrespective of adipose tissue inflammation (18). In fact, circulating MCP-1 levels were found to be increased in type 2 diabetic patients compared with normal subjects (31, 32) or to be correlated with HOMA-IR in type 2 diabetic patients (33). On the other hand, studies by others found no difference or even more infiltrated macrophages in adipose tissues in MCP-1-deficient mice, although the reason for the different results is unknown (34, 35). Recent study by Oh et al. provided evidence by employing a new method for quantitative *in vivo* macrophage tracking, in which monocytes isolated from peripheral blood were labeled *ex vivo* with fluorescent PKH26 dye and then injected into recipient mice (36). Mice receiving CCR2-deficient monocytes were protected from HFD-induced accumulation of macrophages in adipose tissue and the liver, while transplantation of intact monocytes into MCP-1 knockout mice on an HFD did not cause infiltration of macrophages into the tissues (36). These results all suggest that the MCP-1-CCR2 signaling pathway plays an important role in adipose tissue inflammation (17–19, 29, 30, 36), hepatic steatosis (17, 18, 37, 38), and glucose metabolism (17–19, 29, 30, 36–38) in insulin-resistant model mice. Thus, examination of the factors that induce MCP-1 expression in hypertrophied adipocytes is also important. Ito et al. demonstrated that down-regulation of

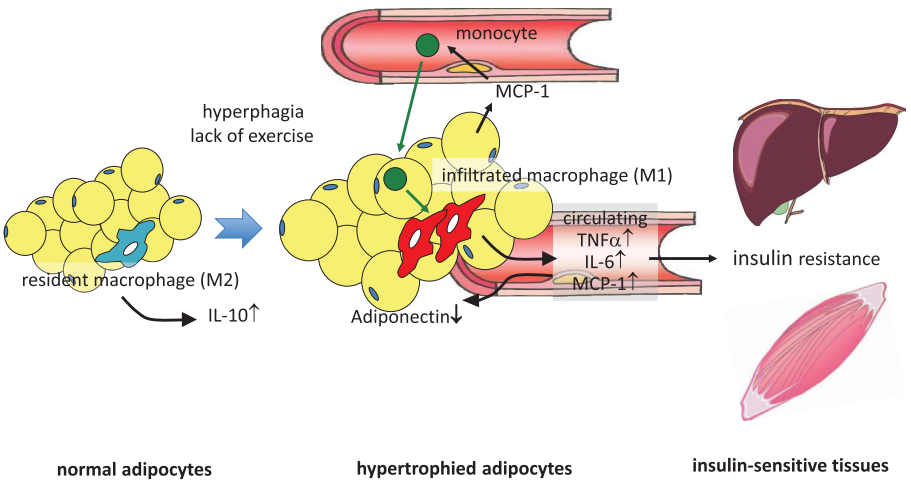


FIGURE 1 | Obesity-induced macrophage infiltration into adipose tissue causes insulin resistance. **(A)** In adipose tissue in a lean state, most resident macrophages are M2 macrophages that contribute to insulin sensitivity by secreting IL-10. **(B)** Hyperphagia and lack of exercise cause hypertrophy of adipocytes, which induces MCP-1 secretion to the circulation, leading to the recruitment of circulating monocytes to adipose tissues. These infiltrated monocytes differentiate into activated M1 macrophages, which robustly secrete proinflammatory cytokines such as TNFα, IL-6, and MCP-1, thus contributing to low-grade inflammation in adipose tissue and a decrease of adiponectin. At the same time, these secreted cytokines cause insulin resistance in liver and skeletal muscle by acting as insulin resistance-inducing adipokines.

mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP-1) increased MCP-1 expression through MAPK activation in cultured adipocytes (39). Furthermore, Kitade et al. demonstrated that the expression of CCR5 in adipose tissue was similarly increased during obesity (40). Genetic deletion of CCR5 in mice resulted in protection against HFD-induced macrophage infiltration, insulin resistance, and hepatic steatosis. Furthermore, alteration of macrophages in adipose tissues was accompanied by polarization to M2. These results were reproduced using a cell-specific approach by employing bone marrow transplantation (40). At present, it is believed that M2 macrophages contribute to maintain insulin sensitivity, while obesity causes a switch to M1 polarization that enhances systemic insulin resistance through the secretion of inflammatory cytokines (41). Subsequently, the contributions of chemokines other than the CCL family, such as CXCL14 (42) or other factors including osteopontin (43), angiopoietin-like protein 2 (Angptl2) (44), serum amyloid A (45), and dietary cholesterol (46), to the accumulation of macrophages in adipose tissue have been demonstrated.

INFLAMMATORY ACTIVATION OF MYELOID CELLS IN THE LIVER

Following the findings for adipose tissue, the issues of whether obesity can cause hepatic inflammation and whether this inflammation can contribute to hepatic or systemic inflammation became important in this field. Obesity-associated nutrient excess has been linked to inflammation in part via activation of inhibitor of κB kinase β (IKKβ) and subsequent nuclear translocation of nuclear factor κB (NF-κB), one of the key transcriptional mediators of inflammation (47–49). Consumption of an HFD clearly induced proinflammatory activation of Kupffer cells, the resident macrophages of the liver, in mice (50, 51). In addition, inflammatory activation of Kupffer cells was implicated in the pathogenesis of obesity-induced insulin resistance and fatty liver disease (50). Deletion of IKKβ in myeloid cells reduced macrophage-mediated inflammation and improved obesity-associated systemic and hepatic insulin sensitivity (47). Furthermore, chemical deletion of Kupffer cells was demonstrated to cause improved insulin sensitivity during HFD feeding (52). Obesity and insulin resistance are often associated with hepatic steatosis in a large proportion of obese patients. We demonstrated mechanically that overexpression of MCP-1 in adipose tissue caused hepatic steatosis along with adipose tissue inflammation, while systemic deletion of MCP-1 inhibited HFD-induced steatosis (17). In addition, chronic increase of plasma MCP-1 level was also sufficient to induce hepatic steatosis and adipose tissue inflammation (18). These findings suggest that an increase of circulating MCP-1 or adipose tissue inflammation may cause hepatic steatosis. Although HFD feeding caused M1 activation of Kupffer cells in the liver (50, 51), it seemed that the number of Kupffer cells was not increased in obesity (53). Using flow cytometry, it was investigated how a population of myeloid cells ($CD11b^+$) changed during obesity or type 2 diabetes. Kupffer cells, defined as $CD45^+$, $F4/80^+$, were a major subset of myeloid cells in the liver. Obesity rather reduced the number of Kupffer cells, while in turn, the proportion of myeloid cells, defined as $CD11b^+$, $CD45^+$, $F4/80^{low}$, doubled, from 10.0 to 19.7% (53). Given that these recruited myeloid cells were also characterized by $CCR2^+$, hepatic expression of CCL2/CCR2, which was increased by HFD, seemed to have originated from infiltrated macrophages. By employing bone marrow transplantation from CCR2-deficient mice, it was further demonstrated that the trafficking of the infiltrated cells was

dependent on CCR2. In addition, adenoviral overexpression of CCL2 in the liver caused the accumulation of myeloid cells coincident with hepatic steatosis (53). CCR2-dependent recruitment of myeloid cells to the liver (36) and CCL2-dependent development of hepatic steatosis (54) were also demonstrated by other studies. These results also underline the role of the CCL2-CCR2 signaling pathway in the recruitment of myeloid cells to the liver. Taking these findings together, the range of immune cells in the liver is thus complex and heterogeneous, but they are thought to play important roles in both insulin resistance and hepatic steatosis.

REGULATION OF KUPFFER CELL ACTIVATION BY ENDOTHELIAL NO PRODUCTION

Local and systemic insulin resistance has been discussed in relation to the interactions between immune cells and parenchymal cells. We have proposed that endothelial cells could be added to those components with which interactions are shown. We have demonstrated that HFD feeding induced proinflammatory activation of Kupffer cells in wild-type (WT) mice coincident with reduced liver endothelial nitric oxide synthase activity and nitric oxide (NO) content while, conversely, the enhancement of cGMP signaling downstream of endogenous NO by phosphodiesterase-5 inhibition protected Kupffer cells against HFD-induced inflammation (51). Furthermore, proinflammatory activation of Kupffer cells was evident in eNos$^{-/-}$ mice, even on a low-fat diet. Targeted deletion of vasodilator-stimulated phosphoprotein (VASP), a key downstream target of endothelially derived NO, similarly led to a predisposition to hepatic and Kupffer cell inflammation and abrogated the protective effect of NO signaling in both macrophages and hepatocytes studied in a cell culture model (51). These results collectively imply a physiological role for endothelial NO to limit obesity-associated inflammation and insulin resistance in hepatocytes and support a model in which Kupffer cell activation during HFD feeding is dependent on reduced NO signaling (51) (**Figure 2**). The NO/cGMP/VASP axis was also shown to be relevant in adipose tissue (55).

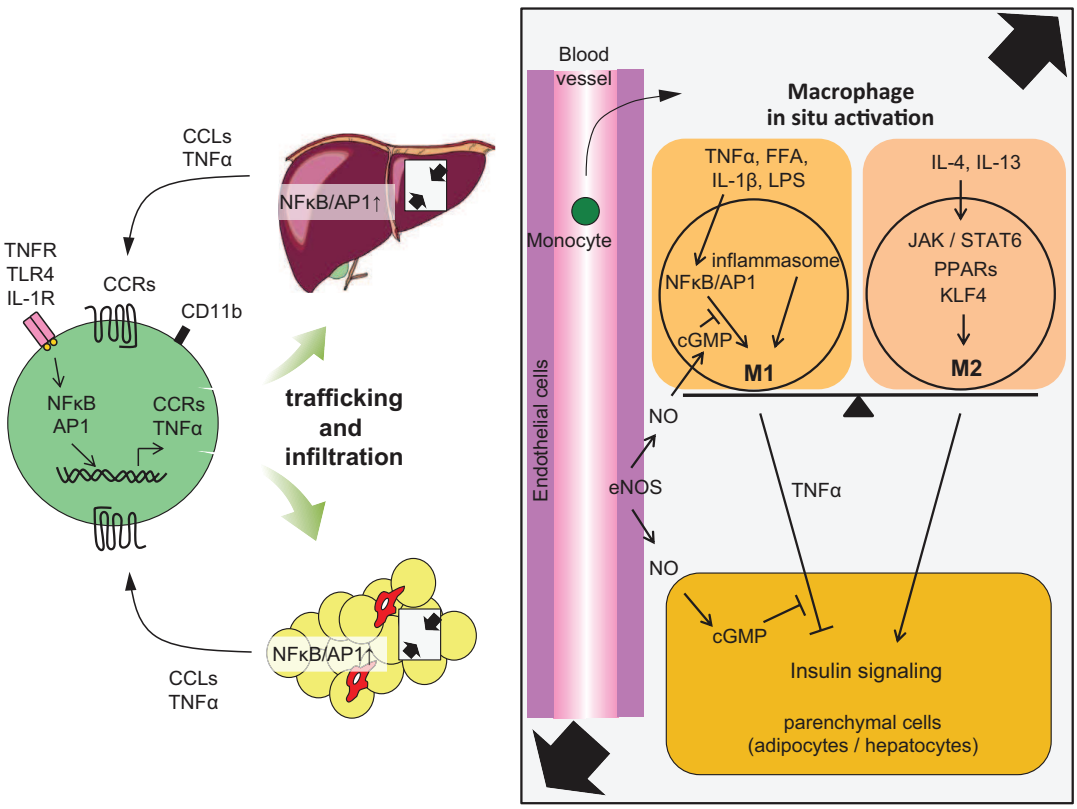


FIGURE 2 | Accumulation of monocytes/macrophages in adipose tissue and liver, and activation in the tissues. Trafficking: During obesity, adipocytes exhibit hypertrophy, while liver incorporates substantial FFAs, both of which cause tissue inflammation, activation of NF-κB, and AP1 signaling, leading to increased secretion of inflammatory chemokines and cytokines, including CCLs and TNFα. Elevated secretion of CCLs (e.g., MCP-1) elicits the accumulation of CCR-positive monocytes to the site of inflammation, particularly CCR2^{+} for adipose and liver, but CCR5^{+} for adipose tissue. *In situ* activation: In a lean state, resident tissue macrophages display the M2 phenotype, which is achieved and sustained through the JAK/STAT6 pathway in response to IL-4 or IL-13 stimuli. These stimuli are derived from resident T_H2 cells, T_{reg} cells, eosinophils, and mast cells. PPARs and KLF4 also induce M2 activation. In turn, obesity and subsequent elevation of tissue FFA or inflammatory cytokines stimulate NF-κB and AP1 signaling, which causes switching of the phenotype to M1, leading to further secretion of TNFα. Signal from inflammasome also activates M1 activation. M1 activation of macrophages can be suppressed by endothelial NO/cGMP signaling. M2 macrophages contribute to insulin sensitivity in neighboring parenchymal cells, while M1 induces insulin resistance, with the M1/M2 balance determining tissue and/or systemic insulin sensitivity.

CONSTITUENT CELLS OTHER THAN MACROPHAGES IN OBESITY-INDUCED INFLAMMATION: INTERACTIONS AMONG IMMUNE CELLS DURING INFLAMMATION IN ADIPOSE TISSUE

The role of macrophages in adipose tissue inflammation has been clearly demonstrated. Besides these cells, additional leukocyte subpopulations have recently been demonstrated to be involved in obesity and insulin resistance, such as T cells, B cells, eosinophils, neutrophils, mast cells, and natural killer cells. The involvement of multiple leukocyte subpopulations underlines the complexity of obesity-associated adipose tissue inflammation (**Figure 3**).

T CELLS

Although macrophage infiltration in adipose tissue has been demonstrated in both mice and humans (56), little is known about the sequence of events that lead to the macrophage accumulation in adipose tissue. Research attempting to investigate which surface antigens of immune cells are associated with inflammation and insulin resistance revealed the involvement of CD11c-positive cells (57). Myeloid-specific deletion of CD11c in mice protected against HFD-induced accumulation of macrophages in adipose tissue and exhibited insulin sensitivity compared with the controls (57). Next, T cells ($CD4^+$, $CD8^+$) were found to be increased in adipose tissue during obesity (58–60). In a lean state, $CD4^+$ helper T cells and regulatory T (T_{reg}) cells ($CD4^+$, $CD25^+$, $Foxp3^+$) were predominant; however, prior to the accumulation of macrophages ($F4/80^+$, $CD11b^-$), $CD8^+$ T cells infiltrated coincidentally with a reduction of the number of T_{reg} (25). The administration of CD8 antibody to WT mice fed an HFD attenuated macrophage infiltration and insulin resistance. Although CD8 knockout mice were protected against HFD-induced accumulation of macrophages, restoration of $CD8^+$ T-cells increased macrophage infiltration. Similar results were obtained by other groups (61, 62). It is now considered that, in a lean state, $CD4^+$ $CD25^+$ $Foxp3^+$ T_{reg} cells induce alternative activation of monocyte/macrophages (63), which is characterized by the expression of macrophage mannose receptor (MMR) or intracellular activity of arginase (64). T helper type 2 (T_H2) cells expressing IL-4 and IL-13 also induce M2 activation of macrophages that secrete IL-10, whereas macrophages are M1-activated through IFNγ by T helper type 1 (T_H1) cells and through IL-17 by T_H17 cells. Recently, peroxisome proliferator-activated receptor γ (PPARγ) activity in T_{reg} cells has been shown to be important to reduce chronic inflammation in adipose tissue (65).

B CELLS

The accumulation of B cells was observed in adipose tissue of mice fed an HFD before macrophage and T-cell accumulation (66). In addition, diet-induced obese mice lacking B cells were protected from metabolic abnormalities despite weight gain (67). B-cell effects on glucose metabolism were associated with the activation of proinflammatory macrophages and T cells and the production of pathogenic IgG antibodies. In fact, treatment of mice fed an HFD with a B-cell-depleting CD20 antibody ameliorated abnormality in glucose metabolism and adipose tissue inflammation, whereas the transfer of IgG from mice with diet-induced obesity rapidly induced insulin resistance and glucose intolerance (67). Recently, obese B-cell-null mice were reported to exhibit decreased systemic inflammation, inflammatory B- and T-cell cytokines, adipose tissue inflammation, and insulin resistance compared with obese WT mice (68). This was associated

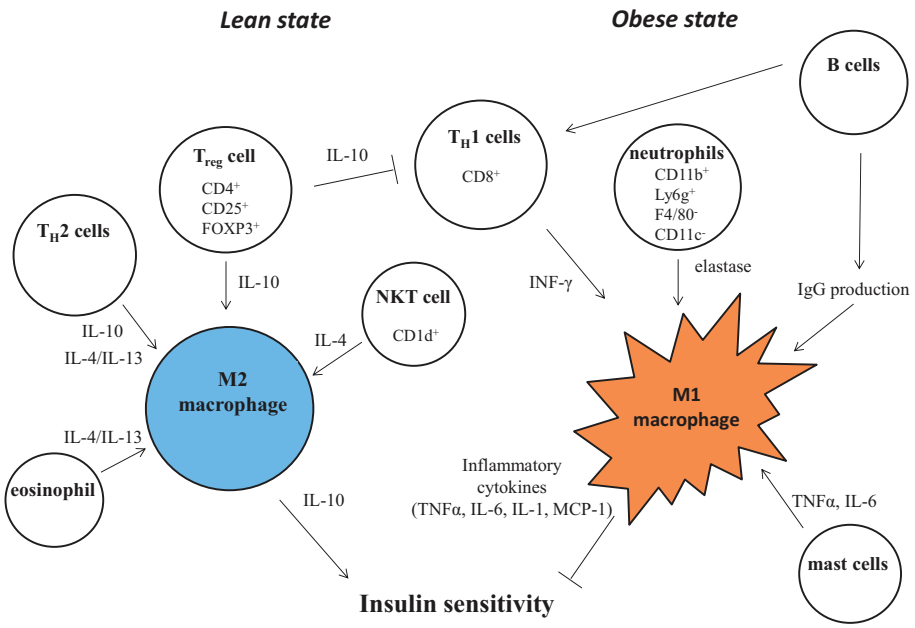


FIGURE 3 | Control of M1/M2 polarization by neighboring immune cells. In a lean state, resident T cells consist of T_H2 cells, T_{reg} cells, and NKT cells. Combined with resident eosinophils, these cells sustain the M2 activation of macrophages through secreting IL-4, IL-10, and IL-13. As obesity progresses, alteration of constituent immune cells occurs, in which the numbers of T_H2 cells and T_{reg} cells decline, while in turn, T_H1 cells and B cells increase. In addition to these more prevalent cells, neutrophils and mast cells induce M1 activation of macrophages by increased secretion of elastase, TNF, IFNγ, IL-6, and pathogenic IgG. B cells also activate T cells.

with an increased percentage of anti-inflammatory regulatory T cells. B cells from type 2 diabetes subjects promote proinflammatory T-cell function through contact-dependent mechanisms, suggesting that B cells regulate inflammation in type 2 diabetes by modulating T-cell functions (68).

EOSINOPHILS

In addition to T_H2 or T_{reg} cells, eosinophils have been shown to exist in lean adipose tissue and participate in the maintenance of M2 activation through secreting IL-4 (69). By using eosinophil-deficient and hypereosinophilic mice, Wu et al. showed that eosinophil-derived IL-4 and IL-13 determined the M2 activation of macrophages in adipose tissue and contributed to insulin sensitivity. Hypereosinophilic mice displayed improved insulin sensitivity, while eosinophil-deficient mice exhibited increased fat together with impaired glucose tolerance and insulin resistance (69).

NEUTROPHILS

Neutrophils are rare in a lean state; however, an HFD elicits the accumulation of neutrophils ($CD11b^+$ $Ly6g^+$ $F4/80^-$ $CD11c^-$), which seem to induce local insulin resistance by secreting elastase (70). The deletion of neutrophil elastase in HFD-induced obese mice led to reduced macrophage content and inflammation. These changes were coincident with improvement of glucose tolerance and increased insulin sensitivity. Intriguingly, neutrophil elastase can degrade IRS-1 protein and cause insulin resistance in adipocytes (70). Similar results were obtained in a very recent study by another group (71). In humans, an increased blood level of myeloperoxidase, a marker of neutrophils, in obese women (72), and increased activity of neutrophils in obese subjects have also been noted (71, 73).

MAST CELLS

Mast cell invasion was also detected in adipose tissue in obese mice (74). Mast cell-deficient mice (*KitW-sh/W-sh* mice) were protected from HFD-induced body weight gain and the increase of proinflammatory cytokines and chemokines along with the improvement of glucose metabolism and energy expenditure due to the up-regulation of UCP-1 expression in BAT (74). Similar effects were observed in the treatment of mice with a mast cell-stabilizing agent. Mast cells were supposed to promote diet-induced obesity and glucose intolerance by the production of IL-6 and IFNγ. Mast cells are also involved in obesity-induced adipose tissue inflammation and insulin resistance. Weight gain of mast-cell-deficient mice during HFD was decreased compared with that of control mice (75). Mechanistically, prostaglandin J2 (PGJ2) produced by mast cells in response to high-glucose enhanced adipocyte differentiation by PPARγ activation, leading to obesity (75).

NATURAL KILLER T CELLS

Natural killer T (NKT) cells are innate-like T lymphocytes that recognize glycolipid antigens and have been implicated in autoimmunity, microbial infection, and cancer and hence represent an important immunotherapeutic target (76). Similar to eosinophils, NKT cells have been shown to reside in lean adipose tissue, in which they contribute to sustain the M2 activation of macrophages by stimulating IL-4/STAT6 signaling (77, 78). Schipper et al. demonstrated that CD1d-null mice whose NKT cells were not activated displayed a distinctive insulin resistance phenotype even on a low-fat diet without overt adipose tissue inflammation (79). Activation of NKT cells has thus been demonstrated to modulate polarization toward M2, resulting in improved glucose metabolism (78–80). Unlike in mouse studies, the role of NKT cells during obesity and adipose tissue inflammation in humans remains unclear. An unaltered number of circulating NKT cells in obesity (80, 81) and significantly lower numbers of circulating NKT cells in obese patients have been documented (82).

CELL SIGNALING IN MACROPHAGES THAT DEFINES M1 AND M2 ACTIVATION

Macrophages are terminally differentiated cells of the mononuclear phagocyte system that include dendritic cells, circulating blood monocytes, and committed myeloid progenitor cells in the bone marrow. Local environmental factors are known to affect the properties, functions, and activation state of macrophages. In general, macrophage activation is defined across two separate polarization states, M1 (proinflammatory) and M2 (anti-inflammatory) states. M1 or "classically activated" macrophages are induced by proinflammatory mediators such as lipopolysaccharide (LPS), TNFα, and IFN-γ. M1 macrophages are also associated with enhanced proinflammatory cytokine production (TNFα, IL-6, IL-1). On the other hand, M2 or "alternatively activated" macrophages have low proinflammatory characteristics and instead generate high levels of anti-inflammatory cytokines, for example, IL-10. Since the attenuation of macrophage M1 activation and the maintenance of M2 activity are believed to be important for intact glucose metabolism, there has been research focusing on intracellular signaling that determines proinflammatory or alternative activation in macrophages (**Figures 2** and **3**).

M1 MACROPHAGES

M1 activation of macrophages is established mainly through the IKKβ/NF-κB and Jun N-terminal kinase (JNK) 1/activator protein 1(AP1) system. Obesity induces adipose tissue inflammation, which results in high levels of proinflammatory cytokines and chemokines. In particular, TNFα is a representative inflammatory cytokine that causes lipolysis in adipose tissue. Thereby, plasma FFA levels are usually elevated in obesity. FFAs released from adipocytes through lipolysis have been shown to be capable of serving as ligands for the toll-like receptor 4 (TLR4) complex (83). TLRs are initially indispensable for innate immune cells to recognize intruding pathogens and trigger an appropriate immune response. Among them, TLR4 is a high-affinity receptor for LPS, which is a component of the cell walls of gram-negative bacteria (84). TLR4 signaling activated by FFA induces the expression of a large number of proinflammatory target genes and drives M1 activation by regulating the transcriptional factors including NF-κB, AP1, and interferon-regulatory factor (IRF) family members. TNFα also drives M1 activation by inducing proinflammatory genes through activating NF-κB and AP1 transcriptional factors. For instance, lipid infusion caused the accumulation of macrophages in adipose tissue accompanied by insulin resistance

in WT control mice, but this was not the case in TLR4-deficient mice (83). Hematopoietic cell-specific deletion of TLR4 in mice attenuated HFD-induced insulin resistance in adipose and the liver (85). Activated TLR4 signaling induced a classical inflammatory response, which led to the recruitment of macrophages. In this way, macrophages activated to M1 by FFA through TLR4-mediated signaling secrete TNFα, which in turn enhances lipolysis in neighboring adipocytes, leading to further production of FFA. This vicious cycle or paracrine loop mediated by TNFα and FFA between adipocytes and macrophages in obese adipose tissue induces further adipose tissue inflammation (86). In addition, TNFα and FFA inhibit insulin receptor signaling via the increase of serine phosphorylation of IRS-1. Recently, a liver secretory glycoprotein, fetuin-A, was demonstrated to play a crucial role as an endogenous ligand for TLR4 in FFA-induced inflammation and insulin resistance in adipocytes (87). The serum concentration of fetuin-A was significantly increased in obese diabetic patients compared with that in non-obese non-diabetic human subjects. Next, myeloid differentiation primary response protein 88 (MYD88), the primary mediator of TLR and IL1 receptor signaling, has been investigated to clarify whether this is also involved in the FFA-induced insulin resistance. However, MyD88 deficiency in mice exacerbated diet-induced glucose intolerance and hyperlipidemia (88). There is therefore a conflict regarding the activity of the TLR4/MyD88 axis in diet-induced obesity and insulin resistance, which remains to be elucidated in future studies.

M2 MACROPHAGES

The activation of M2 macrophages is basically maintained by the signaling of the IL-4/JAK/STAT6 pathway. The administration of IL-4 to mice induces M2 activation of macrophages, thereby attenuating HFD-induced insulin resistance (89). IL-10 secreted by M2 macrophages enhances insulin signaling, including that in the liver, thereby having a protective role against obesity-induced insulin resistance (90). Taking these findings together, the activation of IL-4 signaling is considered to be a promising target to suppress insulin resistance and thus studies to identify molecular mediators are underway. We describe here several factors involved in M2 activation.

Peroxisome proliferator-activated receptor γ

Macrophage-specific deletion of PPARγ in mice impaired M2 activation despite the mice being on a chow diet (91). In these mice, adiponectin expression was decreased. This change was accompanied by reduced oxidative phosphorylation in liver and skeletal muscle, which might have contributed to the insulin resistance in these tissues. Another study demonstrated that macrophage-specific PPARγ-deficient mice showed glucose intolerance and insulin resistance in a lean state. These mice had increased inflammatory markers in adipose tissue, liver, and skeletal muscle and showed decreased effects of thiazolidinediones, indicating a requirement for PPARγ in macrophages for intact insulin sensitivity in muscle/liver and a full antidiabetic effect of thiazolidinediones (92). Odegaard et al. demonstrated that peroxisome proliferator-activated receptor δ (PPARδ) mediated the effects of a Th2 cytokine, IL-4, to direct the expression of the alternative phenotype in Kupffer cells and adipose tissue macrophages of lean mice (50). Adoptive transfer of PPARδ$^{-/-}$ bone marrow into WT mice conversely diminished the alternative activation of hepatic macrophages, causing hepatic dysfunction and systemic insulin resistance (50). Collectively, PPARs are thought to be required for the maturation of M2 activation and the resulting insulin sensitivity.

Krüppel-like factor 4

In addition to PPARs, another nuclear receptor, Krüppel-like factor 4 (KLF4), has been implicated in M2 activation in macrophages (93). In macrophages, KLF4 is suppressed by LPS stimulation, while it is increased by IL-4. Macrophage-specific KLF4 knockout mice display M1 activation and M2 disactivation. Owing to reduced fatty acid oxidation, the mice are susceptible to becoming obese and exhibit glucose intolerance and insulin resistance. In contrast, forced expression of KLF4 in RAW cultured macrophages resulted in M2 activation and resistance to M1 polarization by stimulation of LPS. Importantly, mRNA expression of KLF4 in adipose tissue is reduced in human obesity. Moreover, mRNA expression of KLF4 is not only positively associated with adiponectin expression in adipose tissue but also with well-defined M2 markers, such as CD206 and CCL18 in the stromal vascular fraction of adipose tissue. It has also been documented that IL-4 activates STAT6, leading to transcriptional activation of KLF4 to induce M2 genes (93).

AMP-activated protein kinase

AMP-activated protein kinase (AMPK) is an evolutionarily conserved sensor of cellular energy status that is activated by low energy status (increased cellular AMP/ADP:ATP ratio) and consists of an α catalytic subunit and βγ regulatory subunits. This molecule has also been shown to be crucial for the maintenance of M2 activation (94). Galic et al. tested the effect of AMPK β1 loss in macrophages *in vivo* by transplantation of bone marrow from WT or β1(−/−) mice into WT recipients. When challenged with an HFD, mice that received β1(−/−) bone marrow displayed enhanced adipose tissue macrophage inflammation and liver insulin resistance compared with animals that received WT bone marrow (94). Taking these findings together, the activation of AMPK and increased fatty acid oxidation in macrophages might provide an avenue for the treatment of type 2 diabetes.

Sirtuin 1

Sirtuin 1 (SIRT1), the mammalian homolog of yeast silent information-regulator 2 (Sir2), is an NAD^+-dependent histone deacetylase that has been implicated in the regulation of lifespan under calorie restriction (95) or energy metabolism during fasting (96); thus, it is believed to be a promising target for type 2 diabetes (95, 97). Besides these findings, anti-inflammatory effects have also been demonstrated, showing that SIRT1 deacetylates NFκB and suppresses its transcriptional activity by inhibiting nuclear translocation (98). SIRT1 levels are markedly reduced in adipose tissue of obese humans and mice (99, 100). HFD was also found to result in cleavage of SIRT1 protein (101). In fact, upon the reduction of SIRT1 in fat by antisense oligonucleotides to levels similar to those seen during overnutrition, macrophage recruitment to

adipose tissue was significantly increased. Similar results were obtained in fat-specific SIRT1 knockout mice. In contrast, overexpression of SIRT1 in mice prevented HFD-induced accumulation of macrophages (102). Furthermore, it was found that the SIRT1 expression level in human subcutaneous fat was inversely related to the number of adipose tissue macrophages. Mechanistically, others demonstrated that SIRT1 regulated intracellular inflammatory signaling at the levels of JNK and IKK (103). In addition, AMPK was also reported to regulate lipid-induced inflammation negatively through SIRT1 (104). Taken together, these findings indicate that SIRT1 might exert an insulin-sensitizing effect partially through the suppression of inflammation.

INVOLVEMENT OF INFLAMMASOME IN OBESITY-INDUCED INFLAMMATION

The mechanisms by which obesity induces macrophage activation despite the absence of any infection or autoimmune processes remained unclear. Although some mechanisms including hypoxia (105, 106) and autophagy (107–109) have been proposed for the induction of inflammation, in this review, we would like to focus on a new concept, the involvement of inflammasome in adipose tissue inflammation and insulin resistance. External or internal stimuli are recognized by pattern recognition receptors (PRRs). External stimuli, particularly pathogen-associated molecular patterns (PAMPs), are detected not only by TLRs but also by inflammasome, which is a protein complex consisting of caspase-1, apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and nucleotide-binding oligomerization (NOD)-like receptors (NLRs) (110). Among these components, different pathogens are recognized by distinct constituents of NLRs. For instance, bacterial infection is recognized by nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-1 (NLRP1), NLRP3, NLRP4, and absent in melanoma 2 (AIM2). Viral infection is recognized by NLRP3 and AIM3. Fungal or parasitic infection is recognized by NLRP3 (110). But all of these infections cause the activation of caspase-1, which eventually leads to the processing and secretion of proinflammatory cytokines, including IL-1β and IL-18 (110). A unique feature of inflammasome is its additive ability to recognize internal stimuli as danger signals. For instance, uric acid, silica, fatty acids, and ATP in cytoplasm are detected as non-microbial-originated damage-associated molecular pattern molecules (DAMPs) by NLRs (110). Since mRNA expression of NLRP3 in adipose tissue correlates with IL-1β, body weight, and blood glucose level in rodents and humans (111), Vandanmagsar et al. tested whether NLRP3 played important roles during the development of chronic inflammation in obesity. Using NLRP3 knockout mice, they showed that NLRP3 sensed ceramide as a danger signal that activated caspase-1, which enhanced IL-1β secretion, thereby inducing T-cell activation (111). Target deletion of NLRP3 in mice displayed improved glucose tolerance and increased insulin sensitivity. These results were accompanied by the appearance of small adipocytes, reduced M1 activation, and enhanced insulin signaling in liver, adipose tissue, and skeletal muscle. Elevated ceramide, saturated fatty acid, reactive oxygen species (ROS), and mitochondrial dysfunction caused activation of inflammasome in macrophages (108, 112). The resulting activation of caspase-1 and subsequent secretion of IL-1β then interfere with insulin signaling, whereas inhibition of caspase-1 has been demonstrated to attenuate insulin resistance coincident with improved function of adipocytes (108, 112, 113). In humans, elevated levels of circulating IL-18 in patients with type 2 diabetes have been demonstrated (114), along with a suppressive effect of calorie restriction and resulting weight loss on the reduced expression of adipose NLRP3 in type 2 diabetes (111), and marked reduction of both adipose and liver expression of IL-1β in morbidly obese subjects by laparoscopic adjustable gastric banding surgery (114).

THERAPEUTIC INTERVENTIONS

The basis of therapeutic interventions in inflammation and insulin resistance is to prevent or to ameliorate obesity by physical exercise and diet control. They can also present the beneficial effects to the improvement of inflammation irrespective of body weight loss. In addition, the significance of chronic inflammation and its molecular mechanisms during the development of type 2 diabetes has been demonstrated and, in mice, suppression of inflammation-related molecules has successfully improved glucose intolerance. On the basis of this evidence, clinical trials targeting inflammation-related molecules have started. Thus, at first we would like to introduce the contribution of exercise and diet to the amelioration of inflammation. Next, we describe the current circumstances concerning several clinical applications of anti-inflammatory drugs.

EXERCISE AND DIET

Although exercise is generally admitted to be effective to attenuate obesity and sustain health, single session of exercise has been reported to trigger an increase in proinflammatory cytokine release together with leukocytosis and increased plasma concentration of CRP (115). Regular and chronic exercise, however, has been reported to be associated with reduction of inflammatory markers such as CRP, IL-6, and TNFα (115–118). Physical (aerobic + resistance) exercise was also associated with increase in anti-inflammatory substances, such as IL-4 and IL-10 in type 2 diabetic patients with metabolic syndrome (118). Among many types of exercise, Oliveira et al. compared the effect of 12 weeks training with three different types of exercise (aerobic training, strength training, and combined training) on subjects with type 2 diabetes, demonstrating that the aerobic training program caused significant up-regulation in antioxidant enzymes (119). Accordingly, exercise-dependent improvement of glucose tolerance seems to be related with suppression of inflammation and oxidative stress (116).

Dietary calorie restriction is well recognized to be beneficial to ameliorate obesity-induced inflammation through weight loss. In addition to this, dietary composition has also been demonstrated to be important for the improvement of inflammation. Dietary bioactive compounds, such as polyphenols and certain fatty acids suppress systemic and adipose tissue inflammation. Polyphenols such as resveratrol exhibited anti-inflammatory effects via suppression of NF-κB (120) and extracellular signaling regulated kinase pathway (121) as well as via activating SIRT1 (122). Resveratrol has also been shown to activate AMPK independent of

SIRT1 (123). Therefore, resveratrol may be a promising candidate in anti-inflammatory therapy (124). For instance, resveratrol supplementation for 30 days decreased blood glucose levels and inflammation markers along with improvement of HOMA index in healthy obese men irrespective of body weight (125). In addition, dietary polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) possess anti-inflammatory effects. Mechanistically, these include activation of AMPK and PPARγ (126). These polyunsaturated fatty acids also inhibits NF-κB pathway by activation of G-protein coupled receptor (GPR) 120 (127). In fact, n-3 polyunsaturated fatty acids (EPA and DHA) decreased adipose tissue and systemic inflammation in severe obese non-diabetic patients and improved lipid metabolism (128). EPA was demonstrated to reduce body weight at least by suppressing lipogenesis in the liver (129).

CLINICAL APPLICATIONS OF ANTI-INFLAMMATORY DRUGS

Aspirin/salsalate

It has been reported that high-dose sodium salicylate or acetylsalicylic acid could diminish glycosuria or improve the blood glucose level in diabetic patients (1–4). Given that IKKβ is a key downstream mediator of insulin resistance and its blockade by salicylates attenuated hyperglycemia, hyperinsulinemia, and dyslipidemia in obese rodents (130, 131), Hundal et al. asked whether high-dose aspirin (∼7 g/day) could ameliorate insulin resistance and improve glucose tolerance in patients with type 2 diabetes (132). They demonstrated that this treatment for 2 weeks resulted in marked reduction of metabolic parameters including fasting glucose, basal rate of hepatic glucose production, and insulin-stimulated peripheral glucose uptake, despite no change in body weight. A large randomized trial, the National Institute of Diabetes and Digestive and Kidney Diseases-sponsored Targeting Inflammation with Salsalate, Non-acetylated Prodrug of Salicylate, in Type 2 Diabetes (TINSAL-T2D) trial, recently concluded that salsalate lowered hemoglobin A1c (HbA1c) levels and improved glycemic control in patients with type 2 diabetes (133). In a single-masked run-in period, patients were randomly assigned to receive placebo or salsalate at a dosage of 3.0, 3.5, or 4.0 g/day for 14 weeks (27 patients each) in addition to their current therapy. Mean HbA1c changes were −0.36% ($P = 0.02$) at 3.0 g/day, −0.34% ($P = 0.02$) at 3.5 g/day, and −0.49% ($P = 0.001$) at 4.0 g/day compared with placebo (133). The number of patients studied and the trial duration were insufficient to warrant recommending the use of salsalate for type 2 diabetes; however, it appears warranted to target this molecule in further investigations.

IL-1β

Reducing the activity of inflammasome and suppressing IL-1β secretion might be targets to attenuate insulin resistance in diabetes. Randomized clinical trials have shown that the blockade of IL-1β signaling by anakinra, a recombinant human IL-1 receptor antagonist, reduced systemic inflammation and improved glycemia of type 2 diabetes (134–136).

TNFα

Etanercept is a dimeric recombinant form of the extracellular domain of the human p75 TNFα receptor 2 fused to the Fc fragment of human immunoglobulin G1 (IgG1) and acts as a TNFα antagonist by interfering with the binding of TNFα to its cellular receptors and thus blocks the inflammatory response (137). Several studies have been conducted to test whether this biopharmaceutical improves glucose tolerance in patients with type 2 diabetes; however, despite a suppressive effect on systemic inflammation, the attenuation of glucose tolerance or insulin resistance has not yet been achieved (137–139). These results might be attributable to the distinct role of TNFα between rodents (5) and humans (137–139). Alternatively, antagonism of TNFα by other drugs remains hopeful in future studies.

CONCLUDING REMARKS

Following the discovery of chronic inflammation characterized by macrophage accumulation in adipose tissue, an explosion of studies in the past decade have begun to reveal the contributions of inflammation to the development of insulin resistance and subsequent metabolic abnormalities in other tissues, such as liver (47–52) and most recently brain (140). Adipose tissue, liver, and the hematopoietic system are evolutionarily derived from the same tissue. This developmental heritage can underlie the link between obesity-induced adipose tissue and hepatic inflammation (56) (**Figure 2**). Studies using flow cytometry subsequently identified the relative importance of other immune cells, including T cells, B cells, eosinophils, neutrophils, mast cells, and NKT cells, during the development of chronic inflammation. At present, besides the identification of constituent immune cells, an avenue intended to reveal how these neighboring immune cells modulate the inflammatory signals in macrophages has being created. In order to reveal the significance of inflammation during the development of type 2 diabetes, the identification of both factors that regulate trafficking of macrophages and intracellular molecules that control inflammatory activation in macrophages would be indispensable. Since there might be substantial differences in the nature of inflammation between rodents and humans and since clinical applications have not yet achieved excellent results, the question remains of how much the inhibition of inflammation contributes to improving glucose homeostasis. In future, there is a need for translational research that applies evidence from mice to human subjects. Because chronic inflammation is also involved in the development of atherosclerosis, rheumatoid arthritis, cancers, and neurodegenerative diseases, the suppression of inflammation can be a desirable therapy for type 2 diabetes. However, simple reduction of inflammation cannot be a beneficial approach as innate immunity is a radical form of homeostasis to deal with pathogenic infections. In addition, since the pathophysiology does not develop via a single molecule, multilayered targeting of various molecules without affecting physiological immune function has to be achieved. The location, timing, and degree of suppression all have to be controlled. Although recent studies have shed light on the pathophysiological roles of inflammation in diabetes, substantial efforts are required to achieve clinical application in human subjects.

ACKNOWLEDGMENTS

The authors' work was supported by a grant for the Intellectual Cluster Formation Project and the Twentieth Century COE Program "Center of Excellence for Signal Transduction Disease:

Diabetes Mellitus as a Model" from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (to Masato Kasuga), by the Manpei Suzuki Diabetes Foundation (to Sanshiro Tateya), by the Mochida Memorial Foundation for Medical and Pharmaceutical Research (to Sanshiro Tateya), by The Naito Foundation (to Sanshiro Tateya), by NIH Grant DK-073878 (to Francis Kim), and by a grant from the John L. Locke Jr. Charitable Trust and from the Kenneth H. Cooper Endowed Professorship in Preventive Cardiology (to Francis Kim). Sanshiro Tateya wrote the manuscript, contributed to discussions, and reviewed and edited the manuscript. Francis Kim and Yoshikazu Tamori interpreted the data and wrote, reviewed, and edited the manuscript.

REFERENCES

1. Ebstein W. Zur therapie des diabetes mellitus, insbesondere uber die anwendeng der salicylauren natron bei demselben. *Klin Wochenschr* (1876) **13**:337–40.
2. Williamson RT. On the treatment of glycosuria and diabetes mellitus with sodium salicylate. *Br Med J* (1901) **1**:760–2. doi:10.1136/bmj.1.2100.760
3. Reid J, Macdougall AI, Andrews MM. Aspirin and diabetes mellitus. *Br Med J* (1957) **2**:1071–4. doi:10.1136/bmj.2.5053.1071
4. Reid J, Lightbody TD. The insulin equivalence of salicylate. *Br Med J* (1959) **1**:897–900. doi:10.1136/bmj.1.5126.897
5. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* (1993) **259**:87–91. doi:10.1126/science.7678183
6. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* (1996) **271**:665–8. doi:10.1126/science.271.5249.665
7. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* (1994) **372**:425–32. doi:10.1038/372425a0
8. Hoene M, Weigert C. The role of interleukin-6 in insulin resistance, body fat distribution and energy balance. *Obes Rev* (2008) **9**:20–9.
9. Sabio G, Das M, Mora A, Zhang Z, Jun JY, Ko HJ, et al. A stress signaling pathway in adipose tissue regulates hepatic insulin resistance. *Science* (2008) **322**:1539–43. doi:10.1126/science.1160794
10. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al. The hormone resistin links obesity to diabetes. *Nature* (2001) **409**:307–12. doi:10.1038/35053000
11. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* (2005) **436**:356–62. doi:10.1038/nature03711
12. Yang RZ, Lee MJ, Hu H, Pray J, Wu HB, Hansen BC, et al. Identification of omentin as a novel depot-specific adipokine in human adipose tissue: possible role in modulating insulin action. *Am J Physiol Endocrinol Metab* (2006) **290**:E1253–61. doi:10.1152/ajpendo.00572.2004
13. Goralski KB, McCarthy TC, Hanniman EA, Zabel BA, Butcher EC, Parlee SD, et al. Chemerin, a novel adipokine that regulates adipogenesis and adipocyte metabolism. *J Biol Chem* (2007) **282**:28175–88. doi:10.1074/jbc.M700793200
14. Bozaoglu K, Bolton K, McMillan J, Zimmet P, Jowett J, Collier G, et al. Chemerin is a novel adipokine associated with obesity and metabolic syndrome. *Endocrinology* (2007) **148**:4687–94. doi:10.1210/en.2007-0175
15. Takahashi M, Takahashi Y, Takahashi K, Zolotaryov FN, Hong KS, Kitazawa R, et al. Chemerin enhances insulin signaling and potentiates insulin-stimulated glucose uptake in 3T3-L1 adipocytes. *FEBS Lett* (2008) **582**:573–8. doi:10.1016/j.febslet.2008.01.023
16. Matsubara T, Mita A, Minami K, Hosooka T, Kitazawa S, Takahashi K, et al. PGRN is a key adipokine mediating high fat diet-induced insulin resistance and obesity through IL-6 in adipose tissue. *Cell Metab* (2012) **15**:38–50. doi:10.1016/j.cmet.2011.12.002
17. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest* (2006) **116**:1494–505. doi:10.1172/JCI26498
18. Tateya S, Tamori Y, Kawaguchi T, Kanda H, Kasuga M. An increase in the circulating concentration of monocyte chemoattractant protein-1 elicits systemic insulin resistance irrespective of adipose tissue inflammation in mice. *Endocrinology* (2010) **151**:971–9. doi:10.1210/en.2009-0926
19. Kamei N, Tobe K, Suzuki R, Ohsugi M, Watanabe T, Kubota N, et al. Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance. *J Biol Chem* (2006) **281**:26602–14. doi:10.1074/jbc.M601284200
20. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature* (1997) **389**:610–4. doi:10.1038/39335
21. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* (2003) **112**:1796–808. doi:10.1172/JCI19246
22. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* (2003) **112**:1821–30. doi:10.1172/JCI19451
23. Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* (2002) **8**:731–7. doi:10.1038/nm724
24. Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Matsui J, et al. Disruption of adiponectin causes insulin resistance and neointimal formation. *J Biol Chem* (2002) **277**:25863–6. doi:10.1074/jbc.C200251200
25. Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, et al. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat Med* (2009) **15**:914–20. doi:10.1038/nm.1964
26. Kosteli A, Sugaru E, Haemmerle G, Martin JF, Lei J, Zechner R, et al. Weight loss and lipolysis promote a dynamic immune response in murine adipose tissue. *J Clin Invest* (2010) **120**:3466–79. doi:10.1172/JCI42845
27. Bruserud Ø, Kittang AO. The chemokine system in experimental and clinical hematology. *Curr Top Microbiol Immunol* (2010) **341**:3–12. doi:10.1007/82_2010_18
28. Sartipy P, Loskutoff DJ. Monocyte chemoattractant protein 1 in obesity and insulin resistance. *Proc Natl Acad Sci U S A* (2003) **100**:7265–72. doi:10.1073/pnas.1133870100
29. Weisberg SP, Hunter D, Huber R, Lemieux J, Slaymaker S, Vaddi K, et al. CCR2 modulates inflammatory and metabolic effects of high-fat feeding. *J Clin Invest* (2006) **116**:115–24. doi:10.1172/JCI24335C1
30. Ito A, Suganami T, Yamauchi A, Degawa-Yamauchi M, Tanaka M, Kouyama R, et al. Role of CC chemokine receptor 2 in bone marrow cells in the recruitment of macrophages into obese adipose tissue. *J Biol Chem* (2008) **283**:35715–23. doi:10.1074/jbc.M804220200
31. Piemonti L, Calori G, Mercalli A, Lattuada G, Monti P, Garancini MP, et al. Fasting plasma leptin, tumor necrosis factor-alpha receptor 2, and monocyte chemoattracting protein 1 concentration in a population of glucose-tolerant and glucose-intolerant women: impact on cardiovascular mortality. *Diabetes Care* (2003) **26**:2883–9. doi:10.2337/diacare.26.10.2883
32. Mine S, Okada Y, Tanikawa T, Kawahara C, Tabata T, Tanaka Y. Increased expression levels of monocyte CCR2 and monocyte chemoattractant protein-1 in patients with diabetes mellitus. *Biochem Biophys Res Commun* (2006) **344**:780–5. doi:10.1016/j.bbrc.2006.03.197
33. Kouyama K, Miyake K, Zenibayashi M, Hirota Y, Teranishi T, Tamori Y, et al. Association of serum MCP-1 concentration and MCP-1 polymorphism with insulin resistance in Japanese individuals with obese type 2 diabetes. *Kobe J Med Sci* (2008) **53**:345–54.

34. Kirk EA, Sagawa ZK, McDonald TO, O'Brien KD, Heinecke JW. Monocyte chemoattractant protein deficiency fails to restrain macrophage infiltration into adipose tissue [corrected]. *Diabetes* (2008) **57**:1254–61. doi:10.2337/db07-1061
35. Inouye KE, Shi H, Howard JK, Daly CH, Lord GM, Rollins BJ, et al. Absence of CC chemokine ligand 2 does not limit obesity-associated infiltration of macrophages into adipose tissue. *Diabetes* (2007) **56**:2242–50. doi:10.2337/db07-0425
36. Oh DY, Morinaga H, Talukdar S, Bae EJ, Olefsky JM. Increased macrophage migration into adipose tissue in obese mice. *Diabetes* (2012) **61**:346–54. doi:10.2337/db11-0860
37. Tamura Y, Sugimoto M, Murayama T, Ueda Y, Kanamori H, Ono K, et al. Inhibition of CCR2 ameliorates insulin resistance and hepatic steatosis in db/db mice. *Arterioscler Thromb Vasc Biol* (2008) **28**:2195–201. doi:10.1161/ATVBAHA.108.168633
38. Yang SJ, IglayReger HB, Kadouh HC, Bodary PF. Inhibition of the chemokine (C-C motif) ligand 2/chemokine (C-C motif) receptor 2 pathway attenuates hyperglycaemia and inflammation in a mouse model of hepatic steatosis and lipoatrophy. *Diabetologia* (2009) **52**:972–81. doi:10.1007/s00125-009-1309-8
39. Ito A, Suganami T, Miyamoto Y, Yoshimasa Y, Takeya M, Kamei Y, et al. Role of MAPK phosphatase-1 in the induction of monocyte chemoattractant protein-1 during the course of adipocyte hypertrophy. *J Biol Chem* (2007) **282**:25445–52. doi:10.1074/jbc.M701549200
40. Kitade H, Sawamoto K, Nagashimada M, Inoue H, Yamamoto Y, Sai Y, et al. CCR5 plays a critical role in obesity-induced adipose tissue inflammation and insulin resistance by regulating both macrophage recruitment and M1/M2 status. *Diabetes* (2012) **61**:1680–90. doi:10.2337/db11-1506
41. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* (2007) **117**:175–84. doi:10.1172/JCI29881
42. Nara N, Nakayama Y, Okamoto S, Tamura H, Kiyono M, Muraoka M, et al. Disruption of CXC motif chemokine ligand-14 in mice ameliorates obesity-induced insulin resistance. *J Biol Chem* (2007) **282**:30794–803. doi:10.1074/jbc.M700412200
43. Nomiyama T, Perez-Tilve D, Ogawa D, Gizard F, Zhao Y, Heywood EB, et al. Osteopontin mediates obesity-induced adipose tissue macrophage infiltration and insulin resistance in mice. *J Clin Invest* (2007) **117**:2877–88. doi:10.1172/JCI31986
44. Tabata M, Kadomatsu T, Fukuhara S, Miyata K, Ito Y, Endo M, et al. Angiopoietin-like protein 2 promotes chronic adipose tissue inflammation and obesity-related systemic insulin resistance. *Cell Metab* (2009) **10**:178–88. doi:10.1016/j.cmet.2009.08.003
45. Han CY, Subramanian S, Chan CK, Omer M, Chiba T, Wight TN, et al. Adipocyte-derived serum amyloid A3 and hyaluronan play a role in monocyte recruitment and adhesion. *Diabetes* (2007) **56**:2260–73. doi:10.2337/db07-0218
46. Subramanian S, Han CY, Chiba T, McMillen TS, Wang SA, Haw A, et al. Dietary cholesterol worsens adipose tissue macrophage accumulation and atherosclerosis in obese LDL receptor-deficient mice. *Arterioscler Thromb Vasc Biol* (2008) **28**:685–91. doi:10.1161/ATVBAHA.107.157685
47. Arkan M, Hevener A, Greten F, Maeda S, Li Z, Long J, et al. IKK-beta links inflammation to obesity-induced insulin resistance. *Nat Med* (2005) **11**:191–8. doi:10.1038/nm1185
48. Cai D, Yuan M, Frantz D, Melendez P, Hansen L, Lee J, et al. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med* (2005) **11**:183–90. doi:10.1038/nm1166
49. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest* (2006) **116**:1793–801. doi:10.1172/JCI29069
50. Odegaard J, Ricardo-Gonzalez R, Red Eagle A, Vats D, Morel C, Goforth M, et al. Alternative M2 activation of Kupffer cells by PPARdelta ameliorates obesity-induced insulin resistance. *Cell Metab* (2008) **7**:496–507. doi:10.1016/j.cmet.2008.04.003
51. Tateya S, Rizzo NO, Handa P, Cheng AM, Morgan-Stevenson V, Daum G, et al. Endothelial NO/cGMP/VASP signaling attenuates Kupffer cell activation and hepatic insulin resistance induced by high-fat feeding. *Diabetes* (2011) **60**:2792–801. doi:10.2337/db11-0255
52. Huang W, Metlakunta A, Dedousis N, Zhang P, Sipula I, Dube J, et al. Depletion of liver Kupffer cells prevents the development of diet-induced hepatic steatosis and insulin resistance. *Diabetes* (2010) **59**:347–57. doi:10.2337/db09-0016
53. Obstfeld AE, Sugaru E, Thearle M, Francisco AM, Gayet C, Ginsberg HN, et al. C-C chemokine receptor 2 (CCR2) regulates the hepatic recruitment of myeloid cells that promote obesity-induced hepatic steatosis. *Diabetes* (2010) **59**:916–25. doi:10.2337/db09-1403
54. Nio Y, Yamauchi T, Iwabu M, Okada-Iwabu M, Funata M, Yamaguchi M, et al. Monocyte chemoattractant protein-1 (MCP-1) deficiency enhances alternatively activated M2 macrophages and ameliorates insulin resistance and fatty liver in lipoatrophic diabetic A-ZIP transgenic mice. *Diabetologia* (2012) **55**:3350–8. doi:10.1007/s00125-012-2710-2
55. Handa P, Tateya S, Rizzo NO, Cheng AM, Morgan-Stevenson V, Han CY, et al. Reduced vascular nitric oxide-cGMP signaling contributes to adipose tissue inflammation during high-fat feeding. *Arterioscler Thromb Vasc Biol* (2011) **31**:2827–35. doi:10.1161/ATVBAHA.111.236554
56. Hotamisligil GS. Inflammation and metabolic disorders. *Nature* (2006) **444**:860–7. doi:10.1038/nature05485
57. Patsouris D, Li PP, Thapar D, Chapman J, Olefsky JM, Neels JG. Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. *Cell Metab* (2008) **8**:301–9. doi:10.1016/j.cmet.2008.08.015
58. Wu H, Ghosh S, Perrard XD, Feng L, Garcia GE, Perrard JL, et al. T-cell accumulation and regulated on activation, normal T cell expressed and secreted upregulation in adipose tissue in obesity. *Circulation* (2007) **115**:1029–38. doi:10.1161/CIRCULATIONAHA.106.638379
59. Rausch ME, Weisberg S, Vardhana P, Tortoriello DV. Obesity in C57BL/6J mice is characterized by adipose tissue hypoxia and cytotoxic T-cell infiltration. *Int J Obes (Lond)* (2008) **32**:451–63. doi:10.1038/sj.ijo.0803744
60. Kintscher U, Hartge M, Hess K, Foryst-Ludwig A, Clemenz M, Wabitsch M, et al. T-lymphocyte infiltration in visceral adipose tissue: a primary event in adipose tissue inflammation and the development of obesity-mediated insulin resistance. *Arterioscler Thromb Vasc Biol* (2008) **28**:1304–10. doi:10.1161/ATVBAHA.108.165100
61. Winer S, Chan Y, Paltser G, Truong D, Tsui H, Bahrami J, et al. Normalization of obesity-associated insulin resistance through immunotherapy. *Nat Med* (2009) **15**:921–9. doi:10.1038/nm.2001
62. Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, et al. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nat Med* (2009) **15**:930–9. doi:10.1038/nm.2002
63. Tiemessen MM, Jagger AL, Evans HG, van Herwijnen MJ, John S, Taams LS. CD4+CD25+Foxp3+ regulatory T cells induce alternative activation of human monocytes/macrophages. *Proc Natl Acad Sci U S A* (2007) **104**:19446–51. doi:10.1073/pnas.0706832104
64. Martinez FO, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. *Front Biosci* (2008) **13**:453–61. doi:10.2741/2692
65. Cipolletta D, Feuerer M, Li A, Kamei N, Lee J, Shoelson SE, et al. PPAR-γ is a major driver of the accumulation and phenotype of adipose tissue Treg cells. *Nature* (2012) **486**:549–53. doi:10.1038/nature11132
66. Duffaut C, Galitzky J, Lafontan M, Bouloumié A. Unexpected trafficking of immune cells within the adipose tissue during the onset of obesity. *Biochem Biophys Res Commun* (2009) **384**:482–5. doi:10.1016/j.bbrc.2009.05.002
67. Winer DA, Winer S, Shen L, Wadia PP, Yantha J, Paltser G, et al. B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. *Nat Med* (2011) **17**:610–7. doi:10.1038/nm.2353
68. Defuria J, Belkina AC, Jagannathan-Bogdan M, Snyder-Cappione J, Carr JD, Nersesova

YR, et al. B cells promote inflammation in obesity and type 2 diabetes through regulation of T-cell function and an inflammatory cytokine profile. *Proc Natl Acad Sci U S A* (2013) **110**:5133–8. doi: 10.1073/pnas.1215840110

69. Wu D, Molofsky AB, Liang HE, Ricardo-Gonzalez RR, Jouihan HA, Bando JK, et al. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. *Science* (2011) **332**:243–7. doi: 10.1126/science.1201475
70. Talukdar S, Oh DY, Bandyopadhyay G, Li D, Xu J, McNelis J, et al. Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. *Nat Med* (2012):doi:10.1038/nm.2885
71. Mansuy-Aubert V, Zhou QL, Xie X, Gong Z, Huang JY, Khan AR, et al. Imbalance between neutrophil elastase and its inhibitor α1-antitrypsin in obesity alters insulin sensitivity. inflammation, and energy expenditure. *Cell Metab* (2013) **17**:534–48. doi:10.1016/j.cmet.2013.03.005
72. Andrade VL, Petruceli E, Belo VA, Andrade-Fernandes CM, Caetano Russi CV, Bosco AA, et al. Evaluation of plasmatic MMP-8, MMP-9, TIMP-1 and MPO levels in obese and lean women. *Clin Biochem* (2012) **45**: 412–5. doi:10.1016/j.clinbiochem.2012.01.008
73. Trellakis S, Rydleuskaya A, Fischer C, Canbay A, Tagay S, Scherag A, et al. Low adiponectin, high levels of apoptosis and increased peripheral blood neutrophil activity in healthy obese subjects. *Obes Facts* (2012) **5**:305–18. doi:10.1159/000339452
74. Liu J, Divoux A, Sun J, Zhang J, Clément K, Glickman JN, et al. Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. *Nat Med* (2009) **15**:940–5. doi:10.1038/nm.1994
75. Tanaka A, Nomura Y, Matsuda A, Ohmori K, Matsuda H. Mast cells function as an alternative modulator of adipogenesis through 15-deoxy-delta-12, 14-prostaglandin J2. *Am J Physiol Cell Physiol* (2011) **301**:C1360–7. doi:10.1152/ajpcell.00514.2010
76. Cerundolo V, Silk JD, Masri SH, Salio M. Harnessing invariant NKT cells in vaccination strategies. *Nat Rev Immunol* (2009) **9**:28–38. doi:10.1038/nri2451
77. Ji Y, Sun S, Xu A, Bhargava P, Yang L, Lam KS, et al. Activation of natural killer T cells promotes M2 Macrophage polarization in adipose tissue and improves systemic glucose tolerance via interleukin-4 (IL-4)/STAT6 protein signaling axis in obesity. *J Biol Chem* (2012) **287**: 13561–71. doi:10.1074/jbc.M112.350066
78. Ji Y, Sun S, Xia S, Yang L, Li X, Qi L. Short term high fat diet challenge promotes alternative macrophage polarization in adipose tissue via natural killer T cells and interleukin-4. *J Biol Chem* (2012) **287**: 24378–86. doi:10.1074/jbc.M112.371807
79. Schipper HS, Rakhshandehroo M, van de Graaf SF, Venken K, Koppen A, Stienstra R, et al. Natural killer T cells in adipose tissue prevent insulin resistance. *J Clin Invest* (2012) **122**:3343–54. doi:10.1172/JCI62739
80. Scanga CB, Verde TJ, Paolone AM, Andersen RE, Wadden TA. Effects of weight loss and exercise training on natural killer cell activity in obese women. *Med Sci Sports Exerc* (1998) **30**: 1666–71. doi:10.1097/00005768-199812000-00002
81. Nieman DC, Henson DA, Nehlsen-Cannarella SL, Ekkens M, Utter AC, Butterworth DE, et al. Influence of obesity on immune function. *J Am Diet Assoc* (1999) **99**:294–9. doi: 10.1016/S0002-8223(99)00077-2
82. Lynch LA, O'Connell JM, Kwasnik AK, Cawood TJ, O'Farrelly C, O'Shea DB. Are natural killer cells protecting the metabolically healthy obese patient? *Obesity (Silver Spring)* (2009) **17**:601–5. doi:10.1038/oby.2008.565
83. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. *J Clin Invest* (2006) **116**:3015–25. doi:10.1172/JCI28898
84. Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* (1998) **282**: 2085–8. doi:10.1126/science.282.5396.2085
85. Saberi M, Woods NB, de Luca C, Schenk S, Lu JC, Bandyopadhyay G, et al. Hematopoietic cell-specific deletion of toll-like receptor 4 ameliorates hepatic and adipose tissue insulin resistance in high-fat-fed mice. *Cell Metab* (2009) **10**:419–29. doi:10.1016/j.cmet.2009.09.006
86. Suganami T, Nishida J, Ogawa Y. A paracrine loop between adipocytes and macrophages aggravates inflammatory changes: role of free fatty acids and tumor necrosis factor alpha. *Arterioscler Thromb Vasc Biol* (2005) **25**:2062–8. doi:10.1161/01.ATV.0000183883.72263.13
87. Pal D, Dasgupta S, Kundu R, Maitra S, Das G, Mukhopadhyay S, et al. Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. *Nat Med* (2012):doi:10.1038/nm.2851 [Epub ahead of print].
88. Hosoi T, Yokoyama S, Matsuo S, Akira S, Ozawa K. Myeloid differentiation factor 88 (MyD88)-deficiency increases risk of diabetes in mice. *PLoS ONE* (2010) **5**(9):e12537. doi:10.1371/journal.pone.0012537
89. Ricardo-Gonzalez RR, Red Eagle A, Odegaard JI, Jouihan H, Morel CR, Heredia JE, et al. IL-4/STAT6 immune axis regulates peripheral nutrient metabolism and insulin sensitivity. *Proc Natl Acad Sci U S A* (2010) **107**: 22617–22. doi:10.1073/pnas.1009152108
90. Cintra DE, Pauli JR, Araújo EP, Moraes JC, de Souza CT, Milanski M, et al. Interleukin-10 is a protective factor against diet-induced insulin resistance in liver. *J Hepatol* (2008) **48**: 628–37. doi:10.1016/j.jhep.2007.12.017
91. Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, et al. Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. *Nature* (2007) **447**:1116–20. doi:10.1038/nature05894
92. Hevener AL, Olefsky JM, Reichart D, Nguyen MT, Bandyopadyhay G, Leung HY, et al. Macrophage PPAR gamma is required for normal skeletal muscle and hepatic insulin sensitivity and full antidiabetic effects of thiazolidinediones. *J Clin Invest* (2007) **117**:1658–69. doi:10.1172/JCI31561
93. Liao X, Sharma N, Kapadia F, Zhou G, Lu Y, Hong H, et al. Krüppel-like factor 4 regulates macrophage polarization. *J Clin Invest* (2011) **121**:2736–49. doi:10.1172/JCI45444
94. Galic S, Fullerton MD, Schertzer JD, Sikkema S, Marcinko K, Walkley CR, et al. Hematopoietic AMPK β1 reduces mouse adipose tissue macrophage inflammation and insulin resistance in obesity. *J Clin Invest* (2011) **121**:4903–15. doi:10.1172/JCI58577
95. Houtkooper RH, Pirinen E, Auwerx J. Sirtuins as regulators of metabolism and healthspan. *Nat Rev Mol Cell Biol* (2012) **13**:225–38. doi: 10.1038/nrm3293
96. Liang F, Kume S, Koya D. SIRT1 and insulin resistance. *Nat Rev Endocrinol* (2009) **5**:367–73. doi: 10.1038/nrendo.2009.101
97. Milne JC, Lambert PD, Schenk S, Carney DP, Smith JJ, Gagne DJ, et al. Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. *Nature* (2007) **450**:712–6. doi:10.1038/nature06261
98. Yeung F, Hoberg J, Ramsey C, Keller M, Jones D, Frye R, et al. Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J* (2004) **23**:2369–80. doi:10.1038/sj.emboj.7600244
99. Qiao L, Shao J. SIRT1 regulates adiponectin gene expression through Foxo1-C/enhancer-binding protein alpha transcriptional complex. *J Biol Chem* (2006) **281**:39915–24. doi: 10.1074/jbc.M607215200
100. Costa Cdos S, Hammes TO, Rohden F, Margis R, Bortolotto JW, Padoin AV, et al. SIRT1 transcription is decreased in visceral adipose tissue of morbidly obese patients with severe hepatic steatosis. *Obes Surg* (2010) **20**: 633–9. doi:10.1007/s11695-009-0052-z
101. Chalkiadaki A, Guarente L. High-fat diet triggers inflammation-induced cleavage of SIRT1 in adipose tissue to promote metabolic dysfunction. *Cell Metab* (2012) **16**:180–8. doi:10.1016/j.cmet.2012.07.003
102. Gillum MP, Kotas ME, Erion DM, Kursawe R, Chatterjee P, Nead KT, et al. SirT1 regulates adipose tissue inflammation. *Diabetes* (2011) **60**:3235–45. doi:10.2337/db11-0616
103. Yoshizaki T, Schenk S, Imamura T, Babendure J, Sonoda N, Bae E, et al. SIRT1 inhibits inflammatory pathways in macrophages and modulates insulin sensitivity. *Am J Physiol Endocrinol Metab* (2010) **298**:

E419–28. doi:10.1152/ajpendo.00417.2009
104. Yang Z, Kahn BB, Shi H, Xue BZ. Macrophage alpha1 AMP-activated protein kinase (alpha1AMPK) antagonizes fatty acid-induced inflammation through SIRT1. *J Biol Chem* (2010) **285**:19051–9. doi:10.1074/jbc.M110.123620
105. Hosogai N, Fukuhara A, Oshima K, Miyata Y, Tanaka S, Segawa K, et al. Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. *Diabetes* (2007) **56**:901–11. doi:10.2337/db06-0911
106. Sung HK, Doh KO, Son JE, Park JG, Bae Y, Choi S, et al. Adipose vascular endothelial growth factor regulates metabolic homeostasis through angiogenesis. *Cell Metab* (2013) **17**:61–72. doi:10.1016/j.cmet.2012.12.010
107. Zhang Y, Goldman S, Baerga R, Zhao Y, Komatsu M, Jin S. Adipose-specific deletion of autophagy-related gene 7 (atg7) in mice reveals a role in adipogenesis. *Proc Natl Acad Sci U S A* (2009) **106**:19860–5.
108. Zhou R, Tardivel A, Thorens B, Choi I, Tschopp J. Thioredoxin-interacting protein links oxidative stress to inflammasome activation. *Nat Immunol* (2010) **11**:136–40. doi:10.1038/ni.1831
109. Jansen HJ, van Essen P, Koenen T, Joosten LA, Netea MG, Tack CJ, et al. Autophagy activity is up-regulated in adipose tissue of obese individuals and modulates proinflammatory cytokine expression. *Endocrinology* (2012) **153**:5866–74. doi:10.1210/en.2012-1625
110. Franchi L, Muñoz-Planillo R, Núñez G. Sensing and reacting to microbes through the inflammasomes. *Nat Immunol* (2012) **13**:325–32. doi:10.1038/ni.2231
111. Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, et al. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nat Med* (2011) **17**:179–88. doi:10.1038/nm.2279
112. Stienstra R, Joosten LA, Koenen T, van Tits B, van Diepen JA, van den Berg SA, et al. The inflammasome-mediated caspase-1 activation controls adipocyte differentiation and insulin sensitivity. *Cell Metab* (2010) **12**:593–605. doi:10.1016/j.cmet.2010.11.011
113. Lagathu C, Yvan-Charvet L, Bastard JP, Maachi M, Quignard-Boulangé A, Capeau J, et al. Long-term treatment with interleukin-1beta induces insulin resistance in murine and human adipocytes. *Diabetologia* (2006) **49**:2162–73. doi:10.1007/s00125-006-0335-z
114. Moschen AR, Molnar C, Enrich B, Geiger S, Ebenbichler CF, Tilg H. Adipose and liver expression of interleukin (IL)-1 family members in morbid obesity and effects of weight loss. *Mol Med* (2011) **17**:840–5. doi:10.2119/molmed.2010.00108
115. Kasapis C, Thompson PD. The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review. *J Am Coll Cardiol* (2005) **45**:1563–9. doi:10.1016/j.jacc.2004.12.077
116. de Lemos ET, Oliveira J, Pinheiro JP, Reis F. Regular physical exercise as a strategy to improve antioxidant and anti-inflammatory status: benefits in type 2 diabetes mellitus. *Oxid Med Cell Longev* (2012) **2012**:741545. doi:10.1155/2012/741545
117. Petersen AM, Pedersen BK. The anti-inflammatory effect of exercise. *J Appl Physiol* (2005) **98**:1154–62. doi:10.1152/japplphysiol.00164.2004
118. Balducci S, Zanuso S, Nicolucci A, Fernando F, Cavallo S, Cardelli P, et al. Anti-inflammatory effect of exercise training in subjects with type 2 diabetes and the metabolic syndrome is dependent on exercise modalities and independent of weight loss. *Nutr Metab Cardiovasc Dis* (2010) **20**:608–17. doi:10.1016/j.numecd.2009.04.015
119. de Oliveira VN, Bessa A, Jorge ML, Oliveira RJ, de Mello MT, De Agostini GG, et al. The effect of different training programs on antioxidant status, oxidative stress, and metabolic control in type 2 diabetes. *Appl Physiol Nutr Metab* (2012) **37**:334–44. doi:10.1139/h2012-004
120. Manna SK, Mukhopadhyay A, Aggarwal BB. Resveratrol suppresses TNF-induced activation of nuclear transcription factors NF-kappa B, activator protein-1, and apoptosis: potential role of reactive oxygen intermediates and lipid peroxidation. *J Immunol* (2000) **164**:6509–19.
121. El-Mowafy AM, White RE. Resveratrol inhibits MAPK activity and nuclear translocation in coronary artery smooth muscle: reversal of endothelin-1 stimulatory effects. *FEBS Lett* (1999) **451**:63–7. doi:10.1016/S0014-5793(99)00541-4
122. Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, et al. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature* (2003) **425**:191–6. doi:10.1038/nature01960
123. Dasgupta B, Milbrandt J. Resveratrol stimulates AMP kinase activity in neurons. *Proc Natl Acad Sci U S A* (2007) **104**:7217–22. doi:10.1073/pnas.0610068104
124. Ruderman NB, Xu XJ, Nelson L, Cacicedo JM, Saha AK, Lan F, et al. AMPK and SIRT1: a long-standing partnership? *Am J Physiol Endocrinol Metab* (2010) **298**:E751–60. doi:10.1152/ajpendo.00745.2009
125. Timmers S, Konings E, Bilet L, Houtkooper RH, van de Weijer T, Goossens GH, et al. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. *Cell Metab* (2011) **14**:612–22. doi:10.1016/j.cmet.2011.10.002
126. Figueras M, Olivan M, Busquets S, López-Soriano FJ, Argilés JM. Effects of eicosapentaenoic acid (EPA) treatment on insulin sensitivity in an animal model of diabetes: improvement of the inflammatory status. *Obesity (Silver Spring)* (2011) **19**:362–9. doi:10.1038/oby.2010.194
127. Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell* (2010) **142**:687–98. doi:10.1016/j.cell.2010.07.041
128. Itariu BK, Zeyda M, Hochbrugger EE, Neuhofer A, Prager G, Schindler K, et al. Long-chain n-3 PUFAs reduce adipose tissue and systemic inflammation in severely obese nondiabetic patients: a randomized controlled trial. *Am J Clin Nutr* (2012) **96**:1137–49. doi:10.3945/ajcn.112.037432
129. Sato A, Kawano H, Notsu T, Ohta M, Nakakuki M, Mizuguchi K, et al. Antiobesity effect of eicosapentaenoic acid in high-fat/high-sucrose diet-induced obesity: importance of hepatic lipogenesis. *Diabetes* (2010) **59**:2495–504. doi:10.2337/db09-1554
130. Yin MJ, Yamamoto Y, Gaynor RB. The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. *Nature* (1998) **396**:77–80. doi:10.1038/23948
131. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, et al. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* (2001) **293**:1673–7. doi:10.1126/science.1061620
132. Hundal RS, Petersen KF, Mayerson AB, Randhawa PS, Inzucchi S, Shoelson SE, et al. Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. *J Clin Invest* (2002) **109**:1321–6. doi:10.1172/JCI14955
133. Goldfine AB, Fonseca V, Jablonski KA, Pyle L, Staten MA, Shoelson SE, et al. TDTIUSiTDS: The effects of salsalate on glycemic control in patients with type 2 diabetes: a randomized trial. *Ann Intern Med* (2010) **152**:346–57. doi:10.7326/0003-4819-152-6-201003160-00004
134. Larsen CM, Faulenbach M, Vaag A, Vølund A, Ehses JA, Seifert B, et al. Interleukin-1-receptor antagonist in type 2 diabetes mellitus. *N Engl J Med* (2007) **356**:1517–26. doi:10.1056/NEJMoa065213
135. Osborn O, Brownell SE, Sanchez-Alavez M, Salomon D, Gram H, Bartfai T. Treatment with an Interleukin 1 beta antibody improves glycemic control in diet-induced obesity. *Cytokine* (2008) **44**:141–8. doi:10.1016/j.cyto.2008.07.004
136. Donath MY, Mandrup-Poulsen T. The use of interleukin-1-receptor antagonists in the treatment of diabetes mellitus. *Nat Clin Pract Endocrinol Metab* (2008) **4**:240–1. doi:10.1038/ncpendmet0783
137. Bernstein LE, Berry J, Kim S, Canavan B, Grinspoon SK. Effects of etanercept in patients with the metabolic syndrome.

Arch Intern Med (2006) **166**: 902–8. doi:10.1001/archinte.166.8.902

138. Dominguez H, Storgaard H, Rask-Madsen C, Steffen Hermann T, Ihlemann N, Baunbjerg Nielsen D, et al. Metabolic and vascular effects of tumor necrosis factor-alpha blockade with etanercept in obese patients with type 2 diabetes. *J Vasc Res* (2005) **42**:517–25. doi:10.1159/000088261
139. Lo J, Bernstein LE, Canavan B, Torriani M, Jackson MB, Ahima RS, et al. Effects of TNF-alpha neutralization on adipocytokines and skeletal muscle adiposity in the metabolic syndrome. *Am J Physiol Endocrinol Metab* (2007) **293**:E102–9. doi: 10.1152/ajpendo.00089.2007
140. Thaler JP, Yi CX, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, et al. Obesity is associated with hypothalamic injury in rodents and humans. *J Clin Invest* (2012) **122**:153–62. doi:10.1172/JCI59660

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 10 May 2013; paper pending published: 28 May 2013; accepted: 11 July 2013; published online: 08 August 2013.
*Citation: Tateya S, Kim F and Tamori Y (2013) Recent advances in obesity-induced inflammation and insulin resistance. Front. Endocrinol. **4**:93. doi: 10.3389/fendo.2013.00093*

This article was submitted to Frontiers in Diabetes, a specialty of Frontiers in Endocrinology.
Copyright © 2013 Tateya, Kim and Tamori. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in
ENDOCRINOLOGY

REVIEW ARTICLE
published: 21 August 2013
doi: 10.3389/fendo.2013.00097

Clinical implications of adipocytokines and newly emerging metabolic factors with relation to insulin resistance and cardiovascular health

Sung Hee Choi*, Eun Shil Hong and Soo Lim*

Department of Internal Medicine, Division Endocrinology and Metabolism, Seoul National University College of Medicine, Seoul National University Bundang Hospital, Seongnam, Korea

Edited by:
Tsuguhito Ota, Kanazawa University, Japan

Reviewed by:
Undurti Narasimha Das, UND LIfe Sciences, USA
Carmine Grieco, Glenville State College, USA

****Correspondence:***
Sung Hee Choi and Soo Lim, Department of Internal Medicine, Division Endocrinology and Metabolism, Seoul National University College of Medicine, Seoul National University Bundang Hospital, Gumidong 300, Seongnam, Gyeonggi-do 463-737, Korea
e-mail: drshchoi@snu.ac.kr; limsoo@snu.ac.kr

Adipose tissue is known to secrete hormones actively and produces many biologically active proteins called adipocytokines. Typically, obesity is followed by low-grade inflammation, which is characterized by increased circulating levels of pro-inflammatory cytokines. Macrophages play a role in the inflammatory process by secreting many cytokines such as tumor necrosis factor alpha, interleukin-6, resistin, and retinol binding protein-4. These cytokines and chemokines participate in low-grade pro-inflammatory processes leading to insulin resistance, metabolic impairment, and cardiovascular diseases. More metabolic regulators, such as fibroblast growth factor (FGF)21, FGF19, FGF1, vaspin, and visfatin have now been discovered but their exact roles in human diseases are still unclear. This review focuses on recent research regarding the role of adipokines and new metabolic factors in metabolic derangement or cardiovascular disease.

Keywords: new cytokines, adiponectin, FGFs, biomarker, obesity, diabetes mellitus, insulin resistance

INTRODUCTION

The prevalence of obesity is increasing throughout the world. Obesity is associated with a broad spectrum of cardiometabolic disorders, including hypertension, dyslipidemia, diabetes, and cardiovascular disease (CVD) (1). Obesity is a heterogeneous disorder characterized by multifactorial etiology, which is characterized by various processes: changes in adipocytokines, activation of low-grade inflammation, and production of reactive oxygen species. These factors are linked to endothelial dysfunction, oxidative stress, and inflammatory processes and finally lead to the development of atherosclerosis by multiple interactive pathways (2, 3).

Adipose tissue deposition shows distinct differences between different body areas. These include anatomical, cellular, molecular, physiological, clinical, and prognostic differences. Many studies have suggested that when compared with subcutaneous adipose tissue, visceral adipose tissue and other ectopic fats are more cellular, vascular, and innervated, with a larger number of inflammatory and immune cells, less pre-adipocyte differentiation, and a greater percentage of large adipocytes (4, 5).

As adiposity increases in visceral and ectopic areas, macrophages may increase infiltration (6). This cross-talk between adipose tissue and macrophages is a source of many cytokines such as tumor necrosis factor alpha (TNFα), interleukin (IL)-6, resistin, retinol binding protein-4 (RBP4), which are suspected to participate in low-grade pro-inflammatory processes leading to metabolic disorders, insulin resistance, and CVDs (7). Other adipokines, such as visfatin and vaspin, have been discovered but their exact roles are still unknown. Emerging metabolic regulators such as fibroblast growth factor 21 (FGF21), other FGFs and myonectin appear to play roles in obesity and insulin resistance, from our experience. This review focuses on recent updates regarding the contribution of adipokines and newly discovered metabolic regulators to obesity and insulin resistance.

ADIPONECTIN

Adiponectin has attracted considerable attention among the many adipocytokines secreted from adipose tissue because of its insulin-sensitizing property. Early studies showed that adiponectin levels were low in patients with impaired glucose homeostasis or type 2 diabetes (8, 9). Since then, many studies have demonstrated a significant inverse association between adiponectin and insulin resistance (9). Prospective studies have proved that low levels of adiponectin are associated with an increased incidence of type 2 diabetes (10, 11). Based on this finding, intervention studies focusing on exercise have been tried. A lifestyle intervention study with overweight/obese children for 1 year increased circulating adiponectin levels and insulin sensitivity significantly (12). Our group also proved that adiponectin levels increased significantly after a 10-week aerobic training program in healthy young and middle-aged women, and this was associated with improvements in insulin sensitivity (13).

Adiponectin is closely associated with atherogenesis and the development of CVDs. Low plasma adiponectin level was a predictor of CVD outcome such as myocardial infarction in the general population and among patients with diabetes or end-stage renal disease (14–17). Interestingly, several studies suggested that the

high molecular weight form of adiponectin is a more accurate independent risk factor for CVD than the whole adiponectin level (17–19). However, no significant association between adiponectin and the risk of CVD was found after adjustment for potential confounders (11).

From mechanistic studies in endothelial cells, it was proved that adiponectin strongly inhibits the production of inflammatory cytokines and adhesion molecules, including ICAM-1, VCAM-1, and E-selectin (19). Those results suggest that high levels of adiponectin play a role against the development of atherosclerosis and this has been confirmed in human studies (20, 21).

Thus, many basic and some population-based studies suggest that adiponectin might have a beneficial role in metabolic diseases and atherosclerosis, but some reports are less consistent. This might arise from differences between studies such as variations in populations, confounding factors (or lack thereof) and different isoforms of adiponectin (total vs. the high molecular weight form).

RESISTIN

Initially, resistin was discovered as an adipocytokine in animal models. It was suspected to link obesity with diabetes because it was produced mainly by adipocytes (22). By contrast, adipocytes seem to contribute only a small fraction of the resistin production in humans (23). Instead, inflammatory cells such as macrophages are considered the predominant source of circulating resistin (24).

Some studies have reported that resistin levels are increased in obese individuals (25, 26) while others have not (27, 28). Population-based studies have shown that resistin levels are associated with metabolic impairments and insulin resistance (27, 29, 30) but the association between resistin levels and insulin sensitivity has been inconsistent in humans (25, 31). Resistin levels have also been associated with coronary heart diseases (32) and were correlated with calcification deposition in coronary arteries (28, 33). In contrast, other studies have not shown a significant association between resistin and coronary artery diseases (18, 34). Thus, the evidence linking resistin with decreased insulin sensitivity or increased cardiovascular risk remains inconsistent.

Of note, the secretions of TNFα, IL-6, and other cell adhesion molecules are increased by resistin (35). An *in vitro* study demonstrated that resistin treatment increased the proliferation and migration of vascular smooth muscle and endothelial cells (36). In summary, resistin may participate in cardiovascular physiopathology in humans via the action of macrophages implicated in the inflammatory response related to obesity.

RETINOL BINDING PROTEIN-4

Decades ago, RBP4 was discovered as an adipocytokine that binds specifically to vitamin A (37). RBP4 is produced mainly by the liver and adipose tissue (38). RBP4 levels are closely associated with obesity, particularly visceral adiposity in mice and humans (38, 39). Elevated RBP4 levels were associated with a clustering of components of metabolic syndrome in insulin-resistant subjects (39). In population-based studies, RBP4 levels were positively associated with the obesity index, high blood pressure, and unfavorable lipid profiles (40). RBP4 levels were increased in naive hypertensive women and were correlated with the degree of intima-media thickness, suggesting a participation of this adipocytokine in modulation of the atherosclerotic process and cardio- and cerebrovascular diseases (41, 42). Our group published data showing that regular exercise intervention with a 10-week, moderate-intensity regimen improved cardiorespiratory fitness and adipocytokines including RBP4 levels (13). Weight loss induced by bariatric surgery also decreased RBP4 concentrations (43). In addition, our group also reported that plasma RBP4 levels were significantly higher among patients converting to full diabetes mellitus (DM) from previous gestational DM compared with non-DM converters (44) and plasma RBP4 levels showed significant correlation with cardiovascular risks in patients with subclinical hypothyroidism (45). A recent study with dyslipidemia subjects found that circulating RBP4 concentrations were associated with small dense low-density lipoprotein (LDL) cholesterol and oxidized LDL levels (46).

Although there was robust evidence suggesting role of RBP4 in abnormal glucose metabolism and development of atherosclerosis in mice, several human studies reported that the serum level of RBP4 was not associated with obesity or insulin sensitivity (47, 48). Janke et al. reported discrepancy of relationship of RBP4 with glucose homeostasis between rodents and human (47). Promintzer et al. also demonstrated no increase of plasma RBP4 levels and no correlation with insulin sensitivity in insulin-resistant humans (48).

Since evidence showing relationship of RBP4 with cardiometabolic risk in human is inconsistent, there is still argument on whether elevated RBP4 levels contribute to the pathogenesis of abnormal glucose homeostasis or insulin resistance. More data are needed to clarify the potential role of RBP4 in abnormal metabolic consequences.

C1q TUMOR NECROSIS FACTOR-α-RELATED PROTEIN ISOFORM 5

The C1q TNF-α-related proteins or myonectins have drawn recent attention and C1q TNF-α-related protein isoform 5 (C1QTNF5) has been a focus of research because of its possible association with cardiometabolic risk (49). Structurally, C1QTNF5 is similar to adiponectin in its domain structure. C1QTNF5 belongs to a family of proteins characterized by an N-terminal signal peptide, a collagen repeat domain, and a C-terminal C1q-like globular domain (50). C1QTNFs are expressed in many tissues and have more structural or extracellular matrix-related functions than adiponectin (51). Recently, it was found that the C1QTNF5 level increased in mitochondrial (mt) DNA-depleted myocytes and this was associated with elevated adenosine monophosphate-activated protein kinase (AMPK) activity. In addition, the serum level of C1QTNF5 increased significantly in obese/diabetic animals (52). C1QTNF5 gene was upregulated from the microarray result of subcutaneous fat in obese Pima Indians, suggesting its possible role in developing obesity (53). Our group found that a 10-week exercise training program performed at moderate-intensity decreased C1QTNF5 levels and insulin resistance parameters and increased cardiorespiratory fitness, mtDNA density, and adiponectin level in both young and older groups of women (54). These findings suggest that C1QTNF5 might be an important factor linking mitochondrial dysfunction with insulin resistance. Further research is needed

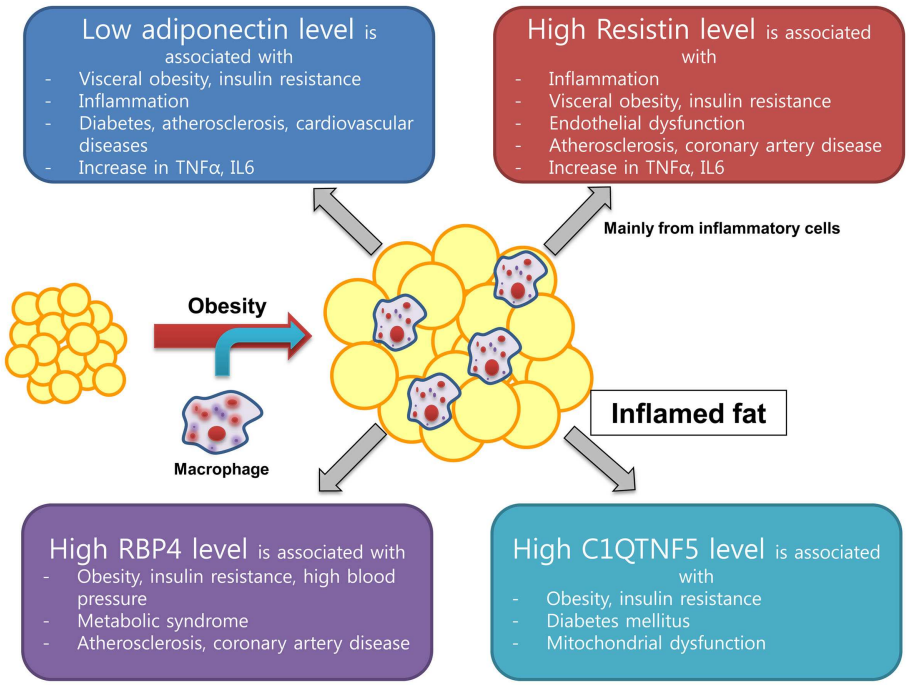


FIGURE 1 | New emerging adipokine from inflamed fat and its association with insulin resistance and cardiovascular health.

to identify the role and molecular mechanism of C1QTNF5 in the development of insulin resistance. Integrated schematic figure from adiponectin to C1QTNF is in **Figure 1**.

FIBROBLAST GROWTH FACTOR 21

Fibroblast Growth Factor (FGF)21, FGF19, and FGF23 belong to the FGF19 family that acts in hormone-like manners unlike other FGF species (55). FGF21 has been highlighted as a new drug candidate for enhancing insulin sensitivity, inducing lipolysis, and preventing diet-induced obesity in many *in vitro* and *in vivo* studies (56–58). FGF21 is mainly produced in the liver but acts on adipose tissue due to its preference for binding to FGF receptor 1 (55, 59). In humans, serum FGF21 levels are paradoxically increased in metabolic diseases such as obesity, diabetes, and CVD (60–62), which infer FGF21 resistance in humans. There are several lines of evidence from animal studies to explain FGF21 resistance in the receptor and in the post-receptor signaling pathway but there is no clear mechanism in humans so far. Including the results of our group, FGF21 excretion in humans is dependent on residual renal function based on data from patients in end-stage renal disease undergoing peritoneal dialysis and hemodialysis (63, 64). We reported that serum FGF21 concentration was significantly associated with altered lipid profiles, especially with hypertriglyceridaemia, insulin resistance, metabolic syndrome, and ectopic fat deposition when adjusted for the body mass index (65). Recently, many interesting features about the role of FGF21 in metabolism have been published. FGF21 regulates PGC1-α protein levels and enhances white adipose tissue browning with upregulation of UCP1 and other thermogenic genes in a cold-exposure mouse model (66). Kim et al. reported that muscle-specific deletion of the *Atg7* (autophagy-related 7) gene in mice produced mitochondrial dysfunction and promoted FGF21 expression, showing a phenotype of being insulin-sensitive and resistant to diet-induced obesity (67). FGF21 enhanced peroxisome proliferator-activated receptor gamma (PPARγ) desumoylation in fat cells to increase its action and showed association with lower bone mass caused by PPARγ activation *in vivo* (68, 69). There are still many unknown aspects of FGF21, especially its role in human metabolism. However, clinical trials of this molecule are ongoing and the results will help explain its effect on glucose, obesity, and lipid metabolism in humans more clearly.

OTHER FGFs

FGF19 in humans and its mouse ortholog FGF15 have been studied for their roles in controlling bile acid synthesis. Recent data showed that activation of the farnesoid X receptor (FXR) by bile acids induced FGF19 and FGF receptor 4-mediated JNK/ERK pathways and inhibited the *CY7A1* gene encoding cholesterol 7α hydroxylase (70). In other study, transintestinal flux of bile acids with diurnal variation to control FGF19 formation in the intestine (71). The peak FGF19 formation was made 90–120 min after postprandial rise of serum bile acids. FGF19 is also a member of the FGF19 family, like FGF21 and FGF23, and acts in a hormone-like manner with a possible role in cholesterol metabolism through bile acid synthesis. In addition, FGF1 has a role in adipose tissue remodeling in mice fed a high-fat diet, being regulated by PPARγ activation. Mice lacking FGF1 showed abnormal adipose tissue with aberrant vasculature and a severe diabetic phenotype in high-fat dietary conditions (72). FGF1 is known for its role in wound healing and development (73), but it is now seen to have a

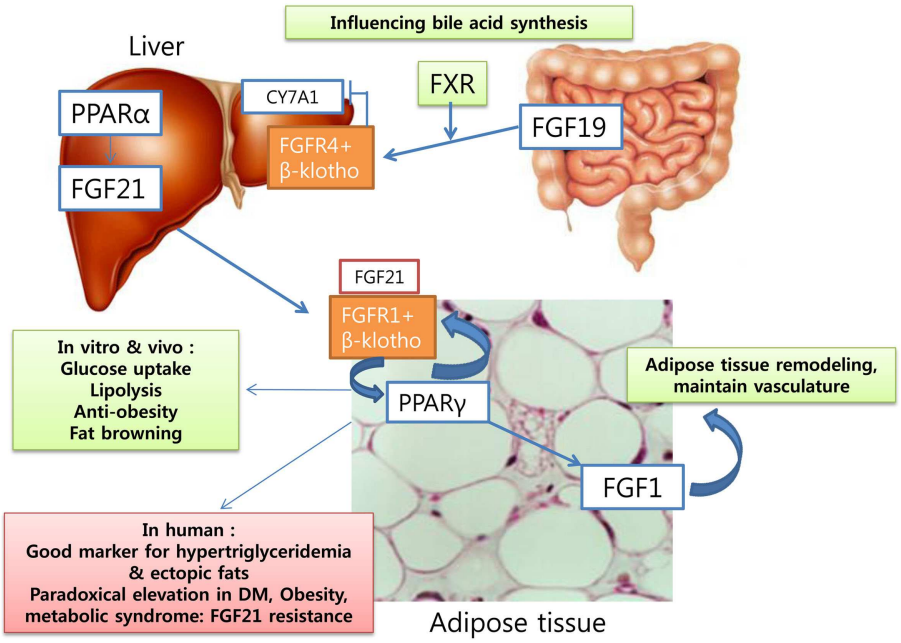


FIGURE 2 | Fibroblast growth factors: as metabolic regulators in human.

role in adipose tissue remodeling and a possible link with obesity. The possible role of FGF 19 family in human is in **Figure 2**.

VASPIN

Vaspin is an adipocytokine isolated from visceral adipose tissue of an animal model of abdominal obesity with type 2 diabetes (OLETF rat). It is increased in the prediabetic stage and decreases when the OLETF rats develop overt diabetes. In animals, vaspin treatment ameliorates insulin sensitivity in high-fat or high sucrose-induced diabetes models (74) and protects against endothelial cell damage caused by free fatty acids through the PI3 kinase/Akt pathway (75). It has been suggested to be a "good" adipocytokine, such as adiponectin. However, serum vaspin levels were paradoxically elevated in human subjects with diabetes and obesity (76). We also reported that the serum vaspin level was higher in women than in men and it was correlated with the metabolic syndrome in men and coronary atherosclerosis in women. However, men with longer duration of diabetes and microvascular complications showed significantly lower levels of vaspin (77). More data are needed to understand the role of vaspin in human diseases such as atherosclerosis, diabetes, and obesity.

VISFATIN

Visfatin was first isolated from the visceral fat of humans and mice and showed insulin-like action by binding to the insulin receptor (78). In carotid artery atheromatous plaques, immunohistochemistry for visfatin showed much higher expression in unstable, symptomatic patients compared with asymptomatic patients, suggesting a role in generating macrophage foam cells in atheromata (79). The serum visfatin level was significantly reduced after gastric bypass surgery in morbidly obese subjects (80). Because visfatin is a pre B cell colony-enhancing factor, it has been studied in modulating systemic inflammation. In CD14+ monocytes, visfatin induced the expression of IL-1β, TNF-α, IL-6, and other CD molecules (81). In addition, the serum visfatin level was higher in patients with diabetes and diabetic nephropathy (82). Visfatin is now regarded as an extracellular nicotinamide phosphoribosyltransferase (eNampt) enzyme and plays an important role in insulin secretion from pancreatic β cell by systemic nicotinamide adenine dinucleotide (NAD) biosynthesis (83). *In vitro* and *in vivo*, visfatin mimics insulin action, but in human studies, it is paradoxically increased in disease conditions and shows correlation with systemic inflammation, vascular complications, and insulin secretion. However, more studies are needed to clarify the role of visfatin in humans.

CONCLUSION

In summary, adipose and muscle tissues are now recognized as important and active endocrine organs. More adipocytokines and metabolic regulators are being discovered continuously and the clinical implications of these molecules are important in understanding the pathophysiology of human obesity, insulin resistance, and CVD. The "classic" adipocytokines such as adiponectin, TNF-α, and IL-6 have been regarded as consistent surrogate markers to reflect cardiovascular risk and other metabolic abnormalities in subjects with insulin resistance. Adiponectin was considered as a good biomarker to protect atherosclerosis and to reduce systemic inflammation from many studies that we mentioned in this review. However, recent studies suggested so called "adiponectin paradox" in many studies, which showed increased adiponectin levels were correlated with higher cardiovascular or all-cause mortality in epidemiological data (84–86). The underlying mechanism behind

this paradox is still unclear, but we can assume that there could some compensatory elevation of adiponectin in patients with metabolic abnormalities resulted in the association with higher mortality in the future.

Some evidence provides linking resistin and RBP4 with insulin resistance or cardiovascular risk. However, there are inconsistent results suggesting no or weak relationship of these factors with obesity and insulin sensitivity. For example, RBP4 was discovered from adipose tissue specific GLUT4 knockout mouse to explain strong insulin resistance in this animal model. However, the inconsistent association with insulin resistance parameters in different clinical settings of various studies which included patients with obesity, DM, different ethnicity, and CVD, it could not convince us to believe the role of RBP4 would be a causality of insulin resistance in metabolic diseases.

As for the emerging metabolic regulators such as FGFs (FGF21, FGF19, and FGF1), adipokines from visceral fat (vaspin and visfatin) and myokines, we need more studies to clarify their role in human diseases. FGF21 has been developed as a new drug for antidiabetic medication and human trial is on-going based on its favorable results from *in vitro* and *in vivo*. We have to wait and see the result of human trials that we could fully understand the mechanism of antidiabetic or any metabolic effects on human body. The possible role of FGF21 through PPAR α and γ in the liver and in fat tissues can be expected to have beneficial effects on insulin signaling pathway to ameliorate metabolic abnormalities. It is still a question whether these new metabolic regulators are just surrogate markers or might be causes of obesity and insulin resistance.

From this perspective, it is too early yet to apply these adipocytokines or metabolic regulators as predictors for cardiometabolic risk in the clinical practice. Up to date, it could be used as a surrogate biomarker to reflect metabolic abnormalities in many metabolic diseases. More mechanistic experiments and long-term outcome studies are warranted to elucidate the active role of these factors in the physiopathology of cardiometabolic disorders to identify their clinical implications at bedside level.

REFERENCES

1. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. *JAMA* (2006) **295**:1549–55. doi:10.1001/jama.295.13.1549
2. Bastard J-P, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur Cytokine Netw* (2006) **17**:4–12.
3. Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. *Gastroenterology* (2007) **132**:2169. doi:10.1053/j.gastro.2007.03.059
4. Kahn BB, Flier JS. Obesity and insulin resistance. *J Clin Invest* (2000) **106**:473–81. doi:10.1172/JCI10842
5. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* (2007) **117**:175–84. doi:10.1172/JCI29881
6. Harman-Boehm I, Blüher M, Redel H, Sion-Vardy N, Ovadia S, Avinoach E, et al. Macrophage infiltration into omental versus subcutaneous fat across different populations: effect of regional adiposity and the comorbidities of obesity. *J Clin Endocrinol Metab* (2007) **92**:2240–7. doi:10.1210/jc.2006-1811
7. Bahceci M, Gokalp D, Bahceci S, Tuzcu A, Atmaca S, Arikan S. The correlation between adiposity and adiponectin, tumor necrosis factor alpha, interleukin-6 and high sensitivity C-reactive protein levels. Is adipocyte size associated with inflammation in adults? *J Endocrinol Invest* (2007) **30**:210.
8. Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* (1996) **271**:10697–703. doi:10.1074/jbc.271.18.10697
9. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* (2000) **20**:1595–9. doi:10.1161/01.ATV.20.6.1595
10. Li S, Shin HJ, Ding EL, van Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA* (2009) **302**:179–88. doi:10.1001/jama.2009.976
11. Karakas M, Zierer A, Herder C, Baumert J, Meisinger C, Koenig W, et al. Leptin, adiponectin, their ratio and risk of Coronary Heart Disease: results from the MONICA/KORA Augsburg Study 1984-2002. *Atherosclerosis* (2010) **209**:220–5. doi:10.1016/j.atherosclerosis.2009.08.020
12. Cambuli VM, Musiu MC, Incani M, Paderi M, Serpe R, Marras V, et al. Assessment of adiponectin and leptin as biomarkers of positive metabolic outcomes after lifestyle intervention in overweight and obese children. *J Clin Endocrinol Metab* (2008) **93**:3051–7. doi:10.1210/jc.2008-0476
13. Lim S, Choi SH, Jeong IK, Kim JH, Moon MK, Park KS, et al. Insulin-sensitizing effects of exercise on adiponectin and retinol binding protein-4 concentrations in young and middle-aged women. *J Clin Endocrinol Metab* (2008) **93**:2263–8. doi:10.1210/jc.2007-2028
14. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA* (2004) **291**:1730–7. doi:10.1001/jama.291.14.1730
15. Zoccali C, Mallamaci F, Tripepi G, Benedetto FA, Cutrupi S, Parlongo S, et al. Adiponectin, metabolic risk factors, and cardiovascular events among patients with end-stage renal disease. *J Am Soc Nephrol* (2002) **13**:134–41.
16. Costacou T, Zgibor JC, Evans RW, Otvos J, Lopes-Virella MF, Tracy RP, et al. The prospective association between adiponectin and coronary artery disease among individuals with type 1 diabetes. The Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetologia* (2005) **48**:41–8. doi:10.1007/s00125-004-1597-y
17. Koenig W, Khuseyinova N, Baumert J, Meisinger C, Lowel H. Serum concentrations of adiponectin and risk of type 2 diabetes mellitus and coronary heart disease in apparently healthy middle-aged men: results from the 18-year follow-up of a large cohort from southern Germany. *J Am Coll Cardiol* (2006) **48**:1369–77. doi:10.1016/j.jacc.2006.06.053
18. Lim S, Koo BK, Cho SW, Kihara S, Funahashi T, Cho YM, et al. Association of adiponectin and resistin with cardiovascular events in Korean patients with type 2 diabetes: the Korean atherosclerosis study (KAS): a 42-month prospective study. *Atherosclerosis* (2008) **196**:398–404. doi:10.1016/j.atherosclerosis.2006.11.017
19. Motoshima H, Wu X, Mahadev K, Goldstein BJ. Adiponectin suppresses proliferation and superoxide generation and enhances eNOS activity in endothelial cells treated with oxidized LDL. *Biochem Biophys Res Commun* (2004) **315**:264–71. doi:10.1016/j.bbrc.2004.01.049
20. Lindberg S, Mogelvang R, Pedersen SH, Bjerre M, Frystyk J, Flyvbjerg A, et al. Relation of serum adiponectin levels to number of traditional atherosclerotic risk factors and all-cause mortality and major adverse cardiovascular events (from the Copenhagen City Heart Study). *Am J Cardiol* (2013) **111**:1139–45. doi:10.1016/j.amjcard.2012.12.043
21. Huang SS, Huang PH, Chen YH, Chiang KH, Chen JW, Lin SJ. Association of adiponectin with future cardiovascular events in patients after acute myocardial infarction. *J Atheroscler Thromb* (2010) **17**:295–303. doi:10.5551/jat.3533
22. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al. The hormone resistin links obesity to diabetes. *Nature* (2001) **409**:307–12. doi:10.1038/35053000
23. Fain JN, Cheema PS, Bahouth SW, Lloyd HM. Resistin release by human adipose tissue explants in primary culture. *Biochem Biophys Res Commun* (2003) **300**:674–8. doi:10.1016/S0006-291X(02)02864-4

24. Patel L, Buckels AC, Kinghorn IJ, Murdock PR, Holbrook JD, Plumpton C, et al. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. *Biochem Biophys Res Commun* (2003) **300**:472–6. doi:10.1016/S0006-291X(02)02841-3
25. Gerber M, Boettner A, Seidel B, Lammert A, Bar J, Schuster E, et al. Serum resistin levels of obese and lean children and adolescents: biochemical analysis and clinical relevance. *J Clin Endocrinol Metab* (2005) **90**:4503–9. doi:10.1210/jc.2005-0437
26. Savage DB, Sewter CP, Klenk ES, Segal DG, Vidal-Puig A, Considine RV, et al. Resistin/Fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor-gamma action in humans. *Diabetes* (2001) **50**:2199–202. doi:10.2337/diabetes.50.10.2199
27. McTernan PG, Fisher FM, Valsamakis G, Chetty R, Harte A, McTernan CL, et al. Resistin and type 2 diabetes: regulation of resistin expression by insulin and rosiglitazone and the effects of recombinant resistin on lipid and glucose metabolism in human differentiated adipocytes. *J Clin Endocrinol Metab* (2003) **88**:6098–106. doi:10.1210/jc.2003-030898
28. Reilly MP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, Rader DJ. Resistin is an inflammatory marker of atherosclerosis in humans. *Circulation* (2005) **111**:932–9. doi:10.1161/01.CIR.0000155620.10387.43
29. Hivert MF, Sullivan LM, Fox CS, Nathan DM, D'Agostino RBSr., Wilson PW, et al. Associations of adiponectin, resistin, and tumor necrosis factor-alpha with insulin resistance. *J Clin Endocrinol Metab* (2008) **93**:3165–72. doi:10.1210/jc.2008-0425
30. Norata GD, Ongari M, Garlaschelli K, Raselli S, Grigore L, Catapano AL. Plasma resistin levels correlate with determinants of the metabolic syndrome. *Eur J Endocrinol* (2007) **156**:279–84. doi:10.1530/eje.1.02338
31. Mojiminiyi OA, Abdella NA. Associations of resistin with inflammation and insulin resistance in patients with type 2 diabetes mellitus. *Scand J Clin Lab Invest* (2007) **67**:215–25. doi:10.1080/00365510601032532
32. Pischon T, Bamberger CM, Kratzsch J, Zyriax BC, Algenstaedt P, Boeing H, et al. Association of plasma resistin levels with coronary heart disease in women. *Obes Res* (2005) **13**:1764–71. doi:10.1038/oby.2005.215
33. Frankel DS, Vasan RS, D'Agostino RBSr., Benjamin EJ, Levy D, Wang TJ, et al. Resistin, adiponectin, and risk of heart failure the Framingham offspring study. *J Am Coll Cardiol* (2009) **53**:754–62. doi:10.1016/j.jacc.2008.07.073
34. Yaturu S, Daberry RP, Rains J, Jain S. Resistin and adiponectin levels in subjects with coronary artery disease and type 2 diabetes. *Cytokine* (2006) **34**:219–23. doi:10.1016/j.cyto.2006.05.005
35. Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A. Resistin, an adipokine with potent proinflammatory properties. *J Immunol* (2005) **174**:5789–95.
36. Calabro P, Samudio I, Willerson JT, Yeh ET. Resistin promotes smooth muscle cell proliferation through activation of extracellular signal-regulated kinase 1/2 and phosphatidylinositol 3-kinase pathways. *Circulation* (2004) **110**:3335–40. doi:10.1161/01.CIR.0000147825.97879.E7
37. Quadro L, Blaner WS, Salchow DJ, Vogel S, Piantedosi R, Gouras P, et al. Impaired retinal function and vitamin A availability in mice lacking retinol-binding protein. *EMBO J* (1999) **18**:4633–44. doi:10.1093/emboj/18.17.4633
38. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* (2005) **436**:356–62. doi:10.1038/nature03711
39. Graham TE, Yang Q, Bluher M, Hammarstedt A, Ciaraldi TP, Henry RR, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *N Engl J Med* (2006) **354**:2552–63. doi:10.1056/NEJMoa054862
40. Meisinger C, Ruckert IM, Rathmann W, Doring A, Thorand B, Huth C, et al. Retinol-binding protein 4 is associated with prediabetes in adults from the general population: the Cooperative Health Research in the Region of Augsburg (KORA) F4 Study. *Diabetes Care* (2011) **34**:1648–50. doi:10.2337/dc11-0118
41. Solini A, Santini E, Madec S, Rossi C, Muscelli E. Retinol-binding protein-4 in women with untreated essential hypertension. *Am J Hypertens* (2009) **22**:1001–6. doi:10.1038/ajh.2009.116
42. Ingelsson E, Sundstrom J, Melhus H, Michaelsson K, Berne C, Vasan RS, et al. Circulating retinol-binding protein 4, cardiovascular risk factors and prevalent cardiovascular disease in elderly. *Atherosclerosis* (2009) **206**:239–44. doi:10.1016/j.atherosclerosis.2009.02.029
43. Haider DG, Schindler K, Prager G, Bohdjalian A, Luger A, Wolzt M, et al. Serum retinol-binding protein 4 is reduced after weight loss in morbidly obese subjects. *J Clin Endocrinol Metab* (2007) **92**:1168–71. doi:10.1210/jc.2006-1839
44. Choi SH, Kwak SH, Youn B-S, Lim S, Park YJ, Lee H, et al. High plasma retinol binding protein-4 and low plasma adiponectin concentrations are associated with severity of glucose intolerance in women with previous gestational diabetes mellitus. *J Clin Endocrinol Metab* (2008) **93**:3142–8. doi:10.1210/jc.2007-1755
45. Choi SH, Lee YJ, Park YJ, Kim KW, Lee EJ, Lim S, et al. Retinol binding protein-4 elevation is associated with serum thyroid-stimulating hormone level independently of obesity in elderly subjects with normal glucose tolerance. *J Clin Endocrinol Metab* (2008) **93**:2313–8. doi:10.1210/jc.2007-2536
46. Wu J, Shi YH, Niu DM, Li HQ, Zhang CN, Wang JJ. Association among retinol-binding protein 4, small dense LDL cholesterol and oxidized LDL levels in dyslipidemia subjects. *Clin Biochem* (2012) **45**:619–22. doi:10.1016/j.clinbiochem.2012.02.022
47. Janke J, Engeli S, Boschmann M, Adams F, Bohnke J, Luft FC, et al. Retinol-binding protein 4 in human obesity. *Diabetes* (2006) **55**:2805–10. doi:10.2337/db06-0616
48. Promintzer M, Krebs M, Todoric J, Luger A, Bischof MG, Nowotny P, et al. Insulin resistance is unrelated to circulating retinol binding protein and protein C inhibitor. *J Clin Endocrinol Metab* (2007) **92**:4306–12. doi:10.1210/jc.2006-2522
49. Schaffler A, Buechler C. CTRP family: linking immunity to metabolism. *Trends Endocrinol Metab* (2012) **23**:194–204. doi:10.1016/j.tem.2011.12.003
50. Wong GW, Krawczyk SA, Kitidis-Mitrokostas C, Revett T, Gimeno R, Lodish HF. Molecular, biochemical and functional characterizations of C1q/TNF family members: adipose-tissue-selective expression patterns, regulation by PPAR-gamma agonist, cysteine-mediated oligomerizations, combinatorial associations and metabolic functions. *Biochem J* (2008) **416**:161–77. doi:10.1042/BJ20081240
51. Tom Tang Y, Hu T, Arterburn M, Boyle B, Bright JM, Palencia S, et al. The complete complement of C1q-domain-containing proteins in *Homo sapiens*. *Genomics* (2005) **86**:100–11. doi:10.1016/j.ygeno.2005.03.001
52. Park SY, Choi JH, Ryu HS, Pak YK, Park KS, Lee HK, et al. C1q tumor necrosis factor alpha-related protein isoform 5 is increased in mitochondrial DNA-depleted myocytes and activates AMP-activated protein kinase. *J Biol Chem* (2009) **284**:27780–9. doi:10.1074/jbc.M109.005611
53. Lee YH, Nair S, Rousseau E, Allison DB, Page GP, Tataranni PA, et al. Microarray profiling of isolated abdominal subcutaneous adipocytes from obese vs non-obese Pima Indians: increased expression of inflammation-related genes. *Diabetologia* (2005) **48**:1776–83. doi:10.1007/s00125-005-1867-3
54. Lim S, Choi SH, Koo BK, Kang SM, Yoon JW, Jang HC, et al. Effects of aerobic exercise training on C1q tumor necrosis factor alpha-related protein isoform 5 (myonectin): association with insulin resistance and mitochondrial DNA density in women. *J Clin Endocrinol Metab* (2012) **97**:E88–93. doi:10.1210/jc.2011-1743
55. Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, et al. FGF-21 as a novel metabolic regulator. *J Clin Invest* (2005) **115**:1627–35. doi:10.1172/JCI23606
56. Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E. Hepatic fibroblast growth factor 21 is regulated by PPARα and is a key mediator of hepatic lipid metabolism in ketotic states. *Cell Metab* (2007) **5**:426–37. doi:10.1016/j.cmet.2007.05.002
57. Coskun T, Bina HA, Schneider MA, Dunbar JD, Hu CC, Chen Y, et al. Fibroblast growth factor 21 corrects obesity in mice. *Endocrinology* (2008) **149**:6018–27. doi:10.1210/en.2008-0816
58. Arner P, Pettersson A, Mitchell PJ, Dunbar JD, Kharitonenkov A, Rydén M. FGF21 attenuates lipolysis in human adipocytes – a possible link to improved insulin sensitivity. *FEBS Lett* (2008) **582**:1725–30. doi:10.1016/j.febslet.2008.04.038
59. Nishimura T, Nakatake Y, Konishi M, Itoh N. Identification of a novel FGF, FGF-21, preferentially

expressed in the liver. *Biochim Biophys Acta* (2000) **1492**:203–6. doi:10.1016/S0167-4781(00)00067-1

60. Chen W, Li L, Yang G, Li K, Qi X, Zhu W, et al. Circulating FGF-21 levels in normal subjects and in newly diagnose patients with type 2 diabetes mellitus. *Exp Clin Endocrinol Diabetes* (2008) **116**:65–8. doi:10.1055/s-2007-985148
61. Eto K, Tumenbayar B, Nagashima S-I, Tazoe F, Miyamoto M, Takahashi M, et al. Distinct association of serum FGF21 or adiponectin levels with clinical parameters in patients with type 2 diabetes. *Diabetes Res Clin Pract* (2010) **89**:52–7. doi:10.1016/j.diabres
62. Zhang X, Yeung DC, Karpisek M, Stejskal D, Zhou ZG, Liu F, et al. Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. *Diabetes* (2008) **57**:1246–53. doi:10.2337/db07-1476
63. Han SH, Choi SH, Cho BJ, Lee Y, Lim S, Park YJ, et al. Serum fibroblast growth factor-21 concentration is associated with residual renal function and insulin resistance in end-stage renal disease patients receiving long-term peritoneal dialysis. *Metabolism* (2010) **59**:1656–62. doi:10.1016/j.metabol.2010.03.018
64. Stein S, Bachmann A, Lössner U, Kratzsch J, Blüher M, Stumvoll M, et al. Serum levels of the adipokine FGF21 depend on renal function. *Diabetes Care* (2009) **32**:126–8. doi:10.2337/dc08-1054
65. Lee Y, Lim S, Hong ES, Kim JH, Moon MK, Chun EJ, et al. Serum FGF21 concentration is associated with hypertriglyceridaemia, hyperinsulinaemia and pericardial fat accumulation, independently of obesity, but not with current coronary artery status. *Clin Endocrinol (Oxf)* (2012) doi:10.1111/cen.12134. [Epub ahead of print].
66. Fisher FM, Kleiner S, Douris N, Fox EC, Mepani RJ, Verdeguer F, et al. FGF21 regulates PGC-1alpha and browning of white adipose tissues in adaptive thermogenesis. *Genes Dev* (2012) **26**:271–81. doi:10.1101/gad.177857.111
67. Kim KH, Jeong YT, Oh H, Kim SH, Cho JM, Kim YN, et al. Autophagy deficiency leads to protection from obesity and insulin resistance by inducing Fgf21 as a mitokine. *Nat Med* (2013) **19**:83–92. doi:10.1038/nm.3014
68. Wei W, Dutchak PA, Wang X, Ding X, Wang X, Bookout AL, et al. Fibroblast growth factor 21 promotes bone loss by potentiating the effects of peroxisome proliferator-activated receptor γ. *Proc Natl Acad Sci U S A* (2012) **109**:3143–8. doi:10.1073/pnas.1200797109
69. Dutchak PA, Katafuchi T, Bookout AL, Choi JH, Yu RT, Mangelsdorf DJ, et al. Fibroblast growth factor-21 regulates PPARγ activity and the antidiabetic actions of thiazolidinediones. *Cell* (2012) **148**:556–67. doi:10.1016/j.cell.2011.11.062
70. Song KH, Li T, Owsley E, Strom S, Chiang JY. Bile acids activate fibroblast growth factor 19 signaling in human hepatocytes to inhibit cholesterol 7α-hydroxylase gene expression. *Hepatology* (2009) **49**:297–305. doi:10.1002/hep.22627
71. Lundasen T, Galman C, Angelin B, Rudling M. Circulating intestinal fibroblast growth factor 19 has a pronounced diurnal variation and modulates hepatic bile acid synthesis in man. *J Intern Med* (2006) **260**:530–6. doi:10.1111/j.1365-2796.2006.01731.x
72. Jonker JW, Suh JM, Atkins AR, Ahmadian M, Li P, Whyte J, et al. A PPARgamma-FGF1 axis is required for adaptive adipose remodelling and metabolic homeostasis. *Nature* (2012) **485**:391–4. doi:10.1038/nature10998
73. Itoh N, Ornitz DM. Evolution of the Fgf and Fgfr gene families. *Trends Genet* (2004) **20**:563–9. doi:10.1016/j.tig.2004.08.007
74. Hida K, Wada J, Eguchi J, Zhang H, Baba M, Seida A, et al. Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. *Proc Natl Acad Sci U S A* (2005) **102**:10610–5. doi:10.1073/pnas.0504703102
75. Jung CH, Lee WJ, Hwang JY, Seol SM, Kim YM, Lee YL, et al. Vaspin protects vascular endothelial cells against free fatty acid-induced apoptosis through a phosphatidylinositol 3-kinase/Akt pathway. *Biochem Biophys Res Commun* (2011) **413**:264–9. doi:10.1016/j.bbrc.2011.08.083
76. Youn BS, Kloting N, Kratzsch J, Lee N, Park JW, Song ES, et al. Serum vaspin concentrations in human obesity and type 2 diabetes. *Diabetes* (2008) **57**:372–7. doi:10.2337/db07-1045
77. Choi SH, Kwak SH, Lee Y, Moon MK, Lim S, Park YJ, et al. Plasma vaspin concentrations are elevated in metabolic syndrome in men and are correlated with coronary atherosclerosis in women. *Clin Endocrinol (Oxf)* (2011) **75**:628–35. doi:10.1111/j.1365-2265.2011.04095.x
78. Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, et al. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science* (2005) **307**:426–30. doi:10.1126/science.1097243
79. Dahl TB, Yndestad A, Skjelland M, Øie E, Dahl A, Michelsen A, et al. Increased expression of visfatin in macrophages of human unstable carotid and coronary atherosclerosis possible role in inflammation and plaque destabilization. *Circulation* (2007) **115**:972–80. doi:10.1161/CIRCULATIONAHA.106.665893
80. Haider DG, Schindler K, Schaller G, Prager G, Wolzt M, Ludvik B. Increased plasma visfatin concentrations in morbidly obese subjects are reduced after gastric banding. *J Clin Endocrinol Metab* (2006) **91**:1578–81. doi:10.1210/jc.2005-2248
81. Moschen AR, Kaser A, Enrich B, Mosheimer B, Theurl M, Niederegger H, et al. Visfatin, an adipocytokine with proinflammatory and immunomodulating properties. *J Immunol* (2007) **178**:1748–58.
82. Kang YS, Song HK, Lee MH, Ko GJ, Cha DR. Plasma concentration of visfatin is a new surrogate marker of systemic inflammation in type 2 diabetic patients. *Diabetes Res Clin Pract* (2010) **89**:141–9. doi:10.1016/j.diabres.2010.03.020
83. Revollo JR, Körner A, Mills KF, Satoh A, Wang T, Garten A, et al. Nampt/PBEF/visfatin regulates insulin secretion in β cells as a systemic NAD biosynthetic enzyme. *Cell Metab* (2007) **6**:363–75. doi:10.1016/j.cmet.2007.09.003
84. Kistorp C, Faber J, Galatius S, Gustafsson F, Frystyk J, Flyvbjerg A, et al. Plasma adiponectin, body mass index, and mortality in patients with chronic heart failure. *Circulation* (2005) **112**:1756–62. doi:10.1161/CIRCULATIONAHA.104.530972
85. Cavusoglu E, Ruwende C, Chopra V, Yanamadala S, Eng C, Clark LT, et al. Adiponectin is an independent predictor of all-cause mortality, cardiac mortality, and myocardial infarction in patients presenting with chest pain. *Eur Heart J* (2006) **27**:2300–9. doi:10.1093/eurheartj/ehl153
86. Wannamethee SG, Whincup PH, Lennon L, Sattar N. Circulating adiponectin levels and mortality in elderly men with and without cardiovascular disease and heart failure. *Arch Intern Med* (2007) **167**:1510–7. doi:10.1001/archinte.167.14.1510

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 11 May 2013; accepted: 26 July 2013; published online: 21 August 2013.
*Citation: Choi SH, Hong ES and Lim S (2013) Clinical implications of adipocytokines and newly emerging metabolic factors with relation to insulin resistance and cardiovascular health. Front. Endocrinol. **4**:97. doi: 10.3389/fendo.2013.00097*
This article was submitted to Diabetes, a section of the journal Frontiers in Endocrinology.
Copyright © 2013 Choi, Hong and Lim. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in
ENDOCRINOLOGY

REVIEW ARTICLE
published: 04 June 2013
doi: 10.3389/fendo.2013.00067

Sphingolipid metabolism and obesity-induced inflammation

Se-Chan Kang, Bo-Rahm Kim, Su-Yeon Lee and Tae-Sik Park*

Department of Life Science, Gachon University, Seongnam, South Korea

Edited by:
Tsuguhito Ota, Kanazawa University, Japan

Reviewed by:
Undurti Narasimha Das, UND Life Sciences, USA
Helena Barbosa Sampaio, State University of Campinas, Brazil

****Correspondence:***
Tae-Sik Park, Department of Life Science, Gachon University, Jinrikwan 304B, Bokjeong-dong, Sujeong-gu, Seongnam, Gyeonggi-do 461-701, South Korea
e-mail: tspark@gachon.ac.kr

Obesity is a metabolic disorder developed by overnutrition and a major cause for insulin resistance and cardiovascular events. Since adipose tissue is one of the major sites for the synthesis and secretion of cytokines, enlarged adipose tissue in obese condition alters inflammatory state leading to pathophysiological conditions such as type 2 diabetes and increased cardiovascular risk. A plausible theory for development of metabolic dysregulation is that obesity increases secretion of inflammatory cytokines from adipose tissue and causes a chronic inflammation in the whole body. Additionally accumulation of lipids in non-adipose tissues elevates the cellular levels of bioactive lipids that inhibit the signaling pathways implicated in metabolic regulation together with activated inflammatory response. Recent findings suggest that obesity-induced inflammatory response leads to modulation of sphingolipid metabolism and these bioactive lipids may function as mediators for increased risk of metabolic dysfunction. Importantly, elucidation of mechanism regarding sphingolipid metabolism and inflammatory disease will provide crucial information to development of new therapeutic strategies for the treatment of obesity-induced pathological inflammation.

Keywords: ceramide, inflammation, obesity, atherosclerosis, cardiomyopathy, fatty liver, diabetes

INTRODUCTION

Obesity is an outcome of overnutrition, less exercise, and sedentary lifestyle. Outcomes of obesity include cardiovascular disease (CVD), diabetes, and hyperlipidemia and contribute to increased mortality and morbidity after myocardial infarction and related complications in diabetic compared with non-diabetic patients (Stone et al., 1989). Even with various current interventions, the occurrence of obesity is increasing and medical expenses associated with curing obesity and its complications are increasing as well (Finkelstein et al., 2009). Deposition of ectopic fat in adipose tissue is associated with increased plasma fatty acids (FA) which are the major contributor to increased lipid contents in non-adipose tissues. Since the adipose tissue is the major place for the pathogenesis of obesity-related metabolic and cardiovascular dysfunction, it has drawn much attention as a target tissue. Obesity worsens tissue functions and contributes to increased risk of development of hypertensions, atherosclerosis, diabetes, and non-alcoholic fatty liver disease (NAFLD) (Flegal et al., 2007). To elucidate the mechanism of pathophysiology of obesity-associated diseases, lipotoxicity and inflammation have been suggested as major contributors to progression of chronic diseases associated with obesity. Among various bioactive lipid metabolites, sphingolipids have been studied due to its implication in development of various chronic metabolic diseases and its bioactive characteristics to modulate cellular signaling pathways (Johns et al., 1998; Shimabukuro et al., 1998; Pettus et al., 2002; Amati et al., 2011). In addition, obesity elevates production of proinflammatory cytokines, chemokines, and coagulation proteins and mediates multiple processes in the body (Hotamisligil et al., 1993, 1995). As a result, inflammation is associated with obese conditions and infiltration of macrophages and T lymphocytes are usually accompanied (Hotamisligil et al., 1993, 1995; Ferrante, 2007). Recent reports demonstrated that sphingolipid metabolism is modulated in obese conditions that alter inflammatory state in adipose tissues and immune cells (Kolak et al., 2007; Holland et al., 2011a). In this review, we will focus on sphingolipid metabolism in the etiology of chronic diseases accompanied with obesity-mediated inflammation. Understanding the role of sphingolipids will provide effective therapeutic targets for obesity-mediated inflammation (Hotamisligil et al., 1993).

ADIPOSE TISSUE INFLAMMATION

The adipose tissues has been considered as a lipid storing organ accumulating triglycerides (TAG) in adipocytes in response to overnutrition and releasing these stored lipids during fasting. However, the notion that adipose tissue produces and releases various cytokines, termed "adipokines," represents this is an active inflammatory organ. The fact that adipose tissue is an active inflammatory organ was initiated from the findings that adipose tissue has increased expression of tumor necrosis factor-α (TNFα) in obese human compared with lean individuals (Hotamisligil et al., 1995). Since this report, it has been reported that adipose tissue from the obese individuals has increased expression and secretion of several proinflammatory cytokines such as tumor necrosis factor-α (TNFα), monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6), iNOS, C-reactive protein (CRP), and plasminogen activator inhibitor type-1 (PAI-1) (**Figure 1**) (Mohamed-Ali et al., 1997; Fried et al., 1998; Visser et al., 1999;

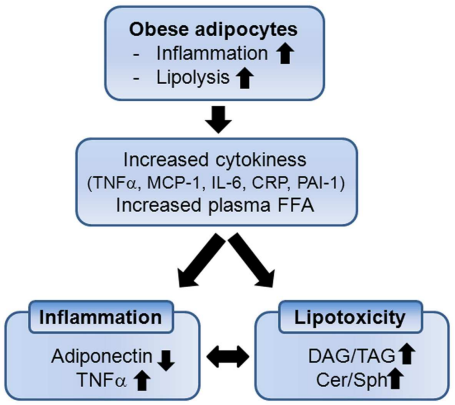


FIGURE 1 | Enlarged adipocytes by obesity contribute to systemic inflammation and lipotoxicity due to increased cytokines and accumulation of lipid metabolites in non-adipocyte tissues. DAG, diacylglycerol; TAG, triacylglycerol; Cer, ceramide; Sph, sphingosine.

Perreault and Marette, 2001; Christiansen et al., 2005). Hypertrophied adipocytes induce infiltration of activated macrophages which mediates increased expression and secretion of a variety of proinflammatory cytokines in systemic circulation (Weisberg et al., 2003).

Various adipokines have been suggested as useful biomarkers for CVD and metabolic dysregulation associated with obesity. Depending on the fat contents in the body, the species and the amounts of adipokines secreted from adipose vary. Expression of anti-inflammatory cytokines is upregulated including adiponectin, leptin and IL-10 in response to decreased fat mass (Yang et al., 2001). These adipokines have beneficial effects by regulating body weight due to reduced food intake/energy expenditure and reducing inflammation (Friedman and Leibel, 1992; Zhang et al., 1994; Friedman and Halaas, 1998; Kadowaki et al., 2006). In contrast, expression of proinflammatory cytokines (such as TNFα, MCP-1, and IL-1β) is upregulated with fat mass increase in adipocytes (Jung et al., 2008). Elevation of these cytokines in circulation promotes insulin resistance in peripheral tissues by inhibition of signaling intermediates (Hotamisligil et al., 1995).

Another outcome of obesity is increased lipid accumulation in non-adipose tissues (Kraegen et al., 2001; Unger, 2003). Saturated fat storage capacity of adipose tissue spills free fatty acids (FFAs) in circulation with lipolysis and leads to accumulation of ectopic fat in the tissues not suited for fat storage (**Figure 1**). Increased FFAs and cytokines activate immune receptors and stress signaling pathways that interfere with insulin signaling in muscle and liver (Holland et al., 2007, 2011a; Hoehn et al., 2008). As non-oxidative pathway of FFAs, intracellular and circulating ceramide are elevated and bioactive sphingolipids such as ceramide, sphingosine, and sphingosine 1-phosphate (S1P) are now known to link overnutrition, inflammation, and metabolic dysregulation.

SPHINGOLIPID METABOLISM IN OBESITY-INDUCED INFLAMMATION

Sphingolipid metabolism is highly regulated by a complex network of interconnected pathways not simply by availability of substrate FFAs. Major bioactive sphingolipids includes ceramide, sphingosine, S1P, and ceramide-1-phosphate (C1P) act as signaling molecules regulating various physiological events such as cell proliferation, apoptosis, and inflammation (Futerman and Hannun, 2004; Hannun and Obeid, 2008; Morad and Cabot, 2013). Ceramide is a major molecule in sphingolipid metabolism and a precursor of complex sphingolipids.

De novo biosynthesis of ceramide is initiated from condensation of serine and palmitoyl CoA by serine palmitoyltransferase (SPT) followed by a series of reactions involving the enzymes 3-ketosphinganine reductase, ceramide synthase (CerS), dihydroceramide desaturase (DES). Another pathway to produce ceramide is through hydrolysis of sphingomyelin (SM) by acid or neutral sphingomyelinase (SMase) (Hannun and Obeid, 2008) (**Figure 2**). Ceramide is further deacylated to generate sphingosine by alkaline or acid ceramidase and sphingosine is phosphorylated to produce S1P by sphingosine kinases. Ceramide kinase phosphorylates ceramide to produce C1P (Sugiura et al., 2002). The sphingolipid biosynthesis pathway affects cellular production of at least four known bioactive lipids: ceramide, sphingosine, S1P, and C1P. These signaling lipids are known to alter various physiological events by regulating signaling pathways.

Accumulating evidence suggest that ceramide synthesis can be activated by increased availability of FFAs, proinflammatory cytokines, oxidative stress, and hormones (Memon et al., 1998; Samad et al., 2006; Schilling et al., 2013). All of these conditions represent the obese conditions of adipose tissue and suggest that ceramide metabolism may be altered in the obese. Indeed, ceramide levels were elevated in skeletal muscle, liver, and hypothalamus in obese rodents and human (Adams et al., 2004; Holland et al., 2007; Reyna et al., 2008). Samad et al. (2006) demonstrated that total SM and ceramide levels were reduced in the adipose tissues from the leptin deficient ob/ob mice. In contrast, plasma SM, ceramide, sphingosine, and S1P were elevated in plasma. Since expression of ceramide synthetic genes including SPT, neutral SMase, and acid SMase is upregulated in adipose tissue, this opposite sphingolipid profiles in plasma and adipose tissue suggest that secretion of ceramide from adipose tissues into circulation is increased.

Obesity elevates TNFα expression in adipose tissues (Hotamisligil and Spiegelman, 1994) and ceramide is elevated via hydrolysis of SM by SMases and SPT-mediated *de novo* synthesis. The findings that intraperitoneal administration of TNFα into C57BL/6J mice upregulates acid SMase, neutral SMase, and SPT suggest increased ceramide synthesis in adipose tissue (Samad et al., 2006). To support this report, Holland et al. (2011a) demonstrated that there is an overlap between inflammatory status and ceramide production converging on the Toll-like receptor 4 (TLR4) pathway independent of TNFα signaling. In mutant mice lacking functional TLR4, increased ceramide production by saturated FA or lipopolysaccharides (LPS) was prevented in skeletal muscle and liver. Saturated fat induces ceramide production and inflammatory response in a TLR4-dependent manner. Recently, Schilling et al. (2013) reported that the combination of LPS and palmitate synergistically activates ceramide production via TLR4-dependent and independent signaling respectively. Thus, correlation of ceramide metabolism and inflammatory state has

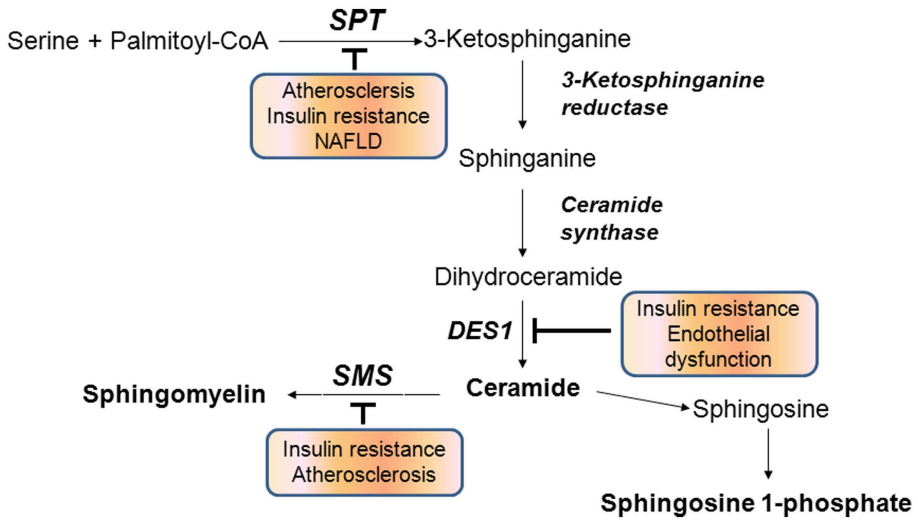


FIGURE 2 | Sphingolipid biosynthetic pathways. Inhibition of indicated biosynthetic enzymes is associated with prevention of chronic metabolic diseases. SPT, serine palmitoyltransferase; DES1, dihydroceramide desaturase 1; SMS, sphingomyelin synthase.

been established by involvement of TLR4 and cytokine-mediated activation of SMase.

ADIPONECTIN, S1P, AND SPHINGOLIPID METABOLISM

Obesity increases systemic inflammation state together with production and secretion of proinflammatory cytokines and reduces production of anti-inflammatory cytokines. While TNFα activates proinflammatory pathways and mediates apoptosis, adiponectin inhibits proinflammatory cytokine-mediated pathways and promotes cell proliferation (Ouchi et al., 2000; Kobayashi et al., 2004). Adiponectin is an anti-inflammatory adipokine usually found in circulation (Fang and Sweeney, 2006; Kadowaki et al., 2008). Adiponectin forms three oligomeric forms that can be cleaved by leukocyte elastase to liberate the globular C-terminal fragment which exerts its biological activity (Waki et al., 2005; Wang et al., 2006). Plasma adiponectin levels are generally reduced in the individuals with increasing obesity and diabetes (Liu et al., 2007). Pathophysiological events including diabetes, inflammation, and atherosclerosis are known to be alleviated by adiponectin (Abel et al., 2008; Shetty et al., 2009). These beneficial effects of adiponectin have been attributed to its insulin-sensitizing and insulin-like effects in skeletal muscle and liver. Adiponectin enhances glucose uptake and acts as a stimulator of FA uptake and oxidation via AMP-dependent kinase pathway (AMPK) (Fang and Sweeney, 2006; Kadowaki et al., 2008; Matsuzawa, 2010). Alleviation of lipotoxicity by adiponectin contributes to reduced metabolic dysregulation.

Recent findings by Holland et al. (2011b) demonstrated the linkage between adiponectin and sphingolipid metabolism. Adiponectin receptors contain an inherent ceramidase activity and reduce ceramide. Activity of ceramidase activity is dependent on amount of adiponectin levels and regulated by its biding to the receptors. Adiponectin binding to its two receptors, AdipoR1 and AdipoR2, stimulates ceramidase activity and formation of sphingosine from ceramide degradation is stimulated. Produced sphingosine is phosphorylated by sphingosine kinase to produce S1P, a major bioactive sphingolipid metabolite exerting its anti-apoptotic and anti-diabetic effects. Formed S1P is transported to extracellular environment, binds to the S1P receptors, elevates intracellular calcium, and activates AMPK. Indeed, insulin tolerance is much improved in adiponectin transgenic mice fed a high fat diet when compared to WT mice fed a high fat diet. In opposite to the action of ceramide, S1P has been known to activate Akt and promotes cell proliferation (Morales-Ruiz et al., 2001; Spiegel and Milstien, 2003; Chavez et al., 2005). These findings suggest that inflammatory cytokines are closely linked to modulation of sphingolipid metabolism. S1P and ceramide has opposite roles and regulate the fate of cells for survival or death, "rheostat theory of sphingolipids" in body metabolism.

CERAMIDE AND HYPOTHALAMIC REGULATION OF FEEDING

Elevated FA in circulation is due to obesity-mediated spillover from adipose tissue. Central nervous system regulates appetite and energy homeostasis and hypothalamus controls food intake and its metabolism (Schwartz et al., 2000). Recent reports clearly show that FA in hypothalamus plays an important role in energy balance (Obici et al., 2003; He et al., 2006; Lopez et al., 2006). Especially, malonyl CoA, a precursor of *de novo* FA biosynthesis, draws attentions as a regulator of hypothalamic control (Loftus et al., 2000; Gao and Lane, 2003; Gao et al., 2007). Leptin, an adipokine regulating food intake and body weight, elevates malonyl CoA levels in hypothalamic arcuate nucleus (Arc) and partly causes its anorexigenic effects (Gao et al., 2007). Carnitine palmitoyltransferase-1 (CPT-1) activity, a key enzyme in mitochondrial FA β-oxidation, is inhibited by malonyl CoA and exerts leptin-mediated anorexia (Wolfgang et al., 2007). Wolfgang et al. (2006) have demonstrated hypothalamic CPT-1c, a brain-specific CPT-1 isoform expressed in hypothalamic Arc neuron, is implicated in energy homeostasis. While CPT-1 liver isoform (CPT-1a and -1b) is a predominant form possessing the acyltransferase activity (Obici et al., 2003),

CPT-1c has a very weak enzyme activity. Importantly, CPT-1c knockout (KO) animals have reduced food intake and weight gain suggesting a critical role of CPT-1c in energy homeostasis (Wolfgang et al., 2006).

Recently, Gao et al. (2011) have shown that adenoviral overexpression of CPT-1c in hypothalamic Arc increases food intake and upregulates orexigenic neuropeptide Y (NPY) and Bsx, a transcription factor of NPY. Interestingly, CPT-1c overexpression elevated ceramide levels and CPT-1c KO resulted in the opposite, a reduced ceramide levels (Gao et al., 2011). The intra-Arc infusion of C6-ceramide, a cell-penetrating ceramide analog, resulted in blockade of leptin- or cerulenin-mediated anorexigenic effects. In contrast, inhibition of *de novo* ceramide synthesis by myriocin resulted in reduced food intake and body weight. Since CPT-1c and SPT are expressed in ER, palmitoyl CoA might be available via CPT-1c action and supplied for *de novo* ceramide biosynthesis in ER. Another possibility is that CPT-1c acts as a transporter of palmitoyl CoA into ER. Taken together, FA metabolism by CPT-1c exerts its anorexigenic regulation by modulating ceramide synthesis. Although the exact mechanism of CPT-1c/ceramide pathway and regulation of energy homeostasis regarding major Arc neurotransmitters should be studied further, these findings suggested the novel role of ceramide in CNS control of appetite and whole body energy metabolism.

INVOLVEMENT OF SPHINGOLIPID METABOLISM IN DEVELOPMENT OF CHRONIC DISEASES BY OBESITY

INSULIN RESISTANCE

Obesity is closely associated with an increased development of insulin resistance. The concept that inflammation elicited by obese conditions contributes to diabetes was initiated from the findings that adipose-released proinflammatory cytokines inhibit insulin signaling in the adipose, skeletal muscle, and liver (Hotamisligil et al., 1993). Insulin regulates glucose homeostasis by activating glucose uptake by the skeletal muscle and adipose tissue and inhibiting hepatic glucose output. In addition, insulin stimulates FA uptake, TAG biosynthesis, and storage in adipose tissue. Insulin resistance is a pathophysiological process associated with reduced response of target tissues, hyperinsulinemia, and elevated blood glucose levels by increased hepatic glucose efflux. Since obese condition results in increased adipose tissue lipolysis leading to increased plasma FFA, lipotoxicity that accumulation of bioactive lipid intermediates inhibits insulin response has gained credibility for development of insulin resistance. Another possibility is that the obesity activates adipose-derived cytokine production and systemic inflammation and these cytokines disrupt signaling pathways in target tissues. Thus, adipose tissue is a primary location for initiation of insulin resistance and subsequent development of type 2 diabetes.

Free fatty acids are going through oxidative pathway to supply the energy for cell metabolism. Another route for FFAs is sphingolipid biosynthetic pathway. Since FFAs are the substrate and major constituents for sphingolipids, ceramide is elevated in the patients with obesity or diabetes and has a positive correlation with severity of insulin resistance (Haus et al., 2009). Accumulating evidences suggest that sphingolipid metabolism is a converging point linking excess FFAs and inflammation aroused by adipose-derived inflammation, and contributes to progression of insulin resistance. Ceramide and sphingosine inhibit insulin actions and signaling by dephosphorylation and inhibition of AKT and AMPK activity in various cell culture systems (Hajduch et al., 2001; Liu et al., 2004; Summers, 2006). Holland et al. (2007) demonstrated that *in vivo* administration of myriocin, a specific SPT inhibitor, improved glucocorticoid, saturated fat, and obesity-induced insulin resistance by inhibiting *de novo* ceramide synthesis. Heterozygous deficiency of dihydroceramide desaturase (DES1) had improved insulin sensitivity and dexamethasone-induced insulin resistance was prevented (Holland et al., 2007). In cultured cells, the mechanisms of ceramide-mediated inhibition of insulin response have been suggested. It has been demonstrated that ceramide antagonized phosphorylation and activation of AKT and tyrosine phosphorylation of insulin receptor substrate (IRS-1) in 3T3-L1 adipocytes and C2C12 myocytes (Summers et al., 1998; Chavez et al., 2003). Ceramide exerts its inhibitory effects by activating protein phosphatase 2A (PP2A) responsible for dephosphorylation of AKT (Dobrowsky et al., 1993). Additionally, ceramide activates PKCζ and inhibits translocation of AKT to the membrane (Powell et al., 2003, 2004). By modulating AKT activity, ceramide inhibits insulin signaling pathway and ultimately the insulin response is altered.

Since the finding that TNFα is linked to insulin resistance, mechanism of obesity-induced inflammation has been suggested. The activity of c-Jun N terminal kinase (JNK) is increased in the obese mice and the lack of JNK showed improved glucose metabolism (Yuan et al., 2001). To support this finding, inhibition of IKKβ by salicylate was effective in ameliorating inflammation-mediated insulin resistance (Yuan et al., 2001). Since JNK and IKKβ are activated by ceramide, occurrence of decreased insulin resistance can be attributed to tissue ceramide levels (Ruvolo, 2003). Especially, the reports that the absence of functional IKKβ by overexpression of a kinase dead IKKβ decreases ceramide levels in myocytes suggest that IKKβ regulates ceramide biosynthesis (Holland et al., 2011a). Additionally, LPS-mediated NFκB activation in macrophage upregulates transcription of enzymes involved in *de novo* ceramide biosynthesis including Sptlc2 and acid SMase (Chang et al., 2011). Thus, therapeutic intervention that lowers inflammation together with *in vivo* ceramide production would be a good target for obesity-mediated insulin resistance.

CERAMIDE AND HEPATIC STEATOSIS

Non-alcoholic fatty liver disease is a component of obesity-mediated complications and defined as excess fat accumulation in the liver (5–10% of liver weight) (Neuschwander-Tetri and Caldwell, 2003). Initial development of NAFLD is the accumulation of TAG in hepatocytes. Benign NAFLD, or hepatic steatosis, at the beginning stage can develop into more malignant conditions exemplified as steatohepatitis and cirrhosis (Farrell and Larter, 2006; Kim and Younossi, 2008). Despite NAFLD is the most common cause of hepatic dysfunction in the United States, its pathogenesis is not completely understood.

Development of NAFLD is associated with overnutrition-mediated obesity. Obesity-induced FFA increase in plasma contributes to approximately 60% of accumulated TAG in the livers of NAFLD patients (Donnelly et al., 2005). In obesity-induced

insulin-resistant states, insulin is not able to inhibit the activity of hormone-sensitive lipase in adipose tissues and release FFA into circulation. Additionally, reduced glycerol-3-phosphate levels by insulin resistance prevent reutilization of FFA for TAG synthesis in adipocytes. Therefore, FFA spill from the adipose tissue to circulation is a major cause of NAFLD prevalence. Accordingly, FFA not utilized for TAG synthesis is shunted for ceramide synthesis and ceramide levels are elevated in the adipose tissues from the patients with NAFLD (Kolak et al., 2007). In obese ob/ob mice, hepatic ceramide levels and the degree of steatosis demonstrated a positive correlation (Yetukuri et al., 2007).

Clinically, association of inflammation with NAFLD was confirmed by the fact that patients with NAFLD have elevated levels of TNFα (Jarrar et al., 2008) and expression of TNFα and TNFα receptor are upregulated in the livers of the patients with NAFLD compared to healthy individuals (Feldstein et al., 2004). On the other hand, circulating adiponectin levels were reduced in diet-induced obese (DIO) mice and hepatic expression of adipoR2, a predominant hepatic adiponectin receptor was downregulated (Peng et al., 2009). Additionally, the report that expression of adiponectin is inversely correlated with expression of SMase implies association of inflammation with sphingolipid biosynthesis (Kolak et al., 2007). LPS treatment resulted in a two-fold upregulation of hepatic Sptlc2 mRNA and activity and led to increased hepatic SM and ceramide by twofold and threefold, respectively (Memon et al., 1998). In this study, treatment of IL-1β and TNFα upregulated Sptlc2 mRNA in hepatocytes and indicated that fatty liver induces inflammation-mediated activation of *de novo* biosynthesis.

Implication of SMase, a salvage pathway of ceramide, has been suggested in NAFLD. SMase is regulated by inflammatory stimuli, including TNFα (Dressler et al., 1992; Schutze et al., 1992, 1995; Chatterjee, 1994). Binding of TNFα to p55 TNFα receptor induced SMase transcription (Vandenabeele et al., 1995). Mice deficient in acid SMase and LDL receptors were protected from high fat diet-induced hepatic TAG accumulation (Deevska et al., 2009). Moreover, hyperglycemia and insulin resistance were prevented despite of elevated SM and other sphingolipids (Deevska et al., 2009). In addition, pharmacological inhibition of SMase in palmitic acid-treated hepatocytes reduced TAG significantly (Deevska et al., 2009). These results suggest that SMase-mediated ceramide production is implicated in hepatic steatosis in response to elevated FA. The role of ceramide biosynthesis in development of hepatic steatosis was demonstrated in DIO mice. SPT inhibition by myriocin reduces hepatic TAG in DIO mice (Yang et al., 2009). Reduced hepatic fat accumulation found in myriocin-treated DIO mice implicates downregulation of SOCS-3, a gene involved in development of hepatic steatosis (Ueki et al., 2004). Therefore, regulation of SOCS-3 by ceramide biosynthesis contributes to the pathophysiology of hepatic steatosis. However, whether ceramide contributes to fatty liver directly or via secondary effects such as increased FFA is not clear and need further studies.

SPHINGOLIPIDS AND ATHEROSCLEROSIS

Obesity-induced inflammation is implicated in increased risk of coronary artery disease. Occurrence of cardiovascular events involves a combined outcome of hyperlipidemia, insulin resistance, hypertension, and heart failure. Recent literature suggests that sphingolipids contribute to pathogenesis of CVD. The fact that sphingolipid metabolism is regulated by inflammatory state suggests that obesity-induced inflammation is the upstream of sphingolipids and risk factors for etiology of various CVD (Holland et al., 2011a). Among them, atherosclerosis is an inflammatory disease characterized by increased production of a wide range of chemokines and cytokines. Early stage of atherogenesis involves the interaction of cholesterol-rich lipoproteins with arterial wall (Ross, 1993). The processes implicated in early atherogenesis include lipoprotein oxidation (Yla-Herttuala et al., 1989; Witztum and Steinberg, 1991), lipoprotein retention and aggregation (Nievelstein et al., 1991; Williams and Tabas, 1995, 1998; Tabas et al., 2007), endothelial alteration, monocyte recruitment, macrophage chemotaxis and foam cell formation, and smooth muscle cell migration and alteration (Ross, 1993). An evidence indicating the importance of SM in atherogenesis is that SM accumulates in atherosclerotic plaques formed in human and animal models (Smith, 1960; Newman et al., 1961; Phillips and Dodge, 1967; Portman and Illingworth, 1976; Hakomori, 1981; Kummerow et al., 2001). LDL extracted from human atherosclerotic lesions has higher SM levels than LDL from plasma (Hoff and Morton, 1985; Guyton and Klemp, 1996; Schissel et al., 1996, 1998). A substantial amount of the SM found in arteries and atherosclerotic lesions appears to arise from SM synthesis in the arterial tissues (Zilversmit et al., 1961; Eisenberg et al., 1969). Plasma SM levels in atherogenic apoE KO mice are fourfold higher than in wild type mice (Jeong et al., 1998) and this may contribute to the increased atherosclerosis (Plump et al., 1992; Zhang et al., 1992). Clinically, Jiang et al. also found that human plasma SM levels and SM/phosphatidylcholine (PC) ratio are independent risk factors for occurrence of coronary heart disease (Jiang et al., 2000; Schlitt et al., 2006).

Park et al. (2004) and Hojjati et al. (2005) have reported that myriocin treatment reduces plasma SM levels and atherosclerosis in apoE KO mice fed normal chow or HFD. While intraperitoneal administration do not alter plasma lipids, myriocin treatment by diet-admix also lower plasma lipid levels in apoE KO mice (Park et al., 2004, 2008b). However, both diet-admix and intraperitoneal administration methods led to reduced atherosclerosis, whereas only oral administration of myriocin lowered plasma cholesterol levels (Park et al., 2004; Hojjati et al., 2005). Oral administration may reduce cholesterol absorption in small intestine (Li et al., 2009). When wild type and apoE KO animals were treated with myriocin, the mice absorbed significantly less cholesterol than controls with no observable pathological changes in the small intestine. Thus, myriocin has direct anti-atherosclerosis vascular effects and also has the potential to function as a plasma lipid-lowering agent. Similar to this study, administration of FTY720, an analog of myriocin, also prevents atherosclerosis in apoE-deficient mice (Liu et al., 2009).

In order to evaluate the role of SM in macrophage, Liu et al. (2009) studied SMS2, SM synthase catalyzing formation of SM from ceramide. In this report, SMS2 KO mouse bone marrow was transplanted into LDL receptor KO *($Ldlr^{-/-}$)* mice. After 3 months on a Western diet, SMS2 deficiency decreased atherosclerotic lesions in the aortic arch, valve, and the entire aorta,

compared with wild type macrophages transplanted into *Ldlr*$^{-/-}$ mice. Moreover, the analysis of plaque morphology demonstrated that SMS2 macrophage deficiency resulted in less necrotic core area and more collagen content in atherosclerotic lesions (Liu et al., 2009). Therefore, SMS2 deficiency in the macrophages reduces atherosclerosis in mice.

CERAMIDE AND CARDIOMYOPATHY

Cardiomyopathy is an outcome of various chronic CVDs that is often found in patients with diabetes. Weakening of the heart is sometimes associated with increased heart content of lipids. Diabetic cardiomyopathy contributes to increased morbidity and mortality after myocardial infarction in diabetic patients compared with non-diabetics (Stone et al., 1989). Inflammation is closely associated with development of cardiac events derived from diabetes. Kawamura et al. (2005) inactivates cardiac NFκB by over-expressing a dominant negative NFκB subunit in cardiac-specific TNFα transgenic mice. Although inactivation of NFκB blockage did not improve myocardial inflammation which is represented by inflammatory cell infiltration, it ameliorates cardiac function and mortality. These findings suggest that activation of NFκB is more important than inflammation-mediated immune reaction in cardiomyopathy. To support this observation, TLR4 deficiency attenuates cardiomyopathy in mice (Riad et al., 2008). These

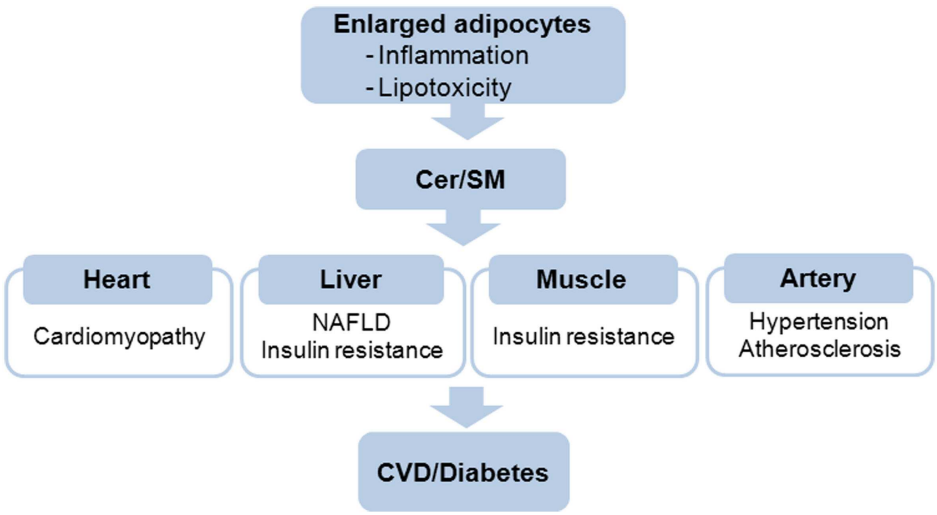


FIGURE 3 | Elevated ceramide and SM in obese adipocytes elicit the pathophysiological events in various tissues and organs. CVD, cardiovascular disease.

Table 1 | Alteration of tissue ceramide levels and related diseases in animal models of obesity and metabolic syndrome.

Animal models	Reference	Tissue	Cer/SM changes	Disease related
TLR4 KO mice	Holland et al. (2011a)	Skeletal muscle, liver	Cer↓	Insulin sensitivity
AdipoR1, AdipoR2 (adenoviral infection into C57BL/6J mice)	Holland et al. (2011b)	Liver	Cer↓	Insulin sensitivity
CPT-1c KO mice	Gao et al. (2011)	Hypothalamus	Cer↓	Anorexia
Adipoq KO mice	Holland et al. (2011b)	Heart	Cer↑	Insulin sensitivity
Des1$^{\pm}$ mice	Holland et al. (2007)	Heart, pancreas, WAT, liver, soleus muscle	Cer↓	Improved insulin sensitivity
Syrian hamsters + LPS	Memon et al. (1998)	Liver	Cer↑	Inflammation
Acidic SMase + LDLr$^{-/-}$ mice	Deevska et al. (2009)	Liver	Cer↑	Steatosis
ApoE KO mice	Jeong et al. (1998)	Plasma	SM↑	Atherosclerosis
SMS2$^{-/-}$, LDLr$^{-/-}$ mice	Liu et al. (2009)	Macrophage	Cer↑ SM↓	Reduced atherosclerosis
DIO mice	Yang et al. (2009)	Plasma, adipose	Cer↑	Hepatic steatosis, insulin resistance
LpL^{GPI} mice	Park et al. (2008a)	Heart	Cer↑	Cardiomyopathy
hSptlc2 KO mice	Lee et al. (2012)	Heart	Cer↓	Cardiomyopathy
ob/ob mice	Samad et al. (2006)	Plasma Adipose	Cer↑, SM↑ Cer↓, SM↓	Obesity, diabetes, atherosclerosis

Cer, ceramide; SM, sphingomyelin.

findings support that an outcome of inflammation is involved in the etiology of inflammation-induced cardiomyopathy.

Toll-like receptor 4 and NFκB, the mediators of inflammatory response, regulate *de novo* sphingolipid biosynthesis (Chang et al., 2011; Holland et al., 2011a; Schilling et al., 2013). Consistent with these findings, Park et al. (2008a) reported the role of ceramide in a lipotoxic cardiomyopathic mice model. Mice with cardiac overexpression of glycosylphosphatidylinositol membrane-anchored LpL mice (LpL^{GPI}) also have increased cardiac ceramide and heart failure markers (Yokoyama et al., 2004). Inhibition of *de novo* ceramide biosynthesis by myriocin or heterozygous deletion of Sptlc1 resulted in decreased expression of some apoptotic markers and ameliorated cardiac contraction in LpL^{GPI} (Park et al., 2008a). In this study, blockage of ceramide biosynthesis appears to modulate mitochondrial substrate oxidation of FA and glucose. A potential mechanism is that decreased ceramide by pharmacological and genetic inhibition of SPT upregulated pyruvate dehydrogenase kinase-4 and decreased the rate of glucose oxidation. However, Lee et al. (2012) reported that ablation of cardiac-specific Sptlc2 (hSptlc2 KO), an essential subunit of SPT, aggravates cardiac function even with reduced ceramide and developed cardiomyopathy. A possible explanation about these inconsistent results is that accumulated FA in hSptlc2 KO hearts due to inhibition of *de novo* ceramide activates ER stress and increases cardiomyocytes apoptosis. Therefore, a single lipid is unlikely to be reduced by inhibition solely and cardiac lipotoxicity is caused by many processes in proper heart function.

CERAMIDE AND VASCULAR DYSFUNCTION

Vascular dysfunction derived from obesity may be mediated by lipotoxic metabolites. A growing body of literature suggests that nitric oxide (NO) is a major modulator to maintain vascular function (Steinberg et al., 2000; Du et al., 2006; Symons et al., 2009). As a ubiquitous signaling molecule, endothelial NO is responsible for regulation of vasodilation (Alderton et al., 2001). Imbalance between production and degradation of NO may lead to occurrence of cardiac events. Obesity mediates increased plasma FFA and increased ceramide contents in various tissues contributing to cardiovascular complications. Especially, ceramide inhibits signaling kinases that phosphorylate endothelial NO synthase (eNOS) at positive regulation site and activates signaling kinases that phosphorylate eNOS at negative regulatory sites (Wu et al., 2007; Chavez and Summers, 2010; Summers, 2010). Recently, Zhang et al. (2012) reported that inhibition of *de novo* ceramide biosynthesis by myriocin ameliorates the blood pressure accrual in mice fed a high fat diet. In this study, prevention of the blood pressure increase by myriocin is endothelium-dependent and via restoration of eNOS phosphorylation at Ser^{1177} (Zhang et al., 2012). Moreover, heterozygous deficiency of dihydroceramide desaturase (des±) partially restored phosphorylation of eNOS, suggesting a major role of ceramide in NO production. A potential mechanism responsible for aggravation of endothelial dysfunction by ceramide is via ceramide-mediated activation of protein phosphatase 2A (PP2A) causing dephosphorylation of eNOS and AKT dissociation. These findings provide the mechanistic link between obesity-induced vascular dysfunction and ceramide.

SUMMARY

Obesity manifests in developed countries and contributes to the prevalence of insulin resistance and cardiovascular risk. As a result, obesity-induced inflammation had placed the adipose tissue at the center of inflammation-related pathophysiology. When storage capacity of adipose tissue exceeds its limit for fat deposition, spillage of FFA and adipokines alters inflammatory states in various tissues causing etiology of type 2 diabetes and vascular disease (**Figure 3**). Due to diverse risk factors for inflammatory disease, it is extremely important to find new approaches for better understanding of the disease. Recent findings suggest that sphingolipids are critical mediators of obesity-mediated inflammation and CVD. SM is implicated as a biochemical modulator of atherosclerosis and ceramide acts as a metabolic switch regulating substrate preference for cardiac energetics and NAFLD (**Table 1**). Although inflammation and sphingolipid metabolism are closely associated, a number of clinical and experimental issues needs further clarification in future. Moreover, new obesity- and sphingolipid-mediated disease may be found. Thus, modulation of sphingolipid biosynthesis in pathophysiological conditions explained in this review will provide a rationale for therapeutic intervention and present new targets for inflammation-induced chronic diseases.

ACKNOWLEDGMENTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (NRF2012047774, NRF20120007124).

REFERENCES

Abel, E. D., Litwin, S. E., and Sweeney, G. (2008). Cardiac remodeling in obesity. *Physiol. Rev.* 88, 389–419. doi:10.1152/physrev.00017.2007

Adams, J. M. II, Pratipanawatr, T., Berria, R., Wang, E., DeFronzo, R. A., Sullards, M. C., et al. (2004). Ceramide content is increased in skeletal muscle from obese insulin-resistant humans. *Diabetes* 53, 25–31. doi:10.2337/diabetes.53.1.25

Alderton, W. K., Cooper, C. E., and Knowles, R. G. (2001). Nitric oxide synthases: structure, function and inhibition. *Biochem. J.* 357, 593–615. doi:10.1042/0264-6021:3570593

Amati, F., Dube, J. J., Alvarez-Carnero, E., Edreira, M. M., Chomentowski, P., Coen, P. M., et al. (2011). Skeletal muscle triglycerides, diacylglycerols, and ceramides in insulin resistance: another paradox in endurance-trained athletes? *Diabetes* 60, 2588–2597. doi:10.2337/db10-1221

Chang, Z. Q., Lee, S. Y., Kim, H. J., Kim, J. R., Kim, S. J., Hong, I. K., et al. (2011). Endotoxin activates de novo sphingolipid biosynthesis via nuclear factor kappa B-mediated upregulation of Sptlc2. *Prostaglandins Other Lipid Mediat.* 94, 44–52. doi:10.1016/j.prostaglandins.2010.12.003

Chatterjee, S. (1994). Neutral sphingomyelinase action stimulates signal transduction of tumor necrosis factor-alpha in the synthesis of cholesteryl esters in human fibroblasts. *J. Biol. Chem.* 269, 879–882.

Chavez, J. A., Holland, W. L., Bar, J., Sandhoff, K., and Summers, S. A. (2005). Acid ceramidase overexpression prevents the inhibitory effects of saturated fatty acids on insulin signaling. *J. Biol. Chem.* 280, 20148–20153. doi:10.1074/jbc.M412769200

Chavez, J. A., Knotts, T. A., Wang, L. P., Li, G., Dobrowsky, R. T., Florant, G. L., et al. (2003). A role for ceramide, but not diacylglycerol, in the antagonism of insulin signal transduction by saturated fatty acids. *J. Biol. Chem.* 278, 10297–10303. doi:10.1074/jbc.M212307200

Chavez, J. A., and Summers, S. A. (2010). Lipid oversupply, selective insulin resistance, and lipotoxicity: molecular mechanisms. *Biochim. Biophys. Acta* 1801, 252–265. doi:10.1016/j.bbalip.2009.09.015

Christiansen, T., Richelsen, B., and Bruun, J. M. (2005). Monocyte chemoattractant protein-1 is produced in isolated adipocytes, associated with adiposity and reduced after weight loss in morbid obese subjects. *Int. J. Obes. (Lond.)* 29, 146–150. doi:10.1038/sj.ijo.0802839

Deevska, G. M., Rozenova, K. A., Giltiay, N. V., Chambers, M. A., White, J., Boyanovsky, B. B., et al. (2009). Acid sphingomyelinase deficiency prevents diet-induced hepatic triacylglycerol accumulation and hyperglycemia in mice. *J. Biol. Chem.* 284, 8359–8368. doi:10.1074/jbc.M807800200

Dobrowsky, R. T., Kamibayashi, C., Mumby, M. C., and Hannun, Y. A. (1993). Ceramide activates heterotrimeric protein phosphatase 2A. *J. Biol. Chem.* 268, 15523–15530.

Donnelly, K. L., Smith, C. I., Schwarzenberg, S. J., Jessurun, J., Boldt, M. D., and Parks, E. J. (2005). Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J. Clin. Invest.* 115, 1343–1351. doi:10.1172/JCI200523621

Dressler, K. A., Mathias, S., and Kolesnick, R. N. (1992). Tumor necrosis factor-alpha activates the sphingomyelin signal transduction pathway in a cell-free system. *Science* 255, 1715–1718. doi:10.1126/science.1313189

Du, X., Edelstein, D., Obici, S., Higham, N., Zou, M. H., and Brownlee, M. (2006). Insulin resistance reduces arterial prostacyclin synthase and eNOS activities by increasing endothelial fatty acid oxidation. *J. Clin. Invest.* 116, 1071–1080. doi:10.1172/JCI23354

Eisenberg, S., Stein, Y., and Stein, O. (1969). Phospholipases in arterial tissue. IV. The role of phosphatide acyl hydrolase, lysophosphatide acyl hydrolase, and sphingomyelin choline phosphohydrolase in the regulation of phospholipid composition in the normal human aorta with age. *J. Clin. Invest.* 48, 2320–2329. doi:10.1172/JCI106198

Fang, X., and Sweeney, G. (2006). Mechanisms regulating energy metabolism by adiponectin in obesity and diabetes. *Biochem. Soc. Trans.* 34, 798–801. doi:10.1042/BST0340798

Farrell, G. C., and Larter, C. Z. (2006). Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 43, S99–S112. doi:10.1002/hep.20973

Feldstein, A. E., Werneburg, N. W., Canbay, A., Guicciardi, M. E., Bronk, S. F., Rydzewski, R., et al. (2004). Free fatty acids promote hepatic lipotoxicity by stimulating TNF-alpha expression via a lysosomal pathway. *Hepatology* 40, 185–194. doi:10.1002/hep.20283

Ferrante, A. W. Jr. (2007). Obesity-induced inflammation: a metabolic dialogue in the language of inflammation. *J. Intern. Med.* 262, 408–414. doi:10.1111/j.1365-2796.2007.01852.x

Finkelstein, E. A., Trogdon, J. G., Cohen, J. W., and Dietz, W. (2009). Annual medical spending attributable to obesity: payer-and service-specific estimates. *Health Aff. (Millwood)* 28, w822–w831. doi:10.1377/hlthaff.28.5.w822

Flegal, K. M., Graubard, B. I., Williamson, D. F., and Gail, M. H. (2007). Cause-specific excess deaths associated with underweight, overweight, and obesity. *JAMA* 298, 2028–2037. doi:10.1001/jama.298.17.2028

Fried, S. K., Bunkin, D. A., and Greenberg, A. S. (1998). Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *J. Clin. Endocrinol. Metab.* 83, 847–850. doi:10.1210/jc.83.3.847

Friedman, J. M., and Halaas, J. L. (1998). Leptin and the regulation of body weight in mammals. *Nature* 395, 763–770. doi:10.1038/27376

Friedman, J. M., and Leibel, R. L. (1992). Tackling a weighty problem. *Cell* 69, 217–220. doi:10.1016/0092-8674(92)90402-X

Futerman, A. H., and Hannun, Y. A. (2004). The complex life of simple sphingolipids. *EMBO Rep.* 5, 777–782. doi:10.1038/sj.embor.7400208

Gao, S., Kinzig, K. P., Aja, S., Scott, K. A., Keung, W., Kelly, S., et al. (2007). Leptin activates hypothalamic acetyl-CoA carboxylase to inhibit food intake. *Proc. Natl. Acad. Sci. U.S.A.* 104, 17358–17363. doi:10.1073/pnas.0708385104

Gao, S., and Lane, M. D. (2003). Effect of the anorectic fatty acid synthase inhibitor C75 on neuronal activity in the hypothalamus and brainstem. *Proc. Natl. Acad. Sci. U.S.A.* 100, 5628–5633. doi:10.1073/pnas.1031698100

Gao, S., Zhu, G., Gao, X., Wu, D., Carrasco, P., Casals, N., et al. (2011). Important roles of brain-specific carnitine palmitoyltransferase and ceramide metabolism in leptin hypothalamic control of feeding. *Proc. Natl. Acad. Sci. U.S.A.* 108, 9691–9696. doi:10.1073/pnas.1103267108

Guyton, J. R., and Klemp, K. F. (1996). Development of the lipid-rich core in human atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 16, 4–11. doi:10.1161/01.ATV.16.1.4

Hajduch, E., Balendran, A., Batty, I. H., Litherland, G. J., Blair, A. S., Downes, C. P., et al. (2001). Ceramide impairs the insulin-dependent membrane recruitment of protein kinase B leading to a loss in downstream signalling in L6 skeletal muscle cells. *Diabetologia* 44, 173–183. doi:10.1007/s001250051596

Hakomori, S. (1981). Glycosphingolipids in cellular interaction, differentiation, and oncogenesis. *Annu. Rev. Biochem.* 50, 733–764. doi:10.1146/annurev.bi.50.070181.003505

Hannun, Y. A., and Obeid, L. M. (2008). Principles of bioactive lipid signalling: lessons from sphingolipids. *Nat. Rev. Mol. Cell Biol.* 9, 139–150. doi:10.1038/nrm2329

Haus, J. M., Kashyap, S. R., Kasumov, T., Zhang, R., Kelly, K. R., Defronzo, R. A., et al. (2009). Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. *Diabetes* 58, 337–343. doi:10.2337/db08-1228

He, W., Lam, T. K., Obici, S., and Rossetti, L. (2006). Molecular disruption of hypothalamic nutrient sensing induces obesity. *Nat. Neurosci.* 9, 227–233. doi:10.1038/nn1626

Hoehn, K. L., Hohnen-Behrens, C., Cederberg, A., Wu, L. E., Turner, N., Yuasa, T., et al. (2008). IRS1-independent defects define major nodes of insulin resistance. *Cell Metab.* 7, 421–433. doi:10.1016/j.cmet.2008.04.005

Hoff, H. F., and Morton, R. E. (1985). Lipoproteins containing apo B extracted from human aortas. Structure and function. *Ann. N. Y. Acad. Sci.* 454, 183–194.

Hojjati, M. R., Li, Z., Zhou, H., Tang, S., Huan, C., Ooi, E., et al. (2005). Effect of myriocin on plasma sphingolipid metabolism and atherosclerosis in apoE-deficient mice. *J. Biol. Chem.* 280, 10284–10289. doi:10.1074/jbc.M412348200

Holland, W. L., Bikman, B. T., Wang, L. P., Yuguang, G., Sargent, K. M., Bulchand, S., et al. (2011a). Lipid-induced insulin resistance mediated by the proinflammatory receptor TLR4 requires saturated fatty acid-induced ceramide biosynthesis in mice. *J. Clin. Invest.* 121, 1858–1870. doi:10.1172/JCI43378

Holland, W. L., Miller, R. A., Wang, Z. V., Sun, K., Barth, B. M., Bui, H. H., et al. (2011b). Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. *Nat. Med.* 17, 55–63. doi:10.1038/nm.2277

Holland, W. L., Brozinick, J. T., Wang, L. P., Hawkins, E. D., Sargent, K. M., Liu, Y., et al. (2007). Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. *Cell Metab.* 5, 167–179. doi:10.1016/j.cmet.2007.01.002

Hotamisligil, G. S., Arner, P., Caro, J. F., Atkinson, R. L., and Spiegelman, B. M. (1995). Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J. Clin. Invest.* 95, 2409–2415. doi:10.1172/JCI117936

Hotamisligil, G. S., Shargill, N. S., and Spiegelman, B. M. (1993). Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 259, 87–91. doi:10.1126/science.7678183

Hotamisligil, G. S., and Spiegelman, B. M. (1994). Tumor necrosis factor alpha: a key component of the obesity-diabetes link. *Diabetes* 43, 1271–1278. doi:10.2337/diabetes.43.11.1271

Jarrar, M. H., Baranova, A., Collantes, R., Ranard, B., Stepanova, M., Bennett, C., et al. (2008). Adipokines and cytokines in non-alcoholic fatty liver disease. *Aliment. Pharmacol. Ther.* 27, 412–421. doi:10.1111/j.1365-2036.2007.03586.x

Jeong, T., Schissel, S. L., Tabas, I., Pownall, H. J., Tall, A. R., and Jiang, X. (1998). Increased sphingomyelin content of plasma lipoproteins in apolipoprotein E knockout mice reflects combined production and catabolic defects and enhances reactivity with mammalian sphingomyelinase. *J. Clin. Invest.* 101, 905–912. doi:10.1172/JCI870

Jiang, X. C., Paultre, F., Pearson, T. A., Reed, R. G., Francis, C. K., Lin, M., et al. (2000). Plasma sphingomyelin level as a risk factor for coronary artery disease. *Arterioscler. Thromb. Vasc. Biol.* 20, 2614–2618. doi:10.1161/01.ATV.20.12.2614

Johns, D. G., Charpie, J. R., and Webb, R. C. (1998). Is ceramide signaling a target for vascular therapeutic intervention? *Curr. Pharm. Des.* 4, 481–488.

Jung, S. H., Park, H. S., Kim, K. S., Choi, W. H., Ahn, C. W., Kim, B. T., et al. (2008). Effect of weight loss on some serum cytokines in human obesity: increase in IL-10 after weight loss. *J. Nutr. Biochem.* 19, 371–375. doi:10.1016/j.jnutbio.2007.05.007

Kadowaki, T., Yamauchi, T., and Kubota, N. (2008). The physiological and pathophysiological role of adiponectin and adiponectin receptors in the peripheral tissues and CNS. *FEBS Lett.* 582, 74–80. doi:10.1016/j.febslet.2007.11.070

Kadowaki, T., Yamauchi, T., Kubota, N., Hara, K., Ueki, K., and Tobe, K. (2006). Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J. Clin. Invest.* 116, 1784–1792. doi:10.1172/JCI29126

Kawamura, N., Kubota, T., Kawano, S., Monden, Y., Feldman, A. M., Tsutsui, H., et al. (2005). Blockade of NF-kappaB improves cardiac function and survival without affecting inflammation in TNF-alpha-induced cardiomyopathy. *Cardiovasc. Res.* 66, 520–529. doi:10.1016/j.cardiores.2005.02.007

Kim, C. H., and Younossi, Z. M. (2008). Nonalcoholic fatty liver disease: a manifestation of the metabolic syndrome. *Cleve. Clin. J. Med.* 75, 721–728. doi:10.3949/ccjm.75.10.721

Kobayashi, H., Ouchi, N., Kihara, S., Walsh, K., Kumada, M., Abe, Y., et al. (2004). Selective suppression of endothelial cell apoptosis by the high molecular weight form of adiponectin. *Circ. Res.* 94, e27–e31. doi:10.1161/01.RES.0000119921.86460.37

Kolak, M., Westerbacka, J., Velagapudi, V. R., Wagsater, D., Yetukuri, L., Makkonen, J., et al. (2007). Adipose tissue inflammation and increased ceramide content characterize subjects with high liver fat content independent of obesity. *Diabetes* 56, 1960–1968. doi:10.2337/db07-0111

Kraegen, E. W., Cooney, G. J., Ye, J. M., Thompson, A. L., and Furler, S. M. (2001). The role of lipids in the pathogenesis of muscle insulin resistance and beta cell failure in type II diabetes and obesity. *Exp. Clin. Endocrinol. Diabetes* 109(Suppl. 2), S189–S201. doi:10.1055/s-2001-18581

Kummerow, F. A., Cook, L. S., Wasowicz, E., and Jelen, H. (2001). Changes in the phospholipid composition of the arterial cell can result in severe atherosclerotic lesions. *J. Nutr. Biochem.* 12, 602–607. doi:10.1016/S0955-2863(01)00181-4

Lee, S. Y., Kim, J. R., Hu, Y., Khan, R., Kim, S. J., Bharadwaj, K. G., et al. (2012). Cardiomyocyte specific deficiency of serine palmitoyltransferase subunit 2 reduces ceramide but leads to cardiac dysfunction. *J. Biol. Chem.* 287, 18429–18439. doi:10.1074/jbc.M111.296947

Li, Z., Park, T. S., Li, Y., Pan, X., Iqbal, J., Lu, D., et al. (2009). Serine palmitoyltransferase (SPT) deficient mice absorb less cholesterol. *Biochim. Biophys. Acta* 1791, 297–306. doi:10.1016/j.bbalip.2009.01.010

Liu, J., Huan, C., Chakraborty, M., Zhang, H., Lu, D., Kuo, M. S., et al. (2009). Macrophage sphingomyelin synthase 2 deficiency decreases atherosclerosis in mice. *Circ. Res.* 105, 295–303. doi:10.1161/CIRCRESAHA.109.194613

Liu, P., Leffler, B. J., Weeks, L. K., Chen, G., Bouchard, C. M., Strawbridge, A. B., et al. (2004). Sphingomyelinase activates GLUT4 translocation via a cholesterol-dependent mechanism. *Am. J. Physiol. Cell Physiol.* 286, C317–C329. doi:10.1152/ajpcell.00073.2003

Liu, Y., Retnakaran, R., Hanley, A., Tungtrongchitr, R., Shaw, C., and Sweeney, G. (2007). Total and high molecular weight but not trimeric or hexameric forms of adiponectin correlate with markers of the metabolic syndrome and liver injury in Thai subjects. *J. Clin. Endocrinol. Metab.* 92, 4313–4318. doi:10.1210/jc.2007-0890

Loftus, T. M., Jaworsky, D. E., Frehywot, G. L., Townsend, C. A., Ronnett, G. V., Lane, M. D., et al. (2000). Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. *Science* 288, 2379–2381. doi:10.1126/science.288.5475.2379

Lopez, M., Lelliott, C. J., Tovar, S., Kimber, W., Gallego, R., Virtue, S., et al. (2006). Tamoxifen-induced anorexia is associated with fatty acid synthase inhibition in the ventromedial nucleus of the hypothalamus and accumulation of malonyl-CoA. *Diabetes* 55, 1327–1336. doi:10.2337/db05-1356

Matsuzawa, Y. (2010). Adiponectin: a key player in obesity related disorders. *Curr. Pharm. Des.* 16, 1896–1901. doi:10.2174/138161210791208893

Memon, R. A., Holleran, W. M., Moser, A. H., Seki, T., Uchida, Y., Fuller, J., et al. (1998). Endotoxin and cytokines increase hepatic sphingolipid biosynthesis and produce lipoproteins enriched in ceramides and sphingomyelin. *Arterioscler. Thromb. Vasc. Biol.* 18, 1257–1265. doi:10.1161/01.ATV.18.8.1257

Mohamed-Ali, V., Goodrick, S., Rawesh, A., Katz, D. R., Miles, J. M., Yudkin, J. S., et al. (1997). Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *J. Clin. Endocrinol. Metab.* 82, 4196–4200. doi:10.1210/jc.82.12.4196

Morad, S. A., and Cabot, M. C. (2013). Ceramide-orchestrated signalling in cancer cells. *Nat. Rev. Cancer* 13, 51–65. doi:10.1038/nrc3398

Morales-Ruiz, M., Lee, M. J., Zollner, S., Gratton, J. P., Scotland, R., Shiojima, I., et al. (2001). Sphingosine 1-phosphate activates Akt, nitric oxide production, and chemotaxis through a Gi protein/phosphoinositide 3-kinase pathway in endothelial cells. *J. Biol. Chem.* 276, 19672–19677. doi:10.1074/jbc.M009993200

Neuschwander-Tetri, B. A., and Caldwell, S. H. (2003). Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 37, 1202–1219. doi:10.1053/jhep.2003.50193

Newman, H. A., McCandless, C. E., and Zilversmit, D. B. (1961). The synthesis of C14-lipids in rabbit atheromatous lesions. *J. Biol. Chem.* 236, 1264–1268.

Nievelstein, P. F., Fogelman, A. M., Mottino, G., and Frank, J. S. (1991). Lipid accumulation in rabbit aortic intima 2 hours after bolus infusion of low density lipoprotein. A deep-etch and immunolocalization study of ultrarapidly frozen tissue. *Arterioscler. Thromb.* 11, 1795–1805. doi:10.1161/01.ATV.11.6.1795

Obici, S., Feng, Z., Arduini, A., Conti, R., and Rossetti, L. (2003). Inhibition of hypothalamic carnitine palmitoyltransferase-1 decreases food intake and glucose production. *Nat. Med.* 9, 756–761. doi:10.1038/nm873

Ouchi, N., Kihara, S., Arita, Y., Okamoto, Y., Maeda, K., Kuriyama, H., et al. (2000). Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. *Circulation* 102, 1296–1301. doi:10.1161/01.CIR.102.11.1296

Park, T. S., Hu, Y., Noh, H. L., Drosatos, K., Okajima, K., Buchanan, J., et al. (2008a). Ceramide is a cardiotoxin in lipotoxic cardiomyopathy. *J. Lipid Res.* 49, 2101–2112. doi:10.1194/jlr.M800147-JLR200

Park, T. S., Rosebury, W., Kindt, E. K., Kowala, M. C., and Panek, R. L. (2008b). Serine palmitoyltransferase inhibitor myriocin induces the regression of atherosclerotic plaques in hyperlipidemic apoE-deficient mice. *Pharmacol. Res.* 58, 45–51. doi:10.1016/j.phrs.2008.06.005

Park, T. S., Panek, R. L., Mueller, S. B., Hanselman, J. C., Rosebury, W. S., Robertson, A. W., et al. (2004). Inhibition of sphingomyelin synthesis reduces atherogenesis in apolipoprotein E-knockout mice. *Circulation* 110, 3465–3471. doi:10.1161/01.CIR.0000148370.60535.22

Peng, Y., Rideout, D., Rakita, S., Sajan, M., Farese, R., You, M., et al. (2009). Downregulation of adiponectin/AdipoR2 is associated with steatohepatitis in obese mice. *J. Gastrointest. Surg.* 13, 2043–2049. doi:10.1007/s11605-009-1032-2

Perreault, M., and Marette, A. (2001). Targeted disruption of inducible nitric oxide synthase protects against obesity-linked insulin resistance in muscle. *Nat. Med.* 7, 1138–1143. doi:10.1038/nm1001-1138

Pettus, B. J., Chalfant, C. E., and Hannun, Y. A. (2002). Ceramide in apoptosis: an overview and current perspectives. *Biochim. Biophys. Acta* 1585, 114–125. doi:10.1016/S1388-1981(02)00331-1

Phillips, G. B., and Dodge, J. T. (1967). Composition of phospholipids and of phospholipid fatty acids of human plasma. *J. Lipid Res.* 8, 676–681.

Plump, A. S., Smith, J. D., Hayek, T., Aalto-Setala, K., Walsh, A., Verstuyft, J. G., et al. (1992). Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell* 71, 343–353. doi:10.1016/0092-8674(92)90362-G

Portman, O. W., and Illingworth, D. R. (1976). Arterial metabolism in primates. *Primates Med.* 9, 145–223.

Powell, D. J., Hajduch, E., Kular, G., and Hundal, H. S. (2003). Ceramide disables 3-phosphoinositide binding to the pleckstrin homology domain of protein kinase B (PKB)/Akt by a PKCzeta-dependent mechanism. *Mol. Cell. Biol.* 23, 7794–7808. doi:10.1128/MCB.23.21.7794-7808.2003

Powell, D. J., Turban, S., Gray, A., Hajduch, E., and Hundal, H. S. (2004). Intracellular ceramide synthesis and protein kinase Czeta activation play an essential role in palmitate-induced insulin resistance in rat L6 skeletal muscle cells. *Biochem. J.* 382, 619–629. doi:10.1042/BJ20040139

Reyna, S. M., Ghosh, S., Tantiwong, P., Meka, C. S., Eagan, P., Jenkinson, C. P., et al. (2008). Elevated toll-like receptor 4 expression and signaling in muscle from insulin-resistant subjects. *Diabetes* 57, 2595–2602. doi:10.2337/db08-0038

Riad, A., Bien, S., Gratz, M., Escher, F., Westermann, D., Heimesaat, M. M., et al. (2008). Toll-like receptor-4

deficiency attenuates doxorubicin-induced cardiomyopathy in mice. *Eur. J. Heart Fail.* 10, 233–243. doi:10.1016/j.ejheart.2008.01.004

Ross, R. (1993). The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 362, 801–809. doi:10.1038/362801a0

Ruvolo, P. P. (2003). Intracellular signal transduction pathways activated by ceramide and its metabolites. *Pharmacol. Res.* 47, 383–392. doi:10.1016/S1043-6618(03)00050-1

Samad, F., Hester, K. D., Yang, G., Hannun, Y. A., and Bielawski, J. (2006). Altered adipose and plasma sphingolipid metabolism in obesity: a potential mechanism for cardiovascular and metabolic risk. *Diabetes* 55, 2579–2587. doi:10.2337/db06-0330

Schilling, J. D., Machkovech, H. M., He, L., Sidhu, R., Fujiwara, H., Weber, K., et al. (2013). Palmitate and lipopolysaccharide trigger synergistic ceramide production in primary macrophages. *J. Biol. Chem.* 288, 2923–2932. doi:10.1074/jbc.M112.419978

Schissel, S. L., Jiang, X., Tweedie-Hardman, J., Jeong, T., Camejo, E. H., Najib, J., et al. (1998). Secretory sphingomyelinase, a product of the acid sphingomyelinase gene, can hydrolyze atherogenic lipoproteins at neutral pH. Implications for atherosclerotic lesion development. *J. Biol. Chem.* 273, 2738–2746. doi:10.1074/jbc.273.5.2738

Schissel, S. L., Tweedie-Hardman, J., Rapp, J. H., Graham, G., Williams, K. J., and Tabas, I. (1996). Rabbit aorta and human atherosclerotic lesions hydrolyze the sphingomyelin of retained low-density lipoprotein. Proposed role for arterial-wall sphingomyelinase in subendothelial retention and aggregation of atherogenic lipoproteins. *J. Clin. Invest.* 98, 1455–1464. doi:10.1172/JCI118934

Schlitt, A., Blankenberg, S., Yan, D., von Gizycki, H., Buerke, M., Werdan, K., et al. (2006). Further evaluation of plasma sphingomyelin levels as a risk factor for coronary artery disease. *Nutr. Metab. (Lond.)* 3, 5.

Schutze, S., Potthoff, K., Machleidt, T., Berkovic, D., Wiegmann, K., and Kronke, M. (1992). TNF activates NF-kappa B by phosphatidylcholine-specific phospholipase C-induced "acidic" sphingomyelin breakdown. *Cell* 71, 765–776. doi:10.1016/0092-8674(92)90553-O

Schutze, S., Wiegmann, K., Machleidt, T., and Kronke, M. (1995). TNF-induced activation of NF-kappa B. *Immunobiology* 193, 193–203. doi:10.1016/S0171-2985(11)80543-7

Schwartz, M. W., Woods, S. C., Porte, D. Jr., Seeley, R. J., and Baskin, D. G. (2000). Central nervous system control of food intake. *Nature* 404, 661–671.

Shetty, S., Kusminski, C. M., and Scherer, P. E. (2009). Adiponectin in health and disease: evaluation of adiponectin-targeted drug development strategies. *Trends Pharmacol. Sci.* 30, 234–239. doi:10.1016/j.tips.2009.02.004

Shimabukuro, M., Higa, M., Zhou, Y. T., Wang, M. Y., Newgard, C. B., and Unger, R. H. (1998). Lipoapoptosis in beta-cells of obese prediabetic fa/fa rats. Role of serine palmitoyltransferase overexpression. *J. Biol. Chem.* 273, 32487–32490. doi:10.1074/jbc.273.49.32487

Smith, E. B. (1960). Intimal and medial lipids in human aortas. *Lancet* 1, 799–803. doi:10.1016/S0140-6736(60)90680-2

Spiegel, S., and Milstien, S. (2003). Sphingosine-1-phosphate: an enigmatic signalling lipid. *Nat. Rev. Mol. Cell Biol.* 4, 397–407. doi:10.1038/nrm1103

Steinberg, H. O., Paradisi, G., Hook, G., Crowder, K., Cronin, J., and Baron, A. D. (2000). Free fatty acid elevation impairs insulin-mediated vasodilation and nitric oxide production. *Diabetes* 49, 1231–1238. doi:10.2337/diabetes.49.7.1231

Stone, P. H., Muller, J. E., Hartwell, T., York, B. J., Rutherford, J. D., Parker, C. B., et al. (1989). The effect of diabetes mellitus on prognosis and serial left ventricular function after acute myocardial infarction: contribution of both coronary disease and diastolic left ventricular dysfunction to the adverse prognosis. The MILIS Study Group. *J. Am. Coll. Cardiol.* 14, 49–57. doi:10.1016/0735-1097(89)90053-3

Sugiura, M., Kono, K., Liu, H., Shimizugawa, T., Minekura, H., Spiegel, S., et al. (2002). Ceramide kinase, a novel lipid kinase. Molecular cloning and functional characterization. *J. Biol. Chem.* 277, 23294–23300. doi:10.1074/jbc.M201535200

Summers, S. A. (2006). Ceramides in insulin resistance and lipotoxicity. *Prog. Lipid Res.* 45, 42–72. doi:10.1016/j.plipres.2005.11.002

Summers, S. A. (2010). Sphingolipids and insulin resistance: the five Ws. *Curr. Opin. Lipidol.* 21, 128–135. doi:10.1097/MOL.0b013e3283373b66

Summers, S. A., Garza, L. A., Zhou, H., and Birnbaum, M. J. (1998). Regulation of insulin-stimulated glucose transporter GLUT4 translocation and Akt kinase activity by ceramide. *Mol. Cell. Biol.* 18, 5457–5464.

Symons, J. D., McMillin, S. L., Riehle, C., Tanner, J., Palionyte, M., Hillas, E., et al. (2009). Contribution of insulin and Akt1 signaling to endothelial nitric oxide synthase in the regulation of endothelial function and blood pressure. *Circ. Res.* 104, 1085–1094. doi:10.1161/CIRCRESAHA.108.189316

Tabas, I., Williams, K. J., and Boren, J. (2007). Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. *Circulation* 116, 1832–1844. doi:10.1161/CIRCULATIONAHA.106.676890

Ueki, K., Kondo, T., Tseng, Y. H., and Kahn, C. R. (2004). Central role of suppressors of cytokine signaling proteins in hepatic steatosis, insulin resistance, and the metabolic syndrome in the mouse. *Proc. Natl. Acad. Sci. U.S.A.* 101, 10422–10427. doi:10.1073/pnas.0402511101

Unger, R. H. (2003). Minireview: weapons of lean body mass destruction: the role of ectopic lipids in the metabolic syndrome. *Endocrinology* 144, 5159–5165. doi:10.1210/en.2003-0870

Vandenabeele, P., Declercq, W., Beyaert, R., and Fiers, W. (1995). Two tumour necrosis factor receptors: structure and function. *Trends Cell Biol.* 5, 392–399. doi:10.1016/S0962-8924(00)89088-1

Visser, M., Bouter, L. M., McQuillan, G. M., Wener, M. H., and Harris, T. B. (1999). Elevated C-reactive protein levels in overweight and obese adults. *JAMA* 282, 2131–2135. doi:10.1001/jama.282.22.2131

Waki, H., Yamauchi, T., Kamon, J., Kita, S., Ito, Y., Hada, Y., et al. (2005). Generation of globular fragment of adiponectin by leukocyte elastase secreted by monocytic cell line THP-1. *Endocrinology* 146, 790–796. doi:10.1210/en.2004-1096

Wang, Y., Lam, K. S., Chan, L., Chan, K. W., Lam, J. B., Lam, M. C., et al. (2006). Post-translational modifications of the four conserved lysine residues within the collagenous domain of adiponectin are required for the formation of its high molecular weight oligomeric complex. *J. Biol. Chem.* 281, 16391–16400. doi:10.1074/jbc.M513907200

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., and Ferrante, A. W. Jr. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* 112, 1796–1808. doi:10.1172/JCI19246

Williams, K. J., and Tabas, I. (1995). The response-to-retention hypothesis of early atherogenesis. *Arterioscler. Thromb. Vasc. Biol.* 15, 551–561. doi:10.1161/01.ATV.15.5.551

Williams, K. J., and Tabas, I. (1998). The response-to-retention hypothesis of atherogenesis reinforced. *Curr. Opin. Lipidol.* 9, 471–474. doi:10.1097/00041433-199810000-00012

Witztum, J. L., and Steinberg, D. (1991). Role of oxidized low density lipoprotein in atherogenesis. *J. Clin. Invest.* 88, 1785–1792. doi:10.1172/JCI115499

Wolfgang, M. J., Cha, S. H., Sidhaye, A., Chohnan, S., Cline, G., Shulman, G. I., et al. (2007). Regulation of hypothalamic malonyl-CoA by central glucose and leptin. *Proc. Natl. Acad. Sci. U.S.A.* 104, 19285–19290. doi:10.1073/pnas.0709778104

Wolfgang, M. J., Kurama, T., Dai, Y., Suwa, A., Asaumi, M., Matsumoto, S., et al. (2006). The brain-specific carnitine palmitoyltransferase-1c regulates energy homeostasis. *Proc. Natl. Acad. Sci. U.S.A.* 103, 7282–7287. doi:10.1073/pnas.0602205103

Wu, Y., Song, P., Xu, J., Zhang, M., and Zou, M. H. (2007). Activation of protein phosphatase 2A by palmitate inhibits AMP-activated protein kinase. *J. Biol. Chem.* 282, 9777–9788. doi:10.1074/jbc.M608310200

Yang, G., Badeanlou, L., Bielawski, J., Roberts, A. J., Hannun, Y. A., and Samad, F. (2009). Central role of ceramide biosynthesis in body weight regulation, energy metabolism, and the metabolic syndrome. *Am. J. Physiol. Endocrinol. Metab.* 297, E211–E224. doi:10.1152/ajpendo.91014.2008

Yang, W. S., Lee, W. J., Funahashi, T., Tanaka, S., Matsuzawa, Y., Chao, C. L., et al. (2001). Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *J. Clin. Endocrinol. Metab.* 86, 3815–3819. doi:10.1210/jc.86.8.3815

Yetukuri, L., Katajamaa, M., Medina-Gomez, G., Seppanen-Laakso, T., Vidal-Puig, A., and Oresic, M. (2007). Bioinformatics strategies for lipidomics analysis: characterization of obesity related hepatic steatosis. *BMC Syst. Biol.* 1:12. doi:10.1186/1752-0509-1-12

Yla-Herttuala, S., Palinski, W., Rosenfeld, M. E., Parthasarathy, S., Carew, T. E., Butler, S., et al. (1989). Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *J. Clin. Invest.* 84, 1086–1095. doi:10.1172/JCI114271

Yokoyama, M., Yagyu, H., Hu, Y., Seo, T., Hirata, K., Homma, S., et al. (2004). Apolipoprotein B production reduces lipotoxic cardiomyopathy: studies in heart-specific lipoprotein lipase transgenic mouse. *J. Biol. Chem.* 279, 4204–4211. doi:10.1074/jbc.M311995200

Yuan, M., Konstantopoulos, N., Lee, J., Hansen, L., Li, Z. W., Karin, M., et al. (2001). Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* 293, 1673–1677. doi:10.1126/science.1061620

Zhang, Q. J., Holland, W. L., Wilson, L., Tanner, J. M., Kearns, D., Cahoon, J. M., et al. (2012). Ceramide mediates vascular dysfunction in diet-induced obesity by PP2A-mediated dephosphorylation of the eNOS-Akt complex. *Diabetes* 61, 1848–1859. doi:10.2337/db11-1399

Zhang, S. H., Reddick, R. L., Piedrahita, J. A., and Maeda, N. (1992). Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science* 258, 468–471. doi:10.1126/science.1411543

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature* 372, 425–432. doi:10.1038/372425a0

Zilversmit, D. B., McCandless, C. E., Jordan, P. H., Henly, W. S., and Ackerman, R. F. (1961). The synthesis of phospholipids in human atheromatous lesions. *Circulation* 23, 370–375. doi:10.1161/01.CIR.23.3.370

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 25 February 2013; accepted: 20 May 2013; published online: 04 June 2013.

*Citation: Kang S-C, Kim B-R, Lee S-Y and Park T-S (2013) Sphingolipid metabolism and obesity-induced inflammation. Front. Endocrinol. **4**:67. doi: 10.3389/fendo.2013.00067*

This article was submitted to Frontiers in Diabetes, a specialty of Frontiers in Endocrinology.

Copyright © 2013 Kang, Kim, Lee and Park. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.

frontiers in
ENDOCRINOLOGY

REVIEW ARTICLE
published: 10 June 2013
doi: 10.3389/fendo.2013.00069

Effects of bariatric surgery on adipokine-induced inflammation and insulin resistance

Zeynep Goktas[1], Naima Moustaid-Moussa[1], Chwan-Li Shen[2], Mallory Boylan[1], Huanbiao Mo[3] and Shu Wang[1]*

[1] *Nutritional Sciences Program, College of Human Science, Texas Tech University, Lubbock, TX, USA*
[2] *Department of Pathology, Texas Tech University Health Sciences Center, Lubbock, TX, USA*
[3] *Department of Nutrition and Food Sciences, Texas Woman's University, Denton, TX, USA*

Edited by:
Tsuguhito Ota, Kanazawa University, Japan

Reviewed by:
Undurti Narasimha Das, Undurti N. Das Life Sciences, USA
Julianne Toohey, University of California, USA

****Correspondence:***
Shu Wang, Nutritional Science Program, College of Human Science, Texas Tech University, P.O. Box: 41240, Lubbock, TX 79409-1240, USA
e-mail: shu.wang@ttu.edu

Over a third of the US population is obese and at high risk for developing type 2 diabetes, insulin resistance, and other metabolic disorders. Obesity is considered a chronic low-grade inflammatory condition that is primarily attributed to expansion and inflammation of adipose tissues. Indeed, adipocytes produce and secrete numerous proinflammatory and anti-inflammatory cytokines known as adipokines. When the balance of these adipokines is shifted toward higher production of proinflammatory factors, local inflammation within adipose tissues and subsequently systemic inflammation occur. These adipokines including leptin, visfatin, resistin, apelin, vaspin, and retinol binding protein-4 can regulate inflammatory responses and contribute to the pathogenesis of diabetes. These effects are mediated by key inflammatory signaling molecules including activated serine kinases such as c-Jun N-terminal kinase and serine kinases inhibitor κB kinase and insulin signaling molecules including insulin receptor substrates, protein kinase B (PKB, also known as Akt), and nuclear factor kappa B. Bariatric surgery can decrease body weight and improve insulin resistance in morbidly obese subjects. However, despite reports suggesting reduced inflammation and weight-independent effects of bariatric surgery on glucose metabolism, mechanisms behind such improvements are not yet well understood. This review article focuses on some of these novel adipokines and discusses their changes after bariatric surgery and their relationship to insulin resistance, fat mass, inflammation, and glucose homeostasis.

Keywords: bariatric surgery, inflammation, obesity, insulin resistance, adipokines

INTRODUCTION

Obesity refers to excess fat mass or adiposity, and is typically defined as a body mass index (BMI) over 30 kg/m^2 or a waist circumference greater than 94 cm for men and 80 cm for women (Nash et al., 2008). It is a complex multifactorial disease that is positively associated with increased risk and onset of numerous chronic diseases including cardiovascular disease and Type 2 diabetes (Sjostrom et al., 2004). According to the World Health Organization report, one billion of the world's adult population is overweight and 300 million of them are obese (Mushtaq et al., 2011). One of three adults is obese in the United States (Bhattacharya and Sood, 2011).

The first recommended treatment option for obesity is a low-calorie diet and regular physical activity. However, these lifestyle interventions have low compliance in general and limited effectiveness in severely obese people (Hell and Miller, 2002; Yermilov et al., 2009). Bariatric surgery has emerged as the approach of choice for weight loss among morbidly obese adults with a BMI over 40 kg/m^2 or those with a BMI over 35 kg/m^2 and existing metabolic risk factors such as hypertension, diabetes, or hypercholesterolemia (Sjostrom et al., 2004; Kulick et al., 2010).

Recent research has established that obesity is a chronic low-grade inflammatory condition (Herder et al., 2007; Liu et al., 2007; Amati et al., 2010; Balistreri et al., 2010; Park et al., 2010; Thompson et al., 2011). Following bariatric surgery, patients usually experience approximately 30% weight loss as well as decreased overall inflammatory responses (Compher and Badellino, 2008). Concomitantly, there are beneficial changes such as improved insulin resistance, reduced cardiovascular risk, and decreased oxidative stress that are achieved through multiple pathways related to systemic and adipocyte inflammation and adipocyte-derived cytokines (Cancello et al., 2005; Holdstock et al., 2005; Mattar et al., 2005; Vazquez et al., 2005; Poitou et al., 2006; Iannelli et al., 2009, 2010; Boesing et al., 2010; Butner et al., 2010; Hofso et al., 2010; Joao Cabrera et al., 2010; Murri et al., 2010; Kalupahana et al., 2011, 2012).

BARIATRIC SURGERY

Different types of bariatric surgeries are used for reducing body weight with various success rates (Franco et al., 2011). In Roux-en-Y gastric bypass, the stomach is divided into two parts: a small proximal pouch (15–20 ml) and a large distal pouch (**Figure 1A**). The small proximal pouch is attached to the proximal jejunum, bypassing the large distal gastric pouch and duodenum (Jaunoo and Southall, 2010). In this procedure energy intake is restricted by the small volume of the stomach pouch (Arceo-Olaiz et al., 2008; Yan et al., 2008). Moreover, bypassing the duodenum decreases the digestion and absorption of food. In vertical banded gastroplasty,

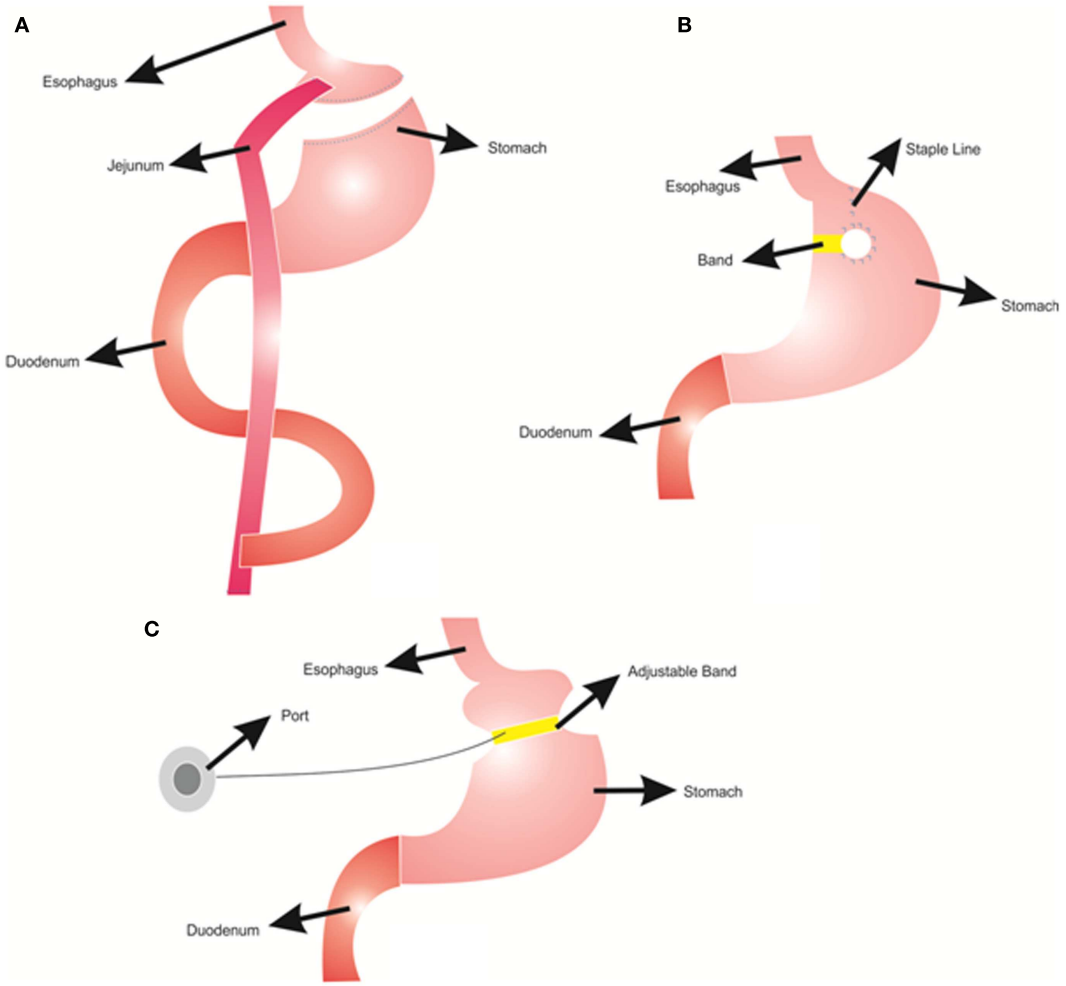


FIGURE 1 | Bariatric surgery procedures; (A) Roux-n-Y Gastric Bypass, (B) Vertical Gastroplasty, (C) Adjustable Gastric Banding.

the proximal stomach is stapled vertically, allowing food draining from the proximal pouch to the distal pouch with the outlet reinforced with a mesh collar to prevent the enlargement of proximal pouch and staple line failures (**Figure 1B**) (Franco et al., 2011). This procedure decreases the energy intake due to reduced stomach size. However, staple line failures are very common problems that may lead to regaining of the lost weight (van Hout et al., 2007). In gastric banding, a cuff band is used to section the stomach into proximal and distal parts (**Figure 1C**). In the adjustable form, there is an inflatable balloon in the cuff band. A reservoir can be placed under the skin and band size can be adjusted by inflating the balloon from this reservoir (Camerini et al., 2004; Spivak et al., 2005; Picot et al., 2009). The Roux-en-Y gastric bypass procedure is more effective for weight loss than the vertical banded gastroplasty and the adjustable gastric banding (Picot et al., 2009).

INFLAMMATION IN OBESITY

Adipose tissue is considered as an endocrine organ, which consists of <50% adipocytes and >50% stromal vascular fraction that contains blood cells, endothelial cells, adipose-tissue precursors and stem cells, macrophages, and other immune cells (Wang and Nakayama, 2010). As an endocrine organ, the adipose-tissue produces and secretes several hormones and cytokines that play important roles in carbohydrate and lipid metabolism, inflammation, blood coagulation, as well as satiety, and hunger signaling (Hajer et al., 2008; Qasim et al., 2008; Wang and Nakayama, 2010). These cytokines, also known as adipocytokines or adipokines, are either secreted by adipocytes and/or by the stromal vascular fraction, especially macrophages, as summarized in **Table 1** (Wang and Nakayama, 2010; Gomez-Illan et al., 2012; Kalupahana et al., 2012). Adipokines can function as classical cytokines, growth factors and proteins that are involved in blood pressure regulation, vascular homeostasis, and lipid and glucose metabolisms (Trayhurn, 2007).

In obesity, expansion of adipose-tissue causes hypoxia and stress, leading to necrosis of adipocytes. More than 90% of macrophages in white adipose tissues of animals are localized to dead adipocytes. The "Crown-Like Structure" (Yudkin, 2007) describes necrotic cells with impaired cell integrity and lipid droplets (Mosser and Edwards, 2008) and the surrounding

Table 1 | Obesity-associated changes in the levels of common cytokines secreted by adipocytes and macrophages.

Cytokines	Changes	Reference
ADIPOCYTE SECRETIONS		
Leptin	↑	Oswal and Yeo (2010)
Adinopectin	↓	Asayama et al. (2003)
Visfatin	↑	Sandeep et al. (2007a)
Vaspin	↑	Li et al. (2008)
Apelin	↑	Boucher et al. (2005)
FABP-4	↓	Queipo-Ortuno et al. (2012)
Perilipin	↓	Wang et al. (2003)
RBP-4	↑	Wolf (2007)
Resistin	↑	Piestrzeniewicz et al. (2008)
Lipocalin 2	↑	Catalan et al. (2009)
MACROPHAGE SECRETIONS		
IL-6	↑	Cesari et al. (2005)
TNF-α	↑	Tzanavari et al. (2010)
HGF	↑	Bell et al. (2006)
IL-10	↓	Blüher et al. (2005)
IL-18	↑	Leick et al. (2007)
PAI-1	↑	Cesari et al. (2005)
CRP	↑	Park et al. (2005)
MCP-1	↑	Panee (2012)
VEGF	↑	Garcia de la Torre et al. (2008)

FABP-4, Fatty Acid Binding Protein-4; RBP-4, Retinol Binding Protein-4; IL-6, Interleukin 6; TNF-α, Tumor necrosis factor-alpha; HGF, Hepatocyte Growth Factor; IL-10, Interleukin 10; IL-18, Interleukin 18; PAI-1, Plasminogen activator inhibitor-1; CRP, C-reactive Protein; MCP-1, Monocyte Chemoattractant Protein-1; VEGF, Vascular Endothelial Growth Factor.

macrophages that serve as scavengers of cell debris and lipid droplets in the necrotic cells.

There are two types of macrophages in white adipose tissues: proinflammatory M1 type and anti-inflammatory M2 type (Rull et al., 2010). M1 type macrophages are mainly recruited and induced by proinflammatory cytokines produced by expanded adipose tissue. After M1 type macrophages infiltrate into the adipose tissue, they secrete more proinflammatory cytokines and produce reactive oxygen species (ROS), which can recruit more macrophages and amplify the inflammatory response. M2 type macrophages are adipose-tissue resident macrophages (Rull et al., 2010). These macrophages are also called as alternatively activated macrophages because they are activated by interleukin (IL)-4. M2 type macrophages secrete anti-inflammatory cytokines and have mannose receptors, scavenger receptors and distinct integrins, which lead to anti-inflammatory functions (Mosser and Edwards, 2008; Rull et al., 2010). With stress and hypoxia in expanded adipose-tissue during obesity, M2 type macrophages lose their IL-4 receptor expression and IL-4-mediated anti-inflammatory functions, and instead are differentiated into M1 type proinflammatory macrophages (Rull et al., 2010). Besides IL-4, decreased expression of IL-10, Ym1, Arginase-1, and increased expression of tumor necrosis factor-alpha (TNF-α) and inducible nitric oxide synthetase (iNOS) induce the switch of adipose-tissue macrophages from a M2 to M1 phenotype.

This switch increases the number of proinflammatory macrophages, resulting in increased production of cytokines and chemokines including monocyte chemoattractant protein (MCP)-1, MCP-2, Regulated upon Activation, Normal T cell Expressed and Secreted (RANTES), and chemokine receptors like CCR2 and CCR5 (Wang and Nakayama, 2010). These inflammatory chemokines further increase macrophage infiltration to the adipose tissue (Malavazos et al., 2005; Trayhurn, 2005).

Kanda et al. found that macrophages and endothelial cells in rat adipose tissue can secrete MCP-1. This chemokine and its receptor CCR2 direct the migration of monocytes into the adipose tissue. Subsequent exposure of the monocytes to macrophage colony-stimulating factor causes differentiation to macrophages, which can secrete more MCP-1 (Kanda et al., 2006). In another study, obese subjects had significantly higher plasma MCP-1 levels than lean subjects, and increased plasma MCP-1 levels were positively associated with insulin resistance (Catalan et al., 2007). In expanded adipose tissue, M1 type macrophages secrete not only MCP-1, but also other proinflammatory cytokines including TNF-α, IL-6, IL-1, IL-1β, and IL-8, which can amplify inflammatory responses (Coppack, 2001; Zeyda and Stulnig, 2009).

THE ROLES OF INFLAMMATION ON INSULIN SIGNALING

The major regulators in the insulin signaling pathway are insulin receptor substrates (IRSs). The binding of insulin to its receptor leads to tyrosine phosphorylation of the receptor. This phosphorylation is recognized by the IRS family that has 6 member proteins, IRS1 to IRS6. IRS1 plays an important role in transmitting signals from the insulin receptor on cell membrane to intracellular phosphatidiylinositol-3-kinase (PI3K)/Akt and extracellular signal-regulated kinases (Erk)/mitogen-activated protein kinase (MAPK) pathways (Cai et al., 2003; Tarantino and Caputi, 2011). Under normal circumstance, the binding of insulin to the insulin receptor induces a conformational change of the receptor, which leads to autophosphorylation of specific tyrosine residues in the cytoplasmic domains of the receptor and further recruitment of adapter proteins IRSs (Nieto-Vazquez et al., 2008). After interacting with IRSs, PI3K is activated. PI3K lipid products can further recruit and activate Akt through phosphorylation on threonine 308 (T308) (Nieto-Vazquez et al., 2008). Akt is an important component of insulin signaling pathways. IRS1 tyrosine phosphorylation leads to a second wave of phosphorylation in the Akt protein. Phosphorylated Akt then initiates more phosphorylation reactions and eventually leads to glucose uptake, glycogen synthesis, protein synthesis, cell survival and gene transcriptions (Siddle, 2011) **(Figure 2)**. Tyrosine phosphorylation is required for IRS1 activation and insulin sensitization, which are blocked by serine phosphorylation on IRS1.

Increased production of TNF-α in expanded adipose-tissue decreases insulin sensitivity (Fantuzzi, 2008). TNF-α binds to TNF receptor-1 (TNFR1) and activates serine kinases including c-Jun N-terminal kinase (JNK), serine kinases inhibitor κB kinase (IKK) and S6 kinase (S6K). These serine kinase can cause serine phosphorylation of IRS1, block activation of downstream signaling molecules, and further decrease insulin sensitivity (Fain, 2010) **(Figure 2)**. JNK and IKK are important components of the activator protein-1 (AP-1) and nuclear factor kappa B (NF-kB)

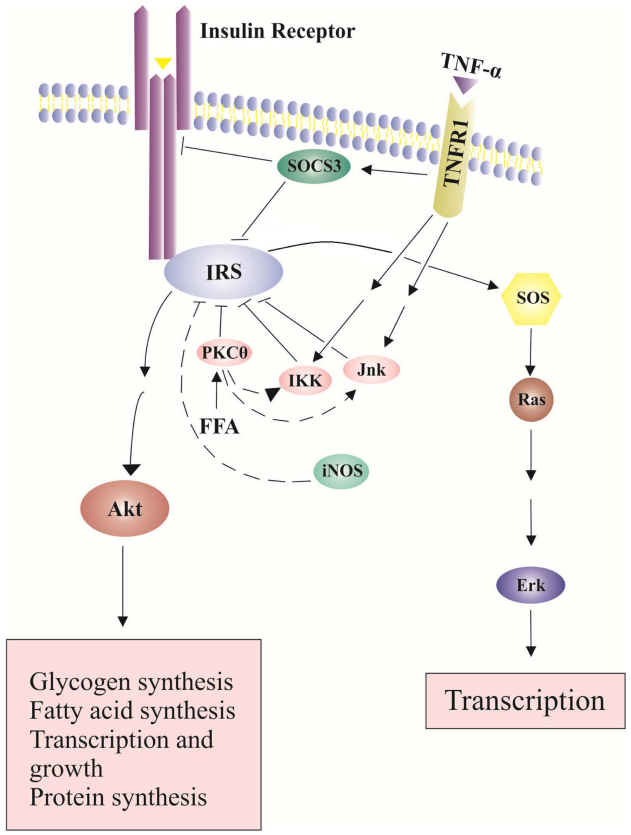


FIGURE 2 | Effects of TNP-α on insulin signaling pathways. Insulin signals tyrosine phosphorylation of IRS and activates SOS (Son of Sevenless) and the downstream Erk pathway, leading to transcription and growth. In addition, insulin-stimulated Akt phosphorylation leads to glucose uptake, glycogen synthesis, protein synthesis, cell survival and gene transcription. The binding of TNF-α to its receptor increases SOCS-3 expression and activates serine kinases including INK and IKK, which inhibit IRS tyrosine phosphorylation and activation.

cascade, respectively (Dhanasekaran and Johnson, 2007; Israel, 2010). NF-kB cascade includes a series of transcription factors that are sequestered by inhibitor of kappa B (IkB) proteins in the cytoplasm under normal conditions. IkB kinase (IKK), an important kinase in the NF-kB cascade, can phosphorylate and inactivate IkB proteins, and activate NF-kB (Israel, 2010). Proinflammatory cytokines like TNF-α can bind to its cell membrane receptor (TNFR1), activate IKK, further phosphorylate and inactivate IkB proteins (Israel, 2010). Subsequently, active NF-kB can translocate into the nucleus, bind on the promoter regions of cytokine genes, and stimulate proinflammatory cytokine expression (Ndisang, 2010). TNF-α also increases the expression of cytokine signaling (SOCS)-1 and SOCS-3 proteins, which can inhibit tyrosine phosphorylation of IRS1 and induce ubiqutinylation and degradation of IRS1 (**Figure 2**). The expression of SOCS-1 and SOCS-3 is also stimulated by IL-6. SOCS-3 blunts hepatocyte insulin signaling by binding to insulin receptors and leading to degradation of IRS proteins (Rasouli and Kern, 2008; Balistreri et al., 2010). In the absence of SOCS-3, IL-6 can have somewhat anti-inflammatory properties (Johnston and O'Shea, 2003). Increased plasma IL-6 concentrations are associated with insulin resistance (Charles et al., 2011). In addition, nitric oxide (NO), an endogenous signaling molecule produced by iNOS, can reduce Akt activity (Rasouli and Kern, 2008).

TNF-α and IL-6 can also alter the protein expression of peroxisome proliferator-activated receptor (PPAR)-γ in adipocytes (Leff et al., 2004; Tilg and Moschen, 2008; Zhou et al., 2008; Fernandez-Veledo et al., 2009). PPARγ is an anti-inflammatory nuclear protein with insulin sensitizing functions (Fernandez-Veledo et al., 2009). PPARγ blunts inflammatory responses and stimulates a switch of adipose-tissue macrophages from a M1 to M2 phenotype (Zeyda and Stulnig, 2009). PPARγ can inhibit the NF-kB signaling pathway, which is an insulin desensitizing pathway because it activates serine kinases and up-regulates the production of proinflammatory cytokines like TNF-α and IL-6 (Sidhu et al., 2003). However, JNK and IKK serine kinases can induce NF-kB activation and further increase TNF-α and IL-6 production, resulting in the suppression of PPARγ in adipocytes (O'Rourke, 2009). This suppression blunts PPARγ insulin sensitizing functions and leads to insulin resistance via the up-regulated NF-kB signaling pathway (Sidhu et al., 2003). Other than the serine kinases, saturated fatty acids can activate the NF-kB signaling pathway by binding to toll-like receptor 2 (TLR2) and 4 (TLR4) in adipocytes. TLR2 and TLR4 are important receptors of the immune system. Saturated fatty acids can bind to TLRs as their ligands and increase proinflammatory cytokine production (Jagannathan et al., 2010). Activation of TLR2 and TLR4 eventually contributes to insulin resistance via activating the NF-kB signaling pathway and increasing proinflammatory cytokine production (Monteiro and Azevedo, 2010).

C-reactive protein (CRP), an acute phase protein, is primarily synthesized in the liver and plays an important role in regulating the innate immune system (Eisenhardt et al., 2009). CRP aggravates the inflammatory status and leads to systemic inflammation (Wang and Nakayama, 2010). Plasma CRP levels are correlated with circulating levels of other inflammatory biomarkers (Kones, 2010). There is also a strong positive correlation between plasma CRP levels and insulin resistance (Pradhan et al., 2003; Pfutzner and Forst, 2006). Hepatic synthesis of CRP is driven at the transcriptional level by IL-6, which is mainly secreted by macrophages, T cells, and adipocytes (Pfutzner and Forst, 2006; Pfutzner et al., 2006). Since CRP mRNA levels rise in expanded adipose tissue, it has been proposed that adipose cells also have some ability to synthesize CRP (Memoli et al., 2007).

THE CHANGES OF NOVEL ADIPOKINES AFTER BARIATRIC SURGERY AND THEIR RELATIONSHIP TO INSULIN RESISTANCE

Other than the classic cytokines and chemokines such as TNF-α, MCP-1, and IL-6, adipocytes also secrete leptin, adiponectin, visfatin, resistin, omentin, apelin, vaspin, perilipin, adipsin, and retinol binding protein-4. All these adipokines can contribute to systemic inflammation and pathogenesis of obesity-associated complications (Balistreri et al., 2010). An evidence-based review by Spector and Shikora (2010) attributed the bariatric surgery-induced insulin sensitization to surgery-specific, metabolic effects

on glucose homeostasis that are independent of weight loss. However, the underlying mechanisms are not yet understood. This review article focuses on novel adipokines including visfatin, resistin, apelin, vaspin, and retinol binding protein-4 (RBP-4) and addresses their changes after bariatric surgery and their relationship to insulin resistance (**Table 2**).

VISFATIN

Visfatin is produced and secreted mainly by adipocytes in visceral adipose tissue (Tokunaga et al., 2008). Visfatin is also produced by a variety of cells including lymphocytes, monocytes, neutrophils, and hepatocytes (Kukla et al., 2011). This peptide adipokine produces insulin-mimetic effects by binding to the insulin receptor. The binding and further activation of the insulin signaling pathway can stimulate glucose uptake and increase insulin sensitivity (Fukuhara et al., 2005; Tilg and Moschen, 2006; Sun et al., 2007; Tokunaga et al., 2008; Fain, 2010). Visfatin and insulin bind to the different sites of the same insulin receptor (Tilg and Moschen, 2006). This difference in binding sites allows visfatin and insulin to work non-competitively. Similar to insulin, visfatin stimulates the phosphorylation of IRS1, IRS2, PI3K binding to IRSs, and activation of Akt and further promotes insulin sensitivity (Fukuhara et al., 2005; Adya et al., 2008a; Tan et al., 2009). Some studies suggested that NF-kB, JNK, and AP-1 upregulate visfatin production (Kim et al., 2007, 2008; Adya et al., 2008b; McGee et al., 2011).

There are many inconclusive data regarding the relationship between serum visfatin levels and body fat percentage or insulin resistance. Several studies suggest that blood visfatin levels are significantly related to type 2 diabetes or insulin resistance, but not to body fat percentage or BMI (Sandeep et al., 2007a; Palin et al., 2008; Retnakaran et al., 2008). One study compared plasma visfatin levels between type 2 diabetes subjects and non-diabetic healthy subjects and found that type 2 diabetes subjects had higher serum visfatin levels than non-diabetic healthy subjects (Sandeep et al., 2007b). However, this positive correlation between serum visfatin levels and diabetes status was no longer significant after adjusting for anthropometrics such as BMI and waist circumference (Dogru et al., 2007; Alghasham and Barakat, 2008; Retnakaran et al., 2008). In contrast, other studies reported that serum visfatin

Table 2 | The origin and roles of some novel adipokines in inflammation and insulin signaling and their responses to obesity, insulin resistance and bariatric surgery.

Adipokine	Origin	Roles in inflammation	Roles in insulin signaling	Response to obesity	Response to insulin resistance	Response to bariatric surgery
Visfatin	Visceral adipose tissue, lymphocytes, monocytes, neutrophils, hepatocytes	Inflammation stimulates its production (Kim et al., 2007)	Insulin-mimetic effects via binding to the insulin receptor and further activating IRSs, PI3K and Akt (Fukuhara et al., 2005)	Inconclusive (Dogru et al., 2007; Botella-Carretero et al., 2008)	Inconclusive (Dogru et al., 2007; Alghasham and Barakat, 2008; Retnakaran et al., 2008)	Elevated (Botella-Carretero et al., 2008)
Resistin	Visceral adipose tissue	Proinflammatory effects via activating NF-kB (Maenhaut and Van de Voorde, 2011)	Insulin desensitizing effects via activating NF-kB and SOCS-3 (Steppan et al., 2005)	Elevated (Moschen et al., 2009)	Inconclusive (Iqbal et al., 2005, Sentinelli et al., 2002; Lee et al., 2003)	Inconclusive (Iqbal et al., 2005; Marantos et al., 2011)
Apelin	Central nervous system, adipose tissue, heart, kidneys, liver, brain	Unknown	Insulin sensitizing effects via activating AMPK, PI3K and Akt (Attane et al., 2011)	Elevated but not entirely due to obesity (Boucher et al., 2005; Castan-Laurell et al., 2011)	Elevated (Boucher et al., 2005; Soriguer et al., 2009; Dray et al., 2010; Yu et al., 2012)	Decreased (Soriguer et al., 2009)
Vaspin	Visceral adipose tissue, subcutaneous adipose tissue	Unknown	Insulin sensitizing effects (Kloting et al., 2006)	Elevated (Youn et al., 2008; Chang et al., 2010)	Elevated (Ye et al., 2009; Kempf et al., 2010)	Decreased (Chang et al., 2010; Handisurya et al., 2010)
RBP-4	Adipose tissue, liver	Unknown	Inhibits PI3K and IRS activation, and GLUT4 protein expression (Wolf, 2007; Esteve et al., 2009)	Mostly elevated (Yao-Borengasser et al., 2007; Bajzova et al., 2008; Gomez-Ambrosi et al., 2008)	Elevated (Graham et al., 2006; Esteve et al., 2009)	Decreased (Janke et al., 2006; von Eynatten et al., 2007; Bajzova et al., 2008)

levels were significantly associated with obesity even after adjusting for age, sex, and diabetes (Fukuhara et al., 2005; Sandeep et al., 2007b). Moreover, serum visfatin levels were more associated with visceral fat mass than subcutaneous fat mass and appear to vary according to the body fat composition (Sun et al., 2007; Botella-Carretero et al., 2008). More studies are required to investigate their relationship.

Surprisingly, plasma visfatin concentrations were elevated after bariatric surgery (Krzyzanowska et al., 2006; Garcia-Fuentes et al., 2007; Botella-Carretero et al., 2008; Friebe et al., 2011). In one study of 53 severely obese persons who underwent biliopancreatic diversion ($n = 38$) and gastric bypass ($n = 15$), plasma visfatin levels were significantly increased 7 months after the surgery, and they were positively correlated with the percentage of waist circumference reduction (Garcia-Fuentes et al., 2007). In another report, there was an almost 50% increase in plasma visfatin levels post-compared to pre-biliopancreatic or laparoscopic bariatric surgery. In this study, multiple regression analysis showed that weight loss, diabetic status, and waist circumference changes were the main contributors to the increased serum visfatin levels (Botella-Carretero et al., 2008). Overall, these results indicate that plasma visfatin levels tend to be increased after weight loss surgeries (Garcia-Fuentes et al., 2007; Botella-Carretero et al., 2008). Given the well documented insulin-mimetic effect visfatin, the reason for the increased visfatin levels after weight loss is unclear, but may indicate a role for visfatin in improved insulin sensitivity after weight loss surgeries.

RESISTIN

Resistin is mainly secreted by adipocytes and macrophages found in visceral adipose tissue. Increased plasma resistin levels lead to over-production of glucose from the liver and inhibit preadipocyte differentiation; however the precise mechanisms are still unclear (Steppan et al., 2001; Steppan and Lazar, 2002; Wolf, 2004). In rats, resistin induces severe hepatic insulin resistance and increases glucose production (Piestrzeniewicz et al., 2008). In human studies the role of resistin in insulin resistance and glucose metabolism is inconclusive (Lee et al., 2003; Qasim et al., 2008). The proinflammatory effects of resistin were attributed to its ability to activate NF-kB signaling pathway and subsequently increase the production of proinflammatory cytokines including TNF-α and IL-6, both of which can impair insulin signaling pathways and lead to insulin resistance (Zeyda and Stulnig, 2009; Singla et al., 2010; Maenhaut and Van de Voorde, 2011). In addition, resistin can activate SOCS-3 protein, which can lead to insulin resistance (Steppan et al., 2005). Although some studies suggest a relationship between resistin and glucose metabolism, others disagree (Sentinelli et al., 2002; Lee et al., 2003; Iqbal et al., 2005). Moreover, several studies reported that increased plasma resistin levels were associated with increased BMI (Filkova et al., 2009; Moschen et al., 2009).

Effects of bariatric surgery on plasma resistin levels are inconclusive. Some studies found that plasma resistin levels were significantly decreased 12 months after bariatric surgery with >10% loss of the excess body weight (Edwards et al., 2010; Jankiewicz-Wika et al., 2011; Marantos et al., 2011). These studies also reported that plasma resistin levels were positively correlated with insulin resistance and glucose intolerance (Moschen et al., 2009; De Luis et al., 2010; Jankiewicz-Wika et al., 2011; Marantos et al., 2011). Other studies showed no difference in plasma resistin levels after significant weight loss caused by bariatric surgery (Wolfe et al., 2004; Iqbal et al., 2005). More studies are required to investigate the relationship between plasma resistin levels and weight loss.

APELIN

Apelin is a recently discovered peptide that is secreted by diverse tissues including central nervous system, adipose, and many other peripheral tissues, such as heart, kidneys, liver, and brain. Apelin is a binding ligand for the orphan G-protein–coupled receptor APJ (Yamamoto et al., 2011). A common 77-amino-acid precursor produces three bioactive apelin, which contain 13 amino acids (apelin-13), 17 amino acids (apelin-17), or 36 amino acids (apelin-36), respectively (Beltowski, 2006; Reinehr et al., 2011). Animal studies showed that apelin-deficient mice are insulin resistant and develop hyperinsulinemia, which can be reversed by administration of exogenous apelin (Yue et al., 2010). Administration of apelin to diabetic (db/db) obese mice can increase glucose uptake and elevate insulin sensitivity (Dray et al., 2008; Yue et al., 2010). Apelin stimulates PI3K/Akt phosphorylation-dependent GLUT 4 translocation, therefore increases glucose uptake by adipocytes (Lee et al., 2006; Zhu et al., 2011). Furthermore, apelin secretion can activate AMP-activated protein kinase (AMPK) pathway and leading to an insulin sensitizing effect (Kadoglou et al., 2010; Attane et al., 2011).

Apelin expression and secretion are increased during adipocyte differentiation and are regulated nutritionally and hormonally (Boucher et al., 2005; Dray et al., 2008). Type 2 diabetes subjects have significantly higher plasma apelin levels than non-diabetic healthy subjects. The increase in plasma apelin levels may be a result of compensatory response to insulin resistance (Boucher et al., 2005; Soriguer et al., 2009; Dray et al., 2010). Obese subjects have significantly higher plasma apelin levels than lean subjects (Boucher et al., 2005; Heinonen et al., 2005; Castan-Laurell et al., 2011). Soriguer et al. (2009) demonstrated that the plasma apelin levels were increased only in obese and diabetic subjects, not in obese and non-diabetic subjects, as compared with control subjects. This indicates that obesity and body fat mass may not be the main factors altering circulating apelin levels (Boucher et al., 2005), and insulin resistance may be more important than obesity in increasing plasma apelin levels in humans. Although there is a strong positive correlation between plasma apelin levels and TNF-α expression in expanded adipose tissue, the role of apelin in regulating inflammatory response is still not clear (Boucher et al., 2005; Daviaud et al., 2006).

Plasma apelin levels can vary according to the diabetic status after weight loss following the bariatric surgery. In a study, diabetic morbidly obese patients had significantly higher plasma apelin levels than non-diabetic non-obese healthy subjects. After bariatric surgery, plasma apelin levels in morbidly obese subjects with impaired blood glucose were significantly decreased (Soriguer et al., 2009). Krist et al. (2013) also demonstrated that bariatric surgery dramatically decreased the apelin expression in adipose tissues and serum apelin levels, which significantly correlated

with improved insulin sensitivity. This correlation is independent of BMI changes. More studies are required to investigate the underlying mechanisms.

VASPIN

Vaspin is a member of the serine protease inhibitor family and is also known as visceral adipose-tissue derived serpin. But human subcutaneous adipose tissue, liver, stomach, and rodent hypothalamus also express vaspin (Lee et al., 2011). Vaspin is also expressed in adipose tissues in Otsuka Long-Evans Tokushima rats, which are used as an animal model for studying type 2 diabetes (El-Mesallamy et al., 2011). Studies suggest that vaspin may have important roles in obesity and insulin resistance (Li et al., 2011). Administration of vaspin to obese mice improved glucose tolerance and elevates insulin sensitivity (Hida et al., 2005; Wada, 2008). However, the underlying mechanisms of improving insulin sensitivity are not known.

Some studies demonstrate that diabetic subjects have higher serum vaspin levels than non-diabetic subjects (Ye et al., 2009; Kempf et al., 2010). Lean subjects have lower serum vaspin levels than overweight subjects (Suleymanoglu et al., 2009). Others reported a positive association between blood vaspin levels and BMI in obesity (Youn et al., 2008; Chang et al., 2010; Bluher, 2012). Blood vaspin levels and BMI correlation is strong in type 2 diabetes or insulin resistant patients. This indicates that obesity-induced insulin resistance may be more important than BMI in regulating circulating vaspin levels (Youn et al., 2008; Chang et al., 2010; Kempf et al., 2010). Since vaspin can improve glucose tolerance and insulin sensitivity, the increase in plasma vaspin levels may be a result of compensatory response to insulin resistance.

Weight loss decreases plasma vaspin levels in humans (Kloting et al., 2006; Li et al., 2008; Youn et al., 2008). Weight loss after bariatric surgery significantly decreases plasma vaspin levels, which also correlates with decreased plasma insulin levels and improved insulin sensitivity (Chang et al., 2010; Handisurya et al., 2010). This decrease might be a compensatory mechanism associated with weight loss and insulin sensitivity. Even though these findings suggest that vaspin may have some roles in regulating glucose metabolism and insulin signaling pathways, the mechanisms are not yet understood (Kloting et al., 2006; Youn et al., 2008).

RETINOL BINDING PROTEIN-4

Retinol binding protein-4 (RBP-4) is secreted predominantly by adipocytes and hepatocytes. In plasma, RBP-4 is the carrier protein of retinol and appears to relate to glucose metabolism and insulin sensitivity (Esteve et al., 2009). Increased RBP-4 levels lead to reduced glucose uptake by muscle cells through inhibiting PI3K signaling pathway and serine phosphorylation of IRS1 (Wolf, 2007), followed by decreased insulin sensitivity. Some studies demonstrated an inverse correlation between glucose transporter 4 (GLUT4) protein expression and plasma RBP-4 levels (Graham et al., 2006; Esteve et al., 2009; Toruner et al., 2010). Decreased GLUT4 expression is accompanied by increased RBP-4 secretion in the adipose tissue of obese subjects and reduced GLUT4 expression can be caused by increased RBP-4 secretion (Yang et al., 2005). In liver, RBP-4 stimulates the expression of phosphoenolpyruvate carboxykinase (PEPCK) enzyme, a gluconeogenic enzyme that is stimulated by glucagon and inhibited by insulin (Quinn and Yeagley, 2005), leading to impaired insulin signaling in hepatocytes (Yang et al., 2005).

Most but not all of the findings suggest that plasma RBP-4 levels are positively associated with body fat percentage and insulin resistance (Janke et al., 2006; von Eynatten et al., 2007; Yao-Borengasser et al., 2007; Bajzova et al., 2008; Gomez-Ambrosi et al., 2008). Obese subjects have higher plasma RBP-4 levels than lean subjects. Plasma RBP-4 levels are positively correlated with insulin resistance in subjects with obesity, impaired glucose tolerance, or type 2 diabetes (Graham et al., 2006). RBP-4 levels were significantly decreased following weight loss after a bariatric surgery (Haider et al., 2007; Gomez-Ambrosi et al., 2008; Tschoner et al., 2008). Barazzoni et al. (2011) showed that high plasma RBP-4 levels was correlated with high systemic inflammatory responses in non-obese, non-diabetic patients with chronic kidney disease (But the correlation was independent of RBP-4 expression in adipose tissue. More studies are required to investigate the roles of RBP-4 in inflammation.

OTHER CHANGES IN GASTROINTESTINAL SYSTEM AFTER BARIATRIC SURGERY

After a meal, gastrointestinal system secretes incretins including glucose dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), which stimulate post-prandial insulin secretion (Holst, 2013; Holst and Deacon, 2013). GIP is secreted by duodenal K cells and GLP-1 is secreted by ileal L cells (Holst, 2013). These incretins have insulinotropic effects, furthermore; GLP-1 delays gastric emptying and decreases appetite leading to increased weight loss and insulin sensitivity (Flint et al., 2001; Laferrère, 2011). GLP-1 is increased after Roux-en-Y gastric bypass and biliopancreatic diversion (Näslund et al., 1997, 1998). Even though inconclusive findings on effects of the bariatric surgery on blood GIP levels are reported (Näslund et al., 1998; Laferrère, 2011, 2012), Laferrère et al. (2007) showed increased blood GIP levels 1 month and 1 year after Roux-en-Y surgery in patients with type 2 diabetes. Näslund et al. (1997) reported that the increase in blood GLP-1 and GIP levels is persistent for 20 years after duodenal jejunal bypass. More studies are required to investigate the effects of GLP-1 and GIP changes on inflammation in obese subjects following bariatric surgery.

There are two hypotheses trying to explain the increase in incretins secretion; foregut; and hindgut hypotheses (Strader et al., 2005; Strader, 2006; Pacheco et al., 2007; Hansen et al., 2011; de Luis et al., 2012). Foregut hypothesis suggests that bypassing duodenum and proximal jejunum increases incretin secretion which improves insulin sensitivity more than weight loss itself (Pacheco et al., 2007; Hansen et al., 2011; de Luis et al., 2012). However, research studies in Roux-en-Y gastric bypass and vertical sleeve gastrectomy which does not bypass duodenum or jejunum reported similar incretin secretion levels, therefore invalidating the foregut hypothesis (Chambers et al., 2011). Hindgut hypothesis suggests that instant stimulation of ileum by the nutrients causes the increase in blood GLP-1 levels (Strader et al., 2005; Strader, 2006). After Roux-en-Y gastric bypass, emptying of the stomach is fast and nutrients reach to ileum rapidly which might be the cause of increased GLP-1 secretion (Morínigo et al., 2006).

Bariatric surgery alters gut microbiota dramatically (Zhang et al., 2009; Furet et al., 2010; Clément, 2011; Kootte et al., 2012; Sweeney and Morton, 2013). Zhang et al. (2009) showed a positive association between Archaea and obesity. They found no Archaea in normal weight subjects and a decreased number in subjects who underwent gastric bypass surgery. Furet et al. (2010) demonstrated a decrease in Prevotellacaea during obesity, but the number of Prevotellacaea was rapidly increased after gastric bypass surgery. Zhang et al. showed obese subjects had more Bacteroidetes than lean subjects. Contrary to Furet et al. findings, Prevotellacaea, which is a subgroup of Bacteroidetes, was enriched in obese subjects. Furthermore, Ley et al. (2006) found no difference in the amount of Bacteroidetes between lean and obese subjects. These inconclusive data indicate that weight loss might be affecting subgroups differently. Effects of gut microbiota changes after bariatric surgery on inflammation, insulin resistance, and diabetes are not completely understood (Kootte et al., 2012). However, even though there might be positive effect of gut microbiota change on insulin sensitivity, this effect probably is overridden by possible malnutrition state after bariatric surgeries (Kootte et al., 2012).

CONCLUSION AND FUTURE DIRECTIONS

Obesity is a chronic low-grade inflammatory disease. In adipose tissue, both adipocytes and macrophages secrete a large number of hormones, proteins, cytokines, and chemokines, collectively called adipokines. These adipokines contribute to the pathogenesis of metabolic syndrome, insulin resistance, type 2 diabetes, and cardiovascular disease, most likely via regulating the inflammatory pathway mediated by TNF-α, IL-6, NF-kB, JNK, and IKK and insulin signaling pathway mediated by IRSs, PI3k/Akt, and SOCSs. Weight loss via surgeries dramatically alters levels of these adipokines and overall increases insulin sensitivity. However, despite ample evidence supporting the role of these adipokines in obesity and insulin resistance, mechanisms mediating their effects are for the most part still unclear. It is also unclear whether or how these adipokines mediate the effect of weight loss surgeries such as bariatric surgery on inflammation and insulin response. Further studies are required to better understand the relationship between those adipokines and insulin resistance and how these are modulated by bariatric surgery. Alteration in the adipokine profile primarily reflects changes in adipose-tissue production and secretion of these cytokines. Thus, additional studies are needed to determine whether reduced adipose-tissue inflammation following bariatric surgery is responsible for reduced systemic inflammation and improved insulin sensitivity. Furthermore, the development of animal models which recapitulate the bariatric surgery model will lead to a better understanding of mechanisms by which this surgery improves metabolic outcomes related to diabetes and obesity. It is especially important to understand whether the beneficial effects of bariatric surgery are solely related to weight loss, improved adipose-tissue inflammation and/or changes in gut physiology and endocrinology. In addition, with an adequately powered sample sizes for cross-sectional or case-control studies, the various types of adipose tissues, including subcutaneous, omental, and mesenteric adipose tissues, along with plasma and other tissues, can be collected from severely obese patients who undergo bariatric surgery. Finally, long term studies are critical to assess whether weight loss surgery and associated metabolic improvements are sustained over a long period of time and whether these are primarily related to weight loss or other beneficial effects of the bariatric surgery.

ACKNOWLEDGMENTS

The work was supported by the College of Human Sciences at Texas Tech University.

REFERENCES

Adya, R., Tan, B. K., Punn, A., Chen, J., and Randeva, H. S. (2008a). Visfatin induces human endothelial VEGF and MMP-2/9 production via MAPK and PI3K/Akt signalling pathways: novel insights into visfatin-induced angiogenesis. *Cardiovasc. Res.* 78, 356–365. doi:10.1093/cvr/cvm111

Adya, R., Tan, B. K., Chen, J., and Randeva, H. S. (2008b). Nuclear factor-kappaB induction by visfatin in human vascular endothelial cells: its role in MMP-2/9 production and activation. *Diabetes Care* 31, 758–760. doi:10.2337/dc07-1544

Alghasham, A. A., and Barakat, Y. A. (2008). Serum visfatin and its relation to insulin resistance and inflammation in type 2 diabetic patients with and without macroangiopathy. *Saudi Med. J.* 29, 185–192.

Amati, L., Marzulli, G., Martulli, M., Chiloiro, M., and Jirillo, E. (2010). Effects of a hypocaloric diet on obesity biomarkers: prevention of low-grade inflammation since childhood. *Curr. Pharm. Des.* 16, 893–897. doi:10.2174/138161210790883525

Arceo-Olaiz, R., Espana-Gomez, M. N., Montalvo-Hernandez, J., Velazquez-Fernandez, D., Pantoja, J. P., and Herrera, M. F. (2008). Maximal weight loss after banded and unbanded laparoscopic Roux-en-Y gastric bypass: a randomized controlled trial. *Surg. Obes. Relat. Dis.* 4, 507–511. doi:10.1016/j.soard.2007.11.006

Asayama, K., Hayashibe, H., Dobashi, K., Uchida, N., Nakane, T., Kodera, K., et al. (2003). Decrease in serum adiponectin level due to obesity and visceral fat accumulation in children. *Obes. Res.* 11, 1072–1079. doi:10.1038/oby.2003.147

Attane, C., Daviaud, D., Dray, C., Dusaulcy, R., Masseboeuf, M., Prevot, D., et al. (2011). Apelin stimulates glucose uptake but not lipolysis in human adipose tissue ex vivo. *J. Mol. Endocrinol.* 46, 21–28. doi:10.1677/JME-10-0105

Bajzova, M., Kovacikova, M., Vitkova, M., Klimcakova, E., Polak, J., Kovacova, Z., et al. (2008). Retinol-binding protein 4 expression in visceral and subcutaneous fat in human obesity. *Physiol. Res.* 57, 927–934.

Balistreri, C. R., Caruso, C., and Candore, G. (2010). The role of adipose tissue and adipokines in obesity-related inflammatory diseases. *Mediators Inflamm.* 2010, 802078. doi:10.1155/2010/802078

Barazzoni, R., Zanetti, M., Semolic, A., Pirulli, A., Cattin, M. R., Biolo, G., et al. (2011). High plasma RBP4 is associated with systemic inflammation independently of low RBP4 adipose expression and is normalized by transplantation in non-obese, non-diabetic patients with chronic kidney disease. *Clin. Endocrinol.* doi:10.1111/j.1365-2265.2011.03990.x. [Epub ahead of print].

Bell, L. N., Ward, J. L., Degawa-Yamauchi, M., Bobenkerk, J. E., Jones, R., Cacucci, B. M., et al. (2006). Adipose tissue production of hepatocyte growth factor contributes to elevated serum HGF in obesity. *Am. J. Physiol. Endocrinol. Metab.* 291, E843–E848. doi:10.1152/ajpendo.00174.2006

Beltowski, J. (2006). Apelin and visfatin: unique "beneficial" adipokines upregulated in obesity? *Med. Sci. Monit.* 12, RA112–RA119.

Bhattacharya, J., and Sood, N. (2011). Who pays for obesity? *J. Econ. Perspect.* 25, 139–158. doi:10.1257/jep.25.1.139

Bluher, M. (2012). Vaspin in obesity and diabetes: pathophysiological and clinical significance. *Endocrine* 41, 176–182. doi:10.1007/s12020-011-9572-0

Blüher, M., Fasshauer, M., Tönjes, A., Kratzsch, J., Schön, M. R., and Paschke, R. (2005). Association of interleukin-6, C-reactive protein, interleukin-10 and adiponectin plasma concentrations with measures of obesity, insulin sensitivity and glucose metabolism. *Exp. Clin. Endocrinol. Diabetes* 113, 534–537. doi:10.1055/s-2005-872851

Boesing, F., Moreira, E. A., Wilhelm-Filho, D., Vigil, S. V., Parizottto, E. B., Inacio, D. B., et al. (2010). Roux-en-Y bypass gastroplasty: markers of oxidative stress 6 months after surgery. *Obes. Surg.* 20, 1236–1244. doi:10.1007/s11695-010-0196-x

Botella-Carretero, J. I., Luque-Ramirez, M., Alvarez-Blasco, F., Peromingo, R., San Millan, J. L., and Escobar-Morreale, H. F. (2008). The increase in serum visfatin after bariatric surgery in morbidly obese women is modulated by weight loss, waist circumference, and presence or absence of diabetes before surgery. *Obes. Surg.* 18, 1000–1006. doi:10.1007/s11695-007-9369-7

Boucher, J., Masri, B., Daviaud, D., Gesta, S., Guigne, C., Mazzucotelli, A., et al. (2005). Apelin, a newly identified adipokine up-regulated by insulin and obesity. *Endocrinology* 146, 1764–1771. doi:10.1210/en.2004-1427

Butner, K. L., Nickols-Richardson, S. M., Clark, S. F., Ramp, W. K., and Herbert, W. G. (2010). A review of weight loss following Roux-en-Y gastric bypass vs restrictive bariatric surgery: impact on adiponectin and insulin. *Obes. Surg.* 20, 559–568. doi:10.1007/s11695-010-0089-z

Cai, D., Dhe-Paganon, S., Melendez, P. A., Lee, J., and Shoelson, S. E. (2003). Two new substrates in insulin signaling, IRS5/DOK4 and IRS6/DOK5. *J. Biol. Chem.* 278, 25323–25330. doi:10.1074/jbc.M212430200

Camerini, G., Adami, G., Marinari, G. M., Gianetta, E., Pretolesi, F., Papadia, F., et al. (2004). Thirteen years of follow-up in patients with adjustable silicone gastric banding for obesity: weight loss and constant rate of late specific complications. *Obes. Surg.* 14, 1343–1348. doi:10.1381/0960892042584049

Cancello, R., Henegar, C., Viguerie, N., Taleb, S., Poitou, C., Rouault, C., et al. (2005). Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. *Diabetes* 54, 2277–2286. doi:10.2337/diabetes.54.8.2277

Castan-Laurell, I., Dray, C., Attane, C., Duparc, T., Knauf, C., and Valet, P. (2011). Apelin, diabetes, and obesity. *Endocrine* 40, 1–9. doi:10.1007/s12020-011-9507-9

Catalan, V., Gomez-Ambrosi, J., Ramirez, B., Rotellar, F., Pastor, C., Silva, C., et al. (2007). Proinflammatory cytokines in obesity: impact of type 2 diabetes mellitus and gastric bypass. *Obes. Surg.* 17, 1464–1474. doi:10.1007/s11695-008-9424-z

Catalan, V., Gomez-Ambrosi, J., Rodriguez, A., Ramirez, B., Silva, C., Rotellar, F., et al. (2009). Increased adipose tissue expression of lipocalin-2 in obesity is related to inflammation and matrix metalloproteinase-2 and metalloproteinase-9 activities in humans. *J. Mol. Med. (Berl.)* 87, 803–813. doi:10.1007/s00109-009-0486-8

Cesari, M., Kritchevsky, S. B., Baumgartner, R. N., Atkinson, H. H., Penninx, B. W., Lenchik, L., et al. (2005). Sarcopenia, obesity, and inflammation–results from the Trial of Angiotensin Converting Enzyme Inhibition and Novel Cardiovascular Risk Factors study. *Am. J. Clin. Nutr.* 82, 428–434.

Chambers, A. P., Stefater, M. A., Wilson-Perez, H. E., Jessen, L., Sisley, S., Ryan, K. K., et al. (2011). Similar effects of roux-en-Y gastric bypass and vertical sleeve gastrectomy on glucose regulation in rats. *Physiol. Behav.* 105, 120–123. doi:10.1016/j.physbeh.2011.05.026

Chang, H. M., Lee, H. J., Park, H. S., Kang, J. H., Kim, K. S., Song, Y. S., et al. (2010). Effects of weight reduction on serum vaspin concentrations in obese subjects: modification by insulin resistance. *Obesity (Silver Spring)* 18, 2105–2110. doi:10.1038/oby.2010.60

Charles, B. A., Doumatey, A., Huang, H., Zhou, J., Chen, G., Shriner, D., et al. (2011). The roles of IL-6, IL-10, and IL-1RA in obesity and insulin resistance in African-Americans. *J. Clin. Endocrinol. Metab.* 96, E2018–2022. doi:10.1210/jc.2011-1497

Clément, K. (2011). Bariatric surgery, adipose tissue and gut microbiota. *Int. J. Obes. (Lond.)* 35(Suppl. 3), S7–15. doi:10.1038/ijo.2011.141

Compher, C., and Badellino, K. O. (2008). Obesity and inflammation: lessons from bariatric surgery. *JPEN J. Parenter. Enteral. Nutr.* 32, 645–647. doi:10.1177/0148607108326070

Coppack, S. W. (2001). Pro-inflammatory cytokines and adipose tissue. *Proc. Nutr. Soc.* 60, 349–356. doi:10.1079/PNS2001110

Daviaud, D., Boucher, J., Gesta, S., Dray, C., Guigne, C., Quilliot, D., et al. (2006). TNFalpha up-regulates apelin expression in human and mouse adipose tissue. *FASEB J.* 20, 1528–1530. doi:10.1096/fj.05-5243fje

de Luis, D., Domingo, M., Romero, A., Gonzalez Sagrado, M., Pacheco, D., Primo, D., et al. (2012). Effects of duodenal-jejunal exclusion on beta cell function and hormonal regulation in Goto-Kakizaki rats. *Am. J. Surg.* 204, 242–247. doi:10.1016/j.amjsurg.2011.07.020

De Luis, D. A., Gonzalez Sagrado, M., Conde, R., Aller, R., and Izaola, O. (2010). Resistin levels and inflammatory markers in patients with morbid obesity. *Nutr. Hosp.* 25, 630–634.

Dhanasekaran, D. N., and Johnson, G. L. (2007). MAPKs: function, regulation, role in cancer and therapeutic targeting. *Oncogene* 26, 3097–3099. doi:10.1038/sj.onc.1210395

Dogru, T., Sonmez, A., Tasci, I., Bozoglu, E., Yilmaz, M. I., Genc, H., et al. (2007). Plasma visfatin levels in patients with newly diagnosed and untreated type 2 diabetes mellitus and impaired glucose tolerance. *Diabetes Res. Clin. Pract.* 76, 24–29. doi:10.1016/j.diabres.2006.07.031

Dray, C., Debard, C., Jager, J., Disse, E., Daviaud, D., Martin, P., et al. (2010). Apelin and APJ regulation in adipose tissue and skeletal muscle of type 2 diabetic mice and humans. *Am. J. Physiol. Endocrinol. Metab.* 298, E1161–1169. doi:10.1152/ajpendo.00598.2009

Dray, C., Knauf, C., Daviaud, D., Waget, A., Boucher, J., Buleon, M., et al. (2008). Apelin stimulates glucose utilization in normal and obese insulin-resistant mice. *Cell Metab.* 8, 437–445. doi:10.1016/j.cmet.2008.10.003

Edwards, C., Hindle, A. K., Fu, S., and Brody, F. (2010). Downregulation of leptin and resistin expression in blood following bariatric surgery. *Surg. Endosc.* 25, 1962–1968. doi:10.1007/s00464-010-1494-z

Eisenhardt, S. U., Thiele, J. R., Bannasch, H., Stark, G. B., and Peter, K. (2009). C-reactive protein: how conformational changes influence inflammatory properties. *Cell Cycle* 8, 3885–3892. doi:10.4161/cc.8.23.10068

El-Mesallamy, H. O., Kassem, D. H., El-Demerdash, E., and Amin, A. I. (2011). Vaspin and visfatin/Nampt are interesting interrelated adipokines playing a role in the pathogenesis of type 2 diabetes mellitus. *Metab. Clin. Exp.* 60, 63–70. doi:10.1016/j.metabol.2010.04.008

Esteve, E., Ricart, W., and Fernandez-Real, J. M. (2009). Adipocytokines and insulin resistance: the possible role of lipocalin-2, retinol binding protein-4, and adiponectin. *Diabetes Care* 32(Suppl. 2), S362–S367. doi:10.2337/dc09-S340

Fain, J. N. (2010). Release of inflammatory mediators by human adipose tissue is enhanced in obesity and primarily by the nonfat cells: a review. *Mediators Inflamm.* 2010, 513948. doi:10.1155/2010/513948

Fantuzzi, G. (2008). Adiponectin and inflammation: consensus and controversy. *J. Allergy Clin. Immunol.* 121, 326–330. doi:10.1016/j.jaci.2007.10.018

Fernandez-Veledo, S., Nieto-Vazquez, I., Vila-Bedmar, R., Garcia-Guerra, L., Alonso-Chamorro, M., and Lorenzo, M. (2009). Molecular mechanisms involved in obesity-associated insulin resistance: therapeutical approach. *Arch. Physiol. Biochem.* 115, 227–239. doi:10.1080/13813450903164330

Filkova, M., Haluzik, M., Gay, S., and Senolt, L. (2009). The role of resistin as a regulator of inflammation: implications for various human pathologies. *Clin. Immunol.* 133, 157–170. doi:10.1016/j.clim.2009.07.013

Flint, A., Raben, A., Ersbøll, A. K., Holst, J. J., and Astrup, A. (2001). The effect of physiological levels of glucagon-like peptide-1 on appetite, gastric emptying, energy and substrate metabolism in obesity. *Int. J. Obes. Relat. Metab. Disord.* 25, 781–792. doi:10.1038/sj.ijo.0801627

Franco, J. V. A., Ruiz, P. A., Palermo, M., and Gagner, M. (2011). A review of studies comparing three laparoscopic procedures in bariatric surgery: sleeve gastrectomy, Roux-en-Y gastric bypass and adjustable gastric banding. *Obes. Surg.* 21, 1458–1468. doi:10.1007/s11695-011-0390-5

Friebe, D., Neef, M., Kratzsch, J., Erbs, S., Dittrich, K., Garten, A., et al. (2011). Leucocytes are a major source of circulating nicotinamide phosphoribosyltransferase (NAMPT)/pre-B cell colony (PBEF)/visfatin linking obesity and inflammation in humans. *Diabetologia* 54, 1200–1211. doi:10.1007/s00125-010-2042-z

Fukuhara, A., Matsuda, M., Nishizawa, M., Segawa, K., Tanaka, M., Kishimoto, K., et al. (2005). Visfatin:

a protein secreted by visceral fat that mimics the effects of insulin. *Science* 307, 426–430. doi:10.1126/science.1097243

Furet, J. P., Kong, L. C., Tap, J., Poitou, C., Basdevant, A., Bouillot, J. L., et al. (2010). Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers. *Diabetes* 59, 3049–3057. doi:10.2337/db10-0253

García de la Torre, N., Rubio, M. A., Bordiú, E., Cabrerizo, L., Aparicio, E., Hernández, C., et al. (2008). Effects of weight loss after bariatric surgery for morbid obesity on vascular endothelial growth factor-A, adipocytokines, and insulin. *J. Clin. Endocrinol. Metab.* 93, 4276–4281. doi:10.1210/jc.2007-1370

Garcia-Fuentes, E., Garcia-Almeida, J. M., Garcia-Arnes, J., Garcia-Serrano, S., Rivas-Marin, J., Gallego-Perales, J. L., et al. (2007). Plasma visfatin concentrations in severely obese subjects are increased after intestinal bypass. *Obesity (Silver Spring)* 15, 2391–2395. doi:10.1038/oby.2007.284

Gomez-Ambrosi, J., Rodriguez, A., Catalan, V., Ramirez, B., Silva, C., Rotellar, F., et al. (2008). Serum retinol-binding protein 4 is not increased in obesity or obesity-associated type 2 diabetes mellitus, but is reduced after relevant reductions in body fat following gastric bypass. *Clin. Endocrinol. (Oxf.)* 69, 208–215. doi:10.1111/j.1365-2265.2007.03156.x

Gomez-Illan, F., Gonzalvez-Ortega, M., Orea-Soler, I., Alcaraz-Tafalla, S., Aragon-Alonso, A., Pascual-Diaz, M., et al. (2012). Obesity and inflammation: change in adiponectin, C-reactive protein, tumor necrosis factor-alpha and Interleukin-6 after bariatric surgery. *Obesity Surgery* 22, 950–955. doi:10.1007/s11695-012-0643-y

Graham, T. E., Yang, Q., Bluher, M., Hammarstedt, A., Ciaraldi, T. P., Henry, R. R., et al. (2006). Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *N. Engl. J. Med.* 354, 2552–2563. doi:10.1056/NEJMoa054862

Haider, D. G., Schindler, K., Prager, G., Bohdjalian, A., Luger, A., Wolzt, M., et al. (2007). Serum retinol-binding protein 4 is reduced after weight loss in morbidly obese subjects. *J. Clin. Endocrinol. Metab.* 92, 1168–1171. doi:10.1210/jc.2006-1839

Hajer, G. R., van Haeften, T. W., and Visseren, F. L. (2008). Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. *Eur. Heart J.* 29, 2959–2971. doi:10.1093/eurheartj/ehn387

Handisurya, A., Riedl, M., Vila, G., Maier, C., Clodi, M., Prikoszovich, T., et al. (2010). Serum vaspin concentrations in relation to insulin sensitivity following RYGB-induced weight loss. *Obes. Surg.* 20, 198–203. doi:10.1007/s11695-009-9882-y

Hansen, E. N., Tamboli, R. A., Isbell, J. M., Saliba, J., Dunn, J. P., Marks-Shulman, P. A., et al. (2011). Role of the foregut in the early improvement in glucose tolerance and insulin sensitivity following Roux-en-Y gastric bypass surgery. *Am. J. Physiol. Gastrointest. Liver Physiol.* 300, G795–G802. doi:10.1152/ajpgi.00019.2011

Heinonen, M. V., Purhonen, A. K., Miettinen, P., Pääkkönen, M., Pirinen, E., Alhava, E., et al. (2005). Apelin, orexin-A and leptin plasma levels in morbid obesity and effect of gastric banding. *Regul. Pept.* 130, 7–13. doi:10.1016/j.regpep.2005.05.003

Hell, E., and Miller, K. (2002). Criteria for selection of patients for bariatric surgery. *Zentralbl. Chir.* 127, 1035–1037. doi:10.1055/s-2002-36375

Herder, C., Schneitler, S., Rathmann, W., Haastert, B., Schneitler, H., Winkler, H., et al. (2007). Low-grade inflammation, obesity, and insulin resistance in adolescents. *J. Clin. Endocrinol. Metab.* 92, 4569–4574. doi:10.1210/jc.2007-0955

Hida, K., Wada, L., Eguchi, J., Zhang, H., Baba, M., Seida, A., et al. (2005). Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. *Proc. Natl. Acad. Sci. U.S.A.* 102, 10610–10615. doi:10.1073/pnas.0504703102

Hofso, D., Nordstrand, N., Johnson, L. K., Karlsen, T. I., Hager, H., Jenssen, T., et al. (2010). Obesity-related cardiovascular risk factors after weight loss: a clinical trial comparing gastric bypass surgery and intensive lifestyle intervention. *Eur. J. Endocrinol.* 163, 735–745. doi:10.1530/EJE-10-0514

Holdstock, C., Lind, L., Engstrom, B. E., Ohrvall, M., Sundbom, M., Larsson, A., et al. (2005). CRP reduction following gastric bypass surgery is most pronounced in insulin-sensitive subjects. *Int. J. Obes. (Lond.)* 29, 1275–1280. doi:10.1038/sj.ijo.0803000

Holst, J. J. (2013). Incretin hormones and the satiation signal. *Int. J. Obes. (Lond.)* doi:10.1038/ijo.2012.208. [Epub ahead of print].

Holst, J. J., and Deacon, C. F. (2013). Is there a place for incretin therapies in obesity and prediabetes? *Trends Endocrinol. Metab.* 24, 145–152. doi:10.1016/j.tem.2013.01.004

Iannelli, A., Anty, R., Piche, T., Dahman, M., Gual, P., Tran, A., et al. (2009). Impact of laparoscopic Roux-en-Y gastric bypass on metabolic syndrome, inflammation, and insulin resistance in super versus morbidly obese women. *Obes. Surg.* 19, 577–582. doi:10.1007/s11695-008-9764-8

Iannelli, A., Anty, R., Schneck, A. S., Tran, A., and Gugenheim, J. (2010). Inflammation, insulin resistance, lipid disturbances, anthropometrics, and metabolic syndrome in morbidly obese patients: a case control study comparing laparoscopic Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy. *Surgery* 149, 364–370. doi:10.1016/j.surg.2010.08.013

Iqbal, N., Seshadri, P., Stern, L., Loh, J., Kundu, S., Jafar, T., et al. (2005). Serum resistin is not associated with obesity or insulin resistance in humans. *Eur. Rev. Med. Pharmacol. Sci.* 9, 161–165.

Israel, A. (2010). The IKK complex, a central regulator of NF-kappaB activation. *Cold Spring Harb. Perspect. Biol.* 2, a000158. doi:10.1101/cshperspect.a000158

Jagannathan, M., McDonnell, M., Liang, Y., Hasturk, H., Hetzel, J., Rubin, D., et al. (2010). Toll-like receptors regulate B cell cytokine production in patients with diabetes. *Diabetologia* 53, 1461–1471. doi:10.1007/s00125-010-1730-z

Janke, J., Engeli, S., Boschmann, M., Adams, F., Bohnke, J., Luft, F. C., et al. (2006). Retinol-binding protein 4 in human obesity. *Diabetes* 55, 2805–2810. doi:10.2337/db06-0616

Jankiewicz-Wika, J., Kolomecki, K., Cywinski, J., Piestrzeniewicz, K., Swietoslawski, J., Stepien, H., et al. (2011). Impact of vertical banded gastroplasty on body weight, insulin resistance, adipocytokine, inflammation and metabolic syndrome markers in morbidly obese patients. *Endokrynol. Pol.* 62, 109–119.

Jaunoo, S. S., and Southall, P. J. (2010). Bariatric surgery. *Int. J. Surg.* 8, 86–89. doi:10.1016/j.ijsu.2009.12.003

Joao Cabrera, E., Valezi, A. C., Delfino, V. D., Lavado, E. L., and Barbosa, D. S. (2010). Reduction in plasma levels of inflammatory and oxidative stress indicators after Roux-en-Y gastric bypass. *Obes. Surg.* 20, 42–49. doi:10.1007/s11695-009-9988-2

Johnston, J. A., and O'Shea, J. J. (2003). Matching SOCS with function. *Nat. Immunol.* 4, 507–509. doi:10.1038/ni0603-507

Kadoglou, N. P., Tsanikidis, H., Kapelouzou, A., Vrabas, I., Vitta, I., Karayannacos, P. E., et al. (2010). Effects of rosiglitazone and metformin treatment on apelin, visfatin, and ghrelin levels in patients with type 2 diabetes mellitus. *Metab. Clin. Exp.* 59, 373–379. doi:10.1016/j.metabol.2009.08.005

Kalupahana, N. S., Claycombe, K. J., and Moustaid-Moussa, N. (2011). (n-3) Fatty acids alleviate adipose tissue inflammation and insulin resistance: mechanistic insights. *Adv. Nutr.* 2, 304–316. doi:10.3945/an.111.000505

Kalupahana, N. S., Moustaid-Moussa, N., and Claycombe, K. J. (2012). Immunity as a link between obesity and insulin resistance. *Mol. Aspects Med.* 33, 26–34. doi:10.1016/j.mam.2011.10.011

Kanda, H., Tateya, S., Tamori, Y., Kotani, K., Hiasa, K., Kitazawa, R., et al. (2006). MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J. Clin. Invest.* 116, 1494–1505. doi:10.1172/JCI26498

Kempf, K., Rose, B., Illig, T., Rathmann, W., Strassburger, K., Thorand, B., et al. (2010). Vaspin (SERPINA12) genotypes and risk of type 2 diabetes: results from the MONICA/KORA studies. *Exp. Clin. Endocrinol. Diabetes* 118, 184–189. doi:10.1055/s-2008-1081499

Kim, S. R., Bae, S. K., Choi, K. S., Park, S. Y., Jun, H. O., Lee, J. Y., et al. (2007). Visfatin promotes angiogenesis by activation of extracellular signal-regulated kinase 1/2. *Biochem. Biophys. Res. Commun.* 357, 150–156. doi:10.1016/j.bbrc.2007.03.105

Kim, S. R., Bae, Y. H., Bae, S. K., Choi, K. S., Yoon, K. H., Koo, T. H., et al. (2008). Visfatin enhances ICAM-1 and VCAM-1 expression through ROS-dependent NF-kappaB activation in endothelial cells. *Biochim. Biophys. Acta* 1783, 886–895. doi:10.1016/j.bbamcr.2008.01.004

Kloting, N., Berndt, J., Kralisch, S., Kovacs, P., Fasshauer, M., Schon, M. R., et al. (2006). Vaspin gene expression in human adipose tissue: association with obesity and type 2 diabetes. *Biochem. Biophys. Res. Commun.* 339, 430–436. doi:10.1016/j.bbrc.2005.11.039

Kones, R. (2010). Rosuvastatin, inflammation, C-reactive protein, JUPITER, and primary prevention of cardiovascular disease – a perspective. *Drug Des. Devel. Ther.* 4, 383–413. doi:10.2147/DDDT.S10812

Kootte, R. S., Vrieze, A., Holleman, F., Dallinga-Thie, G. M., Zoetendal, E. G., de Vos, W. M., et al. (2012). The therapeutic potential of manipulating gut microbiota in obesity and type 2 diabetes mellitus. *Diabetes Obes. Metab.* 14, 112–120. doi:10.1111/j.1463-1326.2011.01483.x

Krist, J., Wieder, K., Klöting, N., Oberbach, A., Kralisch, S., Wiesner, T., et al. (2013). Effects of weight loss and exercise on apelin serum concentrations and adipose tissue expression in human obesity. *Obes. Facts* 6, 57–69. doi:10.1159/000348667

Krzyzanowska, K., Mittermayer, F., Krugluger, W., Kopp, H. P., and Schernthaner, G. (2006). Increase in visfatin after weight loss induced by gastroplastic surgery. *Obesity (Silver Spring)* 14, 1886–1889. doi:10.1038/oby.2006.219

Kukla, M., Mazur, W., Buldak, R. J., and Zwirska-Korczala, K. (2011). Potential role of leptin, adiponectin and the novel adipokines-visfatin, chemerin and vaspin-in chronic hepatitis. *Mol. Med.* 17, 1397–1410. doi:10.2119/molmed.2010.00105

Kulick, D., Hark, L., and Deen, D. (2010). The bariatric surgery patient: a growing role for registered dietitians. *J. Am. Diet. Assoc.* 110, 593–599. doi:10.1016/j.jada.2009.12.021

Laferrère, B. (2011). Do we really know why diabetes remits after gastric bypass surgery? *Endocrine* 40, 162–167. doi:10.1007/s12020-011-9514-x

Laferrère, B. (2012). Gut feelings about diabetes. *Endocrinol. Nutr.* 59, 254–260. doi:10.1016/j.endonu.2012.01.010

Laferrère, B., Heshka, S., Wang, K., Khan, Y., McGinty, J., Teixeira, J., et al. (2007). Incretin levels and effect are markedly enhanced 1 month after Roux-en-Y gastric bypass surgery in obese patients with type 2 diabetes. *Diabetes Care* 30, 1709–1716. doi:10.2337/dc06-1549

Lee, D. K., George, S. R., and O'Dowd, B. F. (2006). Unravelling the roles of the apelin system: prospective therapeutic applications in heart failure and obesity. *Trends Pharmacol. Sci.* 27, 190–194. doi:10.1016/j.tips.2006.02.006

Lee, J. A., Park, H. S., Song, Y. S., Jang, Y. J., Kim, J. H., Lee, Y. J., et al. (2011). Relationship between vaspin gene expression and abdominal fat distribution of Korean women. *Endocr. J.* 58, 639–646. doi:10.1507/endocrj.K11E-073

Lee, J. H., Chan, J. L., Yiannakouris, N., Kontogianni, M., Estrada, E., Seip, R., et al. (2003). Circulating resistin levels are not associated with obesity or insulin resistance in humans and are not regulated by fasting or leptin administration: cross-sectional and interventional studies in normal, insulin-resistant, and diabetic subjects. *J. Clin. Endocrinol. Metab.* 88, 4848–4856. doi:10.1210/jc.2003-030519

Leff, T., Mathews, S. T., and Camp, H. S. (2004). Review: peroxisome proliferator-activated receptor-gamma and its role in the development and treatment of diabetes. *Exp. Diabesity Res.* 5, 99–109. doi:10.1080/15438600490451668

Leick, L., Lindegaard, B., Stensvold, D., Plomgaard, P., Saltin, B., and Pilegaard, H. (2007). Adipose tissue interleukin-18 mRNA and plasma interleukin-18: effect of obesity and exercise. *Obesity* 15, 356–363. doi:10.1038/oby.2007.528

Ley, R. E., Turnbaugh, P. J., Klein, S., and Gordon, J. I. (2006). Microbial ecology: human gut microbes associated with obesity. *Nature* 444, 1022–1023. doi:10.1038/4441022a

Li, Q., Chen, R., Moriya, J., Yamakawa, J., Sumino, H., Kanda, T., et al. (2008). A novel adipocytokine, visceral adipose tissue-derived serine protease inhibitor (vaspin), and obesity. *J. Int. Med. Res.* 36, 625–629. doi:10.1177/147323000803600402

Li, K., Li, L., Yang, M., Liu, H., Liu, D., Yang, H., et al. (2011). Short-term continuous subcutaneous insulin infusion decreases the plasma vaspin levels in patients with type 2 diabetes mellitus concomitant with improvement in insulin sensitivity. *Eur. J. Endocrinol.* 164, 905–910. doi:10.1530/EJE-10-1023

Liu, S., Tinker, L., Song, Y., Rifai, N., Bonds, D. E., Cook, N. R., et al. (2007). A prospective study of inflammatory cytokines and diabetes mellitus in a multiethnic cohort of postmenopausal women. *Arch. Intern. Med.* 167, 1676–1685. doi:10.1001/archinte.167.15.1676

Maenhaut, N., and Van de Voorde, J. (2011). Regulation of vascular tone by adipocytes. *BMC Med.* 9:25. doi:10.1186/1741-7015-9-25

Malavazos, A. E., Cereda, E., Morricone, L., Coman, C., Corsi, M. M., and Ambrosi, B. (2005). Monocyte chemoattractant protein 1: a possible link between visceral adipose tissue-associated inflammation and subclinical echocardiographic abnormalities in uncomplicated obesity. *Eur. J. Endocrinol.* 153, 871–877. doi:10.1530/eje.1.02033

Marantos, G., Daskalakis, M., Karkavitsas, N., Matalliotakis, I., Papadakis, J. A., and Melissas, J. (2011). Changes in metabolic profile and adipoinsular axis in morbidly obese premenopausal females treated with restrictive bariatric surgery. *World J. Surg.* 35, 2022–2030. doi:10.1007/s00268-011-1165-9

Mattar, S. G., Velcu, L. M., Rabinovitz, M., Demetris, A. J., Krasinskas, A. M., Barinas-Mitchell, E., et al. (2005). Surgically-induced weight loss significantly improves nonalcoholic fatty liver disease and the metabolic syndrome. *Ann. Surg.* 242, 610–617. discussion 618-620,

McGee, K. C., Harte, A. L., da Silva, N. F., Al-Daghri, N., Creely, S. J., Kusminski, C. M., et al. (2011). Visfatin is regulated by rosiglitazone in type 2 diabetes mellitus and influenced by NFkappaB and JNK in human abdominal subcutaneous adipocytes. *PLoS ONE* 6:e20287. doi:10.1371/journal.pone.0020287

Memoli, B., Procino, A., Calabro, P., Esposito, P., Grandaliano, G., Pertosa, G., et al. (2007). Inflammation may modulate IL-6 and C-reactive protein gene expression in the adipose tissue: the role of IL-6 cell membrane receptor. *Am. J. Physiol. Endocrinol. Metab.* 293, E1030–1035. doi:10.1152/ajpendo.00697.2006

Monteiro, R., and Azevedo, I. (2010). Chronic inflammation in obesity and the metabolic syndrome. *Mediators Inflamm.* 2010:289645. doi:10.1155/2010/289645

Morínigo, R., Moizé, V., Musri, M., Lacy, A. M., Navarro, S., Marín, J. L., et al. (2006). Glucagon-like peptide-1, peptide YY, hunger, and satiety after gastric bypass surgery in morbidly obese subjects. *J. Clin. Endocrinol. Metab.* 91, 1735–1740. doi:10.1210/jc.2005-0904

Moschen, A. R., Molnar, C., Wolf, A. M., Weiss, H., Graziadei, I., Kaser, S., et al. (2009). Effects of weight loss induced by bariatric surgery on hepatic adipocytokine expression. *J. Hepatol.* 51, 765–777. doi:10.1016/j.jhep.2009.06.016

Mosser, D. M., and Edwards, J. P. (2008). Exploring the full spectrum of macrophage activation. *Nat. Rev. Immunol.* 8, 958–969. doi:10.1038/nri2448

Murri, M., Garcia-Fuentes, E., Garcia-Almeida, J. M., Garrido-Sanchez, L., Mayas, M. D., Bernal, R., et al. (2010). Changes in oxidative stress and insulin resistance in morbidly obese patients after bariatric surgery. *Obes. Surg.* 20, 363–368. doi:10.1007/s11695-009-0021-6

Mushtaq, M. U., Gull, S., Abdullah, H. M., Shahid, U., Shad, M. A., and Akram, J. (2011). Prevalence and socioeconomic correlates of overweight and obesity among Pakistani primary school children. *BMC Public Health* 11:724. doi:10.1186/1471-2458-11-724

Nash, A., Secker, D., Corey, M., Dunn, M., and O'Connor, D. L. (2008). Field testing of the 2006 World Health Organization growth charts from birth to 2 years: assessment of hospital undernutrition and overnutrition rates and the usefulness of BMI. *JPEN J. Parenter. Enteral. Nutr.* 32, 145–153. doi:10.1177/0148607108314386

Näslund, E., Backman, L., Holst, J. J., Theodorsson, E., and Hellström, P. M. (1998). Importance of small bowel peptides for the improved glucose metabolism 20 years after jejunoileal bypass for obesity. *Obes. Surg.* 8, 253–260. doi:10.1381/096089298765554449

Näslund, E., Grybäck, P., Hellström, P. M., Jacobsson, H., Holst, J. J., Theodorsson, E., et al. (1997). Gastrointestinal hormones and gastric emptying 20 years after jejunoileal bypass for massive obesity. *Int. J. Obes. Relat. Metab. Disord.* 21, 387–392. doi:10.1038/sj.ijo.0800418

Ndisang, J. F. (2010). Role of heme oxygenase in inflammation, insulin-signalling, diabetes and obesity. *Mediators Inflamm.* 2010, 359732. doi:10.1155/2010/359732

Nieto-Vazquez, I., Fernandez-Veledo, S., Kramer, D. K., Vila-Bedmar, R., Garcia-Guerra, L., and Lorenzo, M. (2008). Insulin resistance associated to obesity: the link TNF-alpha. *Arch. Physiol. Biochem.* 114, 183–194. doi:10.1080/13813450802181047

O'Rourke, R. W. (2009). Inflammation in obesity-related diseases. *Surgery* 145, 255–259. doi:10.1016/j.surg.2008.08.038

Oswal, A., and Yeo, G. (2010). Leptin and the control of body weight: a review of its diverse central targets, signaling mechanisms, and role in the pathogenesis of obesity. *Obesity* 18, 221–229. doi:10.1038/oby.2009.228

Pacheco, D., de Luis, D. A., Romero, A., González Sagrado, M., Conde, R., Izaola, O., et al. (2007). The

effects of duodenal-jejunal exclusion on hormonal regulation of glucose metabolism in Goto-Kakizaki rats. *Am. J. Surg.* 194, 221–224. doi:10.1016/j.amjsurg.2006.11.015

Palin, M. F., Labrecque, B., Beaudry, D., Mayhue, M., Bordignon, V., and Murphy, B. D. (2008). Visfatin expression is not associated with adipose tissue abundance in the porcine model. *Domest. Anim. Endocrinol.* 35, 58–73. doi:10.1016/j.domaniend

Panee, J. (2012). Monocyte Chemoattractant Protein 1 (MCP-1) in obesity and diabetes. *Cytokine* 60, 1–12. doi:10.1016/j.cyto.2012.06.018

Park, E. J., Lee, J. H., Yu, G. Y., He, G., Ali, S. R., Holzer, R. G., et al. (2010). Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. [a]. *Cell* 140, 197–208. doi:10.1016/j.cell.2009.12.052

Park, H. S., Park, J. Y., and Yu, R. (2005). Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-alpha and IL-6. *Diabetes Res. Clin. Pract.* 69, 29–35. doi:10.1016/j.diabres.2004.11.007

Pfutzner, A., and Forst, T. (2006). High-sensitivity C-reactive protein as cardiovascular risk marker in patients with diabetes mellitus. *Diabetes Technol. Ther.* 8, 28–36. doi:10.1089/dia.2006.8.28

Pfutzner, A., Standl, E., Strotmann, H. J., Schulze, J., Hohberg, C., Lubben, G., et al. (2006). Association of high-sensitive C-reactive protein with advanced stage beta-cell dysfunction and insulin resistance in patients with type 2 diabetes mellitus. *Clin. Chem. Lab. Med.* 44, 556–560. doi:10.1515/CCLM.2006.108

Picot, J., Jones, J., Colquitt, J. L., Gospodarevskaya, E., Loveman, E., Baxter, L., et al. (2009). The clinical effectiveness and cost-effectiveness of bariatric (weight loss) surgery for obesity: a systematic review and economic evaluation. *Health Technol. Assess.* 13, 215–357. doi:10.3310/hta13410

Piestrzeniewicz, K., Luczak, K., Komorowski, J., Maciejewski, M., Jankiewicz-Wika, J., and Goch, J. H. (2008). Resistin increases with obesity and atherosclerotic risk factors in patients with myocardial infarction. *Metab. Clin. Exp.* 57, 488–493. doi:10.1016/j.metabol.2007.11.009

Poitou, C., Coussieu, C., Rouault, C., Coupaye, M., Cancello, R., Bedel, J. F., et al. (2006). Serum amyloid A: a marker of adiposity-induced low-grade inflammation but not of metabolic status. *Obesity (Silver Spring)* 14, 309–318. doi:10.1038/oby.2006.40

Pradhan, A. D., Cook, N. R., Buring, J. E., Manson, J. E., and Ridker, P. M. (2003). C-reactive protein is independently associated with fasting insulin in nondiabetic women. *Arterioscler. Thromb. Vasc. Biol.* 23, 650–655. doi:10.1161/01.ATV.0000065636.15310.9C

Qasim, A., Mehta, N. N., Tadesse, M. G., Wolfe, M. L., Rhodes, T., Girman, C., et al. (2008). Adipokines, insulin resistance, and coronary artery calcification. *J. Am. Coll. Cardiol.* 52, 231–236. doi:10.1016/j.jacc.2008.04.016

Queipo-Ortuño, M. I., Escoté, X., Ceperuelo-Mallafré, V., Garrido-Sanchez, L., Miranda, M., Clemente-Postigo, M., et al. (2012). FABP4 dynamics in obesity: discrepancies in adipose tissue and liver expression regarding circulating plasma levels. *PLoS ONE.* 7:e48605. doi:10.1371/journal.pone.0048605

Quinn, P. G., and Yeagley, D. (2005). Insulin regulation of PEPCK gene expression: a model for rapid and reversible modulation. *Curr. Drug Targets Immune Endocr. Metabol. Disord.* 5, 423–437. doi:10.2174/156800805774912962

Rasouli, N., and Kern, P. A. (2008). Adipocytokines and the metabolic complications of obesity. *J. Clin. Endocrinol. Metab.* 93(11 Suppl. 1), S64–73. doi:10.1210/jc.2008-1613

Reinehr, T., Woelfle, J., and Roth, C. L. (2011). Lack of association between apelin, insulin resistance, cardiovascular risk factors, and obesity in children: a longitudinal analysis. *Metab. Clin. Exp.* 60, 1349–1354. doi:10.1016/j.metabol.2011.02.005

Retnakaran, R., Youn, B. S., Liu, Y., Hanley, A. J., Lee, N. S., Park, J. W., et al. (2008). Correlation of circulating full-length visfatin (PBEF/NAMPT) with metabolic parameters in subjects with and without diabetes: a cross-sectional study. *Clin. Endocrinol. (Oxf)* 69, 885–893. doi:10.1111/j.1365-2265.2008.03264.x

Rull, A., Camps, J., Alonso-Villaverde, C., and Joven, J. (2010). Insulin resistance, inflammation, and obesity: role of monocyte chemoattractant protein-1 (or CCL2) in the regulation of metabolism. *Mediators Inflamm.* 2010:326580. doi:10.1155/2010/326580. [Epub ahead of print].

Sandeep, S., Velmurugan, K., Deepa, R., and Mohan, V. (2007a). Serum visfatin in relation to visceral fat, obesity, and type 2 diabetes mellitus in Asian Indians. *Metab. Clin. Exp.* 56, 565–570. doi:10.1016/j.metabol.2006.12.005

Sandeep, S., Velmurugan, K., Deepa, R., and Mohan, V. (2007b). Serum visfatin in relation to visceral fat, obesity, and type 2 diabetes mellitus in Asian Indians. *Metab. Clin. Exp.* 56, 565–570. doi:10.1016/j.metabol.2006.12.005

Sentinelli, F., Romeo, S., Arca, M., Filippi, E., Leonetti, F., Banchieri, M., et al. (2002). Human resistin gene, obesity, and type 2 diabetes: mutation analysis and population study. *Diabetes* 51, 860–862. doi:10.2337/diabetes.51.3.860

Siddle, K. (2011). Signalling by insulin and IGF receptors: supporting acts and new players. *J. Mol. Endocrinol.* 47, R1–10. doi:10.1530/JME-11-0022

Sidhu, J. S., Cowan, D., and Kaski, J. C. (2003). The effects of rosiglitazone, a peroxisome proliferator-activated receptor-gamma agonist, on markers of endothelial cell activation, C-reactive protein, and fibrinogen levels in non-diabetic coronary artery disease patients. *J. Am. Coll. Cardiol.* 42, 1757–1763. doi:10.1016/j.jacc.2003.04.001

Singla, P., Bardoloi, A., and Parkash, A. A. (2010). Metabolic effects of obesity: a review. *World J. Diabetes* 1, 76–88. doi:10.4239/wjd.v1.i3

Sjostrom, L., Lindroos, A. K., Peltonen, M., Torgerson, J., Bouchard, C., Carlsson, B., et al. (2004). Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *N. Engl. J. Med.* 351, 2683–2693. doi:10.1056/NEJMoa035622

Soriguer, F., Garrido-Sanchez, L., Garcia-Serrano, S., Garcia-Almeida, J. M., Garcia-Arnes, J., Tinahones, F. J., et al. (2009). Apelin levels are increased in morbidly obese subjects with type 2 diabetes mellitus. *Obes. Surg.* 19, 1574–1580. doi:10.1007/s11695-009-9955-y

Spector, D., and Shikora, S. (2010). Neuro-modulation and bariatric surgery for type 2 diabetes mellitus. *Int. J. Clin. Pract. Suppl.* 166, 53–58. doi:10.1111/j.1742-1241.2009.02279.x

Spivak, H., Hewitt, M. F., Onn, A., and Half, E. E. (2005). Weight loss and improvement of obesity-related illness in 500 U.S. patients following laparoscopic adjustable gastric banding procedure. *Am. J. Surg.* 189, 27–32. doi:10.1016/j.amjsurg.2004.06.038

Steppan, C. M., Bailey, S. T., Bhat, S., Brown, E. J., Banerjee, R. R., Wright, C. M., et al. (2001). The hormone resistin links obesity to diabetes. *Nature* 409, 307–312. doi:10.1038/35053000

Steppan, C. M., and Lazar, M. A. (2002). Resistin and obesity-associated insulin resistance. *Trends Endocrinol. Metab.* 13, 18–23. doi:10.1016/S1043-2760(01)00522-7

Steppan, C. M., Wang, J., Whiteman, E. L., Birnbaum, M. J., and Lazar, M. A. (2005). Activation of SOCS-3 by resistin. *Mol. Cell. Biol.* 25, 1569–1575. doi:10.1128/MCB.25.4.1569-1575.2005

Strader, A. D. (2006). Ileal transposition provides insight into the effectiveness of gastric bypass surgery. *Physiol. Behav.* 88, 277–282. doi:10.1016/j.physbeh.2006.05.034

Strader, A. D., Vahl, T. P., Jandacek, R. J., Woods, S. C., D'Alessio, D. A., and Seeley, R. J. (2005). Weight loss through ileal transposition is accompanied by increased ileal hormone secretion and synthesis in rats. *Am. J. Physiol. Endocrinol. Metab.* 288, E447–453. doi:10.1152/ajpendo.00153.2004

Suleymanoglu, S., Tascilar, E., Pirgon, O., Tapan, S., Meral, C., and Abaci, A. (2009). Vaspin and its correlation with insulin sensitivity indices in obese children. *Diabetes Res. Clin. Pract.* 84, 325–328. doi:10.1016/j.diabres.2009.03.008

Sun, G., Bishop, J., Khalili, S., Vasdev, S., Gill, V., Pace, D., et al. (2007). Serum visfatin concentrations are positively correlated with serum triacylglycerols and down-regulated by overfeeding in healthy young men. *Am. J. Clin. Nutr.* 85, 399–404.

Sweeney, T. E., and Morton, J. M. (2013). The human gut microbiome: a review of the effect of obesity and surgically induced weight loss. *JAMA Surg.* doi:10.1001/jamasurg.2013.5. [Epub ahead of print].

Tan, B. K., Chen, J., Brown, J., Adya, R., Ramanjaneya, M., Menon, V., et al. (2009). In vivo and ex vivo regulation of visfatin production by leptin in human and murine adipose tissue: role of mitogen-activated protein kinase and phosphatidylinositol 3-kinase signaling pathways. *Endocrinology* 150, 3530–3539. doi:10.1210/en.2008-1655

Tarantino, G. and Caputi, A. (2011). JNKs, insulin resistance and inflammation: a possible link between NAFLD and coronary artery disease. *World J. Gastroenterol.* 17, 3785–3794. doi:10.3748/wjg.v17.i33.3785

Thompson, A. M., Zhang, Y., Tong, W., Xu, T., Chen, J., Zhao, L., et al. (2011). Association of obesity and biomarkers of inflammation and endothelial dysfunction in adults in Inner Mongolia, China. *Int. J. Cardiol.* 150, 247–252. doi:10.1016/j.ijcard.2010.04.011

Tilg, H., and Moschen, A. R. (2006). Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat. Rev. Immunol.* 6, 772–783. doi:10.1038/nri1937

Tilg, H., and Moschen, A. R. (2008). Inflammatory mechanisms in the regulation of insulin resistance. *Mol. Med.* 14, 222–231. doi:10.2119/2007-00119.Tilg

Tokunaga, A., Miura, A., Okauchi, Y., Segawa, K., Fukuhara, A., Okita, K., et al. (2008). The-1535 promoter variant of the visfatin gene is associated with serum triglyceride and HDL-cholesterol levels in Japanese subjects. *Endocr. J.* 55, 205–212. doi:10.1507/endocrj.K07E-039

Toruner, F., Altinova, A. E., Akturk, M., Kaya, M., Arslan, E., Bukan, N., et al. (2010). The relationship between adipocyte fatty acid binding protein-4, retinol binding protein-4 levels and early diabetic nephropathy in patients with type 2 diabetes. *Diabetes Res. Clin. Pract.* 91, 203–207. doi:10.1016/j.diabres.2010.11.011

Trayhurn, P. (2005). Adipose tissue in obesity – an inflammatory issue. *Endocrinology* 146, 1003–1005. doi:10.1210/en.2004-1597

Trayhurn, P. (2007). Adipocyte biology. *Obes. Rev.* 8(Suppl. 1), 41–44. doi:10.1111/j.1467-789X.2007.00316.x

Tschoner, A., Sturm, W., Engl, J., Kaser, S., Laimer, M., Laimer, E., et al. (2008). Retinol-binding protein 4, visceral fat, and the metabolic syndrome: effects of weight loss. *Obesity (Silver Spring)* 16, 2439–2444. doi:10.1038/oby.2008.391

Tzanavari, T., Giannogonas, P., and Karalis, K. P. (2010). TNF-alpha and obesity. *Curr. Dir. Autoimmun.* 11, 145–156. doi:10.1159/000289203

van Hout, G. C., Jakimowicz, J. J., Fortuin, F. A., Pelle, A. J., and van Heck, G. L. (2007). Weight loss and eating behavior following vertical banded gastroplasty. *Obes. Surg.* 17, 1226–1234. doi:10.1007/s11695-007-9205-0

Vazquez, L. A., Pazos, F., Berrazueta, J. R., Fernandez-Escalante, C., Garcia-Unzueta, M. T., Freijanes, J., et al. (2005). Effects of changes in body weight and insulin resistance on inflammation and endothelial function in morbid obesity after bariatric surgery. *J. Clin. Endocrinol. Metab.* 90, 316–322. doi:10.1210/jc.2003-032059

von Eynatten, M., Lepper, P. M., Liu, D., Lang, K., Baumann, M., Nawroth, P. P., et al. (2007). Retinol-binding protein 4 is associated with components of the metabolic syndrome, but not with insulin resistance, in men with type 2 diabetes or coronary artery disease. *Diabetologia* 50, 1930–1937. doi:10.1007/s00125-007-0743-8

Wada, J. (2008). Vaspin: a novel serpin with insulin-sensitizing effects. *Expert Opin. Investig. Drugs* 17, 327–333. doi:10.1517/13543784.17.3.327

Wang, Y., Sullivan, S., Trujillo, M., Lee, M. J., Schneider, S. H., Brolin, R. E., et al. (2003). Perilipin expression in human adipose tissues: effects of severe obesity, gender, and depot. *Obes. Res.* 11, 930–936. doi:10.1038/oby.2003.128

Wang, Z., and Nakayama, T. (2010). Inflammation, a link between obesity and cardiovascular disease. *Mediators Inflamm.* 2010, 535918. doi:10.1155/2010/535918

Wolf, G. (2004). Insulin resistance and obesity: resistin, a hormone secreted by adipose tissue. *Nutr. Rev.* 62, 389–394. doi:10.1111/j.1753-4887.2004.tb00009.x

Wolf, G. (2007). Serum retinol-binding protein: a link between obesity, insulin resistance, and type 2 diabetes. *Nutr. Rev.* 65, 251–256. doi:10.1111/j.1753-4887.2007.tb00302.x

Wolfe, B. E., Jimerson, D. C., Orlova, C., and Mantzoros, C. S. (2004). Effect of dieting on plasma leptin, soluble leptin receptor, adiponectin and resistin levels in healthy volunteers. *Clin. Endocrinol. (Oxf.)* 61, 332–338. doi:10.1111/j.1365-2265.2004.02101.x

Yamamoto, T., Habata, Y., Matsumoto, Y., Yasuhara, Y., Hashimoto, T., Hamajyo, H., et al. (2011). Apelin-transgenic mice exhibit a resistance against diet-induced obesity by increasing vascular mass and mitochondrial biogenesis in skeletal muscle. *Biochim. Biophys. Acta* 1810, 853–862. doi:10.1016/j.bbagen.2011.05.004

Yan, E., Ko, E., Luong, V., Wang, H. J., Romanova, M., and Li, Z. (2008). Long-term changes in weight loss and obesity-related comorbidities after Roux-en-Y gastric bypass: a primary care experience. *Am. J. Surg.* 195, 94–98. doi:10.1016/j.amjsurg.2007.01.036

Yang, Q., Graham, T. E., Mody, N., Preitner, F., Peroni, O. D., Zabolotny, J. M., et al. (2005). Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* 436, 356–362. doi:10.1038/nature03711

Yao-Borengasser, A., Varma, V., Bodles, A. M., Rasouli, N., Phanavanh, B., Lee, M. J., et al. (2007). Retinol binding protein 4 expression in humans: relationship to insulin resistance, inflammation, and response to pioglitazone. *J. Clin. Endocrinol. Metab.* 92, 2590–2597. doi:10.1210/jc.2006-0816

Ye, Y., Hou, X. H., Pan, X. P., Lu, J. X., and Jia, W. P. (2009). Serum vaspin level in relation to postprandial plasma glucose concentration in subjects with diabetes. *Chin. Med. J.* 122, 2530–2533.

Yermilov, I., McGory, M. L., Shekelle, P. W., Ko, C. Y., and Maggard, M. A. (2009). Appropriateness criteria for bariatric surgery: beyond the NIH guidelines. *Obesity (Silver Spring)* 17, 1521–1527. doi:10.1038/oby.2009.78

Youn, B. S., Kloting, N., Kratzsch, J., Lee, N., Park, J. W., Song, E. S., et al. (2008). Serum vaspin concentrations in human obesity and type 2 diabetes. *Diabetes* 57, 372–377. doi:10.2337/db07-1045

Yu, S., Zhang, Y., Li, M. Z., Xu, H., Wang, Q., Song, J., et al. (2012). Chemerin and apelin are positively correlated with inflammation in obese type 2 diabetic patients. *Chin. Med. J. (Engl.)* 125, 3440–3444.

Yudkin, J. S. (2007). Inflammation, obesity, and the metabolic syndrome. *Horm. Metab. Res.* 39, 707–709. doi:10.1055/s-2007-985898

Yue, P., Jin, H., Aillaud, M., Deng, A. C., Azuma, J., Asagami, T., et al. (2010). Apelin is necessary for the maintenance of insulin sensitivity. *Am. J. Physiol. Endocrinol. Metab.* 298, E59–67. doi:10.1152/ajpendo.00385.2009

Zeyda, M., and Stulnig, T. M. (2009). Obesity, inflammation, and insulin resistance – a mini-review. *Gerontology* 55, 379–386. doi:10.1159/000212758

Zhang, H., DiBaise, J. K., Zuccolo, A., Kudrna, D., Braidotti, M., Yu, Y., et al. (2009). Human gut microbiota in obesity and after gastric bypass. *Proc. Natl. Acad. Sci. U.S.A.* 106, 2365–2370. doi:10.1073/pnas.0812600106

Zhou, Y., Liu, B. L., Liu, K., Tang, N., Huang, J., An, Y., et al. (2008). Establishment of the insulin resistance induced by inflammatory response in 3T3-L1 preadipocytes cell line. *Inflammation* 31, 355–364. doi:10.1007/s10753-008-9086-y

Zhu, S., Sun, F., Li, W., Cao, Y., Wang, C., Wang, Y., et al. (2011). Apelin stimulates glucose uptake through the PI3K/Akt pathway and improves insulin resistance in 3T3-L1 adipocytes. *Mol. Cell. Biochem.* 353, 305–313. doi:10.1007/s11010-011-0799-0

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 01 April 2013; accepted: 23 May 2013; published online: 10 June 2013.
Citation: Goktas Z, Moustaid-Moussa N, Shen C-L, Boylan M, Mo H and Wang S (2013) Effects of bariatric surgery on adipokine-induced inflammation and insulin resistance. Front. Endocrinol. ***4****:69. doi: 10.3389/fendo.2013.00069*
This article was submitted to Frontiers in Diabetes, a specialty of Frontiers in Endocrinology.
Copyright © 2013 Goktas, Moustaid-Moussa, Shen, Boylan, Mo and Wang. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.