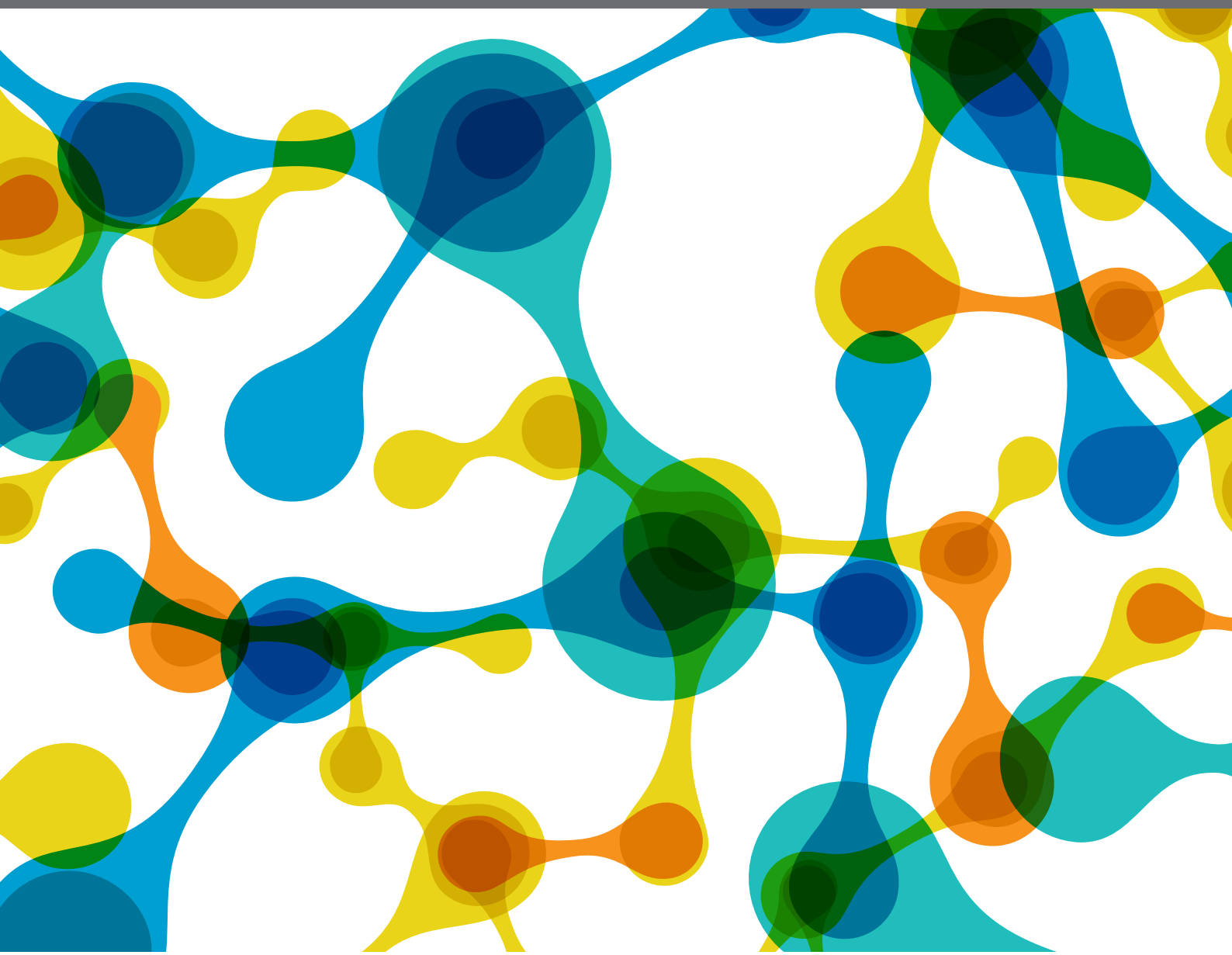
THE ROLE OF BIOACTIVE LIPIDS IN HOMEOSTASIS AND PATHOLOGY

EDITED BY: Chunjiong Wang, Jun Yang and Xu Zhang
PUBLISHED IN: Frontiers in Physiology



frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88974-041-3
DOI 10.3389/978-2-88974-041-3

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: frontiersin.org/about/contact

THE ROLE OF BIOACTIVE LIPIDS IN HOMEOSTASIS AND PATHOLOGY

Topic Editors:
Chunjiong Wang, Tianjin Medical University, China
Jun Yang, University of California, Davis, United States
Xu Zhang, Tianjin Medical University, China

Topic Editor Dr. Jun Yang is a Part-Time Employee of EicOsis LLC (Davis, California, USA). All Other Topic Editors Declare No Competing Interests With Regards to the Research Topic Subject.

Citation: Wang, C., Yang, J., Zhang, X., eds. (2022). The Role of Bioactive Lipids in Homeostasis and Pathology. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88974-041-3

Table of Contents

frontiers in Physiology

EDITORIAL
published: 22 November 2021
doi: 10.3389/fphys.2021.773632

Editorial: The Role of Bioactive Lipids in Homeostasis and Pathology

Chunjiong Wang[1]*, *Jun Yang*[2]* and *Xu Zhang*[1]*

[1] Department of Physiology and Pathophysiology, The Province and Ministry Co-sponsored Collaborative Innovation Center for Medical Epigenetics, Tianjin Medical University, Tianjin, China, [2] Department of Entomology and Nematology, University of California, Davis, Davis, CA, United States

Keywords: bioactive lipid, eicosanoid, oxylipin, sphingolipid, lysophospholipid, metabolic diseases, cardiovascular diseases, respiratory diseases

Editorial on Research Topic:

The Role of Bioactive Lipids in Homeostasis and Pathology

Bioactive lipids, including fatty acids and their metabolic products, acylglycerol derivatives, endocannabinoids, lysophospholipids, sphingolipids, and cholesterol metabolites, etc., play active roles in regulating cellular functions. They are not only products of lipid metabolism, but also important signals in tissue homeostasis and pathology. The functions of a large number of bioactive lipids remain unclear. Moreover, even the same lipid mediator reveals various functions by activating various receptors. Thus, it is important to clarify the receptors and signal transduction capabilities of these lipids. The aim of the current Research Topic is to provide a thorough overview of the function, signal transduction, and regulatory mechanisms of bioactive lipids. This Research Topic will provide insight into their effects on homeostasis and pathology. The current Research Topic includes six reviews and five original research articles. These studies focus on the effects of bioactive lipids in the homeostasis and pathology of the cardiovascular system, respiratory system, liver, and adipose tissue dysfunction as well as others (**Figure 1**).

Arachidonic acid-derived eicosanoids, including prostaglandins (PG), thromboxanes, leukotrienes, lipoxins, hydroxyeicosatetraenoic acids, and epoxyeicosatrienoic acids (EETs), among others, have been the focus of serious interest due to their vital roles in many physiological and pathophysiological processes. The roles of EETs and metabolites of arachidonic acid by cytochrome P450s were reviewed by Lai and Chen. This review suggested that increasing the levels of EETs is a potential therapeutic strategy for cardiovascular disease (Lai and Chen). Cyclopentenone prostaglandins (cyPGs) are a cluster of PGs with a cyclopentenone ring structure. CyPGs (PGA1, PGA2, and PGJ2 and its' metabolites) biosynthesis, mechanism of action, functions, and their effects on virus infection and cancer development were discussed in a review by Lee et al. In addition to arachidonic acid-derived eicosanoids, bioactive metabolites of ω-3 polyunsaturated fatty acids (PUFAs) have also drawn interest in recent years. Duan et al. summarized the effects of ω-3 PUFA-derived oxylipins on metabolic disorders, including diabetes, non-alcoholic fatty liver disease, adipose tissue dysfunction, and atherosclerosis. This review highlighted the importance of these derivatives when exploring the therapeutic effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Duan et al.). Moreover, an original research article reported that ω-3 PUFA treatment improved HHcy-induced insulin resistance and inflammasome activation in adipose tissue. HHcy increased lysophosphatidylcholine (LPC) 16:0 and LPC 18:0 levels in adipose tissue, which were suppressed by ω-3 PUFA treatment. This study linked ω-3 PUFAs to lysophospholipid production (Li et al.). As the major components of edible oil, the roles of ω-6 PUFAs (arachidonic acid and linoleic acid), ω-3 PUFAs (EPA, DPA, DHA, and alpha-linolenic acid), their metabolites, and the role of monounsaturated fatty acids in oxidative stress and

Edited and reviewed by:
Nada A. Abumrad, Washington University in St. Louis, United States

***Correspondence:**
Chunjiong Wang
wangchunjiong@tmu.edu.cn
Jun Yang
junyang@ucdavis.edu
Xu Zhang
xuzhang@tmu.edu.cn

Specialty section:
This article was submitted to Lipid and Fatty Acid Research, a section of the journal Frontiers in Physiology

Received: *10 September 2021*
Accepted: *01 November 2021*
Published: *22 November 2021*

Citation:
Wang C, Yang J and Zhang X (2021) Editorial: The Role of Bioactive Lipids in Homeostasis and Pathology. Front. Physiol. 12:773632. doi: 10.3389/fphys.2021.773632

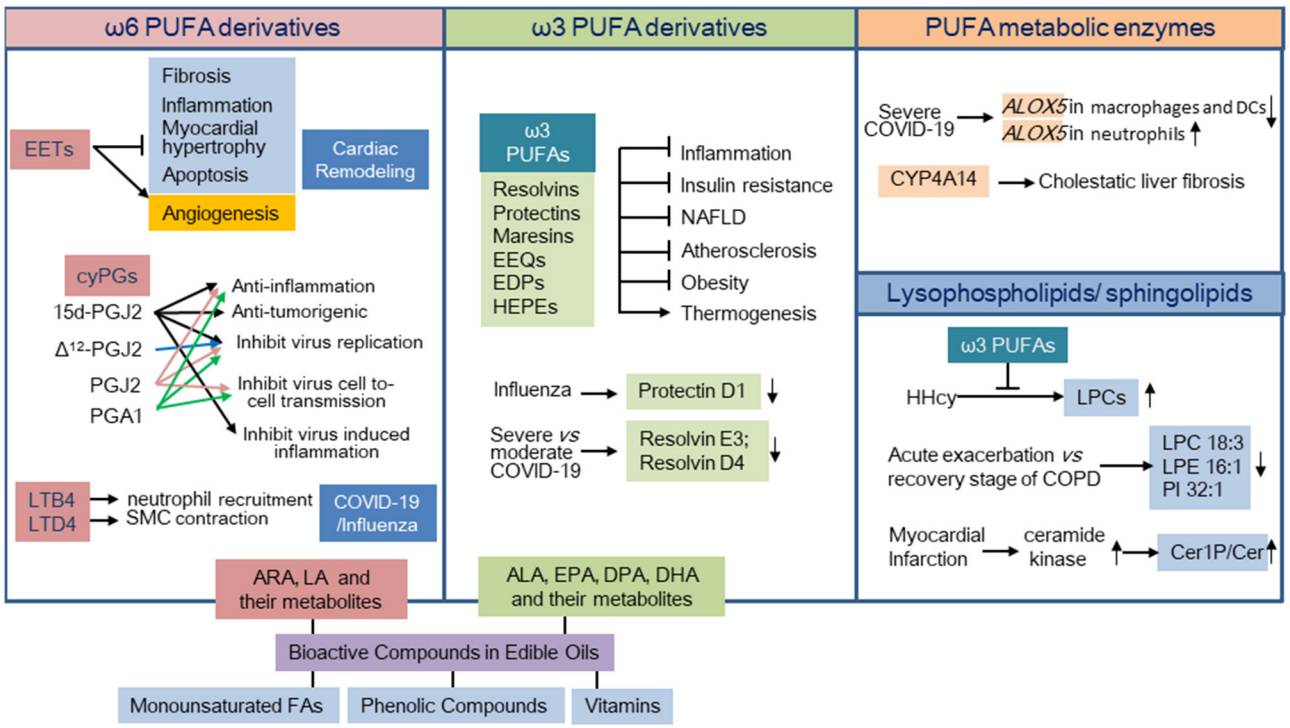


FIGURE 1 | Overview of the contributions included under the Research Topic "Role of Bioactive Lipids in Homeostasis and pathology." PUFA, polyunsaturated fatty acid; CyPGs, cyclopentenone prostaglandins; ARA, arachidonic acid; LA, linoleic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; FA, fatty acid; HHcy, hyperhomocysteinemia; LPC, lysophosphatidylcholin; LPE, lysophosphatidylethanolamine; PI, phosphatidylinositol; Cer1P, ceramide-1-phosphate; Cer, ceramide; SMC, smooth muscle cell; DC, dendritic cell. See text for a brief description and the reference of each contributing article.

inflammation were reviewed by Mazzocchi et al. In addition, this review article discussed the clinical studies conducted with various seed oils and marine animal-derived oils. This review highlighted the fact that high heterogeneity in oil composition plays a significant role in health outcomes (Mazzocchi et al.). In addition, as reported by Liu et al., the metabolite profiles of arachidonic acid and DHA are altered in high iodide-intake-induced hypothyroid offspring rats. Iodide intake adjustment plus 1,25(OH)2D3 supplementation ameliorated hypothyroid and metabolic disturbances along with increased serum EET and hydroxyeicosatetraenoic acid (HETE) levels (Liu et al.).

The effects of bioactive lipids in the respiratory system were also emphasized in this Research Topic. A clinical study reported changes in serum glycerophospholipids in the acute exacerbation of chronic obstructive pulmonary disease (COPD) and various subtypes of COPD (Gai et al.). Sahanic et al. thoroughly reviewed bioactive lipid mediators in COVID-19 and influenza. They further promoted the current knowledge regarding the mediator lipidome in severely affected COVID-19 patients. They accomplished this by investigating a publicly available RNA-seq database of bronchoalveolar lavage cells (Sahanic et al.).

The effects of sphingolipids on the cardiovascular system were reported in a research article from Hua et al. The authors demonstrated that the myocardial sphingolipid profile was altered after myocardial infarction injury. In particular, the ratio of ceramide-1-phosphate/ceramide was increased in the myocardial infarction-injured heart tissue with a higher ceramide kinase expression (Hua et al.).

The function and effects of metabolic enzymes of fatty acids are an important topic in the field of bioactive lipids. Cytochrome P450 omega-hydroxylase 4a14 (Cyp4a14), a homolog of human CYP4A hydroxylase, catalyzes arachidonic acid to produce 20-HETE and mediates the omega-hydroxylation of medium-chain fatty acids in mice. Li et al. found that the reduction of Cyp4a14 expression mediated cholestatic-related liver fibrosis. However, as previously reported, Cyp4a14 was increased in NASH livers and promoted NASH-related fibrosis. This process implied the complex role of cyp4a14 in various liver diseases. Whether 20-HETE is involved in the effects of Cyp4a14 on cholestatic-related liver fibrosis requires further exploration. The effects of transcription factor EB (TFEB) on lipid homeostasis, including lipid degradation and efflux, as well as the regulatory effects on lipid transporters, were summarized by Li et al. In particular, the authors emphasized the role of TFEB in atherosclerosis by regulating lipid metabolism (Li et al.).

Overall, the current Research Topic includes a range of studies and reviews on the effects of bioactive lipids on cardiovascular diseases, metabolic disorders, respiratory diseases, and endocrine disorders (**Figure 1**). Targeting these bioactive lipids or their metabolic enzymes may provide potential therapeutic strategies for these diseases.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

Conflict of Interest: JY is a part-time employee of EicOsis LLC (Davis, California, USA).

The remaining 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.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Physiology

ORIGINAL RESEARCH
published: 12 February 2021
doi: 10.3389/fphys.2021.628122

Omega-3FAs Can Inhibit the Inflammation and Insulin Resistance of Adipose Tissue Caused by HHcy Induced Lipids Profile Changing in Mice

*Jing Li[1], Heng Zhang[1], Yongqiang Dong[2], Xian Wang[2] and Guang Wang[1]**

[1] Department of Endocrinology, Beijing Chao-Yang Hospital, Capital Medical University, Beijing, China, [2] Key Laboratory of Molecular Cardiovascular Science, Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Ministry of Education, Peking University, Beijing, China

OPEN ACCESS

Edited by:
Xu Zhang,
Tianjin Medical University, China

Reviewed by:
Yu-Chen Fan,
Qilu Hospital, Shandong University, China
Liu Yao,
Tianjin Medical University, China

***Correspondence:**
Guang Wang
drwg6688@aliyun.com

Specialty section:
This article was submitted to Lipid and Fatty Acid Research, a section of the journal Frontiers in Physiology

Received: *11 November 2020*
Accepted: *15 January 2021*
Published: *12 February 2021*

Citation:
Li J, Zhang H, Dong Y, Wang X and Wang G (2021) Omega-3FAs Can Inhibit the Inflammation and Insulin Resistance of Adipose Tissue Caused by HHcy Induced Lipids Profile Changing in Mice. Front. Physiol. 12:628122. doi: 10.3389/fphys.2021.628122

The adipose Nod-like receptor protein 3 (NLRP3) inflammasome initiates insulin resistance; however, the mechanism of inflammasome activation in adipose tissue remains elusive. In this study, homocysteine (Hcy) was found to participate in insulin resistance *via* a NLRP3 inflammasome-related process. Hcy-induced activation of NLRP3 inflammasomes were observed in adipose tissue during the generation of insulin resistance *in vivo*. This animal model suggests that diets high in omega-3 fatty acids alter serum and adipose lipid profiles, and in this way, omega-3 fatty acids may reduce adipose tissue inflammation and attenuate insulin resistance.

Keywords: insulin resistance, homocysteine, adipocyte, omega-3 fatty acids, inflammation

INTRODUCTION

Insulin resistance plays a key role in the metabolic syndrome, which includes diabetes, obesity-related atherosclerosis, and lipid disorders (Petersen and Shulman, 2018). Previously, we reported that hyperhomocysteinemia (HHcy) was an independent risk factor for development of insulin resistance (Li Y. et al., 2013). This was confirmed in studies both in humans and in a rodent model. Omega-3 fatty acids (ω-3FAs), primarily eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), exert anti-inflammatory activities in a variety of inflammatory diseases, including obesity, diabetes, atherosclerosis, as well as other metabolic inflammatory disorders (Tortosa-Caparrós et al., 2017). Fish oil, which contains ω-3FAs, is thought to participate in lowering serum concentrations of lipids (Nicholls et al., 2018). In another study, n-3 polyunsaturated fatty acids (PUFAs) regulated hepatic genes such as peroxisome proliferators-activated receptors-alpha (PPAR-alpha) and sterol-regulatory element binding protein-1 (SREBP-1). This may modestly raise hepatic glucose production while also reducing hyperinsulinemia, but without causing peripheral insulin resistance or systemic metabolic dysfunction (Devarshi et al., 2013). In animal models, expression of such kinds of lipids biosynthesis related genes in the liver reduce hepatic steatosis (Rossmeisl et al., 2014). Nevertheless, the mechanisms by which ω-3FAs reduce adipose insulin resistance remain poorly understood.

Homocysteine (Hcy) is a sulfur-containing non-proteinogenic amino acid. Increased plasma Hcy levels [to more than 15 μM, defined as hyperhomocysteinemia (HHcy)] is an independent risk factor for cardiovascular disease and other metabolic inflammation diseases (Hainsworth et al., 2016). Patients with insulin resistance and type 2 diabetes mellitus were found to have increased plasma levels of Hcy (Li et al., 2008; Kundi et al., 2017). Treatments that lower plasma Hcy levels, including folic acid, might improve insulin sensitivity; dietary supplementation vitamin B are more effective in preventing metabolic syndrome and related vascular disease in areas where the population has a normally low folate consumption than in areas with higher dietary folate intake (Earnest et al., 2012). Our previous studies established that mice treated with oral Hcy mimic the increase of atherosclerosis and other general phenotypes in patients with HHcy (Dai et al., 2006). HHcy increases oxidative stress and its downstream signaling pathways, resulting in vascular inflammation stress and endoplasmic reticulum stress (Fu et al., 2018; Majumder et al., 2018). In a mouse model of HHcy, Hcy was enriched in adipose tissue, promoting insulin resistance and metabolic inflammation (Li et al., 2008; Li Y. et al., 2013).

We found that increased plasma levels of Hcy promotes insulin resistance by activating adipocyte and adipose tissue macrophage Nod-like receptor protein 3 inflammasomes (NLRP3). The NLRP3 inflammasome is also involved in Hcy-induced adipose insulin resistance; Hcy acted as a second signal activator of the adipocyte NLRP3 inflammasome, and the adipocyte inflammation participates in lipid disorders and insulin resistance (Zhang et al., 2018). Nevertheless, it remains unknown as to whether ω-3FA intake attenuates adipose metabolic inflammation.

Therefore, in the present study, we focused on whether ω-3FA consumption would attenuate HHcy-induced insulin resistance and tried to explain the mechanism through the histochemical changes caused by ω-3FAs.

We used ultraperformance liquid chromatography coupled to electrospray ionization quadrupole mass spectrometry (UPLC-ESI-QTOFMS)-based metabolomics analysis to measure the changes of bioactive metabolites in HHcy mice undergoing ω-3FA treatment.

MATERIALS AND METHODS

Animals and Housing

All mice were housed under specific pathogen-free conditions in a temperature-controlled room (22°C) with a 12-h light and dark cycle and were given free access to normal chow diet (Cat. 1025, HFK Biosciences, Beijing, China) and drinking water. Wild type (WT) mice were generated from the C57BL/6J background and were obtained from Vital River Laboratories (Beijing, China). Protocols were approved by the Animal Care and Use Committee of Capital Medical University.

HHcy Mouse Models

Mice were given water with or without DL-Hcy (1.8 g/l), and were fed a standard chow diet or a 3.3% omega-3 PUFA (33 mg/g) diet. After 6 weeks, mice were sacrificed, and the metabolic profiles of the adipose tissue were analyzed using UPLC-ESI-QTOFMS-based lipidomics. Expression levels of ceramide metabolism-related genes were measured using quantitative PCR. DL-Hcy was purchased from Sigma-Aldrich Chemicals (Cat. H4628, St. Louis, MO, United States).

Glucose Tolerance Test and Insulin Tolerance Test

For the glucose tolerance test (GTT), mice were fasted for 12 h before the administration of glucose (1.8 g/kg, i.p.). Blood samples were drawn from a cut at the tip of the tail at 0, 30, 60, 90, and 120 min after glucose administration, and blood glucose concentrations were measured immediately. For the insulin tolerance test (ITT), mice were fasted for 4 h before the administration of insulin (1 IU/kg, i.p.). Blood samples were drawn from a cut at the tip of the tail at 0, 30, 60, 90, and 120 min after insulin administration, and blood glucose concentrations were measured immediately.

Immunohistochemistry

F4/80 (Cat.ab16911, Abcam, Abcam Cambridge, United Kingdom) expression in adipose tissue was examined by immunohistochemistry using 7-μm sections of the eWAT. The sections were blocked with 5% bovine serum albumin (BSA) for 1 h and incubated overnight at 4°C with F4/80 antibody (1:500). After washing, the sections were incubated with the Horseradish Peroxidase (HRP)-conjugated anti-rabbit IgG (Cat. sc-2004, Santa Cruz, Dallas, TX, United States) secondary antibody (1:1000) for 1 h. The DAB method was used to detect the F4/80 signal.

Lipidomics Analysis

The serum and adipose sample preparations and the lipidomics analyses were undertaken as described previously (Jiang et al., 2015). In brief, epididymal white adipose tissue (eWAT) (20 mg) were homogenized with ultrapure water (200 μl) and then extracted with chloroform-methanol (2:1) solution (1,000 μl). The samples were incubated at 37°C for 30 min and subsequently centrifuged at 16,000 *g* for 20 min at 4°C. The lower organic phase (approximately 500 μl) was collected and evaporated. The organic residue was dissolved in isopropanol-acetonitrile (1:1) solution (100 μl). Samples were analyzed using the Thermo Scientific Dionex UltiMate 3000 Rapid Separation LC system (Thermo Fisher Scientific, Waltham, MA, United States). Peak extraction and integration were performed using Xcalibur 2.2 SP1.48 software (Thermo Fisher Scientific, Waltham, MA, United States).

Western Blot

Total protein was isolated with RIPA lysis buffer (Cat. P0013C, Beyotime Biotechnology, Shanghai, China). Total protein was subjected to sodium dodecylsulfate polyacrylamide gel electrophoresis on 10 or 12% running gels and then transferred to polyvinylidene fluoride membranes. The membranes were incubated with 10% BSA in Tris Tween-buffered saline at room

temperature for 1 h, with various primary antibodies at 4°C for 12 h and with a HRP-conjugated secondary antibody for 1.5 h. The bands were exposed using the ChemiDOC XRS System (Bio-Rad, Hercules, CA, United States). Anti-NLRP3 (Cat. AF-401-NA) antibody was purchased from R&D Systems (Minneapolis, MN, United States). Anti-β-actin (Cat. 8457) antibody was purchased from Cell Signaling Technology (Danvers, MA, United States). The HRP-conjugated anti-rabbit IgG (Cat. sc-2004), HRP-conjugated anti-goat IgG (Cat. sc-2020), and HRP-conjugated anti-mouse IgG (Cat. sc-2005) secondary antibodies were all purchased from Santa Cruz Biotechnology (Dallas, TX, United States).

Quantitative PCR Measurement of mRNA Levels

Total RNA was isolated using TRIzol Reagent (Cat. 15596018, Thermo Fisher Scientific, Waltham, MA, United States). Total RNA (2 μg) was reverse transcribed using 5X All-In-One RT MasterMix (Cat. G490, abm, Richmond, BC, Canada). Quantitative PCR (qPCR) analysis was performed using RealStar Green Power Mixture (Cat. A314, GenStar, Beijing, China) and run on an Mx3000 Multiplex Quantitative PCR System (Agilent, La Jolla, CA, United States). The amount of the PCR products formed in each cycle was evaluated using the fluorescence of SYBR Green I. The results were analyzed using Stratagene Mx3000 software. The qPCR primer sequences are shown in **Table 1**.

Statistical Analysis

The data were expressed as means ± SD and were analyzed using GraphPad Prism (GraphPad Software, La Jolla, CA, United States). For metabolomics analysis, the data were analyzed using MetaboAnalyst 3.0. One-way ANOVA with Tukey's multiple comparisons test (between multiple groups) and unpaired Student's t test (between two groups) were used as appropriate. $P < 0.05$ was considered significant.

TABLE 1 | The qPCR primer sequences.

Mus musculus hypoxia inducible factor 1, alpha subunit (Hif1a). NM_001313919.1	Forward primer AGGATGAGTTCTGAACGTCGAAA Reverse primer CTGTCTAGACCACCGGCATC
Mus musculus phospholipase A2, group XVI. BC024581.1	Forward primer CGCGTGGGCGAGGAG Reverse primer CTTCACTTGGAGGAGCCAGG
Mus musculus solute carrier family 2 (facilitated glucose transporter), member 1 (Slc2a1). NM_011400.3	Forward primer CGATCTGAGCTACGGGGTCT Reverse primer AGAACTCCTCAATAACCTTCTGGG
Mus musculus pyruvate dehydrogenase kinase, isoenzyme 1 (Pdk1). NM_172665.5	Forward primer CATACAGCCGCAGGTTGGC Reverse primer AGCATTCACTGACCCGAAGT
Mus musculus actin, beta (Actb), NM_007393.5	Forward primer GCCTTCCTTCTTGGGTATGGAA Reverse primer CAGCTCAGTAACAGTCCGCC

RESULTS

ω-3FAs Treatment Improves Hcy-Induced Insulin Resistance in a Mouse Model

To investigate the precise mechanisms underlying the effects fish oil on Hcy-induced insulin resistance, WT mice were given Hcy with or without fish oil in the drinking water (Hcy 1.8 g/l, ω-3FAs 3.3%, 6 weeks). IPGTT and ITT revealed marked glucose intolerance and insulin resistance in the Hcy-treated mice (**Figures 1A,B**), while the body weights were not significantly different from those of controls after fish oil treatment (**Figure 1C**).

ω-3FAs Intake Decreases the Production of Sphingolipids and Free Fatty Acids in Serum and Changes the Lipid Profile of Adipose Tissue Under Normal Diet

To further characterize the exact lipid profile changes because of ω-3FAs treatment, a non-targeted lipidomics assay was performed in serum. ω-3FAs intake decreased the production of sphingolipids and free fatty acids in fat tissue under normal diet ($n = 7$ per group) (**Figures 2A–D**). A heatmap of phospholipids and FAs revealed increased lyso-PC and PUFA levels in the adipose tissue of Hcy-treated mice that represents a lipid metabolic pattern distinct from that of vehicle-treated mice after ω-3FAs intake (**Figure 3**).

ω-3FAs Supplementation Significantly Changes the Lipid Profile in Adipose Tissue

Levels of lyso-PC (16:0), lyso-PC (18:0), lyso-PC (18:1), PC (16:0-22:6), PC (18:1-14:0), PC (18:1-20:4), PC (18:1-22:6), PC (18:0-20:5), PC (16:0-20:0), FA (20:4), and FA (22:4) were altered by Hcy treatment while tending to remain normal after ω-3FA intake (**Figures 4A–C**). The generation of lyso-PC and PUFA depends on phospholipase A2 (PLA2), which hydrolyzes the *sn*-2 position of phosphatidylcholine (PC) (Murakami et al., 2011). In agreement with the lipidomics data, PLA2 activity was elevated in the adipose tissue of Hcy-treated mice (**Figure 4D**).

ω-3FAs Attenuate Hcy-Induced NLRP3 Inflammasome Activation in Adipose Tissue

Homocysteine was previously shown to increase the HIF1α protein levels in podocytes (Li C. et al., 2013). Increased HIF1α levels, and the glycolysis-associated genes, PDK1 and Glut1 mRNA level were also observed in the adipose tissue of Hcy-treated mice (**Figure 4D**). PLA2G16 is a PLA2 and is specifically expressed in adipocytes with a preference toward hydrolysis of PC (Duncan et al., 2008). Expression levels of Pla2g16 were downregulated after ω-3FA intake that was

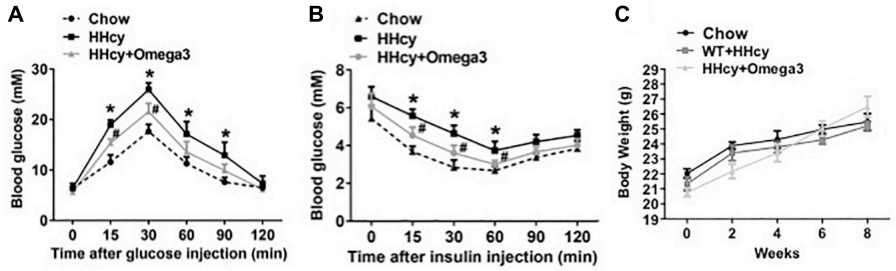


FIGURE 1 | ω-3FAs treatment improves Hcy-induced insulin resistance in a mouse model. **(A)** Plasma glucose levels of Intraperitoneal glucose tolerance test (IPGTT) in a mouse model (n = 7). **(B)** Plasma glucose levels of insulin tolerance test (ITT) in a mouse model (n = 7). **(C)** Body weight in the mouse model (n = 7). All data are presented as the means ± SD. *P < 0.05 vs. control group, #P < 0.05 vs. HHcy group; mice given Hcy (1.8 g/l), ω-3FAs (3.3%) in the drinking water for 6 weeks.

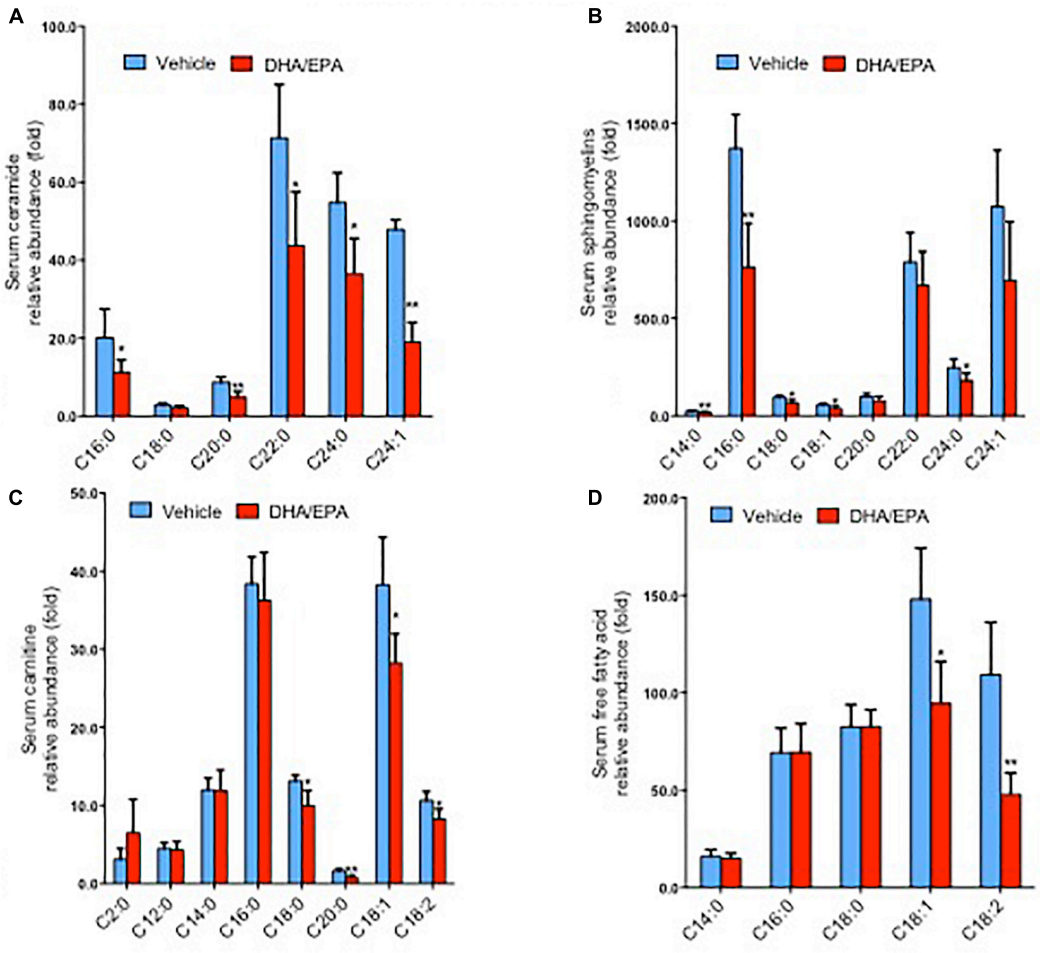


FIGURE 2 | ω-3FAs intake decreases the production of sphingolipids and free fatty acids in serum and adipose under normal diet. **(A)** Lipidomics analysis of the abundance of serum ceramide under normal diet. **(B)** Lipidomics analysis of the abundance of serum sphingomyelins under normal diet. **(C)** Lipidomics analysis of the abundance of serum carnitine under normal diet. **(D)** Lipidomics analysis of the abundance of serum free fatty acids under normal diet. *P < 0.05 vs control group, **P < 0.01 vs control group, n = 7.

highly expressed in Hcy-induced insulin resistance mice while levels of PDK1 and Glut1 decreased (**Figure 4D**). Protein levels of NLRP3 and Act-Casp1 were measured using western blotting. After Hcy treatment, NLRP3 levels were elevated in adipose tissue, and ω-3FAs inhibited levels of Hcy-up-regulated inflammatory markers (**Figure 5A**). In order to verify that fish oil treatment can improve the inflammation in adipose tissue, we carried out the immunohistochemistry of adipose tissue.

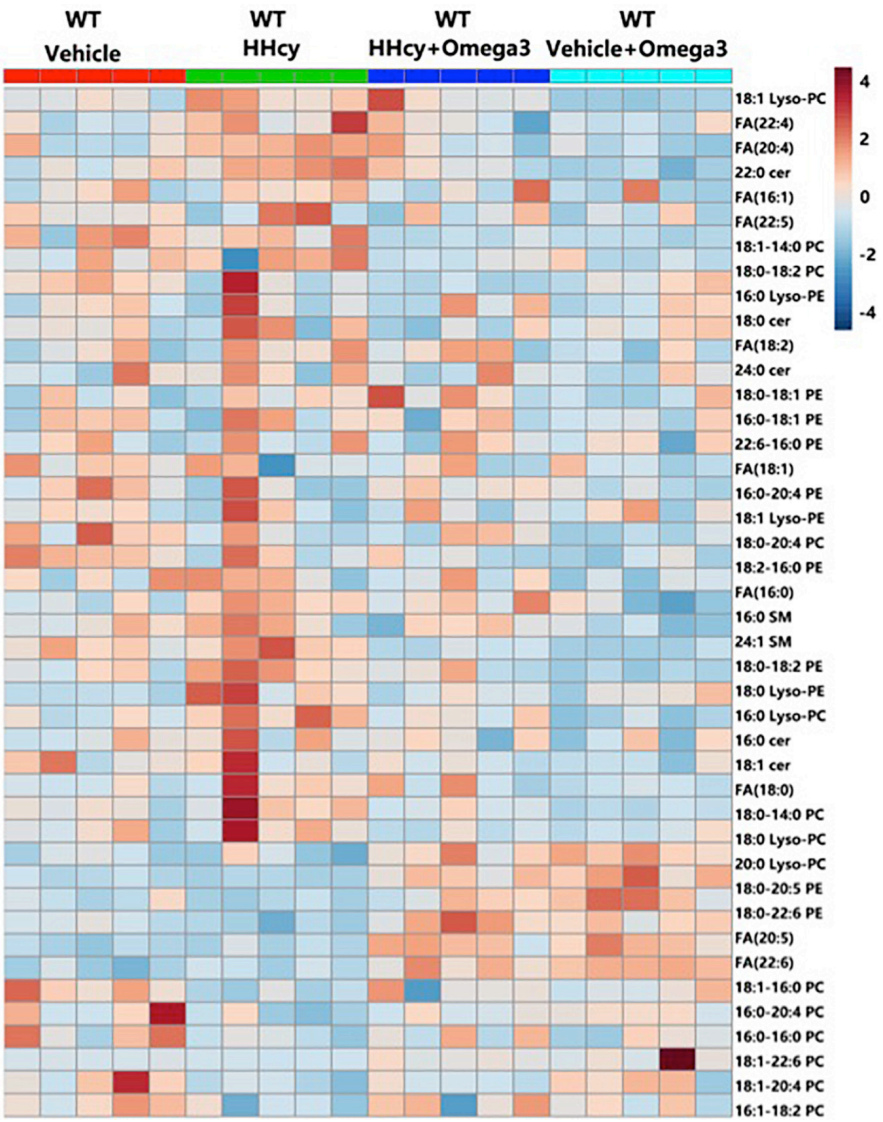


FIGURE 3 | Heatmap of phospholipids and FAs in the adipose tissue of Hcy-treated mice. Seven-week-old WT mice were fed normal chow diet and were given Hcy (1.8 g/L) or vehicle in the drinking water with or without ω-3FA (3.3%) for 6 weeks.

The results showed that after 6 weeks of ω-3FAs treatment, the expression of F4/80 in adipose tissue of mice decreased, indicating that the inflammation of adipose tissue was alleviated (**Figure 5B**).

DISCUSSION

Clinical trials assessing effect of ω-3FAs in primary and secondary prevention of cardiovascular disease have produced conflicting findings. Fish oil rich in ω-3FAs supplements have been widely used and recommended for prevention and treatment of lipid disorders and metabolic disease, and the clinical utility of ω-3FAs in lowering triglyceride levels is well known (Mansoori et al., 2015). There is also evidence suggesting that the Mediterranean diet supports addition of fish to the diet for prevention of cardiovascular disease (Guasch-Ferré et al., 2017). Mechanisms by which ω-3FAs exert their anti-inflammatory effects are not fully understood.

Homocysteine and C2-ceramide cause murine cerebral endothelial cells metabolic disorder by activating the Asm-ceramide pathway (Lee et al., 2013). Oxidative stress and *de novo* ceramide synthesis could induce NLRP3-inflammasome activation. It was found that ω-3FAs reduced nuclear factor kappa B-mediated inflammation in the liver through effects on inflammasome signaling (Feng et al., 2018). One study in 3T3-L1 adipocytes suggested that inhibition of IL-18, IL-1β, and caspase-1 by DHA and EPA were predominantly dependent upon adiponectin (Oster et al., 2010). These findings suggest that Hcy might induce inflammation and ω-3FAs might attenuate

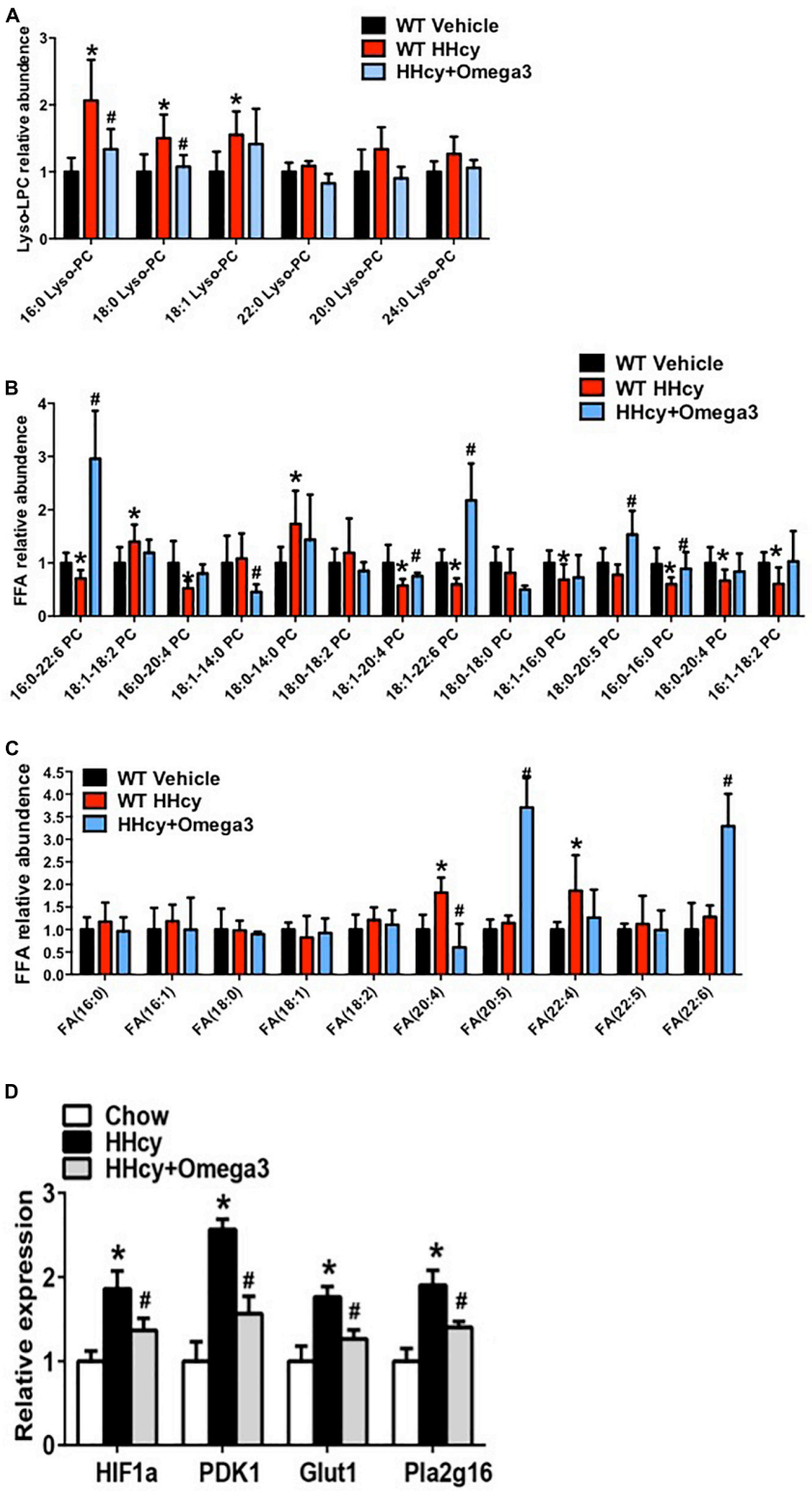


FIGURE 4 | ω-3FAs supplementation significantly changes the lipid profile of adipose tissue. **(A)** Lipidomics analysis of the lyso-PC profile of mouse adipose tissue; (n = 6 per group). **(B)** Lipidomics analysis of the PC profile of mouse adipose tissue; (n = 6 per group). **(C)** Lipidomics analysis of the FA profile of mouse adipose tissue; (n = 6 per group). Seven-week-old WT mice were fed normal chow diet and were given Hcy (1.8 g/L) or vehicle in the drinking water with or without ω-3FAs (3.3%) for 6 weeks. Two-tailed Student's t-test: $^*P < 0.05$ vs. control group, $\#P < 0.05$ vs. HHcy group. **(D)** qPCR analysis of mRNA levels of HIF-1, PDK1, Glut1, and Pla2g16 in mouse adipose tissue; (n = 6 per group).

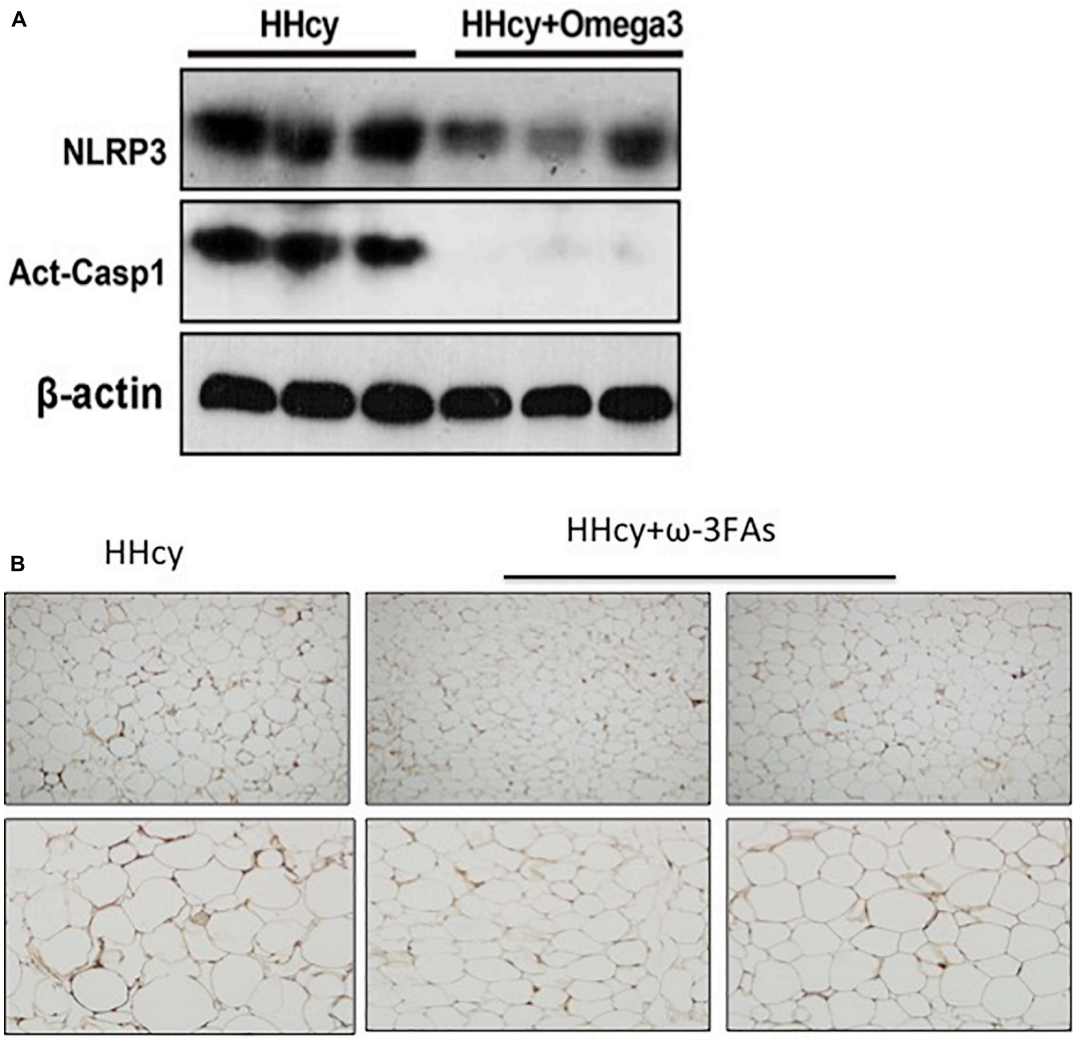


FIGURE 5 | Activation of the Hcy-induced NLRP3 inflammasome was attenuated by ω-3FAs in adipose tissue. **(A)** Western blot analysis of Act-CASP1 and NLRP3 protein levels in adipose tissue; **(B)** Immunohistochemical study of inflammation related molecule F4/80 in mouse adipose tissue, (n = 6 per group). Seven-week-old WT mice were fed normal chow diet and were given Hcy (1.8 g/L) or vehicle in the drinking water with or without ω-3FAs (3.3%) for 6 weeks. Two-tailed Student's t-test: *$P < 0.05$ vs. control group, #$P < 0.05$ vs. HHcy group, $n = 7$.

the inflammation as well as insulin resistance as shown in the present study.

Because the NLRP3 inflammasome is activated in human mesenchymal stem cells *via* lipopolysaccharide and palmitic acid, and plasma (Fu et al., 2019) levels are increased in the context of obesity (Wang et al., 2017), Hcy might be one of the mediators involved in obesity-induced insulin resistance. Oral intake of ω-3FAs as a treatment could turn over HHcy-induced lipid profile alterations in adipose tissue, and may modulate inflammation factors that have potential therapeutic functions in reducing adipose tissue inflammation-induced insulin resistance.

We found that the Hcy induced alterations in adipose tissue lipid profiles, confirming findings of our previous study. The NLRP3 inflammasome is activated by HHcy treatment and promotes insulin resistance and adipose inflammation. Increased expression levels of Pla2g16 mRNA as a marker of inflammatory activation are attenuated after ω-3FAs intake, hinting that adipose insulin resistance might be reduced secondary to the lipid profile modifications, especially in adipose tissue.

C12-16 saturated and monounsaturated fatty acids, ceramide, and lyso-PC have been reported to act as second signal activators of the NLRP3 inflammasome (Matsuzaka et al., 2012; Scheiblich et al., 2017; Zhang et al., 2018). Extracellular lyso-PC has been reported to activate G protein-coupled receptors (GPRs), GPR132 and GPR4, mediating its functions (Meyer zu Heringdorf and Jakobs, 2007) and participating in lyso-PC-induced Ca^{2+} influx and immune cell inflammation (Kabarowski, 2009; Khan et al., 2010), which may be involved in lyso-PC-induced NLRP3 inflammasome activation.

In the present study, lyso-PC was found to activate adipose NLRP3 inflammasomes. Lyso-PC also acts as a lipid mediator in

response to inflammation (Kabarowski, 2009). The contrasting effects of lyso-PC on insulin resistance and adipose tissue inflammation may be due to the different species of lyso-PC. These findings support our finding that Hcy might induce inflammation in a lipid profile-affecting manner, and the alteration in the inflammation-inducing lipid profile might be affected by ω-3FAs administration.

Previous studies showed that adipose tissue insulin resistance is often accompanied by activation of inflammasomes due to hypoxia. PLA2G16 was first recognized as a type II tumor suppressor gene (Sers et al., 1997) and was identified as a PPARγ target gene, expressed specifically in adipose tissue (Hummasti et al., 2008; Uyama et al., 2009). Knockout of *Pla2g16* induced insulin resistance in mice (Jaworski et al., 2009), a finding that appears inconsistent with the present results. In our study, expression levels of HIF-1α, PLA2G16, PDK1, and Glut1 mRNA were upregulated by Hcy treatment, suggesting that Hcy induces inflammation-related insulin resistance in adipocytes, while the abnormally elevated levels of inflammation and insulin resistance were relieved after supplementation with ω-3FAs.

We acknowledge that there are limitations to our studies, including the lack of mechanistic data. Our study focused on adipose and may have reflected mixed contributions from cell types populating adipose tissue (i.e., adipocytes, adipose immune cells, adipose progenitor cells, and vasculature). Finally, in this short-term study, prediction of long-term effects and outcomes was not feasible. Despite these limitations, our study adds to the emerging body of knowledge regarding potential beneficial effects of fish oils and their derivatives.

In summary, this study demonstrated that Hcy activates the NLRP3 inflammasomes in adipocytes in a lyso-PC-dependent manner while HHcy induces insulin resistance in adipose tissue. Addition of ω-3FAs in the diet may modulate inflammatory processes.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Animal Care and Use Committee of Capital Medical University.

AUTHOR CONTRIBUTIONS

JL designed, performed, analyzed, and interpreted the majority of animal and biochemical experiments, and drafted the manuscript. HZ and YD supported the animal experiments, and performed and analyzed the lipidomics analysis. XW and GW designed, planned, and interpreted the study. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Natural Science Foundation of China (Nos. 81200626 and 81670519) and Beijing Hospitals Authority Youth Program (Nos. QML20170302 and QML20190302). These funders had no role in study design, data interpretation, or writing of the manuscript.

REFERENCES

Dai, J., Li, W., Chang, L., Zhang, Z., Tang, C., Wang, N., et al. (2006). Role of redox factor-1 in hyperhomocysteinemia-accelerated atherosclerosis. *Free Radic. Biol. Med.* 41, 1566–1577. doi: 10.1016/j.freeradbiomed.2006.08.020

Devarshi, P. P., Jangale, N. M., Ghule, A. E., Bodhankar, S. L., and Harsulkar, A. M. (2013). Beneficial effects of flaxseed oil and fish oil diet are through modulation of different hepatic genes involved in lipid metabolism in streptozotocin-nicotinamide induced diabetic rats. *Genes Nutr.* 8, 329–342. doi: 10.1007/s12263-012-0326-2

Duncan, R. E., Sarkadi-Nagy, E., Jaworski, K., Ahmadian, M., and Sul, H. S. (2008). Identification and functional characterization of adipose-specific phospholipase A2 (AdPLA). *J. Biol. Chem.* 283, 25428–25436. doi: 10.1074/jbc.m804146200

Earnest, C. P., Kupper, J. S., Thompson, A. M., Guo, W., and Church, T. (2012). Complementary effects of multivitamin and omega-3 fatty acid supplementation on indices of cardiovascular health in individuals with elevated homocysteine. *Int. J. Vitam. Nutr. Res.* 82, 41–52. doi: 10.1024/0300-9831/a000093

Feng, R., Wang, Y., Liu, C., Yan, C., Zhang, H., Su, H., et al. (2018). Acetaminophen-induced liver injury is attenuated in transgenic fat-1 mice endogenously synthesizing long-chain n-3 fatty acids. *Biochem. Pharmacol.* 154, 75–88. doi: 10.1016/j.bcp.2018.04.019

Fu, L., Li, Y. N., Luo, D., Deng, S., and Hu, Y. Q. (2019). Plausible relationship between homocysteine and obesity risk via MTHFR gene: a meta-analysis of 38,317 individuals implementing Mendelian randomization. *Diabetes Metab. Syndr. Obes.* 12, 1201–1212. doi: 10.2147/dmso.s205379

Fu, Y., Wang, X., and Kong, W. (2018). Hyperhomocysteinaemia and vascular injury: advances in mechanisms and drug targets. *Br. J. Pharmacol.* 175, 1173–1189. doi: 10.1111/bph.13988

Guasch-Ferré, M., Hu, F. B., Ruiz-Canela, M., Bulló, M., Toledo, E., Wang, D. D., et al. (2017). Plasma metabolites from choline pathway and risk of cardiovascular disease in the PREDIMED (prevention with Mediterranean diet) Study. *J. Am. Heart Assoc.* 6:e006524.

Hainsworth, A. H., Yeo, N. E., Weekman, E. M., and Wilcock, D. M. (2016). Homocysteine, hyperhomocysteinemia and vascular contributions to cognitive impairment and dementia (VCID). *Biochim. Biophys. Acta* 1862, 1008–1017. doi: 10.1016/j.bbadis.2015.11.015

Hummasti, S., Hong, C., Bensinger, S. J., and Tontonoz, P. (2008). HRASLS3 is a PPARgamma-selective target gene that promotes adipocyte differentiation. *J. Lipid Res.* 49, 2535–2544. doi: 10.1194/jlr.m800269-jlr200

Jaworski, K., Ahmadian, M., Duncan, R. E., Sarkadi-Nagy, E., Varady, K. A., Hellerstein, M. K., et al. (2009). AdPLA ablation increases lipolysis and prevents obesity induced by high-fat feeding or leptin deficiency. *Nat. Med.* 15, 159–168. doi: 10.1038/nm.1904

Jiang, C., Xie, C., Li, F., Zhang, L., Nichols, R. G., Krausz, K. W., et al. (2015). Intestinal farnesoid X receptor signaling promotes nonalcoholic fatty liver disease. *J. Clin. Invest.* 125, 386–402. doi: 10.1172/jci76738

Kabarowski, J. H. (2009). G2A and LPC: regulatory functions in immunity. *Prostaglandins Other Lipid Mediat.* 89, 73–81. doi: 10.1016/j.prostaglandins.2009.04.007

Khan, S. Y., McLaughlin, N. J., Kelher, M. R., Eckels, P., Gamboni-Robertson, F., Banerjee, A., et al. (2010). Lysophosphatidylcholines activate G2A inducing $G(\alpha i)_{-1}$-/$G(\alpha q/)_{11}$-$Ca^2(+)$ flux, G(βγ)-Hck activation and clathrin/β-arrestin-1/GRK6 recruitment in PMNs. *Biochem. J.* 432, 35–45.

Kundi, H., Kiziltunc, E., Ates, I., Cetin, M., Barca, A. N., Ozkayar, N., et al. (2017). Association between plasma homocysteine levels and end-organ damage in newly diagnosed type 2 diabetes mellitus patients. *Endocr. Res.* 42, 36–41. doi: 10.3109/07435800.2016.1171235

Lee, J. T., Peng, G. S., Chen, S. Y., Hsu, C. H., Lin, C. C., Cheng, C. A., et al. (2013). Homocysteine induces cerebral endothelial cell death by activating the acid sphingomyelinase ceramide pathway. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 45, 21–27. doi: 10.1016/j.pnpbp.2013.05.003

Li, C., Xia, M., Abais, J. M., Liu, X., Li, N., Boini, K. M., et al. (2013). Protective role of growth hormone against hyperhomocysteinemia-induced glomerular injury. *Naunyn Schmiedebergs Arch. Pharmacol.* 386, 551–561. doi: 10.1007/s00210-013-0848-1

Li, Y., Jiang, C., Xu, G., Wang, N., Zhu, Y., Tang, C., et al. (2008). Homocysteine upregulates resistin production from adipocytes in vivo and in vitro. *Diabetes* 57, 817–827. doi: 10.2337/db07-0617

Li, Y., Zhang, H., Jiang, C., Xu, M., Pang, Y., Feng, J., et al. (2013). Hyperhomocysteinemia promotes insulin resistance by inducing endoplasmic reticulum stress in adipose tissue. *J. Biol. Chem.* 288, 9583–9592. doi: 10.1074/jbc.m112.431627

Majumder, A., Singh, M., Behera, J., Theilen, N. T., George, A. K., Tyagi, N., et al. (2018). Hydrogen sulfide alleviates hyperhomocysteinemia-mediated skeletal muscle atrophy via mitigation of oxidative and endoplasmic reticulum stress injury. *Am. J. Physiol. Cell Physiol.* 315, C609–C622.

Mansoori, A., Sotoudeh, G., Djalali, M., Eshraghian, M. R., Keramatipour, M., Nasli-Esfahani, E., et al. (2015). Effect of DHA-rich fish oil on PPARγ target genes related to lipid metabolism in type 2 diabetes: a randomized, double-blind, placebo-controlled clinical trial. *J. Clin. Lipidol.* 9, 770–777. doi: 10.1016/j.jacl.2015.08.007

Matsuzaka, T., Atsumi, A., Matsumori, R., Nie, T., Shinozaki, H., Suzuki-Kemuriyama, N., et al. (2012). Elovl6 promotes nonalcoholic steatohepatitis. *Hepatology* 56, 2199–2208. doi: 10.1002/hep.25932

Meyer zu Heringdorf, D., and Jakobs, K. H. (2007). Lysophospholipid receptors: signalling, pharmacology and regulation by lysophospholipid metabolism. *Biochem. Biophys. Res. Commun.* 1768, 923–940. doi: 10.1016/j.bbamem.2006.09.026

Murakami, M., Taketomi, Y., Miki, Y., Sato, H., Hirabayashi, T., and Yamamoto, K. (2011). Recent progress in phospholipase A2 research: from cells to animals to humans. *Prog. Lipid Res.* 50, 152–192. doi: 10.1016/j.plipres.2010.12.001

Nicholls, S. J., Lincoff, A. M., Bash, D., Ballantyne, C. M., Barter, P. J., Davidson, M. H., et al. (2018). Assessment of omega-3 carboxylic acids in statin-treated patients with high levels of triglycerides and low levels of high-density lipoprotein cholesterol: rationale and design of the STRENGTH trial. *Clin. Cardiol.* 41, 1281–1288. doi: 10.1002/clc.23055

Oster, R. T., Tishinsky, J. M., Yuan, Z., and Robinson, L. E. (2010). Docosahexaenoic acid increases cellular adiponectin mRNA and secreted adiponectin protein, as well as PPARγ mRNA, in 3T3-L1 adipocytes. *Appl. Physiol. Nutr. Metab.* 35, 783–789. doi: 10.1139/h10-076

Petersen, M. C., and Shulman, G. I. (2018). Mechanisms of insulin action and insulin resistance. *Physiol. Rev.* 98, 2133–2223. doi: 10.1152/physrev.00063.2017

Rossmeisl, M., Medrikova, D., van Schothorst, E. M., Pavlisova, J., Kuda, O., Hensler, M., et al. (2014). Omega-3 phospholipids from fish suppress hepatic steatosis by integrated inhibition of biosynthetic pathways in dietary obese mice. *Biochim. Biophys. Acta* 1841, 267–278. doi: 10.1016/j.bbalip.2013.11.010

Scheiblich, H., Schlütter, A., Golenbock, D. T., Latz, E., Martinez-Martinez, P., and Heneka, M. T. (2017). Activation of the NLRP3 inflammasome in microglia: the role of ceramide. *J. Neurochem.* 143, 534–550. doi: 10.1111/jnc.14225

Sers, C., Emmenegger, U., Husmann, K., Bucher, K., Andres, A. C., and Schäfer, R. (1997). Growth-inhibitory activity and downregulation of the class II tumor-suppressor gene H-rev107 in tumor cell lines and experimental tumors. *J. Cell Biol.* 136, 935–944. doi: 10.1083/jcb.136.4.935

Tortosa-Caparrós, E., Navas-Carrillo, D., Marín, F., and Orenes-Piñero, E. (2017). Anti-inflammatory effects of omega 3 and omega 6 polyunsaturated fatty acids in cardiovascular disease and metabolic syndrome. *Crit. Rev. Food Sci. Nutr.* 57, 3421–3429. doi: 10.1080/10408398.2015.1126549

Uyama, T., Morishita, J., Jin, X. H., Okamoto, Y., Tsuboi, K., and Ueda, N. (2009). The tumor suppressor gene H-Rev107 functions as a novel Ca2+-independent cytosolic phospholipase A1/2 of the thiol hydrolase type. *J. Lipid Res.* 50, 685–693. doi: 10.1194/jlr.m800453-jlr200

Wang, L., Chen, K., Wan, X., Wang, F., Guo, Z., and Mo, Z. (2017). NLRP3 inflammasome activation in mesenchymal stem cells inhibits osteogenic differentiation and enhances adipogenic differentiation. *Biochem. Biophys. Res. Commun.* 484, 871–877. doi: 10.1016/j.bbrc.2017.02.007

Zhang, S. Y., Dong, Y. Q., Wang, P., Zhang, X., Yan, Y., Sun, L., et al. (2018). Adipocyte-derived lysophosphatidylcholine activates adipocyte and adipose tissue macrophage nod-like receptor protein 3 inflammasomes mediating homocysteine-induced insulin resistance. *EBioMedicine* 31, 202–216. doi: 10.1016/j.ebiom.2018.04.022

Conflict of Interest: 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.

frontiers in Physiology

ORIGINAL RESEARCH
published: 15 February 2021
doi: 10.3389/fphys.2021.646010

Serum Glycerophospholipid Profile in Acute Exacerbation of Chronic Obstructive Pulmonary Disease

Xiaoyan Gai, Chenglin Guo, Linlin Zhang, Lijiao Zhang, Mairipaiti Abulikemu, Juan Wang, Qingtao Zhou, Yahong Chen, Yongchang Sun and Chun Chang**

Department of Respiratory and Critical Care Medicine, Peking University Third Hospital, Beijing, China

Studies have shown that glycerophospholipids are involved in the pathogenesis of chronic obstructive pulmonary disease (COPD). This study adopted targeted metabolomic analysis to investigate the changes in serum glycerophospholipids in acute exacerbation of chronic obstructive pulmonary disease (AECOPD) and their differential expression in patients with different inflammatory subtypes. Patients with AECOPD admitted between January 2015 and December 2017 were enrolled, and their clinical data were collected. The patients' gender, age, body mass index, and lung function were recorded. Routine blood and induced sputum tests were performed. Liquid chromatography-mass spectrometry was used to detect the serum glycerophospholipid metabolic profiles and to analyze the metabolic profile changes between the acute exacerbation and recovery stages as well as the differences between different inflammatory subtypes. A total of 58 patients were hospitalized for AECOPD, including 49 male patients with a mean age of 74.8 ± 10.0 years. In the metabolic profiles, the expression of lysophosphatidylcholine (LPC) 18:3, lysophosphatidylethanolamine (LPE) 16:1, and phosphatidylinositol (PI) 32:1 was significantly reduced in the acute exacerbation stage compared to the recovery stage ($P < 0.05$). The three glycerophospholipids were used to plot the receiver operating characteristic curves to predict the acute exacerbation/recovery stage, and the areas under the curves were all above 70%. There were no differential metabolites between the two groups of patients with blood eosinophil percentage (EOS%) ≥2% and <2% at exacerbation. The expression of LPC 18:3, LPE 16:1, and PI 32:1 was significantly reduced in the acute exacerbation stage compared to the recovery stage in the inflammatory subtype with blood EOS <2% ($P < 0.05$). Abnormalities in the metabolism of glycerophospholipids may be involved in the onset of AECOPD, especially in the non-eosinophilic subtype.

Keywords: glycerophospholipids, metabolomics, chronic obstructive pulmonary disease, acute exacerbation of chronic obstructive pulmonary disease, inflammatory subtype

OPEN ACCESS

Edited by:
Chunjiong Wang,
Tianjin Medical University, China

Reviewed by:
Zhiyi He,
Guangxi Medical University, China
Min Zhou,
Shanghai Jiao Tong University, China
Jiangchao Zhao,
University of Arkansas, United States

****Correspondence:***
Yongchang Sun
suny@bjmu.edu.cn
Chun Chang
doudou_1977@163.com

Specialty section:
This article was submitted to Lipid and Fatty Acid Research, a section of the journal Frontiers in Physiology

Received: *24 December 2020*
Accepted: *27 January 2021*
Published: *15 February 2021*

Citation:
Gai X, Guo C, Zhang L, Zhang L, Abulikemu M, Wang J, Zhou Q, Chen Y, Sun Y and Chang C (2021) Serum Glycerophospholipid Profile in Acute Exacerbation of Chronic Obstructive Pulmonary Disease. Front. Physiol. 12:646010. doi: 10.3389/fphys.2021.646010

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterized by persistent airflow limitation and chronic airway inflammation. It is a common and frequently occurring disease with a global incidence of 10% and has become a worldwide public health problem (Global Initiative for Chronic Obstructive Lung Disease, 2020). The prevalence of COPD in China is 13.7% among people aged 40 years and above, and the total patient population is approximately 100 million

(Zurcher et al., 2020). Furthermore, COPD places a substantial disease burden on individuals and families as well as the society and the economy.

Acute exacerbation (AE) of COPD (AECOPD) refers to the exacerbation of any symptoms (e.g., cough, sputum, and wheezing) in COPD patients. It can be caused by bacterial or viral infection, environmental pollution, cold weather, or interruption of routine treatment. It is a leading cause of hospital admission and death (Berenyi et al., 2020). AECOPD often accelerates disease progression, and each AE worsens the patient's lung function, aggravates complications, and increases the risk of re-hospitalization. Thus, the main goal of COPD treatment is to reduce the frequency of exacerbations. At present, the underlying mechanisms of AECOPD are unclear, and the pathophysiology of the different subtypes is poorly understood. Therefore, metabolomics can be helpful to further investigate the mechanisms and classifications of this disease and explore its biomarkers in greater depth (Kilk et al., 2018; Liang et al., 2019; Ekroos et al., 2020).

Glycerophospholipids are major components of cell membranes, storage materials for bioactive substances, and precursors of informational molecules. They serve important physiological functions in cell growth, migration, signal recognition and transduction, and apoptosis. Glycerophospholipid molecules can be modified by phospholipase A2 to further produce metabolites such as lysophospholipids. Recent studies have shown that glycerophospholipids are involved in the pathogenesis of lung infections (Mander et al., 2002), asthma, and COPD (Telenga et al., 2014; Cruickshank-Quinn et al., 2018; Chen et al., 2019; Gai et al., 2019; Liang et al., 2019) and are associated with the lipid metabolic disorder of alveolar surfactants (Cruickshank-Quinn et al., 2018; Ekroos et al., 2020). However, to date, the differences in the glycerophospholipids profile in the AE stage of COPD and among different inflammatory subtypes have been poorly investigated.

This study aimed to investigate the differences in the serum glycerophospholipid metabolic profiles of AECOPD and among different inflammatory subtypes to provide a basis for further exploring the pathogenesis of AECOPD and identifying precise therapeutic targets for different subtypes.

PATIENTS AND METHODS

Patients

A prospective study was conducted on 58 patients with AECOPD who were admitted to the Department of Respiratory and Critical Care Medicine at Peking University Third Hospital between January 2015 and December 2016.

Inclusion criteria were as follows: (1) age >40 years; (2) COPD diagnosis meeting the GOLD criteria (Global Initiative for Chronic Obstructive Lung Disease, 2020); (3) stable-phase pulmonary function report in the last 6 months; forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) ratio <70% after bronchodilator inhalation and percentage of predicted FEV_1 value (FEV1%pred) <80%; and (4) AECOPD meeting the GOLD diagnostic criteria (Global Initiative for Chronic Obstructive Lung Disease, 2020).

Exclusion criteria were as follows: (1) comorbidity with other lung diseases such as active tuberculosis, bronchiectasis, asthma, interstitial lung disease, pleural effusion from various causes, and lung malignancies; and (2) intravenous or oral glucocorticoid therapy for AEs within 28 days.

This study was approved by the Ethics Committee of Peking University Third Hospital (approval number: LM2016032), and written informed consent was provided by the patients enrolled in the study.

Methods

Clinical Data Collection

Demographic information (e.g., gender, age, body mass index, and smoking history), status of inhaled corticosteroids, number of AEs in the past year, and stable-phase lung function parameters in the past 6 months (e.g., FEV1%pred and FEV1/FVC) were recorded for all patients. Patients' AECOPD symptoms were also recorded, and 2 mL of peripheral blood was collected for serum phospholipid profiling. To further clarify the characteristics of different AECOPD subtypes, serum glycerophospholipid assays were performed on the first day of admission during the AE stage and on the 10–14 days after treatment of AECOPD (before discharge). In addition, patients were classified into different inflammatory subtypes based on whether their peripheral blood eosinophil percentage (EOS%) was ≥2% or <2%, and whether sputum EOS% was ≥3% or <3% to compare the differences in serum glycerophospholipid profiles (**Figure 1**).

Sputum Cytology

Patients were asked to rinse their oral cavity and posterior oropharynx with 3% hypertonic saline prior to sampling. Sputum was then produced spontaneously, or induced sputum was collected. For the collection of induced sputum, patients inhaled an aerosol of 3% saline and coughed up a sufficient amount of sputum within 30 min. The sputum was treated with 0.4% dithiothreitol (DTT, Millipore, Canada), homogenized by shaking at 37°C for 30 min, and stained for cytological examination. The remainder was divided into 0.5 mL portions and stored at −80°C for glycerophospholipid testing (Cao et al., 2006).

Serum Glycerophospholipid Profiling

Liquid chromatography-mass spectrometry (LC-MS) was performed to determine serum glycerophospholipid profiles, as described in our previous publications (Gai et al., 2019; Liang et al., 2019). Briefly, the serum was separated from whole blood and lipid extraction was carried out using the Waters Acquity UPLC system, with a UPLC BEH C18 column (1.7 μm; internal diameter, 100 × 2.1 mm). An AB Sciex 5500 QTRAP mass spectrometer was used with the following specifications: ion source, Turbo Ion Spray electrospray ionization; scan mode, multiple reaction monitoring, with the following ion source parameters: CUR = 40 psi, GS1 = 30 psi, GS2 = 30 psi, IS = −4,500 V, CAD = MEDIUM, and TEMP = 350°C. The glycerophospholipid scanning strategy has been described in

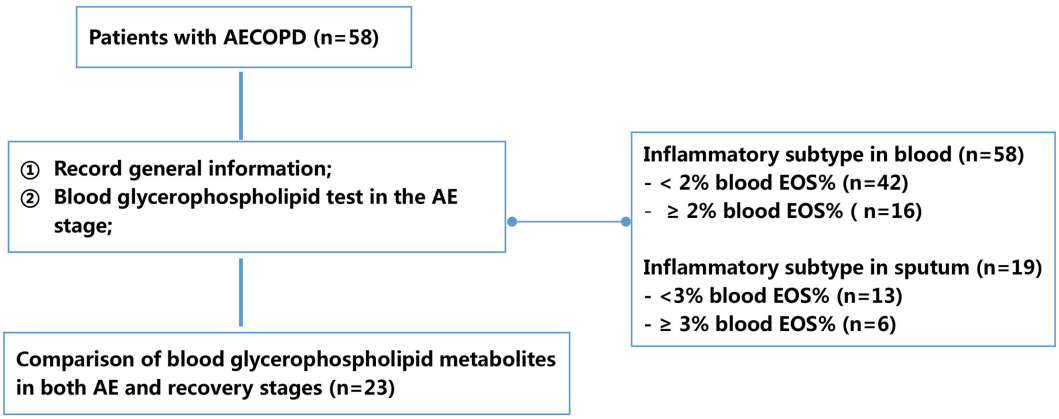


FIGURE 1 | Flowchart of study design. AECOPD, acute exacerbation of chronic obstructive pulmonary disease; EOS, eosinophil percentage.

our previous study (Gai et al., 2019). A total of 14 classes of glycerophospholipids (129 species) were analyzed in this study: phosphatidylcholine (PC, 21 species), alkylphosphatidylcholine [PC(O), 15 species], phosphatidylcholineplasmalogen (PCP, 8 species), phosphatidylethanolamine (PE, 12 species), alkylphosphatidylethanolamine (PEO, 4 species), phosphatidylethanolamine plasmalogen (PEP, 2 species), phosphatidylglycerol (PG, 2 species), phosphatidylinositol (PI, 11 species), lysophosphatidylcholine (LPC, 11 species), lysophosphatidylethanolamine (LPE, 12 species), lysophosphatidylserine (LPS, 5 species), lysophosphatidylglycerol (LPG, 11 species), lysophosphatidylinositol (LPI, 9 species), and lysoalkylphosphatidylcholine (LPCO, 6 species).

Statistical Methods

The metabolic profiles of glycerophospholipid levels in patients with different COPD subtypes were compared using multivariate statistics (Chong et al., 2018). Partial least squares–discrimination analysis (PLS-DA) was performed for the patients' samples, and differential metabolites were screened using methods such as estimation of variable importance in projection values, loading weights, and correlation coefficients. The differential metabolites that contributed to subtyping were further subjected to one-way analysis of variance and Bonferroni multiple comparisons to validate the results obtained by above statistics. Data and statistical analyses were performed using the SPSS 19.0 package. Normally distributed measurement data are expressed as $\bar{x} \pm s$, and the χ^2-test was performed for the comparison of constituent ratios. The intensity of LPC, LPE, and PI were evaluated using the receiver operating characteristic (ROC) curve method. $P \leq 0.05$ was considered statistically significant.

RESULTS

General Information

Fifty-eight patients with AECOPD were included (mean age, 74.8 ± 10.0 years; mean FEV1%pred, 42.6 ± 18.2%; and FEV1/FVC, 49.2 ± 10.4%; **Table 1**).

Metabolic Profile Changes During the AE and Recovery Stages

Among the 58 patients included, serum was collected from 23 patients to compare the glycerophospholipid metabolic profiles between the AE and recovery (7–10 days after admission) stages. PLS-DA indicated that the global glycerophospholipids tends to be distinguishable from the metabolic profiles of AE and recovery stages. The volcano plots (**Figure 2A**) showed that there were three differential glycerophospholipid metabolites after false discovery rate (FDR) correction with significant changes: LPC 18:3, LPE 16:1, and PI 32:1. **Table 2** shows the

TABLE 1 | General information of patients with AECOPD.

Variable	Value
Male/female	49/9
Age (years)	74.8 ± 10.0
BMI (kg/m^2)	22.1 ± 4.9
Current smokers	20 (34.5%)
Former smokers	33 (56.9%)
Never smokers	5 (8.6%)
Smoking (pack-years)	39.1 ± 31.1
COPD duration (years)	14.6 ± 12.0
AEs in the past year	1.57 ± 1.36
Concomitant respiratory failure	25 (41.7%)
ICU on admission (%)	11 (19.0%)
Need for non-invasive ventilation on admission (%)	11 (19.0%)
Need for invasive ventilation on admission (%)	2 (3.4%)
FEV_1%pred	42.6 ± 18.2
FEV_1/FVC (%)	49.2 ± 10.4
Blood EOS count (/10^9/L)	0.04 (0.00, 0.14)
Blood EOS ratio (%)	0.50 (0.10, 2.10)

AECOPD, acute exacerbation of chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; FEV_1%pred, FEV_1 expressed as a percentage of the predicted value; FVC, forced vital capacity; EOS, eosinophil. All data with a normal distribution are shown as the mean ± standard deviation. Non-normally distributed data are expressed as median (25–75%).

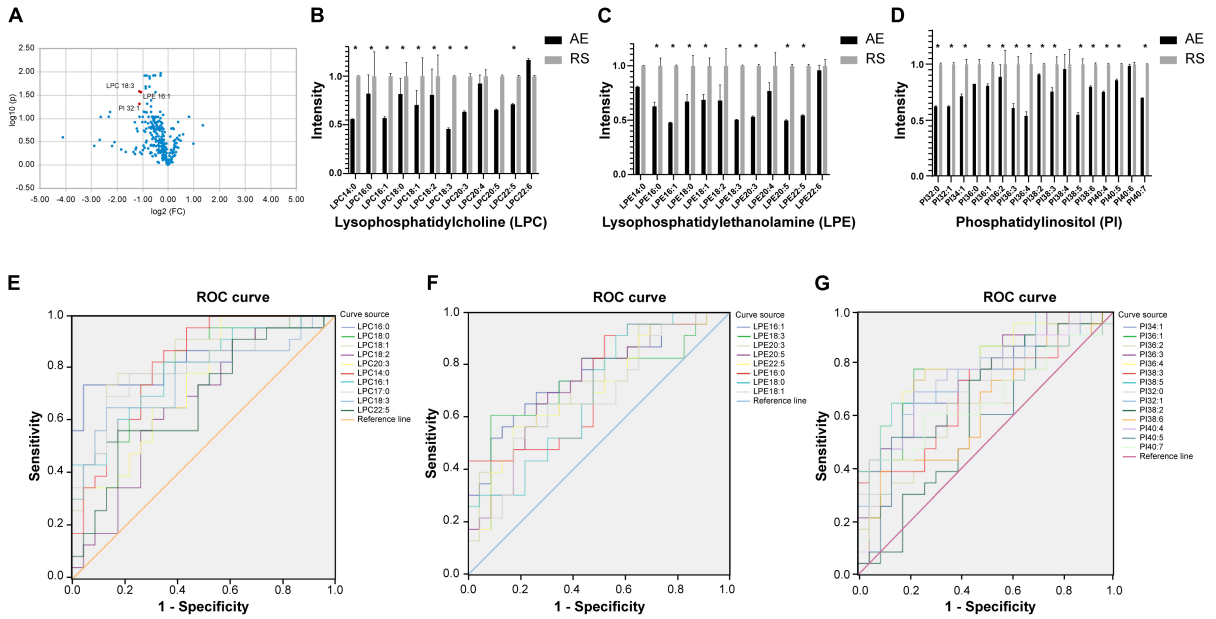


FIGURE 2 | (A) Volcano plot showing glycerophospholipids with significant differences between the AE and recovery stages of COPD. The *x*-axis is the logarithm to the base 2 of the ratio of a given phospholipid in the AE and recovery stages, and the *y*-axis is the logarithm to the base 10 of the *P*-value of that phospholipid in different subgroups. The phospholipids that showed significant changes were LPC 18:3, LPE 16:1, and PI 32:1. **(B–D)** Histograms showing all LPC, LPE, and PI in the metabolic profiles between the AE and recovery stages (*P <0.05). AE, acute exacerbation; RS, recovery stage. **(E–G)** ROC curve analysis of the main differential glycerophospholipids during COPD recovery (LPC, LPE, and PI). ROC curve analysis was performed using the main differential glycerophospholipids above as the test variables and the recovery phase as the state variable. The areas under the curve (AUCs) for LPC 14:0, 16:0, LPC 16:1, LPC 17:0, LPC 18:0, LPC 18:1, LPC 18:2, LPC 18:3, LPC 20:3, and LPC 22:5 were 82.0, 84.5, 79.6, 80.5, 80.7, 83.0, 66.7, 76.4, 74.5, and 68.1%, respectively. The AUCs of LPE 16:0, LPE 16:1, LPE 18:0, LPE 18:1, LPE 18:3, LPE 20:3, LPE 20:5, and LPE 22:5 were 72.4, 76.4, 69.2, 67.9, 73.5, 73.2, 72.4, and 70.1%, respectively. The AUCs of PI 32:0. PI 32:1, PI 34:1, PI 36:1, PI 36:2, PI 36:3, PI 36:4, PI 38:2, PI 38:3, PI 38:5, PI 38:6, PI 40:4, PI 40:5, and PI 40:7 were 72.6, 74.3, 75.4, 75.0, 63.9, 78.4, 81.1, 61.4, 69.8, 80.2, 62.6, 74.1, 63.1, and 66.0%, respectively.

names and fold changes of the differential metabolites identified after data comparison (P <0.05). **Figures 2B–D** show the histograms of all LPC, LPE, and PI species in the metabolic profiles obtained between the AE and recovery stages (*P <0.05). An ROC curve analysis was performed with the three most significantly differential glycerophospholipids as the test variables and recovery stages as the state variable. The area under the curves (AUCs) for LPC 14:0, LPC 16:0, LPC 16:1, LPC 17:0, LPC 18:0, LPC 18:1, LPC 18:2, LPC 18:3, LPC 20:3, and LPC 22:5 were 82.0, 84.5, 79.6, 80.5, 80.7, 83.0, 66.7, 76.4, 74.5, and 68.1%, respectively. The AUCs of LPE 16:0, LPE 16:1, LPE 18:0, LPE 18:1, LPE 18:3, LPE 20:3, LPE 20:5, and LPE 22:5 were 72.4, 76.4, 69.2, 67.9, 73.5, 73.2, 72.4, and 70.1%, respectively. The AUCs of PI 32:0. PI 32:1, PI 34:1, PI 36:1, PI 36:2, PI 36:3, PI 36:4, PI 38:2, PI 38:3, PI 38:5, PI 38:6, PI 40:4, PI 40:5, and PI 40:7 were 72.6, 74.3, 75.4, 75.0, 63.9, 78.4, 81.1, 61.4, 69.8, 80.2, 62.6, 74.1, 63.1, and 66.0%, respectively.

Differences in the Metabolic Profiles of Patients With Different Inflammatory Subtypes During the AE Stage

Routine blood tests were conducted for 58 patients with AECOPD; the results showed that blood eosinophils accounted for <2 and ≥2% of the total white blood cells in 42 and 16 patients, respectively (**Supplementary Table 1**). PLS-DA and FDR correction indicated that there were no differential metabolites between the

TABLE 2 | List of differential metabolites in the AE and recovery stages.

Metabolites	FC (AE/recovery)	log2 (FC)	Corrected *P*-value
LPC 16:0	1.226	0.294	0.011
PI 36:4	1.860	0.895	0.012
LPC 14:0	1.786	0.837	0.012
LPC 16:1	1.765	0.819	0.012
LPC 17:0	1.425	0.511	0.012
LPC 18:1	1.423	0.509	0.012
LPC 18:0	1.229	0.298	0.012
PI 36:3	1.644	0.717	0.014
PI 38:5	1.825	0.868	0.021
LPC 18:3	2.166*	1.115	0.027
LPE 16:1	2.093*	1.066	0.027
PI 34:1	1.402	0.487	0.027
LPC 20:3	1.585	0.664	0.041
PI 32:1	2.178*	1.123	0.048

Three main differential metabolites analyzed using a fold change of two.
AE, acute exacerbation; FC, fold change; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PI, phosphatidylinositol.

two groups of patients with peripheral blood EOS% ≥2 and <2%.

Nineteen patients also underwent sputum examination: based on sputum cell counts, 6 patients were classified as sputum eosinophilic (≥3%) and 13 as non-eosinophilic (<3%) types. PLS-DA showed differences in phospholipid metabolic profiles between the two groups, but no significantly differential metabolites were identified after FDR correction.

Changes in Metabolic Profiles Between AE and Recovery Stage of Different Inflammatory Subtypes

Among the 58 patients included, serum was collected from 23 patients to compare the glycerophospholipid metabolic profiles between the AE and recovery stages, including 8 patients with blood EOS ≥ 2% and 15 patients with EOS% < 2%. In patients with EOS% < 2%, the concentrations of LPC 14:0, LPC 16:0, LPC 16:1, LPC 18:0, LPC18:1, LPC18:3, LPC 20:3, LPE 16:0, LPE 16:1, LPE 18:0, LPE 20:3, LPE 22:5, PI 32:1, PI 34:1, PI 36:1, PI 36:3, PI 36:4, PI 38:3, PI 38:5, PI 40:4, and PI 40:7 were significantly lower in the AE stage than in the recovery stage ($^*P < 0.05$) (**Figures 3A–C**). In patients with EOS% > 2%, only the concentrations of LPC 14:0,LPC 16:1, LPE 16:1, and PI 36:1 were significantly lower in the AE stage than in the recovery stage ($^*P < 0.05$) (**Figures 3D–F**). These findings indicated that the changes in LPC, LPE, and PI between the AE and recovery stages were mainly manifested in AECOPD patients with EOS% < 2%.

DISCUSSION

COPD is a highly heterogeneous disease presenting with different phenotypes. In this study, metabolomic analysis of glycerophospholipids was conducted among patients in the acute and recovery stages of COPD and also for different inflammatory subtypes, involving 129 phospholipids from 14 classes. Comparison of the metabolic profiles between the AE and recovery stages of COPD revealed significant decreases in LPC18:3, LPE 16:1, and PI 32:1 levels, suggesting the involvement of abnormal glycerophospholipid metabolism in the onset of AECOPD.

Glycerophospholipids are important components of the cell membrane and were thought to be related to cell structure and storage compartments. However, it has recently been discovered that glycerophospholipids can participate in mediating airway inflammation through its cleavage by phospholipase A2 to produce lysophospholipids and, hence, become involved in signaling and immune responses. Lysophospholipids are monoacyl hydrolysates of diacyl glycerophospholipid precursor molecules and are so called due to their detergent-like ability to lyse erythrocytes (Makide et al., 2014). In addition to altering the physical structure of cellular lipid bilayers, lysophospholipids can regulate cell signaling pathways by binding directly to membrane G-protein-coupled receptors and indirectly affecting membrane receptors. The main physiological lysophospholipids include LPA, LPS, LPG, and LPC. Recent studies have found that glycerophospholipids are physiologically

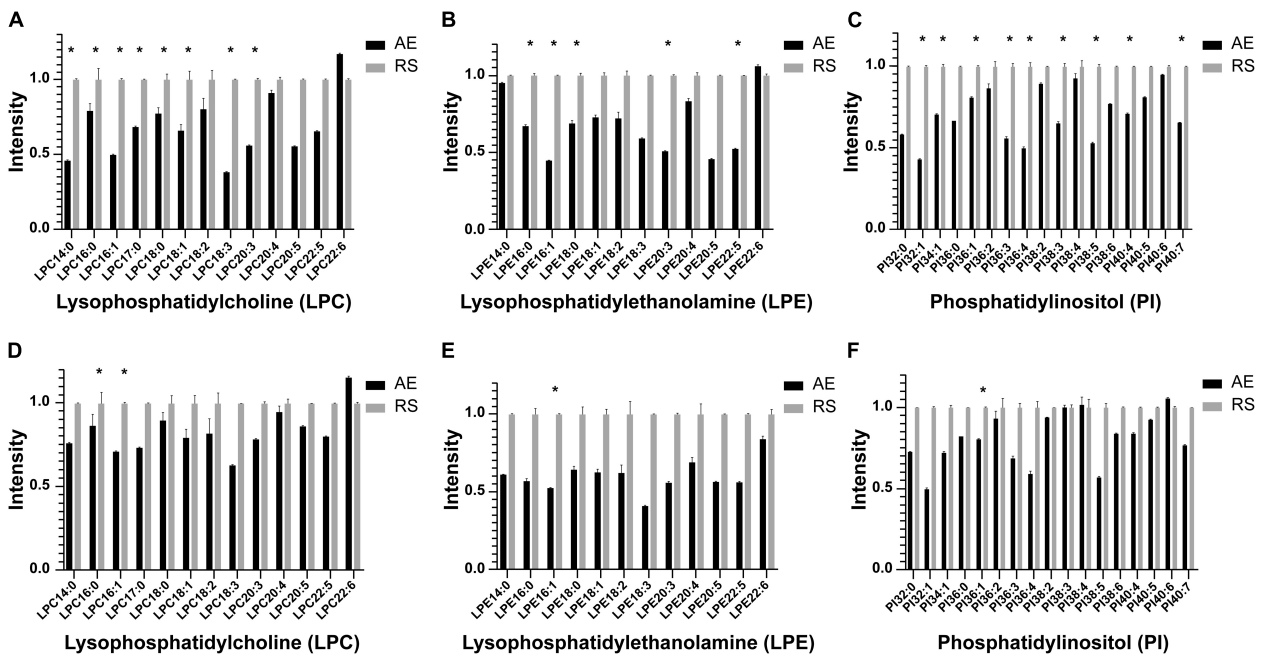


FIGURE 3 | Histogram showing LPC, LPE, and PI species in the metabolic profiles between the AE and recovery stages in different inflammatory subtypes. **(A–C)** In patients with EOS% <2%, the concentrations of LPC 14:0, LPC 16:0, LPC 16:1, LPC 18:0, LPC 18:1, LPC 18:3, LPC 20:3, LPE 16:0, LPE 16:1, LPE 18:0, LPE 20:3, LPE 22:5, PI 32:1, PI 34:1, PI 36:1, PI 36:3, PI 36:4, PI 38:3, PI 38:5, PI 40:4, and PI 40:7 significantly decreased in the AE stage compared to the recovery stage ($^*P < 0.05$). **(D–F)** In patients with EOS% > 2%, only the concentrations of LPC 14:0, LPC 16:1, LPE 16:1, and PI 36:1 were significantly decreased in the AE stage compared to the recovery stage ($^*P < 0.05$).

active intercellular and intracellular lipid mediators involved in the pathogenesis of lung infections (Mander et al., 2002), asthma, and COPD (Sevastou et al., 2013; Drzazga et al., 2014; Telenga et al., 2014; Cruickshank-Quinn et al., 2018; Chen et al., 2019).

LPC is a lysophospholipid with a relatively high concentration in human blood and is produced by the hydrolysis of PC by phospholipase A2; conversely, LPE is produced by the hydrolysis of phosphatidylethanolamine by phospholipase A2. Based on our study results, lipid metabolites, such as LPC, LPE, and PI, were significantly reduced in AECOPD patients. Cigarette smoking is a major cause of COPD. Glycerophospholipids, which are complexes composed of 90% phospholipids and a small amount of protein, are important components of lung surfactants, which may be damaged following smoke exposure (Schurch et al., 1992; Goerke, 1998; Berry et al., 2011). They are secreted into the alveoli by airway epithelial cells to reduce alveolar surface tension and block pathogen invasion. Cruickshank-Quinn et al. used LC-MS to study smokers with COPD and found abnormal expression of several serum glycerophospholipids, among which LPC and PI were significantly negatively correlated with FEV_1%pred and FEV_1/FVC; moreover, they also performed integrated transcriptome/metabolome analysis and showed that the expression of LPC, PI, PE, and LPE gradually decreased with worsening lung function outcomes (Cruickshank-Quinn et al., 2018). Halper-Stromberg et al. (2019) performed LC-MS analysis of bronchoalveolar lavage fluid (BALF) from COPD patients and showed that the concentrations of lipid metabolites, such as LPC, LPE, and PI, were significantly reduced in COPD patients, and that these metabolites negatively correlated with FEV1/FVC. Furthermore, the level of lipid metabolites in BALF correlated with COPD outcomes more closely than that of serum metabolites. These findings suggest that LPC, LPE, and PI are associated with COPD, which is consistent with our results. The lipid metabolism disorder involving alveolar surfactants in patients with COPD (Cruickshank-Quinn et al., 2018; Ekroos et al., 2020) may be a potential therapeutic target for future studies on the restoration of alveolar surfactants. Exposure to cigarette smoke can induce alveolar surfactant dysfunction, alveolar epithelial cell apoptosis, and emphysema. These manifestations may be particularly prominent during AEs. Fatty acids undergo dynamic transformations in the body. The acyl group at the sn-2 site of PC is subjected to continuous deacylation and reacylation, leading to its repeated inversion and re-modification in a process called the Lands cycle (Wang and Tontonoz, 2019). PC is hydrolyzed by phospholipase to form LPC, and lysophosphatidylcholine acyltransferase (LPCAT) is a key enzyme of the Lands cycle. LPCAT is involved in catalyzing the conversion of LPC and acyl-CoA into PC and free CoA, thereby reducing the concentration of endogenous LPC (Wang and Tontonoz, 2019). COPD patients have a high expression of *LPCAT* gene, which correlates with the severity of FEV_1%pred lung function (Cruickshank-Quinn et al., 2018). In addition, chronic airway inflammatory diseases, such as COPD, often involve abnormalities in phospholipase metabolism, which contribute to the differences in phospholipid expression (Wymann and Schneiter, 2008; Azimzadeh Jamalkandi et al., 2015).

In vitro studies have shown that LPC may exhibit pro-inflammatory or anti-inflammatory physiological effects in different pathophysiological conditions. As a representative of pro-inflammatory lysophospholipids, LPC is involved in regulating T-cell function and immune activity, inducing the processing and secretion of IL-1β, and increasing the expression of cytokine-induced interferon gamma (IFN-γ) and transforming growth factor β1 (TGF-β1) (Drzazga et al., 2014). In addition, LPC-dependent NADPH oxidase can stimulate the production of reactive oxygen species, which promotes the conversion of pro-cytokines to their mature, biologically active forms (IL-1β, IL-18, and IL33) (Schilling and Eder, 2010). Furthermore, in addition to their pro-inflammatory effects, polyunsaturated LPCs, such as LPC 20:4, LPC 20:5, and LPC 22:6, can act as potent anti-inflammatory agents against the activity of immune responses induced by saturated LPC (Riederer et al., 2010; Hung et al., 2012). LPCs can also downregulate the formation of pro-inflammatory mediators (IL-5, IL-6, NO, 12-hydroxyeicosatetraenoic acid, and LPC16:0-induced PGE2) and upregulate the expression of anti-inflammatory mediators (IL-4 and IL-10) by reducing leukocyte extravasation and plasma leakage, thereby achieving anti-inflammatory effects (Hung et al., 2012). Thus, the different biological activities of LPCs are related to the body's internal environment, including hypoxia, oxidative stress, T-cell immune homeostasis, and differences in phospholipase metabolism (Murakami et al., 2014); importantly, these activities can vary with the length and saturation of the acyl chain, which also affects the biological properties and activity of the resultant molecules (Drzazga et al., 2014). Our results showed that LPC was decreased in AECOPD, and the mechanism underlying this reduction deserves further study.

Our study showed that LPE was also lower in the AE stage compared to the recovery stage, but the exact mechanism is unclear. Hung et al. found that unsaturated LPE has anti-inflammatory effects (Hung et al., 2011). In their study, zymosan A was used to induce acute peritonitis in mice, and treatment with polyunsaturated acyl-LPE could effectively reduce peritoneal inflammation in mice, while also reducing the formation of LTC4, which is a lipid mediator involved in vascular permeability. Furthermore, the levels of pro-inflammatory mediators (IL-1b, IL-6, TNF-α, and NO) decreased, whereas those of the anti-inflammatory mediator IL-10 increased. Taken together, these results suggest that LPE may have an anti-inflammatory effect. In addition, lysophosphatidyl transferase may be a regulatory factor (Eto et al., 2020). AECOPD is a complex and systemic disease state, and the mechanisms underlying the action of LPC, LPE, and PI in COPD deserve further investigation.

Different inflammatory subtypes can be identified in AECOPD (Yun et al., 2018). The present study defined the eosinophilic subtype as the ratio of peripheral blood EOS to leukocytes $\geq$ 2% and sputum EOS $\geq$ 3%. We found that COPD patients with the peripheral blood eosinophilic subtype

had a shorter duration of COPD, lower rate of concomitant respiratory failure on admission, lower rate of non-invasive ventilation during hospitalization, shorter hospital stay, and fewer AEs in the past year than the sputum EOS < 3% subtype. This finding suggests that there may be a correlation between peripheral blood EOS levels and AECOPD severity in COPD patients (Kostikas et al., 2018). However, this study did not find significant differences in the levels of glycerophospholipid metabolites between the inflammatory subtypes, which may be due to the small sample size. Nevertheless, the results showed that the changes in LPC, LPE, and PI between AE and the recovery stage were more significant in subtypes with blood EOS% < 2% than in those with blood EOS% > 2%. This result indicates that LPC, LPE, and PI may play a role in non-eosinophilic COPD and warrants further study.

This study has some limitations. First, this study had a small sample size since it was primarily an exploratory study. Second, the peripheral blood glycerophospholipid levels measured in this study is a systemic response to chronic airway inflammation and cannot fully represent the local environment in the bronchi and lungs. The next step is to increase the sample size, combine sputum and BALF specimen analysis to further verify its relationship with AECOPD, and conduct in-depth mechanistic studies.

In conclusion, the present metabolomics study used LC-MS to detect the metabolic profiles of serum phospholipids in AECOPD and different subtypes of COPD. LPC, LPE, and PI were significantly reduced in AECOPD and could be used as biomarkers and potential therapeutic targets for treating AECOPD. Metabolomic analysis of glycerophospholipids may become an important research tool that could give rise to new drug targets and new biomarkers for COPD subtypes. Thus, the treatment of AECOPD is expected to evolve rapidly toward phenotypic specificity and individualization.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Peking University Third Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XG, CG, and CC participated in the acquisition, analysis, and interpretation of the data. XG wrote the report. All authors contributed to this article and reviewed and approved the final version.

FUNDING

This research was funded by grants from the 13th National 5-Year Development Plan Program (No. 2016YFC1304100), the National Natural Science Foundation (Nos. 81970028, 81970041, and 82090014), the National Natural Science Foundation of China Youth Fund Project (No. 81700038), the National Natural Science Foundation of China Emergency Management Project (No. 81641153), and the National Key Research and Development Project (No. 2016YFC1304301).

ACKNOWLEDGMENTS

We are indebted to the patients who participated in this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.646010/full#supplementary-material

REFERENCES

Azimzadeh Jamalkandi, S., Mirzaie, M., Jafari, M., Mehrani, H., Shariati, P., and Khodabandeh, M. (2015). Signaling network of lipids as a comprehensive scaffold for omics data integration in sputum of COPD patients. *Biochim. Biophys. Acta* 1851, 1383–1393. doi: 10.1016/j.bbalip.2015.07.005

Berenyi, F., Steinfort, D. P., Abdelhamid, Y. A., Bailey, M. J., Pilcher, D. V., Bellomo, R., et al. (2020). Characteristics and outcomes of critically ill patients with acute exacerbation of chronic obstructive pulmonary disease in Australia and New Zealand. *Ann. Am. Thorac. Soc.* 17, 736–745. doi: 10.1513/AnnalsATS.201911-821OC

Berry, K. A., Li, B., Reynolds, S. D., Barkley, R. M., Gijón, M. A., Hankin, J. A., et al. (2011). MALDI imaging MS of phospholipids in the mouse lung. *J. Lipid Res.* 52, 1551–1560. doi: 10.1194/jlr.M015750

Cao, W. L., Sun, Y. C., Gai, X. Y., and Yao, W. Z. (2006). Safety evaluation of induced sputum in severe persistent asthma. *Chin. J. Respir. Crit. Care* 5, 93–100.

Chen, H., Li, Z., Dong, L., Wu, Y., Shen, H., and Chen, Z. (2019). Lipid metabolism in chronic obstructive pulmonary disease. *Int. J. Chron. Obstruct. Pulm. Dis.* 14, 1009–1018. doi: 10.2147/COPD.S196210

Chong, J., Soufan, O., Li, C., Caraus, I., Li, S., Bourque, G., et al. (2018). MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. *Nucleic Acids Res.* 46, 486–494. doi: 10.1093/nar/gky310

Cruickshank-Quinn, C. I., Jacobson, S., Hughes, G., Powell, R. L., Petrache, I., Kechris, K., et al. (2018). Metabolomics and transcriptomics pathway approach reveals outcome-specific perturbations in COPD. *Sci. Rep.* 8:17132. doi: 10.1038/s41598-018-35372-w

Drzazga, A., Sowińska, A., and Koziołkiewicz, M. (2014). Lysophosphatidylcholine and lysophosphatidylinosiol–novel promissing signaling molecules and their possible therapeutic activity. *Acta Pol. Pharm.* 71, 887–899.

Ekroos, K., Lavrynenko, O., Titz, B., Pater, C., Hoeng, J., and Ivanov, N. V. (2020). Lipid-based biomarkers for CVD, COPD, and aging - a translational perspective. *Prog. Lipid Res.* 78:101030. doi: 10.1016/j.plipres.2020.101030

Eto, M., Shindou, H., Yamamoto, S., Tamura-Nakano, M., and Shimizu, T. (2020). Lysophosphatidylethanolamine acyltransferase 2 (LPEAT2) incorporates DHA into phospholipids and has possible functions for fatty acid-induced cell death. *Biochem. Biophys. Res. Commun.* 526, 246–252. doi: 10.1016/j.bbrc.2020.03.074

Gai, X. Y., Zhang, L. J., Chang, C., Guo, C. L., Abulikemu, M., Li, W. X., et al. (2019). Metabolomic analysis of serum glycerophospholipid levels in eosinophilic and neutrophilic asthma. *Biomed. Environ. Sci.* 32, 96–106. doi: 10.3967/bes2019.013

Global Initiative for Chronic Obstructive Lung Disease (2020). *Global Strategy for the Diagnosis, Management and Prevention of Chronic Obstructive Pulmonary Disease 2021 Report*. Available online at: http://www.goldcopd.org. [Accessed December 15, 2020]

Goerke, J. (1998). Pulmonary surfactant: functions and molecular composition. *Biochim. Biophys. Acta* 1408, 79–89. doi: 10.1016/s0925-4439(98)00060-x

Halper-Stromberg, E., Gillenwater, L., Cruickshank-Quinn, C., O'Neal, W. K., Reisdorph, N., Petrache, I., et al. (2019). Bronchoalveolar lavage fluid from COPD patients reveals more compounds associated with disease than matched plasma. *Metabolites* 9:157. doi: 10.3390/metabo9080157

Hung, N. D., Kim, M. R., and Sok, D. E. (2011). 2-Polyunsaturated acyl lysophosphatidylethanolamine attenuates inflammatory response in zymosan A-induced peritonitis in mice. *Lipids* 46, 893–906. doi: 10.1007/s11745-011-3589-2

Hung, N. D., Sok, D. E., and Kim, M. R. (2012). Prevention of 1-palmitoyllysophosphatidylcholine-induced inflammation by polyunsaturated acyl lysophosphatidylcholine. *Inflam. Res.* 61, 473–483. doi: 10.1007/s00011-012-0434-x

Kilk, K., Aug, A., Ottas, A., Soomets, U., Altraja, S., and Altraja, A. (2018). Phenotyping of chronic obstructive pulmonary disease based on the integration of metabolomes and clinical characteristics. *Int. J. Mol. Sci.* 19:666. doi: 10.3390/ijms19030666

Kostikas, K., Brindicci, C., and Patalano, F. (2018). Blood eosinophils as biomarkers to drive treatment choices in asthma and COPD. *Curr. Drug Targets* 19, 1882–1896. doi: 10.2174/1389450119666180212120012

Liang, Y., Gai, X. Y., Chang, C., Zhang, X., Wang, J., and Li, T. T. (2019). Metabolomic profiling differences among asthma, COPD, and healthy subjects: a LC-MS-based metabolomic analysis. *Biomed. Environ. Sci.* 32, 659–672. doi: 10.3967/bes2019.085

Makide, K., Uwamizu, A., Shinjo, Y., Ishiguro, J., Okutani, M., Inoue, A., et al. (2014). Novel lysophosphoplipid receptors: their structure and function. *J. Lipid Res.* 55, 1986–1995. doi: 10.1194/jlr.R046920

Mander, A., Langton-Hewer, S., Bernhard, W., Warner, J. O., and Postle, A. D. (2002). Altered phospholipid composition and aggregate structure of lung surfactant is associated with impaired lung function in young children with respiratory infections. *Am. J. Respir. Cell Mol. Biol.* 27, 714–721. doi: 10.1165/rcmb.4746

Murakami, M., Taketomi, Y., Miki, Y., Sato, H., Yamamoto, K., and Lambeau, G. (2014). Emerging roles of secreted phospholipase A2 enzymes: the 3rd edition. *Biochimie* 107, 105–113. doi: 10.1016/j.biochi.2014.09.003

Riederer, M., Ojala, P. J., Hrzenjak, A., Graier, W. F., Malli, R., Tritscher, M., et al. (2010). Acyl chain-dependent effect of lysophosphatidylcholine on endothelial prostacyclin production. *J. Lipid Res.* 51, 2957–2966. doi: 10.1194/jlr.M006536

Schilling, T., and Eder, C. (2010). Importance of lipid rafts for lysophosphatidylcholine-induced caspase-1 activation and reactive oxygen species generation. *Cell Immunol.* 265, 87–90. doi: 10.1016/j.cellimm.2010.08.003

Schurch, S., Lee, M., and Gehr, P. (1992). Pulmonary surfactant – surface properties and function of alveolar and airway surfactant. *Pure Appl. Chem.* 64, 1745–1750. doi: 10.1351/pac199264111745

Sevastou, I., Kaffe, E., Mouratis, M. A., and Aidinis, V. (2013). Lysoglycerophospholipids in chronic inflammatory disorders: the PLA2/LPC and ATX/LPA axes. *Biochim. Biophys. Acta* 1831, 42–60. doi: 10.1016/j.bbalip.2012.07.019

Telenga, E. D., Hoffmann, R. F., t'Kindt, R., Hoonhorst, S. J., Willemse, B. W., van Oosterhout, A. J., et al. (2014). Untargeted lipidomic analysis in chronic obstructive pulmonary disease uncovering sphingolipids. *Am. J. Respir. Crit. Care Med.* 190, 155–164. doi: 10.1164/rccm.201312-2210OC

Wang, B., and Tontonoz, P. (2019). Phospholipid remodeling in physiology and disease. *Annu. Rev. Physiol.* 81, 165–188. doi: 10.1146/annurev-physiol-020518-114444

Wymann, M. P., and Schneiter, R. (2008). Lipid signalling in disease. *Nat. Rev. Mol. Cell Biol.* 9, 162–176. doi: 10.1038/nrm2335

Yun, J. H., Lamb, A., Chase, R., Singh, D., Parker, M. M., Saferali, A., et al. (2018). Blood eosinophil count thresholds and exacerbations in patients with chronic obstructive pulmonary disease. *J. Allergy Clin. Immunol.* 141, 2037–2047. doi: 10.1016/j.jaci.2018.04.010

Zurcher, K., Sugi, M. D., Naidu, S. G., Oklu, R., Knuttinen, G., Alzubaidi, S., et al. (2020). Multimodality imaging techniques for performing challenging core biopsies. *RadioGraphics* 40, 910–911. doi: 10.1148/rg.2020190104

Conflict of Interest: 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.

frontiers in Physiology

REVIEW
published: 17 February 2021
doi: 10.3389/fphys.2021.639920

TFEB: A Emerging Regulator in Lipid Homeostasis for Atherosclerosis

Manman Li[1], Zitong Wang[1], Pengyu Wang[1], Hong Li[1] and Liming Yang[2]**

[1] Department of Pathophysiology, School of Basic Medical Sciences, Harbin Medical University, Harbin, China, [2] Department of Pathophysiology, Harbin Medical University-Daqing, Daqing, China

Atherosclerosis, predominantly characterized by the disturbance of lipid homeostasis, has become the main causation of various cardiovascular diseases. Therefore, there is an urgent requirement to explore efficacious targets that act as lipid modulators for atherosclerosis. Transcription factor EB (TFEB), whose activity depends on post-translational modifications, such as phosphorylation, acetylation, SUMOylation, ubiquitination, etc., is significant for normal cell physiology. Recently, increasing evidence implicates a role of TFEB in lipid homeostasis, *via* its functionality of promoting lipid degradation and efflux through mediating lipophagy, lipolysis, and lipid metabolism-related genes. Furthermore, a regulatory effect on lipid transporters and lipid mediators by TFEB is emerging. Notably, TFEB makes a possible therapeutic target of atherosclerosis by regulating lipid metabolism. This review recapitulates the update and current advances on TFEB mediating lipid metabolism to focus on two intracellular activities: a) how cells perceive external stimuli and initiate transcription programs to modulate TFEB function, and b) how TFEB restores lipid homeostasis in the atherosclerotic process. In-depth research is warranted to develop potent agents against TFEB to alleviate or reverse the progression of atherosclerosis.

Keywords: TFEB, lipid homeostasis, atherosclerosis, post-translational modifications, lipid transporters, lipophagy, lipid mediators

OPEN ACCESS

Edited by:
Chunjiong Wang,
Tianjin Medical University, China

Reviewed by:
Luyang Yu,
Zhejiang University, China
Jun Ren,
Fudan University, China

****Correspondence:***
Hong Li
drlihong1971@163.com
Liming Yang
limingyanghmu@163.com

Specialty section:
This article was submitted to Lipid and Fatty Acid Research, a section of the journal Frontiers in Physiology

Received: *10 December 2020*
Accepted: *20 January 2021*
Published: *17 February 2021*

Citation:
Li M, Wang Z, Wang P, Li H and Yang L (2021) TFEB: A Emerging Regulator in Lipid Homeostasis for Atherosclerosis. Front. Physiol. 12:639920. doi: 10.3389/fphys.2021.639920

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ACAT1, acetyl-coenzyme A acyltransferase1; AD, activation domain; AKT, protein kinase B; AMPK, adenosine monophosphate-activated protein kinase; ATGL, adipose triglyceride lipase; bHLH, basic helix-loop-helix; bHLHZIP, basic helix-loop-helix leucine-zipper; CARM1, co-activator-associated arginine methyltransferase 1; ChIP, chromatin immunoprecipitation; CLEAR, coordinated lysosomal expression and regulation; CSCC, cutaneous squamous cell carcinoma; CYP7A1, cholesterol 7α-hydroxylase; DAG, diglyceride; DNA, deoxyribonucleic acid; ERK, extracellular signal-regulated kinase; G, glycerol; GCN5, general control non-repressed protein 5; Gin, glutamine; GSK-3β, glycogen synthase kinase-3β; HDAC2, histone deacetylase 2; HDAC6, histone deacetylase 6; HLH-30, Helix-Loop-Helix-30; HSL, hormone-sensitive lipase; HY-SDT, hypericin-mediated sonodynamic therapy; JAK2, Janus kinase 2; LncRNA, long non-coding RNA; LZ, leucine-zipper; MAG, monoacylglycerol; MAGL, monoacylglycerol lipase; METTL3, methyltransferase like 3; miR-128, microRNA-128; MiT/TFE, microphthalmia/transcription factor E; mTOR, mammalian target of rapamycin; mTORC1, mammalian target of rapamycin complex 1; ox-LDL, oxidized low-density lipoprotein; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α; PKC, protein kinase C; PPARα, peroxisome proliferator-activated receptor α; Pro, proline; ROS, reactive oxygen species; SAHA, suberoylanilide hydroxamic acid; SCD1, stearoyl-CoA desaturase 1; SR-A, scavenger receptor class A; SUMO, small ubiquitin-like modifier; TAG, triglyceride; TFE3, transcription factor E3; TFEB, transcription factor EB; YWHA 14-3-3, phospho-serine/phospho-threonine binding protein.

INTRODUCTION

Cardiovascular diseases cause serious harm to human life and health (Mialet-Perez and Vindis, 2017). Atherosclerosis, characterized by lipid accumulation and inflammatory cell infiltration of the arterial wall, is the main pathological basis of cardiovascular diseases (Libby et al., 2011; Herrington et al., 2016). Upregulated lipid uptake and synthesis as well as deregulated degradation and transport make atherosclerotic cardiovascular events appear (Weber and Noels, 2011). Lipid homeostasis is controlled by the coordinated regulation of various metabolic pathways involving *de novo* synthesis, absorption, storage, transport, and breakdown of lipids (Yuan et al., 2012). Disruption of this balance may lead to lipid accumulation, which in turn gives rise to a multiplicity of life-threatening metabolism-related diseases currently, such as cardiovascular diseases, fatty liver, diabetes (Luo et al., 2020). The ways of regulating lipid metabolism are tightly interlinked and intricately entwined. Multiple lines of evidence have established that making the appropriate adjustments to each link from lipid synthesis to degradation and reuse, paying attention to the key target pathways in which they participate, is crucial to lipid homeostasis (Thelen and Zoncu, 2017).

As a salient transcription factor belonging to the microphthalmia/transcription factor E (MiT/TFE) family, transcription factor EB (TFEB) has been identified to modulate basic intracellular homeostasis (Napolitano and Ballabio, 2016). The activity and function of TFEB are also governed by a quite complex but only partially defined regulatory network consistent with other proteins, including transcription, translation, and the most common post-translational modification. During nutritional deficiency, TFEB translocates into the nucleus combined with downstream target promoters to regulate cell physiological processes, which not only takes part in ontogeny and angiogenesis but also has arisen as a master regulator of autophagy and lysosome biosynthesis (Xu and Ren, 2015; Fan et al., 2018). Evidence has mounted that TFEB is involved in the regulation of lipid metabolism in many diseases. For example, TFEB activates autophagy to indirectly inhibit liver steatosis and against weight gain in obesity (Zhang Y. et al., 2018). TFEB also contributes to being a potential therapeutic target for non-alcoholic fatty liver disease *via* the beneficial effect in regulating lipolysis and lipophagy (Yu et al., 2020). Remarkably, it has been discovered in recent years that TFEB also participates in lipid metabolism through regulating lipid mobilization and oxidation, inhibiting lipid uptake, promoting intracellular lipid degradation and efflux, preventing lipid accumulation by coordinating autophagy-lysosomes and modifying lipid transporters and lipid mediators, thus alleviating the progression of atherosclerosis and stabilizing plaques (Chen L. et al., 2017; **Figure 1**).

Despite the considerable amount of work being conducted to understand the role of TFEB in cell metabolism and lipid homeostasis, several instances of its involvement in the regulation of lipid levels in atherosclerosis and related diseases have only recently been identified. Given the consummated investigations and the unmet challenges, this article focuses on the current cognizance of the several ways to activate TFEB and the integrated network mechanism of TFEB linking lipid homeostasis with atherosclerosis. In addition, we delineate some TFEB activators as potential therapeutic candidates in mitigating the pathological progression of atherosclerosis.

BASIC STRUCTURE AND FUNCTION OF TFEB

Transcription factor EB, consisting of 476 amino acid residues, is a member of MiT/TFE family with high sequence homology whose components include (i) the basic helix-loop-helix leucine-zipper (bHLHZIP) structure, (ii) an acidic transcription activation domain, (iii) glutamic acid-rich domain, (iv) serine-rich domain and other domains (Fisher et al., 1991). Not only can TFEB recognize palindromic CACGTG E-box, but also asymmetric TCATGTG M-box sequence that is unavailable to other bHLHZIP transcription factors (LeBlanc et al., 1998). Moreover, TFEB combines with homologous or heterologous deoxyribonucleic acid (DNA) groups in the form of dimers to initiate transcription of the corresponding gene (Brady et al., 2018a). For instance, the effect of the autophagy process is effectively regulated by TFEB, as it promotes autophagy formation, autolysosome fusion, and degradation through binding to the promoter regions of autophagy-related genes (Shin H. R. et al., 2016). Intriguingly, recent evidence strongly suggests that TFEB overexpression accelerates the degradation of a large number of autophagy substrates such as proteins, lipid droplets, damaged mitochondria, and endoplasmic reticulum, indicating the non-negligible role of TFEB in selective organelle autophagy (Wang et al., 2020; Yao et al., 2020).

Transcription factor EB is also known to be a vital regulator of lysosomal biosynthesis-related genes by connecting with the coordinated lysosomal expression and regulation (CLEAR) motif in the promoter region responsible for lysosome regeneration and biogenesis (Curnock et al., 2019). Another function of TFEB on lysosomes is to induce lysosomal exocytosis, a process in which lysosomes fuse with the plasma membrane, followed by secretion of their contents outside cells (Di Paola and Medina, 2019b). TFEB also exerts emerging effects on lipid metabolism by way of directly or indirectly modulating lipid uptake, transport, and degradation. To realize the above functions, cells need to start a TFEB transcription program in response to various kinds of environmental cues. Therefore, it is important to elucidate how the upstream factors regulate TFEB activity with subsequent enhancement of autophagy and lipid metabolism.

THE SPECIFIC MECHANISM FOR TFEB ACTIVATION

When cells are stressed by factors such as starvation, lysosomal stress, and mitochondrial damage, they must respond quickly by regulating the rapid shift of cytoplasmic TFEB to the nucleus for binding to DNA elements and activating the transcription of

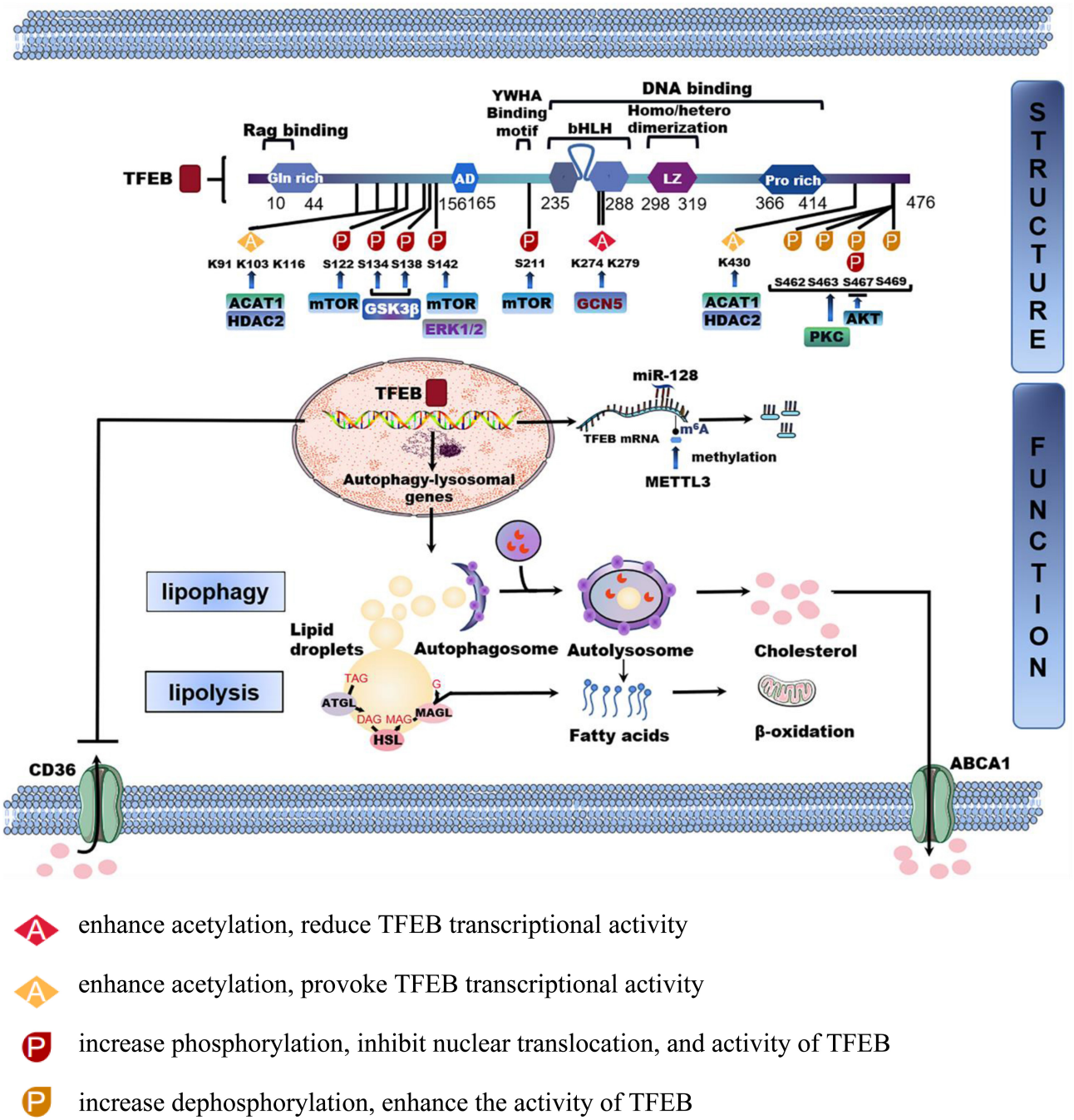


FIGURE 1 | The structure and function of TFEB in lipid homeostasis. TFEB is a protein consisting of 476 amino acid residues, mainly including glutamine-rich (Gln rich), an acidic transcription activation domain (AD), basic helix-loop-helix leucine-zipper (bHLHZIP) structure, and proline-rich motifs (Pro rich). Phosphorylation and acetylation are the most important post-translational modifications to regulate TFEB activity. The phosphorylated or acetylated TFEB sites by different proteins are shown in the figure. TFEB is also regulated by transcription and non-coding RNA. MicroRNA-128 targets TFEB to tune the transcription of autophagy-related genes. Methyltransferase like 3 (METTL3) inhibits the transcriptional activity of TFEB by methylating two m^6A residues. After being activated into the nucleus, TFEB regulates downstream autophagy-lysosomal genes to modulate lipophagy and neutral lipolysis. Furthermore, TFEB also regulates lipid transport processes such as lipid uptake and cholesterol efflux by CD36 and ATP-binding cassette transporter A1(ABCA1).

target genes, so as to adapt to the environment and restore their dynamic balance (Willett et al., 2017). However, the network of mechanisms regulating TFEB activity and function is complex and still not fully defined. At present, there is strong evidence that TFEB is strictly regulated by transcription, translation, post-translational modification, and protein-protein interaction.

Transcriptional Level, Non-coding RNA, and Translation

Transcription factor EB is regulated by different factors at the transcription level. The key stage of gene expression is the initiation of transcription, whose switch is the promoter, a segment of DNA sequence specifically recognized and bound with the ribonucleic acid polymerase. Most promoters are located upstream of the transcriptional starting point of structural genes, controlling the initiation time and the degree of expression to determine the activity of target genes. Some scholars have found that the occurrence of chromosomal translocation results from the fusion of the open reading frame of TFEB on chromosome 6 to the 5′ regulatory region of the non-protein encoding alpha gene on chromosome 11. Consequently, the TFEB promoter is replaced, contributing to the significant up-regulation of the TFEB transcription level and the expression of TFEB protein (Argani et al., 2005, 2016; Zhan et al., 2018). Forkhead box O1, a transcription factor that controls mitochondrial function and morphology, could interact with TFEB, drastically increasing the transcription and protein levels of TFEB through directly binding to its promoter (Liu et al., 2016). Non-receptor kinase Janus kinase 2 (JAK2) knockdown alleviates TFEB promoter activity, expression, and nuclear localization, subsequently blocking autophagy and lysosome function. The observations of *in silico* analysis and chromatin immunoprecipitation (ChIP) assays highlight that activating JAK2 encourages the binding of downstream transcription activator 1 and TFEB promoter, thus increasing the transcription activity of TFEB, achieving the purpose of reversing lysosomal dysfunction and restoring albumin permselectivity in glomerular disease (Alghamdi et al., 2017). Current reports showed that Krüppel-like factor 2 upregulated TFEB expression and promoter activity to exert anti-inflammatory effects in endothelial cells and diabetic mice (Song W. et al., 2019). In a recent study involving analysis by ChIP of primary hepatocytes and whole livers *in vivo* and vitro, hepatic spliced X-box binding protein 1 was found to enhance TFEB transcription and autophagy by occupying the 743 to 523 site of the promoter of TFEB (Zhang et al., 2020).

In addition to the regulation of promoter activity, recent studies have found that microRNA and long non-coding RNA (LncRNA) are involved in regulating the transcription of TFEB. TFEB is the target of microRNA-128 in concomitantly tuning the transcription of autophagy-related genes (Settembre et al., 2011; Tiribuzi et al., 2014). The process of renal cancer caused by high expression of TEFB is related to LncRNA fusion (Malouf et al., 2015; Xia et al., 2015; Pei et al., 2019). Further evidence also links RNA methylation with TFEB regulation. Methyltransferase like 3 (METTL3) reverses the transcriptional activity of TFEB by binding to the 3'-UTR end of TFEB and concomitantly methylating two m^6A residues, thus playing a protective role in ischemic heart disease (Song H. et al., 2019).

A novel modulations of TFEB activity at the translation level has emerged, in which TFEB translation is suppressed by both of MA3 domains within programmed cell death 4, which is a tumor suppressor propitious to lysosome dysfunction related diseases (Chen et al., 2020). Interestingly, salt-inducible kinase 2 (Negoita et al., 2019) and spermidine (Zhang et al., 2019) may affect the expression of TFEB in cells from the perspective of translation. Among the studies on the activity of TFEB, the most concerned is the modification of post-translational proteins, including acetylation, phosphorylation, SUMOylation, and ubiquitination.

Post-translational Modification

Phosphorylation and Dephosphorylation

Our current understanding of TFEB biology posits when cells are under stress, TFEB protein activity, and subcellular localization or its nuclear-cytoplasmic shuttling are driven by phosphorylation modification mainly. The basilic phosphorylation sites include S142, S211, and serine-rich sequences at the end. S122, S134, S138, S462, S463, S467, and S469 phosphorylation also regulates the nuclear translocation of TFEB.

Mammalian target of rapamycin complex 1 (mTORC1) is currently the most thoroughly studied kinases related to TFEB phosphorylation (Nnah et al., 2019). Studies have shown mTORC1 mediates the phosphorylation at the C-terminal S211 amino acid of TFEB (Martina et al., 2012). When nutrients are rich and energy is sufficient, vacuolar H^+-AT-Pase forms a complex interacting with Rag GTPase and other factors. 14-3-3 is a cytoplasmic chaperone protein that keeps TFEB isolated in the cytosol, which masks the nucleus entry signal after binding to TFEB, resulting in the loss of TFEB activity temporarily (Xu et al., 2019). The activated Rags recruit mTORC1 to the lysosome membrane through binding to the mTORC1 component raptor. At this time, the activated mTORC1 phosphorylates the S211 site of TFEB, which promotes 14-3-3 binding sites (Roczniak-Ferguson et al., 2012). Experiments with the above mechanism have proved that, due to rich nutrition, mTORC1 is increased to promote the phosphorylation of TFEB S211, which enhances hepatic steatosis and liver injury in high-fat diet-fed mice. Importantly, studies on hepatic biopsies of patients with non-alcoholic fatty liver disease revealed that the lower the activity of TFEB, the more serious the liver steatosis and lipid aggregation (Zhang H. et al., 2018). Conversely, when the nutrition is deprived, due to the inactivation of the RagGTP enzyme, mTORC1 dissociates from the surface of the lysosome and loses its activity, leading to the dissociation of the TFEB/14-3-3 complex. The rapid dephosphorylation of TFEB is transferred from the cytoplasm to the nucleus, thereby performing transcription function and activating downstream genes to maintain effective homeostasis.

Several other protein kinases have been found to affect the phosphorylation of TFEB. Adenosine monophosphate-activated protein kinase (AMPK), a central regulator of energy homeostasis, is recently shown to attenuate lipid accumulation and inflammation by promoting dephosphorylation and nuclear localization of TFEB independent of mTOR activity (Kim S. H. et al., 2017; Collodet et al., 2019). Extracellular signal-regulated kinase (ERK) phosphorylates the site of TFEB S142 and makes it relocated in the cytoplasm (Yonekawa et al., 2015; Li et al., 2019). Phosphorylation of TFEB S142 by mTOR/ERK inhibits nuclear translocation of TFEB in hepatocytes and thus promotes

hepatic cholesterol accumulation (Wang et al., 2020a). Whereas AKT (protein kinase B) negatively controls TFEB through phosphorylation at S467 (Palmieri et al., 2017). The inactivation of glycogen synthase kinase (GSK)-3β activates TFEB by dephosphorylation and nuclear translocation of TFEB (Li et al., 2018; Theeuwes et al., 2020). GSK-3β coordinates with mTORC1 by phosphorylating TFEB S134 and S138 sites increasing the phosphorylation of S211A with the resultant acceleration of the binding of TFEB to 14-3-3 protein and eventually inhibiting TFEB's nuclear transport. However, protein kinase C (PKC) might increase dephosphorylation and activation of TFEB by inactivating GSK3β. It is likely that PKC activators may be used as an effective treatment for lysosome-related disorders by activating TFEB to accelerate the degradation of accumulated lipid droplets (Li Y. et al., 2016). Besides, in the C-terminus of TFEB, multiple serine residues (i.e., S462, S463, S467, and S469) are phosphorylated by PKCβ for changing the TFEB protein stability instead of influencing subcellular localization (Ferron et al., 2013). As reported, TFEB is dephosphorylated by calcineurin. During starvation and physical exercise, calcineurin induction triggers the TFEB nuclear translocation synergistically with mTORC1 inhibition (Medina et al., 2015; Di Paola and Medina, 2019a; Pan et al., 2020).

Acetylation and Deacetylation

One of the modes of protein modification is acetylation modification, a process by which acetyl groups transfer to amino acid side chain groups (Yang et al., 2020). Researchers have found that acetylation modification of TFEB also affects its activity and localization. Histone acetyltransferase general control non-repressed protein 5 (GCN5) acetylates TFEB pointedly at K274 and K279. This results in the dimerization of TFEB being disrupted and the TFEB transcriptional activity reduced, accompanied by decreasing the biogenesis of autophagy and lysosome-mediated lipid hydrolysis (Scott et al., 2014; Wang et al., 2020b). On the contrary, cytosolic deacetylase histone deacetylase 6 (HDAC6) inhibition enhances the acetylation of TFEB and the TFEB nuclear localization initiating expression of downstream genes. However, the deacetylation site on TFEB and exact mechanisms remain unanswered (Brijmohan et al., 2018). Likewise, suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor that is observed to activate the lysosomal function in human cancer cells. These results disclose a novel form that SAHA provokes TFEB transcriptional activity by recruiting acetyl-coenzyme A acetyltransferase 1 (ACAT1) and histone deacetylase 2 (HDAC2) to TFEB and enhancing TFEB acetylation on the regions containing K91, K103, K116, and K430 (Zhang J. et al., 2018). There is also a report for the inhibitory role of TFEB acetylation at K116 in microglia lysosomal biosynthesis (Bao et al., 2016). The discrepancy about whether acetylation is capable of boosting or constraining the transcriptional activity of TFEB may be due to different research backgrounds including cell types and the nature of the stimuli used. Hence, it remains to be further investigated what the pronounced functionality of TFEB acetylation is in controlling its transcriptional activity. Besides, after being deacetylated into the nucleus, TFEB needs to be appropriately modified in the nucleus before functioning, and it is different from the dephosphorylation as mentioned above.

SUMOylation

Transcription factor EB is also modified by the small ubiquitin-like modifier (SUMO). SUMOylation does not promote protein degradation like ubiquitination, but strengthens protein stability, regulates protein interaction and positioning, affects protein transcription activity, which involves regulating protein functions and cell life activities. The research has mapped that SUMO1 performs SUMOylation on a lysine site of TFEB protein with attenuated transcriptional activity (Miller et al., 2005).

Ubiquitination

Ubiquitination, a post-translational modifier, was discovered to play a part in the TFEB function. As a chaperone-dependent E3 ubiquitin ligase, STIP1 homology and U-Box containing protein 1 interacts with phosphorylated TFEB preferentially and initiate TFEB degradation through a ubiquitin-proteasome pathway. Correspondingly, non-phosphorylated TFEB exposures the nuclear localization signal, which exerts its transcriptional activity to promote the expression of the autophagy–lysosomal genes (Sha et al., 2017).

Others

In addition to the above post-translational modifications, methylation and protein glycosylation are also common post-translational modifications, which are connected with TFEB. Methylation includes DNA methylation, RNA methylation, or protein methylation. There are research reports showing that TFEB may regulate hypo-methylated genes in type 2 diabetes (Liu et al., 2015). It has been mentioned previously that TFEB undergoes RNA methylation *via* the m^6A modification of its mRNA adenylate catalyzed by METTL3 (Song H. et al., 2019). Besides, TFEB activity is also regulated by a protein methyltransferase. Research has shown that under nutrient starvation, co-activator-associated arginine methyltransferase 1 (CARM1) and TFEB exhibit mutual combination and subsequently increases the levels of histone H3 Arg17 dimethylation and autophagy. CARM1 exerts transcriptional co-activator function through binding to the transcriptional activation domain of TFEB, whereas TFEB combines with the methyltransferase domain of CARM1 (Shin H. J. et al., 2016; Shin H. R. et al., 2016). An emerging phenomenon reveals that TFEB is an important upstream activator of some glycoproteins, which may be associated with increased levels of N-glycosylation and altered lysosomal protein activity in pancreatic ductal adenocarcinoma (Pan et al., 2014). However, it remains unclear on the specific mechanism responsible for TFEB regulating glycosylation and changes in the glycosylation levels of certain glycoproteins affecting the activity of TFEB.

As mentioned above, we briefly summarized those that are currently studied. It remains unclear how different pathways communicate with each other to regulate the activity of TFEB, and whether all of these different levels of modification ultimately affect lipid metabolism by regulating TFEB activity.

THE FUNCTION AND MOLECULAR MECHANISM OF TFEB REGULATING LIPID METABOLISM IN ATHEROSCLEROSIS

The Role of TFEB in Lipid Homeostasis

After the transcriptional activity of TFEB itself is regulated by many other factors, it is activated into the nucleus and further regulates downstream targets to change intracellular metabolic changes, such as lipid metabolism (Settembre et al., 2013). The adaptive response of organisms to changes in nutritional status is related to major transcriptional and metabolic changes, one of which prominently observed during nutrient deprivation is the increase in lipid catabolism. During this period, lipid metabolism is regulated by many transcription factors, nuclear receptors, and non-coding RNA, which quickly adapt to the current environment to maintain the basic homeostasis and support the energy requirements (Li et al., 2017). TFEB is one of the transcription factors that efficaciously regulate lipid metabolism. It regulates not only the lipid transport processes such as lipid uptake and cholesterol efflux but also the lipophagy mediated by intracellular lysosomal lipolysis and neutral lipolysis adjusted by fatty acid oxidation-related proteins (Zechner et al., 2012). Microarray analysis identified numerous genes of lipid and fatty acid catabolic process, fatty acid-binding, and transport, fatty acid oxidation, sphingolipid catabolic process, steroid catabolic process et al., such the expression of lipid metabolic process genes are perturbed by TFEB overexpression (Settembre et al., 2013). Furthermore, TFEB has been found to also work cooperatively with transcription factor E3 (TFE3) in controlling whole-body metabolism, including lipid catabolism, energy metabolism, glucose homeostasis, and mitochondrial β-oxidation (Pastore et al., 2017). Accordingly, if the focus of basic research is turned to the transcriptional regulation of lipid metabolism by TFEB, it sheds light on the more thorough understanding of lipid trafficking pathways and homeostasis.

TFEB and Lipid Transporters

Transcription factor EB influences the expression of CD36, scavenger receptor class A (SR-A), ATP-binding cassette transporter A1 (ABCA1), and other proteins to regulate lipid transport. The level of these transporters, which is responsible for cholesterol uptake and efflux on the surface of macrophages, is dynamically changed to deal with the environment stimulation (Ye et al., 2019). The results have proved that hypericin-mediated sonodynamic therapy (HY-SDT) promotes the nuclear translocation of TFEB by generating reactive oxygen species (ROS) in macrophages, with a resultant increase in the level of macrophage cholesterol efflux and the expression of ABCA1 protein, and also inhibition at the mRNA levels of CD36 and SR-A. These findings indicate that TFEB is a momentous regulator that promotes lipid degradation and efflux of macrophages, and exhibits the potential therapeutic effect of inhibiting lipid uptake in response to atherogenic lipid stressors. Besides, this result also unveils that TFEB is a crucial regulator that promotes autophagy activation and lysosomal regeneration to ultimately reduce lipid accumulation in macrophages and ameliorate lipid overload in atherosclerotic plaques (Li X. et al., 2016).

TFEB and Lipid Degradation

When nutrients are lacking, lipid droplets are broken down to release energy. Maintaining the dynamic balance of lipid flux requires the synergistic regulation of autophagy-dependent lipolysis and neutral lipolysis (Zechner et al., 2017; Khawar et al., 2019). Autophagy-dependent lipolysis is the major way to adapt to environmental challenges, allowing removal of excess substrates and repair of damaged organelles (Maan et al., 2018). While neutral lipolysis is the key basis for lipid degradation, which provides necessary lipids for autophagic cell membranes (Tall, 2017; Schott et al., 2019). Additionally, neutral lipolysis may be a powerful effector of autophagy-dependent lipolysis as a result of making oxidative phosphorylation more allowable for mitochondrion.

Autophagy is a long-term evolutionary and reserved degradation system in cells that maintains the stability of the intracellular environment by degrading protein aggregates and damaged organelles, playing a central role in lipid metabolism (Martinez-Lopez and Singh, 2015). The lipid droplets accumulated in the cytoplasm are wrapped by a double-layer membrane structure to form autophagosomes, which then are fused with the lysosomes. Thereafter, the lipid droplets shuttle to the lysosome and are then degraded by acid lipase to produce free cholesterol and free fatty acids (Chen K. et al., 2017). Among them, the free cholesterol flows out of the cytoplasm through the mediation of transmembrane protein ABCA1 and is metabolized along with the blood circulation back to the liver to participate in the process of reverse cholesterol transport (Ouimet et al., 2019). Whereas free fatty acids are effluxed and re-uptake into mitochondria, where they undergo β oxidation to produce energy or ketone bodies for supporting cellular energy requirements. This selective autophagy process, also specifically known as lipophagy, is the lipid conversion of intracytoplasmic lipid droplets through the autophagy pathway, and the main way for lipid metabolism caused by long-term hunger in the body. As TFEB is a key regulatory factor for autophagy and lysosome, some compelling evidence suggests that TFEB modulates the degradation and efflux of intracellular lipids by regulating lipophagy-related genes after transferring from the cytoplasm to the nucleus (Thomes et al., 2019).

This is substantiated by studies demonstrating that TFEB inhibition abolishes lipophagy and makes for the decomposition of cellular lipid in anti-non-alcoholic fatty liver disease (Wang et al., 2019). Furthermore, in mouse models of obesity, suppression of TFEB, and Atg7 by liver-specific gene deletion facilitate liver steatosis and weight-gain (Yang et al., 2010). Helix-Loop-Helix-30 (HLH-30), the TFEB ortholog as transcriptional switches, couple autophagy and lysosomal lipolysis to nutritional changes through mediating the efficient lipid clearance in Caenorhabditis Elegans, resulting in additive effects with controlling fat storage (O'Rourke and Ruvkun, 2013; Franco-Juárez et al., 2018). In agreement with this, a similar conserved pathway is replicable in human cells (Ji et al., 2020) and murine models (Visvikis et al., 2014; Nakamura and Yoshimori, 2018),

underscoring that HLH-30 or TFEB is conservative in linking autophagy to lipid homeostasis and lifespan. There are also reports for regulation by TFEB of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) (Finck and Kelly, 2006) and peroxisome proliferator-activated receptor α (PPARα) (Tan H. W. S. et al., 2019), the main regulatory factors of lipid metabolism. Support for this concept is the finding that PGC-1α activation promoted by TFEB targets the downstream PPARα and controls lipid degradation (Evans et al., 2019). In macrophages infected by mycobacteria, PPARα agonists promote the transcription of TFEB, lipid catabolism, fatty acid β-oxidation, and inhibit lipid formation for antimycobacterial effect (Kim Y. S. et al., 2017), which emphasizes the interplay between autophagy and lipid metabolism at the transcriptional level. Recent studies show that TFEB participates in store-operated calcium entry-controlled lipid metabolism, inducing lipophagy, and mobilization of fatty acid from lipid droplets (Maus et al., 2017; Zhu et al., 2018). Besides, TFEB plays a part in lipid catabolism mediated by phosphatidylinositol-5-phosphate 4-kinases required for autophagosome-lysosome fusion (Lundquist et al., 2018). These reliable reports provide proof that TFEB is connected closely with lipophagy *via* the autophagy-lysosome pathway. But the key of lipophagy is sufficient autophagic flux and complete autophagy-lysosome form and fusion pathway to produce enough autophagy cycles for phagocytosing excess lipids.

Neutral lipolysis is the process in which cytoplasmic lipases such as the key cytoplasmic lipase, adipose triglyceride lipase (ATGL), and hormone-sensitive lipase (HSL) exert the hydrolytic effect by directly attaching to the surface of lipid droplets, and serving as another capital intracellular lipid degradation mode. Together with lipophagy, lipolysis constitutes the key to the regulation of lipid droplet catabolism (Kloska et al., 2020). Studies have shown that lipase-driven decomposition of lipid droplets has proved part of its process depends on autophagy. Since it was observed that ATGL and HSL both contain motifs that directly interact with LC3, it is speculated that the resulting up-regulation of hepatic lipid phagocytosis has a synergistic effect with lipolysis (Martinez-Lopez et al., 2016; Cui et al., 2020). Similarly, ATGL activity may also be an influential checkpoint for regulating lipid phagocytosis, and ATGL overexpression may increase liver fat phagocytosis *via* sirtuin-1 (Sathyanarayan et al., 2017). Therefore, lipophagy and lipolysis are closely related. As the role of TFEB in lipophagy is becoming more and more clear, it is also important to explore the role of TFEB in the lipolysis process. While there are few related reports at present and further investigation is needed.

In addition to the aforementioned approaches involving autophagy-related targets in regulating lipid metabolism, TFEB also binds directly to cholesterol 7α-hydroxylase (CYP7A1) promoter in human and mouse hepatocytes, which augments bile acid synthesis resulting in cholesterol catabolism and degradation. This mechanism may contribute to the prevention of hypercholesterolemia and metabolic disorders. Bile acid-induced fibroblast growth factor 19 mediates feedback inhibition of TFEB activity and nuclear translocation *via* activating the mTOR/ERK pathway and phosphorylating TFEB (Wang et al., 2020a).

TFEB and Bioactive Lipid Mediators

It is well known that lipids metabolism produces many small molecules, acting as bioactive lipid mediators to participate in cell signaling pathways and mediate numerous biological effects (Huang and Freter, 2015). Such key bioactive lipids include fatty acids, diacylglycerol, sphingolipids, ceramide, sphingosine, sphingosine-1-phosphate, and others. TFEB promotes fatty acid oxidation in mitochondria and peroxisomes and inhibits fatty acid biosynthesis. Strikingly, TFEB action is a result of the different fatty acid types and duration of exposure to exogenous fatty acids in murine cardiomyocytes (Trivedi et al., 2020). TFEB content is decreased in a concentration- and time-dependent manner by palmitate, the saturated fatty acid, rather than polyunsaturated fatty acids. Animal models show that a high-fat and high-sucrose diet induces a temporary decline in nuclear TFEB, concomitant with the molecular events that lipid deposition is induced by marked elevation of diacylglycerol and triacylglycerol. Early studies show that some lysosomal enzymes that regulate the degradation of sphingolipids and glycogen become direct targets of TFEB (Palmieri et al., 2011), implying the indirect regulation of TFEB on lipid mediators. In murine (C57BL/6) lungs, TFEB/lipophagy induction substantially reduces cigarette smoke-mediated intracellular-ceramide-accumulation to normal levels (Bodas et al., 2019).

The Pathological Process of Atherosclerosis

Atherosclerosis is a chronic inflammation-related pathological process caused by various reasons (Yang M. Y. et al., 2018; Ray et al., 2019). The hallmarks of atherosclerosis are lipid accumulation and chronic inflammation of that arterial wall. Under the action of various risk factors such as smoking, obesity, hypertension, and dyslipidemia, the oxidized low-density lipoprotein (ox-LDL) causes endothelial damage and dysfunction ascribed to the state of hyperlipidemia (Negre-Salvayre et al., 2020), leading to increased levels of adhesion factors and chemokines. These cytokines promote the monocytes to be converted into macrophages to devour lipids through the adhesion at the injury site of arteries and migration to the subendothelial layer. The degree of lipid accumulation in macrophages represents the balance between extracellular lipid uptake and lipid reverse transport. When the influx and esterification of intracellular lipids are greater than the outflow, the accumulation of lipids occurs, and the excess lipids are stored in the lipid droplets. Therefore, macrophage-derived foam cells promote the formation of lipid striae and become the kernel of plaques (Guerrini and Gennaro, 2019). In parallel, smooth muscle cells of the medial membrane migrate into the subendothelial layer and gradually accumulate to form a new fibrous cap of atherosclerotic plaque, which enlarges the plaque to rupture injury. If the body is unable to promptly clear the apoptotic foam cells, a thrombus, and an inflammatory necrosis

core is formed, eventually leading to acute coronary syndrome (Paone et al., 2019).

The Importance of Lipid Homeostasis for Atherosclerosis

Many factors promote atherosclerosis, among which lipid metabolism disorder is a conspicuous pathological factor contributing to the formation and progression of atherosclerosis. It is generally believed that foam cells are the typical features of early atherosclerosis (Maguire et al., 2019). The main lipid types in these foam cells are free cholesterol and cholesterol esters, which are stored in the form of lipid droplets. In mammals, lipid droplets play a standout role in intracellular lipid balance. Damaged lipophagy causes lipid accumulation and deranges lipid metabolism, which aggravates atherosclerosis progress. Therefore, reducing lipid uptake, or mobilizing the release of cholesterol in the cytoplasm from lipid droplets, and enhancing lipid catabolism and efflux are principal for understanding the physiological mechanisms regulating lipid homeostasis and reversing lipid content of plaques (Yang M. et al., 2018). It provides a prominent theoretical basis for us to develop effective drug targets to reduce the formation of foam cells constituting an attractive method for the prevention and treatment of atherosclerosis. Over the past years, with the study of lipid homeostasis in atherosclerosis showing the situation of the explosive growth, the regulatory role of TFEB has also been gradually explored.

TFEB, Lipid Homeostasis, and Atherosclerosis

Transcription factor EB is ubiquitously expressed in multifarious cell lines, performing the functions of regulating lipid homeostasis in many diseases. For the reason that lipid metabolism is closely related to atherosclerosis, it is hypothesized that TFEB also regulates lipid metabolism in atherosclerosis. Therefore, the transcription function of TFEB has attracted the attention of scientific researchers in the atherosclerosis field.

Many studies have confirmed that TFEB inhibits the progression of atherosclerosis mainly by promoting lipid degradation. During the development of atherosclerosis, lipids are catabolized in lysosomes. Adequate lipid catabolism has the effect of delaying the progression of atherosclerosis. However, excessive lipid accumulation in lysosomes may cause progressive dysregulation of lysosomes and the autophagy function. Therefore, lysosomes show the characteristics of larger volume and decreased protein degradation ability, which aggravates the deterioration of the plaque microenvironment and accelerates the plaque rupture. It is reported that the promotion of macrophage autophagy metabolizes lipid droplets into free fatty acids, which is of stupendous significance in promoting lipid efflux in atherosclerosis.

As a regulator of the autophagy-lysosome pathway, TFEB up-regulates the expression of 2/3 autophagy-lysosome-related genes. Studies have found that TFEB overexpression promotes the synthesis of lysosomes and related enzymes to rescue damaged lysosomes. Enhancing autophagy promotes the clearance of damaged proteins, alleviates lipid-induced macrophage lysosomal dysfunction, and provokes lipid metabolism (Emanuel et al., 2014). In vascular smooth muscle cells, proteomic analysis, and database searching identified an appealing protein stearoyl-CoA desaturase 1 (SCD1). Overexpressed SCD1 has a promotional effect on the TFEB nuclear translocation and its reporter activity, mediating lipophagy for inhibiting foam cell formation (Pi et al., 2019). Analogously, in $APOE^{-/-}$ mice with macrophage depletion, sorting nexin 10 deficiency enhances TFEB nuclear translocation exerting the effect of promoting activity of lysosomal acid lipase and mitochondrial fatty acid oxidation, coinciding with interrupting the internalization of CD36 to prevent the uptake of lipids, thereby inhibiting foam cell formation (You et al., 2020). TFEB overexpression also inhibits atherosclerosis plaques from the aspect of promoting lipid transport. Li et al. demonstrated that TFEB was shown to promote cholesterol and fatty acid efflux by enhancing the expression of ABCA1 while inhibiting lipid uptake by suppression of CD36 expression (Li X. et al., 2016).

APPLICATION OF TFEB AS A TARGET IN DISEASE TREATMENT

Based on the understanding of the above regulatory mechanisms, the function of TFEB in lipid metabolism is increasingly clear. Recent research effort has devoted into developing drugs targeting the regulation of TFEB to treat several diseases in which a defective lipid and energy metabolism is a key contributor to the disease pathogenesis. Indeed, some gratifying progress has been made. Three lead compounds as TFEB efficacious agonists have been identified through a nanotechnology-enabled high-throughput screening strategy, embracing digoxin, ikarugamycin, and alexidine dihydrochloride, which belong to a small cohort of clinically approved drugs, the marine-derived natural product, and synthetic small molecules, respectively. These compounds engage TFEB activation pathways *via* three distinct mobilizations of $Ca2^{+}$-dependent mechanisms, which improve lipid metabolism with relieviated steatosis in C57BL/6 mice fed a high-fat diet (Wang et al., 2017). Procyanidin B2, a naturally occurring phenolic compound, holds the promise to be developed as a novel drug for the treatment of non-alcoholic fatty liver disease, as its function in modulating lysosomal pathway and redox state mediated by TFEB to attenuate free fatty acids-induced hepatic steatosis (Su et al., 2018). The cholesterol-lowering agent ezetimibe is widely used for hypercholesterolemia. In mice treated with a methionine- and choline-deficient diet, ezetimibe attenuates lipid accumulation and steatohepatitis by autophagy activation through phosphorylating AMPK and promoting TFEB nuclear translocation (Kim S. H. et al., 2017). As a natural product, formononetin has been shown to protect against hepatosteatosis by promoting TFEB-mediated lipophagy (Wang et al., 2019). A very recent study reveals that dihydromyricetin initiates autophagic cutaneous squamous cell carcinoma (CSCC) cell death through inducing TFEB (S142) dephosphorylation and

increasing the activity of TFEB reporter, which is conducive to CSCC chemotherapy (Tan M. et al., 2019).

As noted above, some small-molecule substances regulate the activity of TFEB in atherosclerosis. Disaccharide trehalose is shown to exhibit atheroprotective effects. As a beneficial inducer of TFEB, disaccharide trehalose orchestrates the functional autophagy-lysosome system for lipid degradation in the atherosclerotic plaque (Sergin et al., 2017; Evans et al., 2018). HY-SDT was found to be effective in improving the progression of atherosclerosis through triggering TFEB nuclear translocation and promoting lipid degradation and efflux, decreasing the lipid content (Li X. et al., 2016). The research on TFEB linked with atherosclerosis is still in the early stage. Current research is only limited to the discovery of several small molecular substances that might be used as TFEB agonists to inhibit plaques in mice. Studies have proved that the overexpression of TFEB reduces the samples of human atherosclerotic plaques (Sergin et al., 2017), but no agent targeting TFEB has been found to treat atherosclerosis patients. Nevertheless, these findings are encouraging in terms of potential therapeutic application, as an interventional treatment after disease onset would be a valuable advantage.

CONCLUDING REMARKS

As an important transcription factor that regulates cell homeostasis and body pathogenesis, TFEB has attracted tremendous attention in various fields credited with its transcription function system. The unambiguous mechanism of its activity remains to be elucidated. Although post-translational modification is considered to be the main way to regulate TFEB activity and intracellular localization, currently only phosphorylation modification has received thorough research, whereas the other powerful regulation machinery, such as acetylation, SUMOylation, and ubiquitination, need to be explored. It is also necessary to determine the particular sites of post-translational modifications regulating the TFEB function, which is also challenging. Another vital issue to be solved is how TFEB is transferred to the cytoplasm once it enters the nucleus, and how TFEB export from the nucleus is regulated and whether it exerts additional roles.

Under stress conditions, TFEB undergoes nuclear translocation and binds to the corresponding DNA fragments to regulate the expression of proteins related to the autophagy-lysosome pathway. The information on environmental cues is transmitted to the nucleus to trigger a transcription response. Particularly, TFEB links different organelles such as lysosomes and the nucleus to enhance overall metabolism. There have been many data proving the importance of TFEB in all levels of the body, but it is still necessary to clarify the idiographic cellular function mediated by TFEB under different research background such as different types of cells or tissue and the nature of external stress conditions.

Due to its involvement in the intracellular clearance pathway, TFEB has been shown as an essential factor for lipid degradation and has made preliminary progress in regulating lipid transporters and lipid mediators. However, more high-quality, in-depth study of its definite mechanisms are still needed, and the comprehensive regulatory network of TFEB in lipid metabolism awaits perfection, considering the limited understanding of TFEB in lipid synthesis, mobilization, esterification, transport. Lipid disorders and inflammation are the two main characteristics of atherosclerosis. The regulation mechanism of TFEB in immune inflammation has been roughly determined (Brady et al., 2018b), but the regulation of lipid homeostasis of TFEB in atherosclerosis needs to be fully studied.

With the continuous deepening of molecular biology research, various mechanisms regulating TFEB and targeted drugs have been understood to a certain extent. However, the current research is only limited to the transcription level and the protein level and mainly focuses on the dephosphorylation of TFEB which gets into the nucleus and increases its activity. There are no intervention drugs to be able to modulate the acetylation of TFEB protein. Undoubtedly, it is a demand to explore effective drugs targeting TFEB continuously. In conclusion, the transformation from TFEB basic research into clinical applications is crucial. An in-depth exploration of the explicit mode of action of TFEB on lipid metabolism and the dialogue mechanism with other signaling pathways will improve our understanding on the regulation mechanism of lipid metabolism in atherosclerosis and provide new perspectives and strategies for the prevention and treatment of related metabolic diseases.

AUTHOR CONTRIBUTIONS

ML, HL, and LY designed and wrote the manuscript. All authors edited the manuscript.

FUNDING

This review was funded by the National Natural Science Foundation of China (91939104), National Natural Science Foundation of China (82070465), Open Project of National Key Laboratory of Cardiovascular Disease (2019kf-02), Open Project of Key Laboratory of the Ministry of Education of Myocardial Ischemia (KF201908), Scientific Research Project of Heilongjiang Health and Health Commission (2020-075), National Undergraduate Innovation and Entrepreneurship Training Program (202010226014), The Innovation and Entrepreneurship Training Program for Harbin Medical University Students (201910226002), and Postgraduate academic exchange program of Harbin medical university.

REFERENCES

Alghamdi, T. A., Majumder, S., Thieme, K., Batchu, S. N., White, K. E., Liu, Y., et al. (2017). Janus kinase 2 regulates transcription factor eb expression and autophagy completion in glomerular podocytes. *J. Am. Soc. Nephrol.* 28, 2641–2653. doi: 10.1681/asn.2016111208

Argani, P., Laé, M., Hutchinson, B., Reuter, V. E., Collins, M. H., Perentesis, J., et al. (2005). Renal carcinomas with the t(6;11)(p21;q12): clinicopathologic features and demonstration of the specific alpha-TFEB gene fusion by immunohistochemistry, RT-PCR, and DNA PCR. *Am. J. Surg. Pathol.* 29, 230–240. doi: 10.1097/01.pas.0000146007.54092.37

Argani, P., Reuter, V. E., Zhang, L., Sung, Y. S., Ning, Y., Epstein, J. I., et al. (2016). TFEB-amplified renal cell carcinomas: an aggressive molecular subset demonstrating variable melanocytic marker expression and morphologic heterogeneity. *Am. J. Surg. Pathol.* 40, 1484–1495. doi: 10.1097/pas.0000000000000720

Bao, J., Zheng, L., Zhang, Q., Li, X., Zhang, X., Li, Z., et al. (2016). Deacetylation of TFEB promotes fibrillar Aβ degradation by upregulating lysosomal biogenesis in microglia. *Protein Cell* 7, 417–433. doi: 10.1007/s13238-016-0269-2

Bodas, M., Pehote, G., Silverberg, D., Gulbins, E., and Vij, N. (2019). Autophagy augmentation alleviates cigarette smoke-induced CFTR-dysfunction, ceramide-accumulation and COPD-emphysema pathogenesis. *Free Radic. Biol. Med.* 131, 81–97. doi: 10.1016/j.freeradbiomed.2018.11.023

Brady, O. A., Jeong, E., Martina, J. A., Pirooznia, M., Tunc, I., and Puertollano, R. (2018a). The transcription factors TFE3 and TFEB amplify p53 dependent transcriptional programs in response to DNA damage. *Elife* 7:e40856. doi: 10.7554/eLife.40856

Brady, O. A., Martina, J. A., and Puertollano, R. (2018b). Emerging roles for TFEB in the immune response and inflammation. *Autophagy* 14, 181–189. doi: 10.1080/15548627.2017.1313943

Brijmohan, A. S., Batchu, S. N., Majumder, S., Alghamdi, T. A., Thieme, K., McGaugh, S., et al. (2018). HDAC6 inhibition promotes transcription factor EB activation and is protective in experimental kidney disease. *Front. Pharmacol.* 9:34. doi: 10.3389/fphar.2018.00034

Chen, K., Yuan, R., Zhang, Y., Geng, S., and Li, L. (2017). Tollip deficiency alters atherosclerosis and steatosis by disrupting lipophagy. *J. Am. Heart Assoc.* 6:e004078. doi: 10.1161/jaha.116.004078

Chen, L., Wang, K., Long, A., Jia, L., Zhang, Y., Deng, H., et al. (2017). Fasting-induced hormonal regulation of lysosomal function. *Cell Res.* 27, 748–763. doi: 10.1038/cr.2017.45

Chen, X., Guan, Y., Zhang, Y., Jia, Y., Li, W., Guo, C., et al. (2020). Programmed cell death 4 modulates lysosomal function by inhibiting TFEB translation. *Cell Death Differ.* doi: 10.1038/s41418-020-00646-2 [Epub ahead of print].

Collodet, C., Foretz, M., Deak, M., Bultot, L., Metairon, S., Viollet, B., et al. (2019). AMPK promotes induction of the tumor suppressor FLCN through activation of TFEB independently of mTOR. *FASEB J.* 33, 12374–12391. doi: 10.1096/fj.201900841R

Cui, W., Sathyanarayan, A., Lopresti, M., Aghajan, M., Chen, C., and Mashek, D. G. (2020). Lipophagy-derived fatty acids undergo extracellular efflux via lysosomal exocytosis. *Autophagy* doi: 10.1080/15548627.2020.1728097 [Epub ahead of print].

Curnock, R., Calcagni, A., Ballabio, A., and Cullen, P. J. (2019). TFEB controls retromer expression in response to nutrient availability. *J. Cell Biol.* 218, 3954–3966. doi: 10.1083/jcb.201903006

Di Paola, S., and Medina, D. L. (2019a). Ca(2+)-dependent regulation of TFEB and lysosomal function. *Methods Mol. Biol.* 1925, 145–155. doi: 10.1007/978-1-4939-9018-4_13

Di Paola, S., and Medina, D. L. (2019b). TRPML1-/TFEB-dependent regulation of lysosomal exocytosis. *Methods Mol. Biol.* 1925, 143–144. doi: 10.1007/978-1-4939-9018-4_12

Emanuel, R., Sergin, I., Bhattacharya, S., Turner, J., Epelman, S., Settembre, C., et al. (2014). Induction of lysosomal biogenesis in atherosclerotic macrophages can rescue lipid-induced lysosomal dysfunction and downstream sequelae. *Arterioscler. Thromb. Vasc. Biol.* 34, 1942–1952. doi: 10.1161/atvbaha.114.303342

Evans, T. D., Jeong, S. J., Zhang, X., Sergin, I., and Razani, B. (2018). TFEB and trehalose drive the macrophage autophagy-lysosome system to protect against atherosclerosis. *Autophagy* 14, 724–726. doi: 10.1080/15548627.2018.1434373

Evans, T. D., Zhang, X., Jeong, S. J., He, A., Song, E., Bhattacharya, S., et al. (2019). TFEB drives PGC-1α expression in adipocytes to protect against diet-induced metabolic dysfunction. *Sci. Signal.* 12:eaau2281. doi: 10.1126/scisignal.aau2281

Fan, Y., Lu, H., Liang, W., Garcia-Barrio, M. T., Guo, Y., Zhang, J., et al. (2018). Endothelial TFEB (transcription factor EB) positively regulates postischemic angiogenesis. *Circ. Res.* 122, 945–957. doi: 10.1161/circresaha.118.312672

Ferron, M., Settembre, C., Shimazu, J., Lacombe, J., Kato, S., Rawlings, D. J., et al. (2013). A RANKL-PKCβ-TFEB signaling cascade is necessary for lysosomal biogenesis in osteoclasts. *Genes Dev.* 27, 955–969. doi: 10.1101/gad.213827.113

Finck, B. N., and Kelly, D. P. (2006). PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. *J. Clin. Invest.* 116, 615–622. doi: 10.1172/jci27794

Fisher, D. E., Carr, C. S., Parent, L. A., and Sharp, P. A. (1991). TFEB has DNA-binding and oligomerization properties of a unique helix-loop-helix/leucine-zipper family. *Genes Dev.* 5, 2342–2352. doi: 10.1101/gad.5.12a.2342

Franco-Juárez, B., Mejía-Martínez, F., Moreno-Arriola, E., Hernández-Vázquez, A., Gómez-Manzo, S., Marcial-Quino, J., et al. (2018). A high glucose diet induces autophagy in a HLH-30/TFEB-dependent manner and impairs the normal lifespan of C. elegans. *Aging (Albany N. Y.)* 10, 2657–2667. doi: 10.18632/aging.101577

Guerrini, V., and Gennaro, M. L. (2019). Foam cells: one size doesn't fit all. *Trends Immunol.* 40, 1163–1179. doi: 10.1016/j.it.2019.10.002

Herrington, W., Lacey, B., Sherliker, P., Armitage, J., and Lewington, S. (2016). Epidemiology of atherosclerosis and the potential to Reduce the global burden of atherothrombotic disease. *Circ. Res.* 118, 535–546. doi: 10.1161/circresaha.115.307611

Huang, C., and Freter, C. (2015). Lipid metabolism, apoptosis and cancer therapy. *Int. J. Mol. Sci.* 16, 924–949. doi: 10.3390/ijms16010924

Ji, Y. J., Ugolino, J., Zhang, T., Lu, J., Kim, D., and Wang, J. (2020). C9orf72/ALFA-1 controls TFEB/HLH-30-dependent metabolism through dynamic regulation of Rag GTPases. *PLoS Genet.* 16:e1008738. doi: 10.1371/journal.pgen.1008738

Khawar, M. B., Gao, H., and Li, W. (2019). Autophagy and lipid metabolism. *Adv. Exp. Med. Biol.* 1206, 359–374. doi: 10.1007/978-981-15-0602-4_17

Kim, S. H., Kim, G., Han, D. H., Lee, M., Kim, I., Kim, B., et al. (2017). Ezetimibe ameliorates steatohepatitis via AMP activated protein kinase-TFEB-mediated activation of autophagy and NLRP3 inflammasome inhibition. *Autophagy* 13, 1767–1781. doi: 10.1080/15548627.2017.1356977

Kim, Y. S., Lee, H. M., Kim, J. K., Yang, C. S., Kim, T. S., Jung, M., et al. (2017). PPAR-α activation mediates innate host defense through induction of tfeb and lipid catabolism. *J. Immunol.* 198, 3283–3295. doi: 10.4049/jimmunol.1601920

Kloska, A., Wêsierska, M., Malinowska, M., Gabig-Cimińska, M., and Jakóbkiewicz-Banecka, J. (2020). Lipophagy and lipolysis status in lipid storage and lipid metabolism diseases. *Int. J. Mol. Sci.* 21:6113. doi: 10.3390/ijms21176113

LeBlanc, J. F., McLane, K. E., Parren, P. W., Burton, D. R., and Ghazal, P. (1998). Recognition properties of a sequence-specific DNA binding antibody. *Biochemistry* 37, 6015–6022. doi: 10.1021/bi9730792

Li, L., Friedrichsen, H. J., Andrews, S., Picaud, S., Volpon, L., Ngeow, K., et al. (2018). A TFEB nuclear export signal integrates amino acid supply and glucose availability. *Nat. Commun.* 9:2685. doi: 10.1038/s41467-018-04849-7

Li, S., Song, Y., Quach, C., Guo, H., Jang, G. B., Maazi, H., et al. (2019). Transcriptional regulation of autophagy-lysosomal function in BRAF-driven melanoma progression and chemoresistance. *Nat. Commun.* 10:1693. doi: 10.1038/s41467-019-09634-8

Li, X., Zhang, X., Zheng, L., Kou, J., Zhong, Z., Jiang, Y., et al. (2016). Hypericin-mediated sonodynamic therapy induces autophagy and decreases lipids in THP-1 macrophage by promoting ROS-dependent nuclear translocation of TFEB. *Cell Death Dis.* 7:e2527. doi: 10.1038/cddis.2016.433

Li, Y., Ma, Z., Jiang, S., Hu, W., Li, T., Di, S., et al. (2017). A global perspective on FOXO1 in lipid metabolism and lipid-related diseases. *Prog. Lipid Res.* 66, 42–49. doi: 10.1016/j.plipres.2017.04.002

Li, Y., Xu, M., Ding, X., Yan, C., Song, Z., Chen, L., et al. (2016). Protein kinase C controls lysosome biogenesis independently of mTORC1. *Nat. Cell Biol.* 18, 1065–1077. doi: 10.1038/ncb3407

Libby, P., Ridker, P. M., and Hansson, G. K. (2011). Progress and challenges in translating the biology of atherosclerosis. *Nature* 473, 317–325. doi: 10.1038/nature10146

Liu, F., Sun, Q., Wang, L., Nie, S., and Li, J. (2015). Bioinformatics analysis of abnormal DNA methylation in muscle samples from monozygotic twins discordant for type 2 diabetes. *Mol. Med. Rep.* 12, 351–356. doi: 10.3892/mmr.2015.3452

Liu, L., Tao, Z., Zheng, L. D., Brooke, J. P., Smith, C. M., Liu, D., et al. (2016). FoxO1 interacts with transcription factor EB and differentially regulates mitochondrial uncoupling proteins via autophagy in adipocytes. *Cell Death Discov.* 2:16066. doi: 10.1038/cddiscovery.2016.66

Lundquist, M. R., Goncalves, M. D., Loughran, R. M., Possik, E., Vijayaraghavan, T., Yang, A., et al. (2018). Phosphatidylinositol-5-phosphate 4-kinases regulate cellular lipid metabolism by facilitating autophagy. *Mol. Cell* 70, 531–544.e539. doi: 10.1016/j.molcel.2018.03.037

Luo, J., Yang, H., and Song, B. L. (2020). Mechanisms and regulation of cholesterol homeostasis. *Nat. Rev. Mol. Cell Biol* 21, 225–245. doi: 10.1038/s41580-019-0190-7

Maan, M., Peters, J. M., Dutta, M., and Patterson, A. D. (2018). Lipid metabolism and lipophagy in cancer. *Biochem. Biophys. Res. Commun.* 504, 582–589. doi: 10.1016/j.bbrc.2018.02.097

Maguire, E. M., Pearce, S. W. A., and Xiao, Q. (2019). Foam cell formation: a new target for fighting atherosclerosis and cardiovascular disease. *Vascul. Pharmacol.* 112, 54–71. doi: 10.1016/j.vph.2018.08.002

Malouf, G. G., Zhang, J., Yuan, Y., Compérat, E., Rouprêt, M., Cussenot, O., et al. (2015). Characterization of long non-coding RNA transcriptome in clear-cell renal cell carcinoma by next-generation deep sequencing. *Mol. Oncol.* 9, 32–43. doi: 10.1016/j.molonc.2014.07.007

Martina, J. A., Chen, Y., Gucek, M., and Puertollano, R. (2012). MTORC1 functions as a transcriptional regulator of autophagy by preventing nuclear transport of TFEB. *Autophagy* 8, 903–914. doi: 10.4161/auto.19653

Martinez-Lopez, N., Garcia-Macia, M., Sahu, S., Athonvarangkul, D., Liebling, E., Merlo, P., et al. (2016). Autophagy in the CNS and periphery coordinate lipophagy and lipolysis in the brown adipose tissue and liver. *Cell Metab.* 23, 113–127. doi: 10.1016/j.cmet.2015.10.008

Martinez-Lopez, N., and Singh, R. (2015). Autophagy and lipid droplets in the liver. *Annu. Rev. Nutr.* 35, 215–237. doi: 10.1146/annurev-nutr-071813-105336

Maus, M., Cuk, M., Patel, B., Lian, J., Ouimet, M., Kaufmann, U., et al. (2017). Store-operated Ca(2+) entry controls induction of lipolysis and the transcriptional reprogramming to lipid metabolism. *Cell Metab.* 25, 698–712. doi: 10.1016/j.cmet.2016.12.021

Medina, D. L., Di Paola, S., Peluso, I., Armani, A., De Stefani, D., Venditti, R., et al. (2015). Lysosomal calcium signalling regulates autophagy through calcineurin and TFEB. *Nat. Cell Biol.* 17, 288–299. doi: 10.1038/ncb3114

Mialet-Perez, J., and Vindis, C. (2017). Autophagy in health and disease: focus on the cardiovascular system. *Essays Biochem.* 61, 721–732. doi: 10.1042/ebc20170022

Miller, A. J., Levy, C., Davis, I. J., Razin, E., and Fisher, D. E. (2005). Sumoylation of MITF and its related family members TFE3 and TFEB. *J. Biol. Chem.* 280, 146–155. doi: 10.1074/jbc.M411757200

Nakamura, S., and Yoshimori, T. (2018). Autophagy and longevity. *Mol. Cells* 41, 65–72. doi: 10.14348/molcells.2018.2333

Napolitano, G., and Ballabio, A. (2016). TFEB at a glance. *J. Cell Sci.* 129, 2475–2481. doi: 10.1242/jcs.146365

Negoita, F., Säll, J., Morén, B., Stenkula, K., and Göransson, O. (2019). Salt-inducible kinase 2 regulates TFEB and is required for autophagic flux in adipocytes. *Biochem. Biophys. Res. Commun.* 508, 775–779. doi: 10.1016/j.bbrc.2018.11.177

Negre-Salvayre, A., Guerby, P., Gayral, S., Laffargue, M., and Salvayre, R. (2020). Role of reactive oxygen species in atherosclerosis: lessons from murine genetic models. *Free Radic. Biol. Med.* 149, 8–22. doi: 10.1016/j.freeradbiomed.2019.10.011

Nnah, I. C., Wang, B., Saqcena, C., Weber, G. F., Bonder, E. M., Bagley, D., et al. (2019). TFEB-driven endocytosis coordinates MTORC1 signaling and autophagy. *Autophagy* 15, 151–164. doi: 10.1080/15548627.2018.1511504

O'Rourke, E. J., and Ruvkun, G. (2013). MXL-3 and HLH-30 transcriptionally link lipolysis and autophagy to nutrient availability. *Nat. Cell Biol.* 15, 668–676. doi: 10.1038/ncb2741

Ouimet, M., Barrett, T. J., and Fisher, E. A. (2019). HDL and reverse cholesterol transport. *Circ. Res.* 124, 1505–1518. doi: 10.1161/circresaha.119.312617

Palmieri, M., Impey, S., Kang, H., di Ronza, A., Pelz, C., Sardiello, M., et al. (2011). Characterization of the CLEAR network reveals an integrated control of cellular clearance pathways. *Hum. Mol. Genet.* 20, 3852–3866. doi: 10.1093/hmg/ddr306

Palmieri, M., Pal, R., Nelvagal, H. R., Lotfi, P., Stinnett, G. R., Seymour, M. L., et al. (2017). mTORC1-independent TFEB activation via Akt inhibition promotes cellular clearance in neurodegenerative storage diseases. *Nat. Commun.* 8:14338. doi: 10.1038/ncomms14338

Pan, B., Li, J., Parajuli, N., Tian, Z., Wu, P., Lewno, M. T., et al. (2020). The calcineurin-TFEB-p62 pathway mediates the activation of cardiac macroautophagy by proteasomal malfunction. *Circ. Res.* 127, 502–518. doi: 10.1161/circresaha.119.316007

Pan, S., Chen, R., Tamura, Y., Crispin, D. A., Lai, L. A., May, D. H., et al. (2014). Quantitative glycoproteomics analysis reveals changes in N-glycosylation level associated with pancreatic ductal adenocarcinoma. *J. Proteome Res.* 13, 1293–1306. doi: 10.1021/pr4010184

Paone, S., Baxter, A. A., Hulett, M. D., and Poon, I. K. H. (2019). Endothelial cell apoptosis and the role of endothelial cell-derived extracellular vesicles in the progression of atherosclerosis. *Cell.Mol. Life Sci.* 76, 1093–1106. doi: 10.1007/s00018-018-2983-9

Pastore, N., Vainshtein, A., Klisch, T. J., Armani, A., Huynh, T., Herz, N. J., et al. (2017). TFE3 regulates whole-body energy metabolism in cooperation with TFEB. *EMBO Mol. Med.* 9, 605–621. doi: 10.15252/emmm.201607204

Pei, J., Cooper, H., Flieder, D. B., Talarchek, J. N., Al-Saleem, T., Uzzo, R. G., et al. (2019). NEAT1-TFE3 and KAT6A-TFE3 renal cell carcinomas, new members of MiT family translocation renal cell carcinoma. *Mod. Pathol.* 32, 710–716. doi: 10.1038/s41379-018-0191-7

Pi, H., Wang, Z., Liu, M., Deng, P., Yu, Z., Zhou, Z., et al. (2019). SCD1 activation impedes foam cell formation by inducing lipophagy in oxLDL-treated human vascular smooth muscle cells. *J. Cell. Mol. Med.* 23, 5259–5269. doi: 10.1111/jcmm.14401

Ray, K. K., Corral, P., Morales, E., and Nicholls, S. J. (2019). Pharmacological lipid-modification therapies for prevention of ischaemic heart disease: current and future options. *Lancet* 394, 697–708. doi: 10.1016/s0140-6736(19)31950-6

Roczniak-Ferguson, A., Petit, C. S., Froehlich, F., Qian, S., Ky, J., Angarola, B., et al. (2012). The transcription factor TFEB links mTORC1 signaling to transcriptional control of lysosome homeostasis. *Sci. Signal.* 5:ra42. doi: 10.1126/scisignal.2002790

Sathyanarayan, A., Mashek, M. T., and Mashek, D. G. (2017). ATGL promotes autophagy/lipophagy via SIRT1 to control hepatic lipid droplet catabolism. *Cell Rep* 19, 1–9. doi: 10.1016/j.celrep.2017.03.026

Schott, M. B., Weller, S. G., Schulze, R. J., Krueger, E. W., Drizyte-Miller, K., Casey, C. A., et al. (2019). Lipid droplet size directs lipolysis and lipophagy catabolism in hepatocytes. *J. Cell Biol* 218, 3320–3335. doi: 10.1083/jcb.201803153

Scott, I., Webster, B. R., Chan, C. K., Okonkwo, J. U., Han, K., and Sack, M. N. (2014). GCN5-like protein 1 (GCN5L1) controls mitochondrial content through coordinated regulation of mitochondrial biogenesis and mitophagy. *J. Biol. Chem.* 289, 2864–2872. doi: 10.1074/jbc.M113.521641

Sergin, I., Evans, T. D., Zhang, X., Bhattacharya, S., Stokes, C. J., Song, E., et al. (2017). Exploiting macrophage autophagy-lysosomal biogenesis as a therapy for atherosclerosis. *Nat. Commun.* 8:15750. doi: 10.1038/ncomms15750

Settembre, C., De Cegli, R., Mansueto, G., Saha, P. K., Vetrini, F., Visvikis, O., et al. (2013). TFEB controls cellular lipid metabolism through a starvation-induced autoregulatory loop. *Nat. Cell Biol.* 15, 647–658. doi: 10.1038/ncb2718

Settembre, C., Di Malta, C., Polito, V. A., Garcia Arencibia, M., Vetrini, F., Erdin, S., et al. (2011). TFEB links autophagy to lysosomal biogenesis. *Science* 332, 1429–1433. doi: 10.1126/science.1204592

Sha, Y., Rao, L., Settembre, C., Ballabio, A., and Eissa, N. T. (2017). STUB1 regulates TFEB-induced autophagy-lysosome pathway. *EMBO J.* 36, 2544–2552. doi: 10.15252/embj.201796699

Shin, H. J., Kim, H., Oh, S., Lee, J. G., Kee, M., Ko, H. J., et al. (2016). AMPK-SKP2-CARM1 signalling cascade in transcriptional regulation of autophagy. *Nature* 534, 553–557. doi: 10.1038/nature18014

Shin, H. R., Kim, H., Kim, K. I., and Baek, S. H. (2016). Epigenetic and transcriptional regulation of autophagy. *Autophagy* 12, 2248–2249. doi: 10.1080/15548627.2016.1214780

Song, H., Feng, X., Zhang, H., Luo, Y., Huang, J., Lin, M., et al. (2019). METTL3 and ALKBH5 oppositely regulate m(6)A modification of TFEB mRNA, which dictates the fate of hypoxia/reoxygenation-treated cardiomyocytes. *Autophagy* 15, 1419–1437. doi: 10.1080/15548627.2019.1586246

Song, W., Zhang, C. L., Gou, L., He, L., Gong, Y. Y., Qu, D., et al. (2019). Endothelial TFEB (transcription factor EB) restrains IKK (IκB Kinase)-p65 pathway to attenuate vascular inflammation in diabetic db/db Mice. *Arterioscler. Thromb. Vasc. Biol.* 39, 719–730. doi: 10.1161/atvbaha.119.312316

Su, H., Li, Y., Hu, D., Xie, L., Ke, H., Zheng, X., et al. (2018). Procyanidin B2 ameliorates free fatty acids-induced hepatic steatosis through regulating TFEB-mediated lysosomal pathway and redox state. *Free Radic. Biol. Med.* 126, 269–286. doi: 10.1016/j.freeradbiomed.2018.08.024

Tall, A. R. (2017). Increasing lipolysis and reducing atherosclerosis. *N. Engl. J. Med.* 377, 280–283. doi: 10.1056/NEJMe1706907

Tan, H. W. S., Anjum, B., Shen, H. M., Ghosh, S., Yen, P. M., and Sinha, R. A. (2019). Lysosomal inhibition attenuates peroxisomal gene transcription via suppression of PPARA and PPARGC1A levels. *Autophagy* 15, 1455–1459. doi: 10.1080/15548627.2019.1609847

Tan, M., Jiang, B., Wang, H., Ouyang, W., Chen, X., Wang, T., et al. (2019). Dihydromyricetin induced lncRNA MALAT1-TFEB-dependent autophagic cell death in cutaneous squamous cell carcinoma. *J. Cancer* 10, 4245–4255. doi: 10.7150/jca.32807

Theeuwes, W. F., Gosker, H. R., Schols, A., Langen, R. C. J., and Remels, A. H. V. (2020). Regulation of PGC-1α expression by a GSK-3β-TFEB signaling axis in skeletal muscle. *Biochim. Biophys. Acta Mol. Cell Res.* 1867:118610. doi: 10.1016/j.bbamcr.2019.118610

Thelen, A. M., and Zoncu, R. (2017). Emerging roles for the lysosome in lipid metabolism. *Trends Cell Biol.* 27, 833–850. doi: 10.1016/j.tcb.2017.07.006

Thomes, P. G., Rasineni, K., Yang, L., Donohue, T. M. Jr., Kubik, J. L., McNiven, M. A., et al. (2019). Ethanol withdrawal mitigates fatty liver by normalizing lipid catabolism. *Am. J. Physiol. Gastrointest. Liver Physiol.* 316, G509–G518. doi: 10.1152/ajpgi.00376.2018

Tiribuzi, R., Crispoltoni, L., Porcellati, S., Di Lullo, M., Florenzano, F., Pirro, M., et al. (2014). miR128 up-regulation correlates with impaired amyloid β(1-42) degradation in monocytes from patients with sporadic Alzheimer's disease. *Neurobiol. Aging* 35, 345–356. doi: 10.1016/j.neurobiolaging.2013.08.003

Trivedi, P. C., Bartlett, J. J., Mercer, A., Slade, L., Surette, M., Ballabio, A., et al. (2020). Loss of function of transcription factor EB remodels lipid metabolism and cell death pathways in the cardiomyocyte. *Biochim. Biophys. Acta Mol. Basis Dis.* 1866:165832. doi: 10.1016/j.bbadis.2020.165832

Visvikis, O., Ihuegbu, N., Labed, S. A., Luhachack, L. G., Alves, A. F., Wollenberg, A. C., et al. (2014). Innate host defense requires TFEB-mediated transcription of cytoprotective and antimicrobial genes. *Immunity* 40, 896–909. doi: 10.1016/j.immuni.2014.05.002

Wang, C., Niederstrasser, H., Douglas, P. M., Lin, R., Jaramillo, J., Li, Y., et al. (2017). Small-molecule TFEB pathway agonists that ameliorate metabolic syndrome in mice and extend C. elegans lifespan. *Nat. Commun.* 8:2270. doi: 10.1038/s41467-017-02332-3

Wang, S., Chen, X., Zeng, B., Xu, X., Chen, H., Zhao, P., et al. (2020). Knockout of macrophage migration inhibitory factor accentuates side-stream smoke exposure-induced myocardial contractile dysfunction through dysregulated mitophagy. *Pharmacol. Res.* 157:104828. doi: 10.1016/j.phrs.2020.104828

Wang, Y., Gunewardena, S., Li, F., Matye, D. J., Chen, C., Chao, X., et al. (2020a). An FGF15/19-TFEB regulatory loop controls hepatic cholesterol and bile acid homeostasis. *Nat. Commun.* 11:3612. doi: 10.1038/s41467-020-17363-6

Wang, Y., Huang, Y., Liu, J., Zhang, J., Xu, M., You, Z., et al. (2020b). Acetyltransferase GCN5 regulates autophagy and lysosome biogenesis by targeting TFEB. *EMBO Rep.* 21:e48335. doi: 10.15252/embr.201948335

Wang, Y., Zhao, H., Li, X., Wang, Q., Yan, M., Zhang, H., et al. (2019). Formononetin alleviates hepatic steatosis by facilitating TFEB-mediated lysosome biogenesis and lipophagy. *J. Nutr. Biochem.* 73:108214. doi: 10.1016/j.jnutbio.2019.07.005

Weber, C., and Noels, H. (2011). Atherosclerosis: current pathogenesis and therapeutic options. *Nat. Med.* 17, 1410–1422. doi: 10.1038/nm.2538

Willett, R., Martina, J. A., Zewe, J. P., Wills, R., Hammond, G. R. V., and Puertollano, R. (2017). TFEB regulates lysosomal positioning by modulating TMEM55B expression and JIP4 recruitment to lysosomes. *Nat. Commun.* 8, 1580. doi: 10.1038/s41467-017-01871-z

Xia, Q., Shi, S., Shen, Q., Wei, X., Wang, X., Ma, H., et al. (2015). [Renal cell carcinoma with t(6;11)(p21.2;q13)/MALAT1-TFEB fusion: a clinical and pathological analysis]. *Zhonghua Bing Li Xue Za Zhi* 44, 895–899.

Xu, H., and Ren, D. (2015). Lysosomal physiology. *Annu. Rev. Physiol.* 77, 57–80. doi: 10.1146/annurev-physiol-021014-071649

Xu, Y., Ren, J., He, X., Chen, H., Wei, T., and Feng, W. (2019). YWHA/14-3-3 proteins recognize phosphorylated TFEB by a noncanonical mode for controlling TFEB cytoplasmic localization. *Autophagy* 15, 1017–1030. doi: 10.1080/15548627.2019.1569928

Yang, L., Li, P., Fu, S., Calay, E. S., and Hotamisligil, G. S. (2010). Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. *Cell Metab.* 11, 467–478. doi: 10.1016/j.cmet.2010.04.005

Yang, M., Zhang, Y., and Ren, J. (2018). Autophagic regulation of lipid homeostasis in cardiometabolic syndrome. *Front. Cardiovasc. Med.* 5:38. doi: 10.3389/fcvm.2018.00038

Yang, M., Zhang, Y., and Ren, J. (2020). Acetylation in cardiovascular diseases: molecular mechanisms and clinical implications. *Biochim. Biophys. Acta Mol. Basis Dis.* 1866:165836. doi: 10.1016/j.bbadis.2020.165836

Yang, M. Y., Wang, Y. B., Han, B., Yang, B., Qiang, Y. W., Zhang, Y., et al. (2018). Activation of aldehyde dehydrogenase 2 slows down the progression of atherosclerosis via attenuation of ER stress and apoptosis in smooth muscle cells. *Acta Pharmacol. Sin.* 39, 48–58. doi: 10.1038/aps.2017.81

Yao, R. Q., Ren, C., Xia, Z. F., and Yao, Y. M. (2020). Organelle-specific autophagy in inflammatory diseases: a potential therapeutic target underlying the quality control of multiple organelles. *Autophagy* doi: 10.1080/15548627.2020.1725377 [Epub ahead of print].

Ye, M., Zhou, J., Zhong, Y., Xu, J., Hou, J., Wang, X., et al. (2019). SR-A-targeted phase-transition nanoparticles for the detection and treatment of atherosclerotic vulnerable plaques. *ACS Appl. Mater. Interfaces* 11, 9702–9715. doi: 10.1021/acsami.8b18190

Yonekawa, T., Gamez, G., Kim, J., Tan, A. C., Thorburn, J., Gump, J., et al. (2015). RIP1 negatively regulates basal autophagic flux through TFEB to control sensitivity to apoptosis. *EMBO Rep.* 16, 700–708. doi: 10.15252/embr.201439496

You, Y., Bao, W. L., Zhang, S. L., Li, H. D., Li, H., Dang, W. Z., et al. (2020). Sorting Nexin 10 mediates metabolic reprogramming of macrophages in atherosclerosis through the Lyn-dependent TFEB signaling pathway. *Circ. Res.* 127, 534–549. doi: 10.1161/circresaha.119.315516

Yu, S., Wang, Z., Ding, L., and Yang, L. (2020). The regulation of TFEB in lipid homeostasis of non-alcoholic fatty liver disease: molecular mechanism and promising therapeutic targets. *Life Sci.* 246, 117418. doi: 10.1016/j.lfs.2020.117418

Yuan, Y., Li, P., and Ye, J. (2012). Lipid homeostasis and the formation of macrophage-derived foam cells in atherosclerosis. *Protein Cell* 3, 173–181. doi: 10.1007/s13238-012-2025-6

Zechner, R., Madeo, F., and Kratky, D. (2017). Cytosolic lipolysis and lipophagy: two sides of the same coin. *Nat. Rev. Mol. Cell Biol.* 18, 671–684. doi: 10.1038/nrm.2017.76

Zechner, R., Zimmermann, R., Eichmann, T. O., Kohlwein, S. D., Haemmerle, G., Lass, A., et al. (2012). FAT SIGNALS–lipases and lipolysis in lipid metabolism and signaling. *Cell Metab.* 15, 279–291. doi: 10.1016/j.cmet.2011.12.018

Zhan, H. Q., Li, S. T., Shu, Y., Liu, M. M., Qin, R., Li, Y. L., et al. (2018). Alpha gene upregulates TFEB expression in renal cell carcinoma with t(6;11) translocation, which promotes cell canceration. *Int. J. Oncol.* 52, 933–944. doi: 10.3892/ijo.2018.4239

Zhang, H., Alsaleh, G., Feltham, J., Sun, Y., Napolitano, G., Riffelmacher, T., et al. (2019). Polyamines control eIF5A hypusination, TFEB translation, and autophagy to reverse B cell senescence. *Mol. Cell* 76, 110–125.e119. doi: 10.1016/j.molcel.2019.08.005

Zhang, H., Yan, S., Khambu, B., Ma, F., Li, Y., Chen, X., et al. (2018). Dynamic MTORC1-TFEB feedback signaling regulates hepatic autophagy, steatosis and

liver injury in long-term nutrient oversupply. *Autophagy* 14, 1779–1795. doi: 10.1080/15548627.2018.1490850

Zhang, J., Wang, J., Zhou, Z., Park, J. E., Wang, L., Wu, S., et al. (2018). Importance of TFEB acetylation in control of its transcriptional activity and lysosomal function in response to histone deacetylase inhibitors. *Autophagy* 14, 1043–1059. doi: 10.1080/15548627.2018.1447290

Zhang, Y., Sowers, J. R., and Ren, J. (2018). Targeting autophagy in obesity: from pathophysiology to management. *Nat. Rev. Endocrinol.* 14, 356–376. doi: 10.1038/s41574-018-0009-1

Zhang, Z., Qian, Q., Li, M., Shao, F., Ding, W. X., Lira, V. A., et al. (2020). The unfolded protein response regulates hepatic autophagy by sXBP1-mediated activation of TFEB. *Autophagy* doi: 10.1080/15548627.2020.1788889 [Epub ahead of print].

Zhu, Z. D., Yu, T., Liu, H. J., Jin, J., and He, J. (2018). SOCE induced calcium overload regulates autophagy in acute pancreatitis via calcineurin activation. *Cell Death Dis.* 9:50. doi: 10.1038/s41419-017-0073-9

Conflict of Interest: 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.

frontiers in Physiology

REVIEW
published: 24 February 2021
doi: 10.3389/fphys.2021.642470

The Role of Epoxyeicosatrienoic Acids in Cardiac Remodeling

*Jinsheng Lai and Chen Chen**

Division of Cardiology, Tongji Hospital, Tongji Medical College and Hubei Key Laboratory of Genetics and Molecular Mechanisms of Cardiologic Disorders, Huazhong University of Science and Technology, Wuhan, China

Epoxyeicosatrienoic acids (EETs) are metabolites of arachidonic acid by cytochrome P450 (CYP) epoxygenases, which include four regioisomers: 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET. Each of them possesses beneficial effects against inflammation, fibrosis, and apoptosis, which could combat cardiovascular diseases. Numerous studies have demonstrated that elevation of EETs by overexpression of CYP2J2, inhibition of sEH, or treatment with EET analogs showed protective effects in various cardiovascular diseases, including hypertension, myocardial infarction, and heart failure. As is known to all, cardiac remodeling is the major pathogenesis of cardiovascular diseases. This review will begin with the introduction of EETs and their protective effects in cardiovascular diseases. In the following, the roles of EETs in cardiac remodeling, with a particular emphasis on myocardial hypertrophy, apoptosis, fibrosis, inflammation, and angiogenesis, will be summarized. Finally, it is suggested that upregulation of EETs is a potential therapeutic strategy for cardiovascular diseases. The EET-related drug development against cardiac remodeling is also discussed, including the overexpression of CYP2J2, inhibition of sEH, and the analogs of EET.

Keywords: EET, AA, cytochrome P450 epoxygenases, epoxyeicosatrienoic acids, cardiac remodeling, cardiovascular disease, heart failure, drug development

OPEN ACCESS

Edited by:
Chunjiong Wang,
Tianjin Medical University, China

Reviewed by:
Yan Zhang,
Peking University, China
Jun-Yan Liu,
Chongqing Medical University, China
Sofia Bibli,
Goethe University Frankfurt, Germany

***Correspondence:**
Chen Chen
chenchen@tjh.tjmu.edu.cn;
aids_0_111@126.com

Specialty section:
This article was submitted to Lipid and Fatty Acid Research, a section of the journal Frontiers in Physiology

Received: *16 December 2020*
Accepted: *25 January 2021*
Published: *24 February 2021*

Citation:
Lai J and Chen C (2021) The Role of Epoxyeicosatrienoic Acids in Cardiac Remodeling. Front. Physiol. 12:642470. doi: 10.3389/fphys.2021.642470

Abbreviations: AA, arachidonic acids; ACF, aortocaval fistula; Ang II, angiotensin II; AMPK, adenosine 5'-monophosphate (AMP)-activated protein kinase; ANP, atrial natriuretic peptide; APOE, apolipoprotein E; AR, aortic regurgitation; ATF-6, activating transcription factor; BAX, BCL2-associated X; BCL2, B-cell lymphoma-2; BNP, brain natriuretic peptide; CAD, coronary artery disease; CHF, chronic heart failure; CHOP, C/EBP-homologous protein; COX, cyclooxygenase; CVD, cardiovascular disease; CYP, cytochrome P450; DHET, dihydroxyeicosatrienoic acid; DiHOME, dihydroxyoctadecenoic acid; EDHF, endothelium-derived hyperpolarizing factor; EDPs, epoxydocosapentaenoic acid; EEQs, epoxyeicosatetraenoic acid; EETs, epoxyeicosatrienoic acids; EpOMEs, epoxyoctadecanoic acids; ER, endoplasmic reticulum; ET-1, endothelin 1; GRP-78, glucose-regulated protein 78; HDL, high density lipoprotein; HETE, hydroxy eicosatetraenoic acid; HF, heart failure; HFD, high-fat diet; HO-1, heme oxygenase-1; ICAM-1, intercellular cell adhesion molecule 1; IGF, insulin-like growth factor; IL-1α, interleukin 1 alpha; ISO, isoprenaline; JAK, Janus kinase; LAD, left anterior descending; LDL, low-density lipoprotein; LOX, lipoxygenase; LPS, lipopolysaccharide; LTX, leukotoxin; MAPK, mitogen-activated protein kinase; MI, myocardial infarction; MMP9, matrix metalloproteinase 9; MR, mitral regurgitation; NADPH, nicotinamide adenine dinucleotide phosphate; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor kappa B; NO, nitric oxide; NUDSA, e(*S*)-2-(11-(nonyloxy)undec-8(*Z*)-enamido)succinic acid; PI3K, phosphatidylinositol 3 kinase; PLA, phospholipase; PPAR-γ, peroxisome proliferator-activated receptor gamma; rAAV, recombinant adeno-associated virus; RAS, renin–angiotensin system; ROS, reactive oxygen species; sEH, soluble epoxide hydrolase; STAT, signal transducers and activators of transcription; TAC, transverse aortic constriction; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

INTRODUCTION

Cardiovascular disease (CVD) is recognized as a leading cause of mortality worldwide (Roth et al., 2015). Heart failure (HF) is the final stage of CVD, which has become a burgeoning problem that tortures about 23 million people (Mozaffarian et al., 2015; Murphy et al., 2020). Exploring the pathophysiological etiologies of HF is essential to identify novel therapeutic strategies. Dramatically, during the progress of HF, cardiac remodeling is one of the key pathophysiological processes. It is a maladaptive change including changes in wall thickness, ventricular volumes, and cardiac mass to various internal and (or) external pathological stimulus, which ultimately causes noncompensatory HF (Opie et al., 2006; Braunwald, 2013; Yang et al., 2020).

Up to now, the underlying mechanisms of cardiac remodeling are not completely elucidated; several pathological changes are involved, including cardiac hypertrophy, cardiomyocytes apoptosis, cardiac fibrosis, inflammation, oxidative stress, and endothelial dysfunction (Wu et al., 2017; Zhou et al., 2019). Traditionally, it is believed that cardiac remodeling is irreversible. Once it develops into HF, there is no effective therapy. Thus, cardiac remodeling is becoming an important therapeutic target for HF (Kim et al., 2018). The overall goal of HF therapy is to ameliorate symptoms, decrease hospitalization rates, and prevent premature death. Although there are several drugs targeting HF, such as angiotensin-converting enzyme (ACE) inhibitors, beta blockers, and mineralocorticoid receptor antagonists, which are able to slow down the progress of cardiac remodeling, the morbidity remains at a very high level (Tham et al., 2015). Thus, more therapeutic targets are urgently needed.

Recently, epoxyeicosatrienoic acids (EETs) have been reported as novel agents against cardiac remodeling (Alsaad et al., 2013; Althurwi et al., 2015; Romashko et al., 2016). EETs are the metabolites of arachidonic acids (AAs) through the cytochrome P450 (CYP) epoxygenase pathway (Capdevila et al., 2000), and it has been proved that the levels of EETs could be elevated by several strategies. *In vivo* and *in vitro* studies suggested that EETs acted as protective effectors against cardiac remodeling and ameliorated HF (Xu et al., 2011). Meanwhile, the levels of EETs could be elevated by several approaches, for example, soluble epoxide hydrolase (sEH) inhibitors. Thus, it could be a promising therapy target. Of course, more work needs to be done to translate it from experimental studies to the clinic. In the present review, we highlight the effects of EETs on cardiac remodeling in CVD.

EETs AND ITS EFFECTS IN CVD

CYP is a superfamily of membrane-bound, NADPH-dependent monooxygenases, which plays a vital role in the oxidation of both xenobiotics and endogenous compounds (Aliwarga et al., 2018). Up to now, 57 different genes, arranged in 18 families and 43 subfamilies, were found in the human CYP superfamily (Guengerich, 2006). Among them, the CYP2J and CYP2C families are the most important enzymes in the synthesis of EETs. CYP2J2 is the only human CYP2J epoxygenase, which is dominantly expressed in the heart, especially in the endothelium, and is receiving increasing attention due to its metabolites (Wu et al., 1996). CYP2J2 converted AA into four regioisomeric EETs, including 5, 6-, 8, 9-, 11, 12-, and 14,15-EET (Zeldin, 2001; Rand et al., 2017). Among them, more than 90% of the products are 14,15-EET and 11,12-EET (Sudhahar et al., 2010). Once synthesized, EETs can be hydrated *in vivo* into dihydroxyeicosatrienoic acids (DHETs) by sEH, especially 11,12-EET and 14,15-EET (**Figure 1**; Zeldin et al., 1995; Zeldin, 2001; Sudhahar et al., 2010). sEH is the production of gene EPHX2, which is ubiquitous in human and animal tissues. So far, sEH has been identified as an important target to upregulate the level of EETs *in vivo* (Yu et al., 2000). In addition, AA could be metabolized into prostanoids by cyclooxygenase (COX) or hydroxy eicosatetraenoic acid (HETE) and leukotrienes by lipoxygenase (LOX) (**Figure 1**; Zeldin, 2001).

In 1981, Capdevila et al. (1981) firstly reported that AA could be metabolized to EETs by CYP in the liver. Since then, plenty of studies have demonstrated that EETs played important roles in several diseases, such as kidney diseases (Hye Khan et al., 2016; Deng et al., 2017; Wang et al., 2019), neurodegenerative diseases (Atone et al., 2020; Pallas et al., 2020), rheumatic arthritis (Hoxha, 2018; Hoxha and Zappacosta, 2020), and especially CVD (Xu et al., 2011; Chen and Wang, 2013).

In 1980s, EETs were reported to have vasodilator effects and could lower blood pressure in animals (Zeldin, 2001). Since then, accumulated data revealed that EETs showed protective effects against various CVDs. It has been well established that EETs are potent vasodilators *in vivo*, which are independent of nitric oxide (NO) in response to bradykinin (Imig et al., 2001). Furthermore, evidence from various studies showed that EETs acted as endothelium-derived hyperpolarizing factor (EDHF), which mediated the vasodilation of vascular smooth muscle by activating Ca^{2+}-activated K^+ channels (Campbell and Fleming, 2010). In the animal models of hypertension, synthesis of EETs was inhibited (Capdevila et al., 2007), and EET levels were upregulated by sEH inhibitors or overexpression of CYP genes, which in turn decreased blood pressure *in vivo* (Imig et al., 2002; Lee et al., 2010). Data from our laboratory also confirmed these effects of 14,15-EET in spontaneously hypertensive rats by enhancing atrial natriuretic peptide (ANP) (Xiao et al., 2010). Besides, EET analogs that mimic the endogenous EETs showed blood pressure-lowering effects in spontaneously hypertensive rats and angiotensin-associated hypertension (Imig et al., 2010; Hye Khan et al., 2014). These beneficial roles of EETs are attributed to anti-inflammation, vasodilation, and natriuresis (Imig, 2019).

Atherosclerosis is defined as a chronic vascular inflammatory disease induced by chronic inflammation and abnormalities in cholesterol metabolism. It is the main pathologic change in coronary artery disease (CAD) and myocardial infarction (MI), which are responsible for majority of cardiac death. Interestingly, EETs attenuated the expression of pro-inflammatory genes and proteins (Schmelzer et al., 2005), while sEH inhibition ameliorated lipid metabolism disorder by reducing cholesterol and low-density lipoprotein (LDL) levels and increasing high-density lipoprotein (HDL) levels (Zhang et al., 2009;

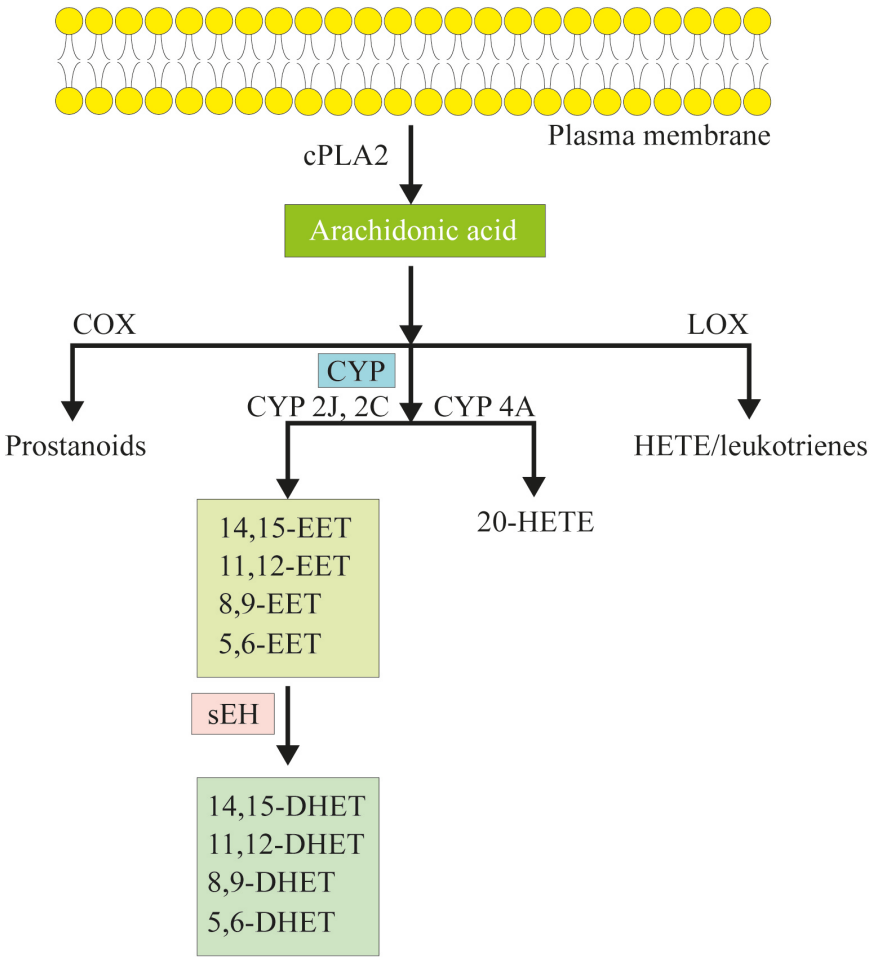


FIGURE 1 | The cascade of arachidonic acid (AA). AA is a polyunsaturated omega-6 fatty acid which is released from the membrane phospholipids in the presence of phospholipase a2 (cPLA2). AA can be metabolized to eicosanoids through three major pathways: the cyclooxygenase (COX) pathway, the lipoxygenase (LOX) pathway, and the cytochrome P450 (CYP) pathway. In the CYP pathway, AA is converted to epoxyeicosatrienoic acids (EETs) and 20-hydroxy eicosatetraenoic acid (HETE) by CYP epoxygenases and CYP ω-hydroxylases. Following, EETs can be hydrated *in vivo* to dihydroxyeicosatrienoic acids (DHETs) by soluble epoxide hydrolase (sEH).

Wang et al., 2010). Thus, EETs could act as anti-atherosclerosis factors in CAD and MI. Substantial studies have proved that EETs prevented atherosclerosis in several models, including ApoE$^{-/-}$ mice and high-fat diet (HFD)-induced models (Wang et al., 2010; Liu et al., 2016). Besides, Oni-Orisan et al. (2016) reported that lower EET levels including 8,9-EET, 11,12-EET, and 14,15-EET were observed in obstructed CAD patients compared with patients with no apparent CAD, which was consistent with the results reported by Theken et al. (2012).

Moreover, EETs offer protective effects on MI. Previous studies provided evidence that treatments with sEH inhibitors could reduce the infarct size and chronic cardiac remodeling post MI, which was beneficial in preventing electrical remodeling and cardiac arrhythmias post MI, as well as reducing inflammation (Li et al., 2009). Concomitantly, EET agonist, e(*S*)-2-(11-(nonyloxy)undec-8(*Z*)-enamido)succinic acid (NUDSA), restored cardiac function, promoted angiogenesis, and ameliorated fibrosis in MI mice induced by left anterior descending ligation (Pullen et al., 2020). These effects were mediated by increasing the canonical Wnt1 signaling cascade with the subsequent increase in heme oxygenase-1 (HO-1) (Cao et al., 2015).

HF is the end-stage of all kinds of CVD due to cardiac remodeling. As mentioned above, once HF progressed, it is always irreversible and lacks effective treatments. Researches showed that elevation of EETs might represent a promising therapeutic strategy combating HF. In a recent review including various studies employing HF animal models, consistent results were observed, wherein upregulation of EETs by sEH inhibitors improved cardiac function in different HF models (Qiu et al., 2011). In line with that, our laboratory also proved that CYP2J2-derived EETs attenuated cardiac function detected by echocardiography and invasive pressure–volume analysis (Wang et al., 2014). It is believed that inhibition of endoplasmic reticulum (ER) stress and oxidative stress was involved in the effects of overexpression of CYP2J2

and exogenous 11,12-EET, which finally ameliorated cardiac hypertrophy (Wang et al., 2014).

Nevertheless, it is evident that inflammation is present in HF (Shirazi et al., 2017). In HF models induced by isoprenaline (ISO) (Harrison and Cai, 2003) or angiotensin II (Ang II), the expression levels of inflammatory cytokines were significantly increased as a result of NF-κB pathway activation, while overexpression of CYP2J2 reduced the levels of inflammatory cytokines and improved cardiac function (Yang et al., 2015).

Collectively, EETs are metabolites of AA via the CYP pathway and play a vital role in CVD, such as hypertension, CAD, and HF. However, more studies are needed to elucidate the underlying mechanisms and further translation to clinical use.

THE ROLE OF EETs IN DIFFERENT MODELS OF CARDIAC REMODELING

Cardiac remodeling is an adaptive process to the cardiac overload caused by different stimuli. Typically, it could be divided into two types: one is pressure overload, while another is volume overload. In pressure overload diseases, such as hypertension, the cardiomyocytes increase in thickness more than in length to reduce the ventricular wall stress. Thus, the heart develops concentric hypertrophy with wall thickness. In volume overload diseases, such as MI or dilated cardiomyopathy, cardiomyocytes might reduce and rearrange with ventricular dilation. Therefore, the heart develops eccentric hypertrophy and ventricular dilation with lengthening of cardiomyocytes as a result of decreased cardiomyocyte number and rearrangement of surviving cardiomyocytes.

Actually, there might not be only two patterns of remodeling existing. For example, in HF induced by hypertension, at the early stage, there is mainly pressure overload, which makes the heart undergo hypertrophy, while in the late stage, the heart develops eccentric hypertrophy, so volume overload will also happen. These two patterns of cardiac remodeling are related closely. Nonetheless, there are differences between the two models, including different pathological changes and mechanisms (Toischer et al., 2010; You et al., 2018). In pressure overload heart, fibrosis and apoptosis are more significant with activation in calcium/calmodulin-dependent protein kinase II-dependent altered calcium cycling, whereas angiogenesis was more frequently in the volume overload model with the activated AKT pathway (Toischer et al., 2010; Kim et al., 2019).

Numerous studies have reported that EETs ameliorated cardiac remodeling, including both pressure overload- and volume overload-induced cardiac remodeling. In the following section, we will review the roles of EETs in different models of cardiac remodeling.

EETs in Pressure Overload-Induced Cardiac Remodeling

Pressure overload is the main inducible factor of cardiac remodeling. It could be involved in several diseases, such as hypertension, aortic stenosis, and aortic coarctation, which finally results in HF. There are several models employed, and EETs showed a protective effect on pressure overload-induced cardiac remodeling.

In the pressure overload-induced cardiac remodeling, drugs, such as Ang II, ISO, and endothelin 1 (ET-1), were always used. Among them, Ang II is the most common one, which acts as a potent vessel constrictor that elevates blood pressure (afterload to the heart). Interestingly, it is demonstrated that the expression of sEH was upregulated, and the level of EETs was downregulated in Ang II-induced hypertension (Ai et al., 2007; Pang et al., 2011). Given that, what would happen if the expression of EETs was restored? sEH is well known as a hydrolase of EETs. In Ang II-treated hearts, the size of cardiomyocytes and the expression of hypertrophy markers, including the atrial natriuretic factor and myosin heavy chain, were significantly elevated, while administration with a potent sEH inhibitor, AEPU, prevented this pathogenesis (Ai et al., 2009). In addition, CYP2J2-derived EETs also played a protective role in Ang II-induced cardiac remodeling. In mice treated with Ang II, cardiac hypertrophy was verified by echocardiography and invasive pressure–volume analysis, with increases in the ratio of heart weight to body weight and cardiomyocyte apoptosis, as well as ER stress. Overexpression of CYP2J2 showed an elevation in 14,15-DHET, the metabolite of 14,15-EET, which inhibited the expression of ER stress molecules, GRP-78, CHOP, and cleaved ATF-6. As a result, CYP2J2 transgenic mice showed a reversed heart function in the presentation of Ang II (Wang et al., 2014). Our recent data also showed that 11,12-EET and 14,15-EET ameliorated cardiac remodeling by attenuating oxidative stress via PPAR-γ activation (He et al., 2015) and by suppressing transmission of pro-inflammation from cardiomyocytes to macrophages in the heart (Yang et al., 2015).

The transverse aortic constriction (TAC) model is another model commonly used to investigate cardiac remodeling upon pressure overload. In 1991, Rockman et al. (1991) first reported this model to study the mechanism underlying cardiac hypertrophy. At the early stage, the heart develops cardiac hypertrophy in the presence of dramatically increased afterload, while it finally causes HF with eccentric hypertrophy (Zi et al., 2019; Bosch et al., 2020). Cardiac remodeling is involved throughout the process, and the calcineurin-NFAT pathway is one of the most important signaling pathways (Rohini et al., 2010). A recent study carried out by Li et al. proved it again in mice. Mice with hypertrophy induced by TAC appeared with an impaired cardiac function and abundant fibrosis subsequent to the activation of the calcineurin/NFAT and TGF-β/Smad pathway. However, these effects could be suppressed by overexpression of CYP2J2 mediated by the recombinant adeno-associated virus (rAAV) and treatment with 11,12-EET (Li et al., 2020a). Coincidentally, TAC induced a severe hypertrophic response and decline of cardiac function in wild-type mice, while these features were ameliorated in CYP2J2 transgenic mice. Dramatically, mice with an overexpression of CYP2J2 showed a significant decrease in mortality after TAC (6%) compared with the wild-type mice (42%) (Westphal et al., 2013).

Furthermore, the effects of EETs by inhibition or deletion of sEH are explored in the TAC-induced cardiac remodeling model. The human sEH is the product of gene EPHX2 with

19 exons on chromosome 8 (Sandberg and Meijer, 1996; Qiu et al., 2011). Four weeks after TAC, the EPHX2$^{-/-}$ mice did not present a hypertrophic phenotype compared with the control mice, indicated by having no change in the ratio of heart weight to body weight and in wall thickness as evaluated by echocardiography (Zhang et al., 2014). This effect was related to the deletion of the sEH enzyme, while inhibition of sEH remained controversial. Xu et al. (2006) reported that treatment with sEH inhibitors, AEPU or AUDA, prevented the development of cardiac hypertrophy after 3 weeks of TAC in mice. Furthermore, in the presence of cardiac hypertrophy by TAC, treatment with AEPU or AUDA for another 3 weeks showed a reversible effect against cardiac hypertrophy. It was demonstrated that these compounds potently block the NF-κB pathway activation in cardiomyocytes (Xu et al., 2006). However, another study employed two novel synthesis compounds, GSK2188931 and GSK2256294, which showed a potent inhibition on sEH by increasing the leukotoxin (LTX)/LTX diol ratio and reducing plasma 14,15-DHET. Unexpectedly, GSK2188931 and GSK2256294 exhibited no influence on mortality, tissue weights, hypertrophic biomarkers, fibrosis level, and cardiac function and morphology after TAC (Morgan et al., 2013). These disparate results indicate that the role of EETs and sEH in cardiac remodeling is complex and model dependent, and the physical and chemical properties of different chemical structures of sEH inhibitors are also different, which needs further work to explore the underlying mechanism.

EETs in Volume Overload-Induced Cardiac Remodeling

Other than pressure overload-induced cardiac remodeling, volume overload-induced cardiac remodeling is characterized by wall thinning and chamber dilatation with cardiomyocyte lengthening. It is involved in several diseases, such as aortic regurgitation (AR) and mitral regurgitation (MR). In rodents, aortocaval fistula (ACF) is most widely used for inducing volume overload. It is operated by puncturing the shared midwall between the aorta and inferior vena cava with a sharp needle (Liu et al., 1991). There are three stages of HF progression in response to volume overload: acute stress (12 h–7 days), compensatory remodeling (3–10 weeks), and decompensated HF (>15 weeks) (Hutchinson et al., 2010).

The effects of EETs in ACF-induced volume overload-related cardiac remodeling have been studied previously (Cervenka et al., 2015a,b; Sporkova et al., 2017; Kala et al., 2018; Vackova et al., 2019a,b). It is demonstrated that the level of EETs is downregulated in the heart of ACF animals, while the expression of sEH is upregulated (Cervenka et al., 2015a,b). Restoration of EETs by sEH inhibition showed a protective effect against chronic HF by 10 weeks in Ren-2 transgenic rats combined with ACF. The conclusion was confirmed by pressure–volume analyses. It is shown that sEH inhibitor treatment significantly lowered left ventricular peak pressure in chronic HF (CHF) animals, which finally remarkably increased survival rate from 14 to 41% (Cervenka et al., 2015a). However, in Han SD rats, treatment with sEH inhibitors did not prevent the development of CHF, and these rats presented with a similar death curve to that of control groups (Cervenka et al., 2015b). The only difference between the studies is the rat strain. One is a Ren-2 transgenic rat and another is a Han SD rat. The Ren-2 transgenic rat is a model of Ang II-related hypertension, which means that the different effects of sEH inhibition between the Ren-2 transgenic and Han SD rats revealed the importance of the interaction of hypertension, renin–angiotensin system (RAS), and CYP-derived metabolites in the progression of CHF-related mortality. Dramatically, a combination of ACE inhibitor and sEH inhibitor did not elicit a significant elevation in survival rate (53%), while ACE inhibitor alone showed a significant effect on the course of CHF with a survival rate of 84% (Kala et al., 2018). Since these results lack probable explanations, further studies are needed to address these issues.

It is known that MI contributes to the development of cardiac remodeling and HF. MI-related HF is viewed as a kind of volume overload cardiac remodeling because it is characterized with ventricular dilation and decline in ejection fraction (Pfeffer and Braunwald, 1990). Once MI occurred, cardiomyocytes died due to inadequate blood supply. Although ischemia induces angiogenesis in the infarcted area, this process is insufficient (van der Laan et al., 2009). Thus, drugs promoting angiogenesis are thought to be effective in MI. Dozens of studies have proved that EETs are potent inducible factors of angiogenesis (Pozzi et al., 2005; Fleming, 2007; Rand et al., 2019). Theoretically, elevation of EETs can be an important strategy of MI-induced cardiac remodeling. Thus, scientists did make great efforts in exploring the effects of EETs on MI-related cardiac remodeling.

Recently, evidence revealed that overexpression of CYP2J2 and 11,12-EET significantly increased vascular endothelial growth factor (VEGF), which enhanced myocardial angiogenesis and improved cardiac function in MI-induced HF (Zhao et al., 2018). Using the same transgenic mice, Aliwarga et al. (2020) reported that improved cardiac performance was observed in the CYP2J2 transgenic mice following MI. Besides, transgenic mice ameliorated myocardial remodeling induced by MI, evidenced by higher fractional shortening, smaller infarct size, lower reactive oxygen species (ROS) formation, reduced fibrosis and apoptosis, and lower pulmonary edema. Interestingly, treatment with EET agonist (Cao et al., 2015) or EET analog (Neckar et al., 2019) both increased the expression of HO-1 and attenuated post-MI cardiac remodeling. HO-1 is a cytoprotective enzyme, which protects the heart from remodeling after MI (Lakkisto et al., 2011).

More convincing studies were conducted with sEH inhibitors, and similar results were observed (Seubert et al., 2006; Zhao et al., 2012). Administration with TPPU, a potent sEH inhibitor-activated AKT pathway, promoted angiogenesis and restored blood supply, which finally inhibited ventricular enlargement and improved cardiac function after MI (Guo et al., 2018). Oxidative stress is involved in cardiac remodeling and responsible for apoptosis in the stressed heart. In an established chronic HF model by coronary ligation, the heart function was reduced, as evidenced by impaired parameters in LV hemodynamics, increased oxidative stress with increasing ROS level, and reduced GSH-to-GSSG ratio. Fortunately, these impairments induced by MI could be reversed by sEH inhibition with AUDA

(Merabet et al., 2012). EPHX2 has been recognized as an HF susceptibility gene. In ischemic HF patients, the expression of EPHX2 was significantly decreased compared with that in the control group who maintained a high level of 14,15-EET (Monti et al., 2008). However, sEH deletion activated cardiac ATP-sensitive K^+ channels, enhanced L-type calcium currents, and improved cardiac function after ischemia/reperfusion injury (Seubert et al., 2006). Consistently, sEH deletion in EPHX2-null mice limited cardiac functional decline following MI in both aged and young mice following MI by preserving mitochondrial bioenergetics (Jamieson et al., 2017b). These effects were in proportion to the inhibition tensity of sEH. In EPHX2-null mice, plasma levels of 8, 9-, 11, 12-, and 14,15-DHET were reduced by 38, 44, and 67%, while in dual EPHX1- and EPHX2-null mice, the levels of the DHETs were reduced by 100, 99, and 96%, respectively. In addition, compared to EPHX2-null mice, dual EPHX1- and EPHX2-null mice showed a better heart function recovery induced by MI (Edin et al., 2018).

Collectively, EETs played important roles in both pressure overload- and volume overload-induced cardiac remodeling, and the underlying mechanisms seems to be different from each other (**Table 1**). Although EETs seem to have similar effects against both types of cardiac remodeling, few studies focused on the different effects. The effects of EETs on cardiac remodeling could be affected by several factors including gender. It is well known that gender is an important factor in CVD. The differences between male and female in cardiovascular health could be affected by many factors, such as sex hormones, gene expression, and sociocultural aspects. It has been reported that the level of EETs, especially 11,12-EET in female mice, was significantly higher than that in male mice when challenged by MI, which was beneficial to the female mice in cardiac recovery (Pullen et al., 2020). Interestingly, sEH gene expression was also different between genders. Qin et al. (2016) reported that the expression of sEH in female mice was significantly lower compared to that in males. These might also contribute to the different responses between males and females during cardiac diseases. It indicates that deeper studies need to be carried out to understand the underlying mechanism.

TABLE 1 | The mechanisms involved in the protective effects of EETs in cardiac remodeling.

Pressure-overload related cardiac remodeling
Activation of PPARγ pathway
Inhibition of ER stress
Prohibiting the transmission of pro-inflammation
Inhibition of calcineurin/NFAT pathway
Reducing fibrosis by inhibiting TGF-β/Smad pathway
Volume-overload related cardiac remodeling
Decreasing apoptosis
Induction of VEGF
Activation of AKT pathway
Increasing the expression of HO-1
Regulating the expression of ion channels
Inhibition of oxidative stress

THE ROLE OF EETs IN THE PATHOLOGICAL CHANGES OF CARDIAC REMODELING

Cardiac remodeling mainly refers to a maladaptive process to pressure overload or volume overload, which is characterized by myocardial hypertrophy, inflammation, apoptosis, fibrosis, and vascular dysfunction. During the processes, these pathogeneses interact with each other and finally cause HF (Zhou et al., 2019). EETs have been reported to have protective factors against cardiac remodeling, including these pathogeneses.

EETs and Myocardial Hypertrophy in Cardiac Remodeling

Myocardial hypertrophy is the major pathologic change in cardiac remodeling. The role of EETs in myocardial hypertrophy has been reviewed previously (Wang et al., 2013). Xu et al. reported that increasing the level of EETs by sEH inhibitors could prevent pressure-induced cardiac hypertrophy. In addition, inhibition of sEH reversed the development of cardiac hypertrophy caused by chronic pressure overload (Xu et al., 2006). In an *in vitro* study, ISO induced hypertrophic phenotype in cardiomyocytes by elevating the hypertrophic markers (ANP and BNP), which could be inhibited by sEH inhibition (Althurwi et al., 2013, 2015).

Moreover, CYP2J2-derived EETs also showed protective effects on cardiac hypertrophy (Alsaad et al., 2013). In the ISO-induced cardiac hypertrophy model, hypertrophy was initiated 72 h after ISO treatment. In the hypertrophic heart, the levels of EETs were observed, while restoring EET levels by overexpression of CYP2J2 prevented the initiation of cardiac hypertrophy through NF-κB-mediated mechanism (Althurwi et al., 2015). However, Tse et al. (2013) reported that the expression of CYP enzymes was increased after treatment with ISO, which also mediated the increase in the cell surface area. Interestingly, 14,15-EET significantly attenuated the ISO-mediated induction of cardiac hypertrophy. Coincidentally, our laboratory also showed that overexpression of CYP2J2 and 11,12-EET attenuated cardiac hypertrophy elicited with Ang II, which was mediated through the activation of AMPK-α2 (Wang et al., 2016).

EETs and Apoptosis in Cardiac Remodeling

A large number of experimental data support the presence of apoptosis in the progress of HF. Apoptosis is also called programmed cell death, which can be found in all the cardiac remodeling models, such as aortic constriction model and coronary artery occlusion model (Chen and Tu, 2002). It has been demonstrated that the resulting reduction of cardiomyocytes would finally lead to HF (Olivetti et al., 1997; Williams, 1999). Increasing data revealed that EETs inhibited apoptosis mediated by different pathways in cardiac remodeling. Oxidative stress and ER stress are initiators of cardiomyocyte apoptosis. In HF models induced by Ang II, increased levels of oxidative stress and ER stress were noticed in the heart compared with the control animals, which was responsible for

the increased apoptosis level confirmed by TUNEL staining. Excitingly, all these effects were abolished by overexpression of CYP2J2, while 14,15-EET significantly ameliorated ER stress and subsequently apoptosis in cultured cardiomyocytes (Wang et al., 2014). In another *in vitro* study, 11,12-EET is involved in cardioprotection effects by inhibiting apoptosis via a caspase-dependent pathway, indicated by the ratio change of apoptotic protein expression (Bcl2 and Bax) and activation of pro-apoptotic caspase-3 (Wang et al., 2012). Moreover, in ethanol-induced HF models, cardiac remodeling was evidenced by cardiac dilation and dysfunction. In addition, the levels of apoptosis and oxidative stress were increased in ethanol-treated hearts, while overexpression of CYP2J2 and exogenous 11,12-EET ameliorated apoptosis and oxidative stress in ethanol-induced HF (Zhou et al., 2018). The phosphatidylinositol 3-kinase (PI3K)/AKT pathway is one of the strongest anti-apoptotic signaling systems. In an *in vitro* study, cardiomyocytes were subjected with hypoxia/anoxia to mimic ischemia/reperfusion injury. Abundant apoptosis of cardiomyocytes was recorded via inhibition of the PI3K/AKT pathway, indicated by activation of caspase 9 and caspase 3. Treatment with EETs (8,9-EET, 11,12-EET, and 14,15-EET) significantly increased the phosphorylation level of AKT and reduced the cardiomyocyte's apoptosis level (Dhanasekaran et al., 2008).

Despite the apoptosis of cardiomyocytes, the apoptosis of endothelial cells in the heart plays a vital role in cardiac remodeling. In adult mice, endothelial cells accounted for 64% of non-cardiomyocytes (Pinto et al., 2016). Endothelial cells participate in the regulation of cardiac remodeling after pathological stress via secreting various biological molecules, such as adhesion molecules (ICAM-1 and tenascin-C) and angiogenetic factors (VEGF and IGF) (Yang et al., 2020). However, endothelial cells exhibited apoptosis when exposed to stimulus. TNF-α induced apoptosis in endothelial cells was analyzed with flow cytometry after Annexin V/PI staining, accompanied with activation of caspase 3 and downregulation of Bcl-2. CYP2J2 overexpression significantly inhibited caspase 3 activity and downregulated Bcl-2 expression. The anti-apoptotic effects of CYP2J2 overexpression in endothelial cells were attenuated by activation of the PI3K/Akt and inhibition of MAPK signaling pathways (Yang et al., 2007).

Besides, the cross talk between the endothelium and cardiomyocytes has been studied in EETs against apoptosis. As reported, the cardiomyocytes presented with apoptosis after ischemia. Interestingly, CYP2J2-specific overexpression in endothelial cells showed protective effects from apoptosis of cardiomyocytes after ischemia, which finally ameliorated cardiac function (Zhao et al., 2018).

EETs and Fibrosis in Cardiac Remodeling

Fibrosis always occurs in both cardiac remodeling models. In pressure overload-induced cardiac remodeling, fibrosis is mainly presented in interstitial substance without cardiomyocyte deletion. However, in volume overload-induced cardiac remodeling, the cardiomyocytes were dramatically reduced, and fibrosis is evoked, especially in MI.

Four weeks after TAC, the mice developed maladaptive cardiac hypertrophy with abundant fibrosis and increased collagen level in the heart. Overexpression of CYP2J2 significantly inhibited fibrosis in TAC mice and protected against HF (Li et al., 2020a). In fibrosis, the TGF-β/Smad pathway is one of the most studied pro-fibrosis signaling pathways. It was proven that the TGF-β/Smad pathway was involved in the process (Li et al., 2020a). They also found that similar effects were observed in the abdominal aortic constriction model, which is another pressure overload-induced cardiac remodeling model (Li et al., 2020b). Besides, Ang II-induced cardiac remodeling is always accompanied by cardiac fibrosis. He et al. exposed CYP2J2 transgenic mice to Ang II treatment for 2 weeks continuously to induce HF. Results show that Ang II elicited cardiac fibrosis by enhancing collagen accumulation and increasing expression of α-SMA and collagen I, which are the markers of cardiac fibrosis. Overexpression of CYP2J2 and 11,12-EET inhibited cardiac fibrosis via inhibition of Gα12/13/RhoA/ROCK signaling (He et al., 2017). At the same time, the effects against fibrosis in Ang II-induced HF were confirmed by Yang et al. (2015), which were mediated by suppressing the transmission of pro-inflammation from cardiomyocytes to macrophages in the heart.

Ischemic cardiomyopathy is a common cause of HF, and fibrosis is involved in ischemia-induced cardiac remodeling. The key roles played by EETs in MI-related cardiac remodeling have been reviewed previously. Treatment with sEH inhibitor limited the cardiac fibrosis after MI, which is dependent on the drug dose. The higher the dose used, the smaller the fibrosis area observed in the heart (Guo et al., 2018). Besides, both EET agonist (Cao et al., 2015) and EET analog (Neckar et al., 2019) showed anti-fibrosis effects determined by histochemical stain.

EETs and Inflammation in Cardiac Remodeling

Inflammation is an important pathological process of diseases, including HF. It is evident that inflammation is present in both acute and chronic HF, and a higher level of pro-inflammation factors is related to poorer prognosis. Thus, exploring anti-inflammatory therapy is a new target of HF. It was found that EETs are important anti-inflammatory mediators (Inceoglu et al., 2007). Node et al. (1999) first reported the anti-inflammatory effects of EETs in response to several inflammatory mediators, such as TNF-α and interleukin 1α (IL-1α), by inhibition of the NF-κB pathway. NF-κB is a nuclear transcription factor that regulates expression of a plenty of genes that are critical for inflammation. Both 11,12-EET (Bystrom et al., 2011) and 14,15-EET (Morin et al., 2008) inhibited NF-κB activation *in vitro*. Our laboratory also revealed that in challenge to Ang II, mice exhibited cardiac remodeling with NF-κB pathway activation, while CYP2J2 transgenic mice ameliorated cardiac function with reduced NF-κB p65 nuclear translocation (He et al., 2015). It is well established that PPAR-γ is a key factor in the anti-inflammation process. EETs have been reported as ligands for PPAR-γ and increased PPAR-γ transcription activity in endothelial cells and 3T3-L1 preadipocytes (Liu et al., 2005). The anti-inflammation effects of EETs could be abolished by

PPAR-γ antagonist both *in vivo* and *in vitro* (Liu et al., 2005; He et al., 2015). Besides, a PPAR-γ-independent pathway was also involved in Ang II-related inflammation. In our recent study, Ang II induced the activation of the JAK2/STAT3 pathway and subsequently resulted in inflammation and fibrosis of the aorta. Overexpression of CYP2J2 induced the expression of SOCS3, which inhibited the activation of the JAK2/STAT3 pathway independent of the PPAR-γ/NF-κB pathway (Zhou et al., 2016).

HO-1 is the rate-limiting enzyme in the catabolism of heme. It plays an important role in the amelioration of cardiac remodeling and inflammation. The overlapping effects of EETs and HO-1 led researchers to figure out the relationship between them. Several studies were carried out, and results showed that EETs could be an inducer of HO-1 expression (Sacerdoti et al., 2007; Li et al., 2009; Aliwarga et al., 2020). Moreover, in obesity-related cardiomyopathy, proinflammatory adiponectin and damaged cardiac function were observed, while treatment with EET analog ameliorated the expression of proinflammatory adiponectin and prevented HF. These effects may be due to the induction of HO-1 by the EET analog (Cao et al., 2017).

Other studies indicated that sEH was a target for the inhibition of inflammation (Schmelzer et al., 2005). AUDA, a potent sEH inhibitor, protected the mice from inflammation via inhibiting the NF-κB pathway (Liu et al., 2005). The effects were also observed in cardiac remodeling models. Stevenson et al. evaluated the inflammation cytokines (including MMP9, CCL5, CCL4, and IL-16) in human ischemic cardiomyopathy, results showed that these inflammation cytokines were upregulated in ischemic cardiomyopathy hearts in both mRNA and protein levels. In an *in vivo* study, HF induced by LAD ligation was elicited, and the inflammation cytokine CCL5 was markedly increased after LAD ligation in the hearts. Importantly, the expression of sEH in ligation hearts was significantly greater than that in hearts from wild-type mice. In addition, the inflammation factors were ameliorated when treated with sEH inhibitors (Stevenson et al., 2019). In another HF model induced by LPS, the cardiac function was decreased, as evaluated by hemodynamic analysis and echocardiography, while sEH deficiency attenuated LPS-induced cardiac dysfunction. It was also revealed that sEH deficiency lowered levels of TNFα and MCP-1 cytokines induced by LPS (Samokhvalov et al., 2018), which suggested that these protective effects of EETs against HF were beneficial for inhibition of inflammatory responses.

EETs and Angiogenesis in Cardiac Remodeling

Capillary density is a crucial factor controlling the development of cardiac remodeling. Vascular endothelial growth factor (VEGF) is an important angiogenic factor involved in the maintenance of myocardial capillary density. This process involves proliferation, invasion, migration of endothelial cells, and tube formation, which is termed angiogenesis. Overexpression of CYP2J2 increased the expression of VEGF and promoted angiogenesis in the heart after ischemia (Zhao et al., 2018). The effects of EETs on angiogenesis were confirmed by Xu et al. with sEH inhibitors. A study carried out by Xu et al. revealed that sEH inhibition promoted a dose-dependent migration and tube formation of endothelial cell from MI patients, which could be abolished by a PPAR-γ antagonist, GW9662.

The effects of EETs on angiogenesis were reviewed by Fleming (2007) and updated by Imig (2016). The underlying cell signaling was explored by *in vitro* studies. In cultured endothelial cells, both 11,12-EET and 14,15-EET treatment increased cell proliferation and the formation of a tube-like structure by activating the AKT and MAPK pathways, which were downstream of VEGF-stimulated angiogenesis (Yang et al., 2009). Despite VEGF, fibroblast growth factor-2 participated in the angiogenesis promoted by EETs (Zhang et al., 2006).

Cardiac remodeling is a complex process in the challenge of stimulus, including a lot of pathological changes. Among them, inflammation, apoptosis, cardiac hypertrophy, fibrosis, and angiogenesis are the major changes. These changes are independent and closely related with each other. Besides, there are some other pathology changes such as oxidative stress and ER stress, which also play important roles in the pathogenesis and progress of cardiac remodeling. Research work carried out in our laboratory showed that elevation of EETs by overexpression or treatment with 11,12-EET or 14,5-EET showed protective effects against oxidative stress and ER stress (Wang et al., 2014; He et al., 2015; Zhou et al., 2018).

In summary, elevation of endogenous EETs by overexpression of CYP2J2 and sEH inhibition, as well as EET analogs, showed protective effects against cardiac remodeling, which was mediated mainly by prohibiting inflammation, apoptosis, fibrosis, and cardiac hypertrophy and promoting angiogenesis.

DRUG DEVELOPMENT-RELATED EETs AGAINST CARDIAC REMODELING

Given the pathophysiological role of EETs in cardiac remodeling, the increase in EET availability emerges as a new opportunity to prevent and treat HF. Elevation of EETs has been viewed as a potential strategy for drug development. However, the half-life of EETs is very short, and its solubility is poor, which limits its clinical use.

Over the past decades, sEH inhibitors have been shown to increase the levels of endogenous EETs and showed a potent effects against cardiac remodeling (Imig, 2019). The key point to developing effective inhibitors is to optimize the absorption, distribution, metabolism, and excretion *in vivo*, as well as ease of formulation. In the early stage, AUDA was synthesized and showed a protective role in developing HF (Merabet et al., 2012). However, the drug is difficult to dissolve in water and even in several organic solvents with poor metabolic stability. Subsequently, AEPU was synthesized instead of AUDA, which showed a higher water solubility and is able to pass through the cell membranes freely. Recently, scientists found two novel compounds, TPAU and *t*-AUCB. These drugs participated in the attenuation of cardiac remodeling via oral administration (Cervenka et al., 2015a). Among all these sEH inhibitors, TPAU and t-AUCB showed an advantage in water solubility and metabolic stability. Up to now, all the studies were carried

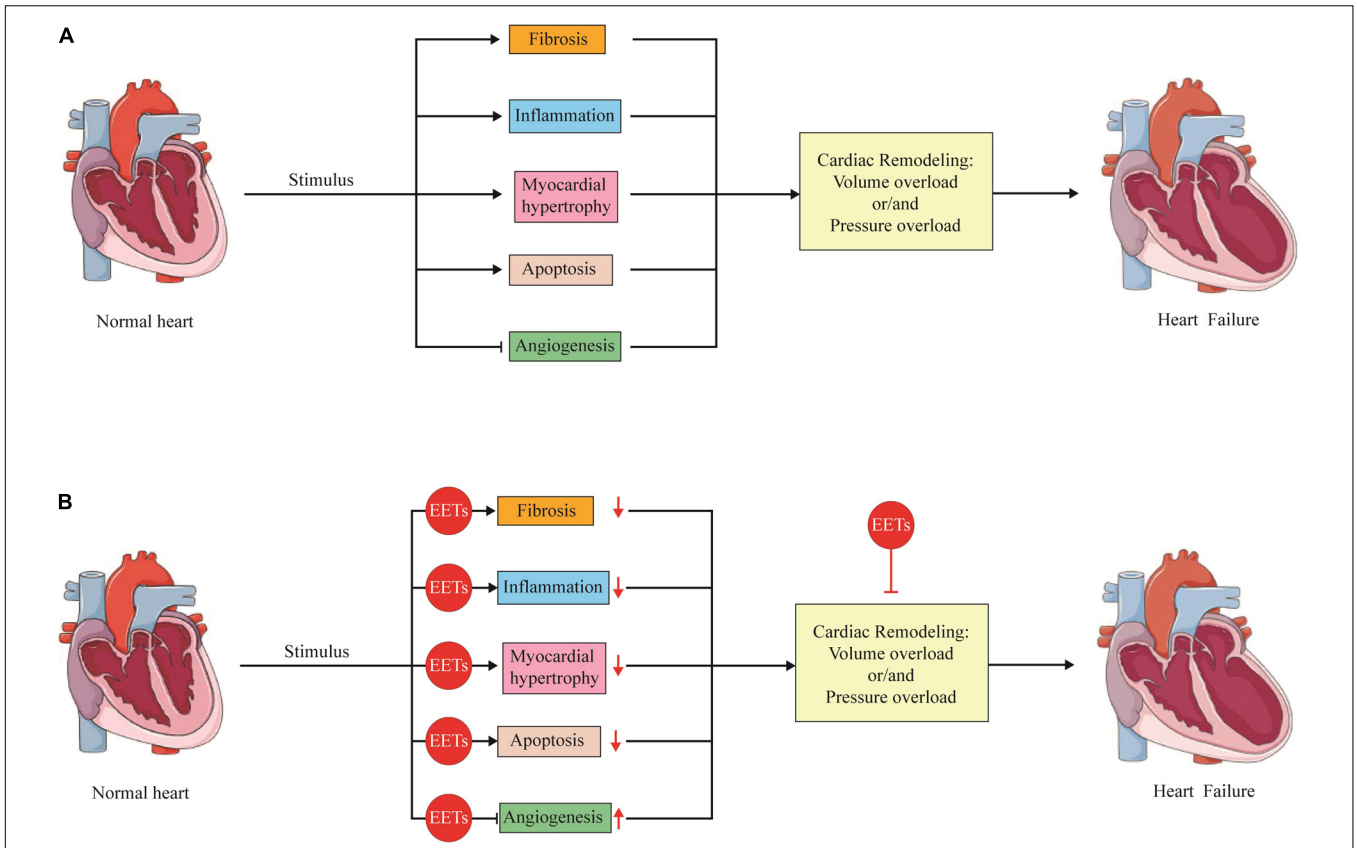


FIGURE 2 | The role of EETs in the pathological changes of cardiac remodeling. **(A)** Under various stimulus, the heart will go through cardiac remodeling, which is an adaptive process to volume overload or/and pressure overload. During the process, several pathological changes including fibrosis, inflammation, myocardial hypertrophy, apoptosis as well as angiogenesis are involved, which finally caused heart failure. **(B)** EETs are able to attenuate cardiac remodeling with ameliorated myocardial hypertrophy, reduction in apoptosis and fibrosis, decreased inflammation as well as promotion in angiogenesis.

out in rodents, and the translation of sEH inhibitors needs further studies.

As described above, it has been well learned that sEH played an important role in the metabolism of AA (one of omega-6 polyunsaturated fatty acid, PUFA) into EETs. Interestingly, inhibition of sEH also increased the metabolites of omega-3 PUFA [including epoxyeicosatetraenoic (EEQs) and epoxydocosapentaenoic acids (EDPs) and linoleic acid (epoxyoctadecanoic acids (EpOMEs)]. Cristina López-Vicario et al. proved that sEH inhibition by t-TUCB increased the level of EDP and EEQ, as well as the level of EETs (Lopez-Vicario et al., 2015). In 1979, the protective effects of omega-3 PUFA were firstly reported in Greenland Inuits, who had higher fish oil intake (Dyerberg and Bang, 1979). The metabolites of omega-3 PUFA, EDP and EEQ were reported to play a similar role in the health (Jamieson et al., 2017a). In addition, EpOMEs were found as the metabolites of linoleic acid by CYP enzymes, which could be further metabolized to dihydroxyoctadecenoic acid (DiHOME) by sEH (Newman et al., 2005). Recent studies showed that DiHOMEs might be harmful to cardiovascular system (Hildreth et al., 2020). Thus, beneficial effects of sEH inhibitors might be induced not only by EETs, but also the elevated EDPs and EEQs, as well as the reduction of DiHOMEs.

Besides, EETs analogs were designed and synthesized. Up to now, a series of approximately 50 EET analogs were developed (Campbell et al., 2017). Among them, EET-A and EET-B are most studied in cardiovascular system. Cardiac beneficial effects for EET analogs have been revealed in both pressure overload- and volumed overload-induced cardiac remodeling (Hye Khan et al., 2014; Neckar et al., 2019; Vackova et al., 2019a). In addition, EET agonist, NUDSA, which is another kind of EET analog, ameliorated cardiac failure induced by ischemia (Cao et al., 2015).

EETs is the metabolite of AA via CYP enzymes, especially CYP2J2. The protective effects of CYP2J2 overexpression on cardiac remodeling are validated previously (Wang et al., 2014; He et al., 2015; Yang et al., 2015). Interestingly, besides of the direct beneficial biological effects of increased EETs, recent study reported that CYP2J2 also played an important role in transcriptional programs in adult human cardiomyocytes. CYP2J2 silencing resulted in the expression change of the genes involved in ion channel signaling, development, extracellular matrix, as well as metabolism (Evangelista et al., 2020). There are maybe two methods to regulate CYP 2J2 derived EETs. One is overexpression of CYP 2J2 in the tissue directly by gene therapy utilizing several vectors, such as recombinant adeno associated

virus system. This system is approached by U.S. Food and Drug Administration to treat several diseases (Gruntman and Flotte, 2018). On the other hand, with the development of computer technique, the molecular secret of CYP 2J2 could be deeply discovered and potential drugs enhancing their activities might be a potential strategy for therapeutic design (Das et al., 2020).

CONCLUSION AND FURTHER PERSPECTIVE

In summary, cardiac remodeling is the main pathogenesis of HF, which occurs in all kinds of CVD. Generally, there were two different models of cardiac remodeling depending on the overload. Pressure overload-induced cardiac remodeling is characterized with concentric hypertrophy, while volume overload mainly induces dicentric hypertrophy. In both cardiac remodeling, myocardial hypertrophy, apoptosis, fibrosis, inflammation as well as angiogenesis are involved. Once initiated, cardiac remodeling is hardly to be reversed and finally caused HF. In the past 20 years, EETs has attracted numerous attentions because of its protection effects against cardiac remodeling. A plenty of studies were carried out, and most results revealed that increase the level of EETs by different methods attenuated cardiac remodeling with ameliorated myocardial hypertrophy, reduction in apoptosis and fibrosis, decreased inflammation as well as promotion in angiogenesis (**Figure 2**).

Because of its instability, it is impossible for EETs to develop as exogenous drugs. Encouragingly, a lot of efforts have been made to explore the effective strategy to increase the level of endogenous EETs, including gene therapy, sEH inhibitor and EET analogs. Among them, sEH might be the most promising treatment. sEH inhibitor has been tested in a phase 2a clinical setting for its effectiveness in reducing blood pressure. Besides, the gene therapy has been largely developed recently, which could be prospective in upregulating the expression of CYP2J2 or deletion of EPHX2 in the future. Although, it is still a long way to translation into clinical use and further studies are needed to address these issues.

AUTHOR CONTRIBUTIONS

JL conceived and wrote the manuscript. CC supervised and wrote the manuscript. Both authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by grants from the National Natural Science Foundation of China (Grant Nos. 81790624 and 51707076). The funders had no role in study design, data collection and analysis, manuscript preparation, or decision to publish.

REFERENCES

Ai, D., Fu, Y., Guo, D., Tanaka, H., Wang, N., Tang, C., et al. (2007). Angiotensin II up-regulates soluble epoxide hydrolase in vascular endothelium in vitro and in vivo. *Proc. Natl. Acad. Sci. U.S.A.* 104, 9018–9023. doi: 10.1073/pnas.0703229104

Ai, D., Pang, W., Li, N., Xu, M., Jones, P. D., Yang, J., et al. (2009). Soluble epoxide hydrolase plays an essential role in angiotensin II-induced cardiac hypertrophy. *Proc. Natl. Acad. Sci. U.S.A.* 106, 564–569. doi: 10.1073/pnas.0811022106

Aliwarga, T., Evangelista, E. A., Sotoodehnia, N., Lemaitre, R. N., and Totah, R. A. (2018). Regulation of CYP2J2 and EET levels in cardiac disease and diabetes. *Int. J. Mol. Sci.* 19:1916. doi: 10.3390/ijms19071916

Aliwarga, T., Guo, X., Evangelista, E. A., Lemaitre, R. N., Sotoodehnia, N., Gharib, S. A., et al. (2020). Higher epoxyeicosatrienoic acids in cardiomyocytes-specific CYP2J2 transgenic mice are associated with improved myocardial remodeling. *Biomedicines* 8:144. doi: 10.3390/biomedicines8060144

Alsaad, A. M., Zordoky, B. N., Tse, M. M., and El-Kadi, A. O. (2013). Role of cytochrome P450-mediated arachidonic acid metabolites in the pathogenesis of cardiac hypertrophy. *Drug Metab. Rev.* 45, 173–195. doi: 10.3109/03602532.2012.754460

Althurwi, H. N., Maayah, Z. H., Elshenawy, O. H., and El-Kadi, A. O. (2015). Early changes in cytochrome P450s and their associated arachidonic acid metabolites play a crucial role in the initiation of cardiac hypertrophy induced by isoproterenol. *Drug Metab. Dispos.* 43, 1254–1266. doi: 10.1124/dmd.115.063776

Althurwi, H. N., Tse, M. M., Abdelhamid, G., Zordoky, B. N., Hammock, B. D., and El-Kadi, A. O. (2013). Soluble epoxide hydrolase inhibitor, TUPS, protects against isoprenaline-induced cardiac hypertrophy. *Br. J. Pharmacol.* 168, 1794–1807. doi: 10.1111/bph.12066

Atone, J., Wagner, K., Hashimoto, K., and Hammock, B. D. (2020). Cytochrome P450 derived epoxidized fatty acids as a therapeutic tool against neuroinflammatory diseases. *Prostaglandins Other Lipid Mediat.* 147:106385. doi: 10.1016/j.prostaglandins.2019.106385

Bosch, L., de Haan, J. J., Bastemeijer, M., van der Burg, J., van der Worp, E., Wesseling, M., et al. (2020). The transverse aortic constriction heart failure animal model: a systematic review and meta-analysis. *Heart Fail Rev.* doi: 10.1007/s10741-020-09960-w [Epub ahead of print],

Braunwald, E. (2013). Heart failure. *JACC Heart Fail.* 1, 1–20. doi: 10.1016/j.jchf.2012.10.002

Bystrom, J., Wray, J. A., Sugden, M. C., Holness, M. J., Swales, K. E., Warner, T. D., et al. (2011). Endogenous epoxygenases are modulators of monocyte/macrophage activity. *PLoS One* 6:e26591. doi: 10.1371/journal.pone.0026591

Campbell, W. B., and Fleming, I. (2010). Epoxyeicosatrienoic acids and endothelium-dependent responses. *Pflugers Arch.* 459, 881–895. doi: 10.1007/s00424-010-0804-6

Campbell, W. B., Imig, J. D., Schmitz, J. M., and Falck, J. R. (2017). Orally active epoxyeicosatrienoic acid analogs. *J. Cardiovasc. Pharmacol.* 70, 211–224. doi: 10.1097/FJC.0000000000000523

Cao, J., Singh, S. P., McClung, J. A., Joseph, G., Vanella, L., Barbagallo, I., et al. (2017). EET intervention on Wnt1, NOV, and HO-1 signaling prevents obesity-induced cardiomyopathy in obese mice. *Am. J. Physiol. Heart Circ. Physiol.* 313, H368–H380. doi: 10.1152/ajpheart.00093.2017

Cao, J., Tsenovoy, P. L., Thompson, E. A., Falck, J. R., Touchon, R., Sodhi, K., et al. (2015). Agonists of epoxyeicosatrienoic acids reduce infarct size and ameliorate cardiac dysfunction via activation of HO-1 and Wnt1 canonical pathway. *Prostaglandins Other Lipid Mediat.* 11, 76–86. doi: 10.1016/j.prostaglandins.2015.01.002

Capdevila, J., Chacos, N., Werringloer, J., Prough, R. A., and Estabrook, R. W. (1981). Liver microsomal cytochrome P-450 and the oxidative metabolism of arachidonic acid. *Proc. Natl. Acad. Sci. U.S.A.* 78, 5362–5366. doi: 10.1073/pnas.78.9.5362

Capdevila, J. H., Falck, J. R., and Harris, R. C. (2000). Cytochrome P450 and arachidonic acid bioactivation. Molecular and functional properties of the arachidonate monooxygenase. *J. Lipid Res.* 41, 163–181. doi: 10.1016/s0022-2275(20)32049-6

Capdevila, J. H., Falck, J. R., and Imig, J. D. (2007). Roles of the cytochrome P450 arachidonic acid monooxygenases in the control of systemic blood pressure and experimental hypertension. *Kidney Int.* 72, 683–689. doi: 10.1038/sj.ki.5002394

Cervenka, L., Melenovsky, V., Huskova, Z., Skaroupkova, P., Nishiyama, A., and Sadowski, J. (2015a). Inhibition of soluble epoxide hydrolase counteracts the development of renal dysfunction and progression of congestive heart failure in Ren-2 transgenic hypertensive rats with aorto-caval fistula. *Clin. Exp. Pharmacol. Physiol.* 42, 795–807. doi: 10.1111/1440-1681.12419

Cervenka, L., Melenovsky, V., Huskova, Z., Sporkova, A., Burgelova, M., Skaroupkova, P., et al. (2015b). Inhibition of soluble epoxide hydrolase does not improve the course of congestive heart failure and the development of renal dysfunction in rats with volume overload induced by aorto-caval fistula. *Physiol. Res.* 64, 857–873. doi: 10.33549/physiolres.932977

Chen, C., and Wang, D. W. (2013). CYP epoxygenase derived EETs: from cardiovascular protection to human cancer therapy. *Curr. Top. Med. Chem.* 13, 1454–1469. doi: 10.2174/1568026611313120007

Chen, Q. M., and Tu, V. C. (2002). Apoptosis and heart failure: mechanisms and therapeutic implications. *Am. J. Cardiovasc. Drugs* 2, 43–57. doi: 10.2165/00129784-200202010-00006

Das, A., Weigle, A. T., Arnold, W. R., Kim, J. S., Carnevale, L. N., and Huff, H. C. (2020). CYP2J2 molecular recognition: a new axis for therapeutic design. *Pharmacol. Ther.* 215:107601. doi: 10.1016/j.pharmthera.2020.107601

Deng, B. Q., Luo, Y., Kang, X., Li, C. B., Morisseau, C., Yang, J., et al. (2017). Epoxide metabolites of arachidonate and docosahexaenoate function conversely in acute kidney injury involved in GSK3beta signaling. *Proc. Natl. Acad. Sci. U.S.A.* 114, 12608–12613. doi: 10.1073/pnas.1705615114

Dhanasekaran, A., Gruenloh, S. K., Buonaccorsi, J. N., Zhang, R., Gross, G. J., Falck, J. R., et al. (2008). Multiple antiapoptotic targets of the PI3K/Akt survival pathway are activated by epoxyeicosatrienoic acids to protect cardiomyocytes from hypoxia/anoxia. *Am. J. Physiol. Heart Circ. Physiol.* 294, H724–H735. doi: 10.1152/ajpheart.00979.2007

Dyerberg, J., and Bang, H. O. (1979). Lipid metabolism, atherogenesis, and haemostasis in Eskimos: the role of the prostaglandin-3 family. *Haemostasis* 8, 227–233. doi: 10.1159/000214314

Edin, M. L., Hamedani, B. G., Gruzdev, A., Graves, J. P., Lih, F. B., Arbes, S. J. III, et al. (2018). Epoxide hydrolase 1 (EPHX1) hydrolyzes epoxyeicosanoids and impairs cardiac recovery after ischemia. *J. Biol. Chem.* 293, 3281–3292. doi: 10.1074/jbc.RA117.000298

Evangelista, E. A., Aliwarga, T., Sotoodehnia, N., Jensen, P. N., McKnight, B., Lemaitre, R. N., et al. (2020). CYP2J2 modulates diverse transcriptional programs in adult human cardiomyocytes. *Sci. Rep.* 10:5329. doi: 10.1038/s41598-020-62174-w

Fleming, I. (2007). Epoxyeicosatrienoic acids, cell signaling and angiogenesis. *Prostaglandins Other Lipid Mediat.* 82, 60–67. doi: 10.1016/j.prostaglandins.2006.05.003

Gruntman, A. M., and Flotte, T. R. (2018). The rapidly evolving state of gene therapy. *FASEB J.* 32, 1733–1740. doi: 10.1096/fj.201700982R

Guengerich, F. P. (2006). Cytochrome P450s and other enzymes in drug metabolism and toxicity. *AAPS J.* 8, E101–E111. doi: 10.1208/aapsj080112

Guo, Y., Luo, F., Zhang, X., Chen, J., Shen, L., Zhu, Y., et al. (2018). TPPU enhanced exercise-induced epoxyeicosatrienoic acid concentrations to exert cardioprotection in mice after myocardial infarction. *J. Cell Mol. Med.* 22, 1489–1500. doi: 10.1111/jcmm.13412

Harrison, D. G., and Cai, H. (2003). Endothelial control of vasomotion and nitric oxide production. *Cardiol. Clin.* 21, 289–302. doi: 10.1016/s0733-8651(03)00073-0

He, Z., Yang, Y., Wen, Z., Chen, C., Xu, X., Zhu, Y., et al. (2017). CYP2J2 metabolites, epoxyeicosatrienoic acids, attenuate Ang II-induced cardiac fibrotic response by targeting Galpha12/13. *J. Lipid Res.* 58, 1338–1353. doi: 10.1194/jlr.M074229

He, Z., Zhang, X., Chen, C., Wen, Z., Hoopes, S. L., Zeldin, D. C., et al. (2015). Cardiomyocyte-specific expression of CYP2J2 prevents development of cardiac remodelling induced by angiotensin II. *Cardiovasc. Res.* 105, 304–317. doi: 10.1093/cvr/cvv018

Hildreth, K., Kodani, S. D., Hammock, B. D., and Zhao, L. (2020). Cytochrome P450-derived linoleic acid metabolites EpOMEs and DiHOMEs: a review of recent studies. *J. Nutr. Biochem.* 86:108484. doi: 10.1016/j.jnutbio.2020.108484

Hoxha, M. (2018). A systematic review on the role of eicosanoid pathways in rheumatoid arthritis. *Adv. Med. Sci.* 63, 22–29. doi: 10.1016/j.advms.2017.06.004

Hoxha, M., and Zappacosta, B. (2020). CYP-derived eicosanoids: Implications for rheumatoid arthritis. *Prostaglandins Other Lipid Mediat.* 146:106405. doi: 10.1016/j.prostaglandins.2019.106405

Hutchinson, K. R., Stewart, J. A. Jr., and Lucchesi, P. A. (2010). Extracellular matrix remodeling during the progression of volume overload-induced heart failure. *J. Mol. Cell Cardiol.* 48, 564–569. doi: 10.1016/j.yjmcc.2009.06.001

Hye Khan, M. A., Fish, B., Wahl, G., Sharma, A., Falck, J. R., Paudyal, M. P., et al. (2016). Epoxyeicosatrienoic acid analogue mitigates kidney injury in a rat model of radiation nephropathy. *Clin. Sci. (Lond.)* 130, 587–599. doi: 10.1042/CS20150778

Hye Khan, M. A., Pavlov, T. S., Christain, S. V., Neckar, J., Staruschenko, A., Gauthier, K. M., et al. (2014). Epoxyeicosatrienoic acid analogue lowers blood pressure through vasodilation and sodium channel inhibition. *Clin. Sci. (Lond.)* 127, 463–474. doi: 10.1042/CS20130479

Imig, J. D. (2016). Epoxyeicosatrienoic acids and 20-hydroxyeicosatetraenoic acid on endothelial and vascular function. *Adv. Pharmacol.* 77, 105–141. doi: 10.1016/bs.apha.2016.04.003

Imig, J. D. (2019). Epoxyeicosanoids in hypertension. *Physiol Res.* 68, 695–704. doi: 10.33549/physiolres.934291

Imig, J. D., Elmarakby, A., Nithipatikom, K., Wei, S., Capdevila, J. H., Tuniki, V. R., et al. (2010). Development of epoxyeicosatrienoic acid analogs with in vivo anti-hypertensive actions. *Front. Physiol.* 1:157. doi: 10.3389/fphys.2010.00157

Imig, J. D., Falck, J. R., Wei, S., and Capdevila, J. H. (2001). Epoxygenase metabolites contribute to nitric oxide-independent afferent arteriolar vasodilation in response to bradykinin. *J. Vasc. Res.* 38, 247–255. doi: 10.1159/000051053

Imig, J. D., Zhao, X., Capdevila, J. H., Morisseau, C., and Hammock, B. D. (2002). Soluble epoxide hydrolase inhibition lowers arterial blood pressure in angiotensin II hypertension. *Hypertension* 39(Pt 2), 690–694. doi: 10.1161/hy0202.103788

Inceoglu, B., Schmelzer, K. R., Morisseau, C., Jinks, S. L., and Hammock, B. D. (2007). Soluble epoxide hydrolase inhibition reveals novel biological functions of epoxyeicosatrienoic acids (EETs). *Prostaglandins Other Lipid Mediat.* 82, 42–49. doi: 10.1016/j.prostaglandins.2006.05.004

Jamieson, K. L., Endo, T., Darwesh, A. M., Samokhvalov, V., and Seubert, J. M. (2017a). Cytochrome P450-derived eicosanoids and heart function. *Pharmacol. Ther.* 179, 47–83. doi: 10.1016/j.pharmthera.2017.05.005

Jamieson, K. L., Samokhvalov, V., Akhnokh, M. K., Lee, K., Cho, W. J., Takawale, A., et al. (2017b). Genetic deletion of soluble epoxide hydrolase provides cardioprotective responses following myocardial infarction in aged mice. *Prostaglandins Other Lipid Mediat.* 132, 47–58. doi: 10.1016/j.prostaglandins.2017.01.001

Kala, P., Sedlakova, L., Skaroupkova, P., Kopkan, L., Vanourkova, Z., Taborsky, M., et al. (2018). Effect of angiotensin-converting enzyme blockade, alone or combined with blockade of soluble epoxide hydrolase, on the course of congestive heart failure and occurrence of renal dysfunction in Ren-2 transgenic hypertensive rats with aorto-caval fistula. *Physiol. Res.* 67, 401–415. doi: 10.33549/physiolres.933757

Kim, G. H., Uriel, N., and Burkhoff, D. (2018). Reverse remodelling and myocardial recovery in heart failure. *Nat. Rev. Cardiol.* 15, 83–96. doi: 10.1038/nrcardio.2017.139

Kim, K. H., Kim, H. M., Park, J. S., and Kim, Y. J. (2019). Differential transcriptome profile and exercise capacity in cardiac remodeling by pressure overload versus volume overload. *J. Cardiovasc. Imaging* 27, 50–63. doi: 10.4250/jcvi.2019.27.e4

Lakkisto, P., Siren, J. M., Kyto, V., Forsten, H., Laine, M., Pulkki, K., et al. (2011). Heme oxygenase-1 induction protects the heart and modulates cellular and extracellular remodelling after myocardial infarction in rats. *Exp. Biol. Med. (Maywood)* 236, 1437–1448. doi: 10.1258/ebm.2011.011148

Lee, C. R., Imig, J. D., Edin, M. L., Foley, J., DeGraff, L. M., Bradbury, J. A., et al. (2010). Endothelial expression of human cytochrome P450 epoxygenases lowers blood pressure and attenuates hypertension-induced renal injury in mice. *FASEB J.* 24, 3770–3781. doi: 10.1096/fj.10-160119

Li, N., Liu, J. Y., Timofeyev, V., Qiu, H., Hwang, S. H., Tuteja, D., et al. (2009). Beneficial effects of soluble epoxide hydrolase inhibitors in myocardial

infarction model: insight gained using metabolomic approaches. *J. Mol. Cell Cardiol.* 47, 835–845. doi: 10.1016/j.yjmcc.2009.08.017

Li, X., Chu, G., Zhu, F., Zheng, Z., Wang, X., Zhang, G., et al. (2020a). Epoxyeicosatrienoic acid prevents maladaptive remodeling in pressure overload by targeting calcineurin/NFAT and Smad-7. *Exp. Cell Res.* 386:111716. doi: 10.1016/j.yexcr.2019.111716

Li, X., Zhu, F., Meng, W., Zhang, F., Hong, J., Zhang, G., et al. (2020b). CYP2J2/EET reduces vulnerability to atrial fibrillation in chronic pressure overload mice. *J. Cell Mol. Med.* 24, 862–874. doi: 10.1111/jcmm.14796

Liu, W., Wang, T., He, X., Liu, X., Wang, B., Liu, Y., et al. (2016). CYP2J2 overexpression increases EETs and protects against HFD-induced atherosclerosis in ApoE-/- mice. *J. Cardiovasc. Pharmacol.* 67, 491–502. doi: 10.1097/FJC.0000000000000371

Liu, Y., Zhang, Y., Schmelzer, K., Lee, T. S., Fang, X., Zhu, Y., et al. (2005). The antiinflammatory effect of laminar flow: the role of PPARgamma, epoxyeicosatrienoic acids, and soluble epoxide hydrolase. *Proc. Natl. Acad. Sci. U.S.A.* 102, 16747–16752. doi: 10.1073/pnas.0508081102

Liu, Z., Hilbelink, D. R., Crockett, W. B., and Gerdes, A. M. (1991). Regional changes in hemodynamics and cardiac myocyte size in rats with aortocaval fistulas. 1. Developing and established hypertrophy. *Circ. Res.* 69, 52–58. doi: 10.1161/01.res.69.1.52

Lopez-Vicario, C., Alcaraz-Quiles, J., Garcia-Alonso, V., Rius, B., Hwang, S. H., Titos, E., et al. (2015). Inhibition of soluble epoxide hydrolase modulates inflammation and autophagy in obese adipose tissue and liver: role for omega-3 epoxides. *Proc. Natl. Acad. Sci. U.S.A.* 112, 536–541. doi: 10.1073/pnas.1422590112

Merabet, N., Bellien, J., Glevarec, E., Nicol, L., Lucas, D., Remy-Jouet, I., et al. (2012). Soluble epoxide hydrolase inhibition improves myocardial perfusion and function in experimental heart failure. *J. Mol. Cell Cardiol.* 52, 660–666. doi: 10.1016/j.yjmcc.2011.11.015

Monti, J., Fischer, J., Paskas, S., Heinig, M., Schulz, H., Gosele, C., et al. (2008). Soluble epoxide hydrolase is a susceptibility factor for heart failure in a rat model of human disease. *Nat. Genet.* 40, 529–537. doi: 10.1038/ng.129

Morgan, L. A., Olzinski, A. R., Upson, J. J., Zhao, S., Wang, T., Eisennagel, S. H., et al. (2013). Soluble epoxide hydrolase inhibition does not prevent cardiac remodeling and dysfunction after aortic constriction in rats and mice. *J. Cardiovasc. Pharmacol.* 61, 291–301. doi: 10.1097/FJC.0b013e31827fe59c

Morin, C., Sirois, M., Echave, V., Gomes, M. M., and Rousseau, E. (2008). EET displays anti-inflammatory effects in TNF-alpha stimulated human bronchi: putative role of CPI-17. *Am. J. Respir. Cell Mol. Biol.* 38, 192–201. doi: 10.1165/rcmb.2007-0232OC

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., et al. (2015). Heart disease and stroke statistics–2015 update: a report from the American Heart Association. *Circulation* 131, e29–e322. doi: 10.1161/CIR.0000000000000152

Murphy, S. P., Ibrahim, N. E., and Januzzi, J. L. Jr. (2020). Heart failure with reduced ejection fraction: a review. *JAMA* 324, 488–504. doi: 10.1001/jama.2020.10262

Neckar, J., Hye Khan, M. A., Gross, G. J., Cyprova, M., Hrdlicka, J., Kvasilova, A., et al. (2019). Epoxyeicosatrienoic acid analog EET-B attenuates post-myocardial infarction remodeling in spontaneously hypertensive rats. *Clin. Sci. (Lond.)* 133, 939–951. doi: 10.1042/CS20180728

Newman, J. W., Morisseau, C., and Hammock, B. D. (2005). Epoxide hydrolases: their roles and interactions with lipid metabolism. *Prog. Lipid Res.* 44, 1–51. doi: 10.1016/j.plipres.2004.10.001

Node, K., Huo, Y., Ruan, X., Yang, B., Spiecker, M., Ley, K., et al. (1999). Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science* 285, 1276–1279. doi: 10.1126/science.285.5431.1276

Olivetti, G., Abbi, R., Quaini, F., Kajstura, J., Cheng, W., Nitahara, J. A., et al. (1997). Apoptosis in the failing human heart. *N. Engl. J. Med.* 336, 1131–1141. doi: 10.1056/NEJM199704173361603

Oni-Orisan, A., Edin, M. L., Lee, J. A., Wells, M. A., Christensen, E. S., Vendrov, K. C., et al. (2016). Cytochrome P450-derived epoxyeicosatrienoic acids and coronary artery disease in humans: a targeted metabolomics study. *J. Lipid Res.* 57, 109–119. doi: 10.1194/jlr.M061697

Opie, L. H., Commerford, P. J., Gersh, B. J., and Pfeffer, M. A. (2006). Controversies in ventricular remodelling. *Lancet* 367, 356–367. doi: 10.1016/S0140-6736(06)68074-4

Pallas, M., Vazquez, S., Sanfeliu, C., Galdeano, C., and Grinan-Ferre, C. (2020). Soluble Epoxide Hydrolase Inhibition to Face Neuroinflammation in Parkinson's disease: a new therapeutic strategy. *Biomolecules* 10:703. doi: 10.3390/biom10050703

Pang, W., Li, N., Ai, D., Niu, X. L., Guan, Y. F., and Zhu, Y. (2011). Activation of peroxisome proliferator-activated receptor-gamma downregulates soluble epoxide hydrolase in cardiomyocytes. *Clin. Exp. Pharmacol. Physiol.* 38, 358–364. doi: 10.1111/j.1440-1681.2011.05492.x

Pfeffer, M. A., and Braunwald, E. (1990). Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. *Circulation* 81, 1161–1172. doi: 10.1161/01.cir.81.4.1161

Pinto, A. R., Ilinykh, A., Ivey, M. J., Kuwabara, J. T., D'Antoni, M. L., Debuque, R., et al. (2016). Revisiting cardiac cellular composition. *Circ. Res.* 118, 400–409. doi: 10.1161/CIRCRESAHA.115.307778

Pozzi, A., Macias-Perez, I., Abair, T., Wei, S., Su, Y., Zent, R., et al. (2005). Characterization of 5,6- and 8,9-epoxyeicosatrienoic acids (5,6- and 8,9-EET) as potent in vivo angiogenic lipids. *J. Biol. Chem.* 280, 27138–27146. doi: 10.1074/jbc.M501730200

Pullen, A. B., Kain, V., Serhan, C. N., and Halade, G. V. (2020). Molecular and cellular differences in cardiac repair of male and female mice. *J. Am. Heart Assoc.* 9:e015672. doi: 10.1161/JAHA.119.015672

Qin, J., Le, Y., Froogh, G., Kandhi, S., Jiang, H., Luo, M., et al. (2016). Sexually dimorphic adaptation of cardiac function: roles of epoxyeicosatrienoic acid and peroxisome proliferator-activated receptors. *Physiol. Rep.* 4:e12838. doi: 10.14814/phy2.12838

Qiu, H., Li, N., Liu, J. Y., Harris, T. R., Hammock, B. D., and Chiamvimonvat, N. (2011). Soluble epoxide hydrolase inhibitors and heart failure. *Cardiovasc. Ther.* 29, 99–111. doi: 10.1111/j.1755-5922.2010.00150.x

Rand, A. A., Barnych, B., Morisseau, C., Cajka, T., Lee, K. S. S., Panigrahy, D., et al. (2017). Cyclooxygenase-derived proangiogenic metabolites of epoxyeicosatrienoic acids. *Proc. Natl. Acad. Sci. U.S.A.* 114, 4370–4375. doi: 10.1073/pnas.1616893114

Rand, A. A., Rajamani, A., Kodani, S. D., Harris, T. R., Schlatt, L., Barnych, B., et al. (2019). Epoxyeicosatrienoic acid (EET)-stimulated angiogenesis is mediated by epoxy hydroxyeicosatrienoic acids (EHETs) formed from COX-2. *J. Lipid Res.* 60, 1996–2005. doi: 10.1194/jlr.M094219

Rockman, H. A., Ross, R. S., Harris, A. N., Knowlton, K. U., Steinhelper, M. E., Field, L. J., et al. (1991). Segregation of atrial-specific and inducible expression of an atrial natriuretic factor transgene in an in vivo murine model of cardiac hypertrophy. *Proc. Natl. Acad. Sci. U.S.A.* 88, 8277–8281. doi: 10.1073/pnas.88.18.8277

Rohini, A., Agrawal, N., Koyani, C. N., and Singh, R. (2010). Molecular targets and regulators of cardiac hypertrophy. *Pharmacol. Res.* 61, 269–280. doi: 10.1016/j.phrs.2009.11.012

Romashko, M., Schragenheim, J., Abraham, N. G., and McClung, J. A. (2016). Epoxyeicosatrienoic acid as therapy for diabetic and ischemic cardiomyopathy. *Trends Pharmacol. Sci.* 37, 945–962. doi: 10.1016/j.tips.2016.08.001

Roth, G. A., Nguyen, G., Forouzanfar, M. H., Mokdad, A. H., Naghavi, M., and Murray, C. J. (2015). Estimates of global and regional premature cardiovascular mortality in 2025. *Circulation* 132, 1270–1282. doi: 10.1161/CIRCULATIONAHA.115.016021

Sacerdoti, D., Colombrita, C., Di Pascoli, M., Schwartzman, M. L., Bolognesi, M., Falck, J. R., et al. (2007). 11,12-epoxyeicosatrienoic acid stimulates heme-oxygenase-1 in endothelial cells. *Prostaglandins Other Lipid Mediat.* 82, 155–161. doi: 10.1016/j.prostaglandins.2006.07.001

Samokhvalov, V., Jamieson, K. L., Darwesh, A. M., Keshavarz-Bahaghighat, H., Lee, T. Y. T., Edin, M., et al. (2018). Deficiency of soluble epoxide hydrolase protects cardiac function impaired by LPS-induced acute inflammation. *Front. Pharmacol.* 9:1572. doi: 10.3389/fphar.2018.01572

Sandberg, M., and Meijer, J. (1996). Structural characterization of the human soluble epoxide hydrolase gene (EPHX2). *Biochem. Biophys. Res. Commun.* 221, 333–339. doi: 10.1006/bbrc.1996.0596

Schmelzer, K. R., Kubala, L., Newman, J. W., Kim, I. H., Eiserich, J. P., and Hammock, B. D. (2005). Soluble epoxide hydrolase is a therapeutic target for acute inflammation. *Proc. Natl. Acad. Sci. U.S.A.* 102, 9772–9777. doi: 10.1073/pnas.0503279102

Seubert, J. M., Sinal, C. J., Graves, J., DeGraff, L. M., Bradbury, J. A., Lee, C. R., et al. (2006). Role of soluble epoxide hydrolase in postischemic recovery of heart

contractile function. *Circ. Res.* 99, 442–450. doi: 10.1161/01.RES.0000237390.92932.37

Shirazi, L. F., Bissett, J., Romeo, F., and Mehta, J. L. (2017). Role of inflammation in heart failure. *Curr. Atheroscler. Rep.* 19:27. doi: 10.1007/s11883-017-0660-3

Sporkova, A., Huskova, Z., Skaroupkova, P., Rami Reddy, N., Falck, J. R., Sadowski, J., et al. (2017). Vasodilatory responses of renal interlobular arteries to epoxyeicosatrienoic acids analog are not enhanced in Ren-2 transgenic hypertensive rats: evidence against a role of direct vascular effects of epoxyeicosatrienoic acids in progression of experimental heart failure. *Physiol. Res.* 66, 29–39. doi: 10.33549/physiolres.933350

Stevenson, M. D., Canugovi, C., Vendrov, A. E., Hayami, T., Bowles, D. E., Krause, K. H., et al. (2019). NADPH Oxidase 4 regulates inflammation in ischemic heart failure: role of soluble epoxide hydrolase. *Antioxid. Redox Signal.* 31, 39–58. doi: 10.1089/ars.2018.7548

Sudhahar, V., Shaw, S., and Imig, J. D. (2010). Epoxyeicosatrienoic acid analogs and vascular function. *Curr. Med. Chem.* 17, 1181–1190. doi: 10.2174/092986710790827843

Tham, Y. K., Bernardo, B. C., Ooi, J. Y., Weeks, K. L., and McMullen, J. R. (2015). Pathophysiology of cardiac hypertrophy and heart failure: signaling pathways and novel therapeutic targets. *Arch. Toxicol.* 89, 1401–1438. doi: 10.1007/s00204-015-1477-x

Theken, K. N., Schuck, R. N., Edin, M. L., Tran, B., Ellis, K., Bass, A., et al. (2012). Evaluation of cytochrome P450-derived eicosanoids in humans with stable atherosclerotic cardiovascular disease. *Atherosclerosis* 222, 530–536. doi: 10.1016/j.atherosclerosis.2012.03.022

Toischer, K., Rokita, A. G., Unsold, B., Zhu, W., Kararigas, G., Sossalla, S., et al. (2010). Differential cardiac remodeling in preload versus afterload. *Circulation* 122, 993–1003. doi: 10.1161/CIRCULATIONAHA.110.943431

Tse, M. M., Aboutabl, M. E., Althurwi, H. N., Elshenawy, O. H., Abdelhamid, G., and El-Kadi, A. O. (2013). Cytochrome P450 epoxygenase metabolite, 14,15-EET, protects against isoproterenol-induced cellular hypertrophy in H9c2 rat cell line. *Vascul. Pharmacol.* 58, 363–373. doi: 10.1016/j.vph.2013.02.004

Vackova, S., Kikerlova, S., Melenovsky, V., Kolar, F., Imig, J. D., Kompanowska-Jezierska, E., et al. (2019a). Altered renal vascular responsiveness to vasoactive agents in rats with angiotensin II-dependent hypertension and congestive heart failure. *Kidney Blood Press. Res.* 44, 792–809. doi: 10.1159/000501688

Vackova, S., Kopkan, L., Kikerlova, S., Huskova, Z., Sadowski, J., Kompanowska-Jezierska, E., et al. (2019b). Pharmacological blockade of soluble epoxide hydrolase attenuates the progression of congestive heart failure combined with chronic kidney disease: insights from studies with fawn-hooded hypertensive rats. *Front. Pharmacol.* 10:18. doi: 10.3389/fphar.2019.00018

van der Laan, A. M., Piek, J. J., and van Royen, N. (2009). Targeting angiogenesis to restore the microcirculation after reperfused MI. *Nat. Rev. Cardiol.* 6, 515–523. doi: 10.1038/nrcardio.2009.103

Wang, B., Zeng, H., Wen, Z., Chen, C., and Wang, D. W. (2016). CYP2J2 and its metabolites (epoxyeicosatrienoic acids) attenuate cardiac hypertrophy by activating AMPKalpha2 and enhancing nuclear translocation of Akt1. *Aging Cell* 15, 940–952. doi: 10.1111/acel.12507

Wang, H. X., Zhang, D. M., Zeng, X. J., Mu, J., Yang, H., Lu, L. Q., et al. (2012). Upregulation of cytochrome P450 2J3/11,12-epoxyeicosatrienoic acid inhibits apoptosis in neonatal rat cardiomyocytes by a caspase-dependent pathway. *Cytokine* 60, 360–368. doi: 10.1016/j.cyto.2012.04.029

Wang, T., Fu, X., Chen, Q., Patra, J. K., Wang, D., Wang, Z., et al. (2019). Arachidonic Acid Metabolism and Kidney Inflammation. *Int. J. Mol. Sci.* 20:3683. doi: 10.3390/ijms20153683

Wang, X., Ni, L., Yang, L., Duan, Q., Chen, C., Edin, M. L., et al. (2014). CYP2J2-derived epoxyeicosatrienoic acids suppress endoplasmic reticulum stress in heart failure. *Mol. Pharmacol.* 85, 105–115. doi: 10.1124/mol.113.087122

Wang, Y. X., Ulu, A., Zhang, L. N., and Hammock, B. (2010). Soluble epoxide hydrolase in atherosclerosis. *Curr. Atheroscler. Rep.* 12, 174–183. doi: 10.1007/s11883-010-0108-5

Wang, Z. H., Davis, B. B., Jiang, D. Q., Zhao, T. T., and Xu, D. Y. (2013). Soluble epoxide hydrolase inhibitors and cardiovascular diseases. *Curr. Vasc. Pharmacol.* 11, 105–111.

Westphal, C., Spallek, B., Konkel, A., Marko, L., Qadri, F., DeGraff, L. M., et al. (2013). CYP2J2 overexpression protects against arrhythmia susceptibility in cardiac hypertrophy. *PLoS One* 8:e73490. doi: 10.1371/journal.pone.0073490

Williams, R. S. (1999). Apoptosis and heart failure. *N. Engl. J. Med.* 341, 759–760. doi: 10.1056/NEJM199909023411012

Wu, Q. Q., Xiao, Y., Yuan, Y., Ma, Z. G., Liao, H. H., Liu, C., et al. (2017). Mechanisms contributing to cardiac remodelling. *Clin. Sci. (Lond.)* 131, 2319–2345. doi: 10.1042/CS20171167

Wu, S., Moomaw, C. R., Tomer, K. B., Falck, J. R., and Zeldin, D. C. (1996). Molecular cloning and expression of CYP2J2, a human cytochrome P450 arachidonic acid epoxygenase highly expressed in heart. *J. Biol. Chem.* 271, 3460–3468. doi: 10.1074/jbc.271.7.3460

Xiao, B., Li, X., Yan, J., Yu, X., Yang, G., Xiao, X., et al. (2010). Overexpression of cytochrome P450 epoxygenases prevents development of hypertension in spontaneously hypertensive rats by enhancing atrial natriuretic peptide. *J. Pharmacol. Exp. Ther.* 334, 784–794. doi: 10.1124/jpet.110.167510

Xu, D., Li, N., He, Y., Timofeyev, V., Lu, L., Tsai, H. J., et al. (2006). Prevention and reversal of cardiac hypertrophy by soluble epoxide hydrolase inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* 103, 18733–18738. doi: 10.1073/pnas.0609158103

Xu, X., Zhang, X. A., and Wang, D. W. (2011). The roles of CYP450 epoxygenases and metabolites, epoxyeicosatrienoic acids, in cardiovascular and malignant diseases. *Adv. Drug Deliv. Rev.* 63, 597–609. doi: 10.1016/j.addr.2011.03.006

Yang, D., Liu, H. Q., Liu, F. Y., Tang, N., Guo, Z., Ma, S. Q., et al. (2020). The roles of noncardiomyocytes in cardiac remodeling. *Int. J. Biol. Sci.* 16, 2414–2429. doi: 10.7150/ijbs.47180

Yang, L., Ni, L., Duan, Q., Wang, X., Chen, C., Chen, S., et al. (2015). CYP epoxygenase 2J2 prevents cardiac fibrosis by suppression of transmission of pro-inflammation from cardiomyocytes to macrophages. *Prostaglandins Other Lipid Mediat.* 116-117, 64–75. doi: 10.1016/j.prostaglandins.2015.01.004

Yang, S., Lin, L., Chen, J. X., Lee, C. R., Seubert, J. M., Wang, Y., et al. (2007). Cytochrome P-450 epoxygenases protect endothelial cells from apoptosis induced by tumor necrosis factor-alpha via MAPK and PI3K/Akt signaling pathways. *Am. J. Physiol. Heart Circ. Physiol.* 293, H142–H151. doi: 10.1152/ajpheart.00783.2006

Yang, S., Wei, S., Pozzi, A., and Capdevila, J. H. (2009). The arachidonic acid epoxygenase is a component of the signaling mechanisms responsible for VEGF-stimulated angiogenesis. *Arch. Biochem. Biophys.* 489, 82–91. doi: 10.1016/j.abb.2009.05.006

You, J., Wu, J., Zhang, Q., Ye, Y., Wang, S., Huang, J., et al. (2018). Differential cardiac hypertrophy and signaling pathways in pressure versus volume overload. *Am. J. Physiol. Heart Circ. Physiol.* 314, H552–H562. doi: 10.1152/ajpheart.00212.2017

Yu, Z., Xu, F., Huse, L. M., Morisseau, C., Draper, A. J., Newman, J. W., et al. (2000). Soluble epoxide hydrolase regulates hydrolysis of vasoactive epoxyeicosatrienoic acids. *Circ. Res.* 87, 992–998. doi: 10.1161/01.res.87.11.992

Zeldin, D. C. (2001). Epoxygenase pathways of arachidonic acid metabolism. *J. Biol. Chem.* 276, 36059–36062. doi: 10.1074/jbc.R100030200

Zeldin, D. C., Wei, S., Falck, J. R., Hammock, B. D., Snapper, J. R., and Capdevila, J. H. (1995). Metabolism of epoxyeicosatrienoic acids by cytosolic epoxide hydrolase: substrate structural determinants of asymmetric catalysis. *Arch. Biochem. Biophys.* 316, 443–451. doi: 10.1006/abbi.1995.1059

Zhang, B., Cao, H., and Rao, G. N. (2006). Fibroblast growth factor-2 is a downstream mediator of phosphatidylinositol 3-kinase-Akt signaling in 14,15-epoxyeicosatrienoic acid-induced angiogenesis. *J. Biol. Chem.* 281, 905–914. doi: 10.1074/jbc.M503945200

Zhang, H., Wang, T., Zhang, K., Liu, Y., Huang, F., Zhu, X., et al. (2014). Deletion of soluble epoxide hydrolase attenuates cardiac hypertrophy via down-regulation of cardiac fibroblasts-derived fibroblast growth factor-2. *Crit. Care Med.* 42, e345–e354. doi: 10.1097/CCM.0000000000000226

Zhang, L. N., Vincelette, J., Cheng, Y., Mehra, U., Chen, D., Anandan, S. K., et al. (2009). Inhibition of soluble epoxide hydrolase attenuated atherosclerosis, abdominal aortic aneurysm formation, and dyslipidemia. *Arterioscler Thromb. Vasc. Biol.* 29, 1265–1270. doi: 10.1161/ATVBAHA.109.186064

Zhao, Q., Huang, J., Wang, D., Chen, L., Sun, D., and Zhao, C. (2018). Endothelium-specific CYP2J2 overexpression improves cardiac dysfunction by promoting angiogenesis via Jagged1/Notch1 signaling. *J. Mol. Cell Cardiol.* 123, 118–127. doi: 10.1016/j.yjmcc.2018.08.027

Zhao, T. T., Wasti, B., Xu, D. Y., Shen, L., Du, J. Q., and Zhao, S. P. (2012). Soluble epoxide hydrolase and ischemic cardiomyopathy. *Int. J. Cardiol.* 155, 181–187. doi: 10.1016/j.ijcard.2011.05.067

Zhou, C., Huang, J., Chen, J., Lai, J., Zhu, F., Xu, X., et al. (2016). CYP2J2-Derived EETs attenuated angiotensin II-induced adventitial remodeling via reduced inflammatory response. *Cell Physiol. Biochem.* 39, 721–739. doi: 10.1159/000445663

Zhou, C., Huang, J., Li, Q., Zhan, C., Xu, X., Zhang, X., et al. (2018). CYP2J2-derived EETs attenuated ethanol-induced myocardial dysfunction through inducing autophagy and reducing apoptosis. *Free Radic. Biol. Med.* 117, 168–179. doi: 10.1016/j.freeradbiomed.2018.02.009

Zhou, H., Wang, B., Yang, Y. X., Jia, Q. J., Zhang, A., Qi, Z. W., et al. (2019). Long noncoding RNAs in pathological cardiac remodeling: a review of the update literature. *Biomed. Res. Int.* 2019:7159592. doi: 10.1155/2019/7159592

Zi, M., Stafford, N., Prehar, S., Baudoin, F., Oceandy, D., Wang, X., et al. (2019). Cardiac hypertrophy or failure? – A systematic evaluation of the transverse aortic constriction model in C57BL/6NTac and C57BL/6J substrains. *Curr. Res. Physiol.* 1, 1–10. doi: 10.1016/j.crphys.2019.10.001

Conflict of Interest: 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.

frontiers in Physiology

ORIGINAL RESEARCH
published: 12 March 2021
doi: 10.3389/fphys.2021.663480

Lipidomics Revealed Alteration of Sphingolipid Metabolism During the Reparative Phase After Myocardial Infarction Injury

Tong Hua[1†], Qiankun Bao[2†], Xue He[1], Wenbin Cai[1] and Jinlong He[1]*

[1] Tianjin Key Laboratory of Metabolic Diseases, Collaborative Innovation Center of Tianjin for Medical Epigenetics, Department of Physiology and Pathophysiology, Tianjin Medical University, Tianjin, China, [2] Tianjin Key Laboratory of Ionic-Molecular Function of Cardiovascular Disease, Department of Cardiology, Tianjin Institute of Cardiology, The Second Hospital of Tianjin Medical University, Tianjin, China

Aberrant sphingolipid metabolism contributes to cardiac pathophysiology. Emerging evidence found that an increased level of ceramide during the inflammatory phase of post-myocardial infarction (MI) served as a biomarker and was associated with cardiac dysfunction. However, the alternation of the sphingolipid profile during the reparative phase after MI is still not fully understood. Using a mouse model of the left anterior descending ligation that leads to MI, we performed metabolomics studies to assess the alternations of both plasma and myocardial sphingolipid profiles during the reparative phase post-MI. A total number of 193 sphingolipid metabolites were detected. Myocardial sphingolipids but not plasma sphingolipids showed marked change after MI injury. Ceramide-1-phosphates, which were accumulated after MI, contributed highly to the difference in sphingolipid profiles between groups. Consistently, the expression of ceramide kinase, which phosphorylates ceramides to generate ceramide-1-phosphates, was upregulated in heart tissue after MI injury. Our findings revealed the altering sphingolipid metabolism during the reparative phase post-MI and highlighted the potential role of ceramide kinase/ceramide-1-phosphate in ischemic heart disease.

Keywords: lipidomics, sphingolipid, myocardial infarction, ceramide kinase(Cerk), ceramide-1-phosphate(C1P)

OPEN ACCESS

Edited by:
Jun Yang,
University of California, Davis, United States

Reviewed by:
Ying Liang,
University of Arizona, United States
Qiulun Lu,
Nanjing Medical University, China

****Correspondence:***
Jinlong He
hejinlong@tmu.edu.cn

[†] These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Lipid and Fatty Acid Research, a section of the journal Frontiers in Physiology

Received: *03 February 2021*
Accepted: *23 February 2021*
Published: *12 March 2021*

Citation:
Hua T, Bao Q, He X, Cai W and He J (2021) Lipidomics Revealed Alteration of Sphingolipid Metabolism During the Reparative Phase After Myocardial Infarction Injury. Front. Physiol. 12:663480. doi: 10.3389/fphys.2021.663480

INTRODUCTION

Cardiovascular diseases were the most common underlying cause of death in the world, accounting for an estimated 31.5% of all global deaths (Benjamin et al., 2019). Heart failure (HF) after myocardial infarction (MI) is the major driver of late morbidity, mortality, and healthcare cost (Cahill and Kharbanda, 2017). Since both the incidence and prevalence of post-MI leading to HF have continually climbed (Benjamin et al., 2019), it is important to identify treatable conditions potentially contributive to HF progression.

Emerging evidence suggest that aberrant sphingolipid metabolism and signaling play a crucial role in cardiac pathophysiology (Kovilakath and Cowart, 2020; Kovilakath et al., 2020). Sphingolipids, including ceramide (Cer), ceramide-1-phosphate (Cer1P), sphingomyelins (SM), glycosphingolipids, and so on, are defined by their sphingoid base, which is generated by

condensation of an amino acid with an acyl-CoA (Kovilakath and Cowart, 2020). Sphingolipids, not only act as the essential components of the eukaryotic cell membrane, but also serve as signaling molecules to regulate many cellular processes including cell survival, proliferation, apoptosis, and protein synthesis (Spiegel et al., 1998; Hannun and Obeid, 2018). For example, studies demonstrated that increased Cer levels in mammalian heart tissues during acute MI (AMI) were associated with higher cell death rates in the left ventricle and deteriorated cardiac function (Yu et al., 2015; Hadas et al., 2020). Hence, increased attention has been focused on perturbed sphingolipid metabolism associated with cardiovascular diseases (Laaksonen et al., 2016; Parveen et al., 2019), highlighting the importance to study the alteration of sphingolipid profile in a certain condition and the underlying mechanism.

Cardiac remodeling is a dynamic and time-dependent process, with changes occurring in both the infarcted and non-infarcted regions of the ventricle (Olivetti et al., 1990; Yang et al., 2002). There are two well-defined phases of healing after MI in the mouse model. The inflammatory phase (peak day ∼3–4 post-MI) is associated with acute inflammation with intense cellular infiltration. The reparative phase (peak day ∼7 post-MI) is the following resolution and repair period with active resolution of inflammation, quiescence of cell activity, and scar stabilization and maturation over 14 days in mice (Prabhu and Frangogiannis, 2016). Cer, as the key metabolite of sphingolipids, has been reported to be highly related to MI. Plasma Cer was found markedly increased at day 1 and day 2 after AMI in patients (de Carvalho et al., 2018). Similarly, myocardial Cer concentration was increased at 4 h post-MI in rats compared to sham groups (de Carvalho et al., 2018). However, the sphingolipid metabolism of the heart during the reparative phase post-MI is still not fully understood.

In the present study, we investigated the metabolomic profile of plasma and myocardium of mice at the reparative phase after MI surgery using a liquid chromatography/mass spectrometry (LC-MS) based approach. Our results indicated that myocardial Cer1P derived from Cer was upregulated and contributed highly to the alteration of sphingolipid profile after MI injury. Accordingly, this change might attribute to the upregulation of Cerk expression in heart tissue post-MI.

MATERIALS AND METHODS

Animal Myocardial Infarction Models

Study protocols involving the use of animals were approved by Institutional Animal Care and Use Committee of Tianjin Medical University. Eight-week-old C57BL/6 mice were purchased from Vital River Laboratory Animal Technology (Beijing). For all the experiments, male mice were used because of gender difference of lipidomic profile (Barrier et al., 2010). All mice were maintained under a 12:12 h light/dark cycle before and throughout experiments. MI surgery was performed as previously described (Ding et al., 2017). Briefly, mice were anesthetized in an induction chamber with 3% isoflurane mixed with 1 L/min of 100% O_2, while the anesthesia during the surgery was maintained at 1.5% isoflurane. The mice were operated on a heating plate (36°C), then the left anterior descending branch of the coronary artery (LAD) was permanently ligated above branching using a 6.0 silk suture, about 1 mm below the tip of the left auricle. In sham surgery, only the chest and pericardium were opened but no LAD ligation was carried out. The mouse thoracic cavity was then closed and sutured.

Echocardiography

Cardiac function was assessed by transthoracic echocardiography. Echocardiographic monitoring was performed at baseline (before surgery) and 7 days post-surgery before the tissue was harvested via using an ultrasound system with a linear transducer with 32–55 MHz frequency combined with Vevo 2100 software (VisualSonics Inc., Toronto, Canada). B-mode tracings in the long-axis views were recorded, and dimensions of systolic and diastolic myocardium were measured. Parameters are calculated according to the VisualSonics standard measurements and calculations.

Triphenyl Tetrazolium Chloride Staining

Triphenyl tetrazolium chloride (TTC) staining was performed as previously reported (Ding et al., 2017). Briefly, mouse heart was dissected after infusion of 10% Alcian blue (Cat No. A3157, Sigma-Aldrich) and frozen at −20°C for 20 min, then cut into 1-mm-thick slices from apex to base. The slices were incubated in 1.5% TTC at 37°C for 15 min.

LC-MS Method for Metabolomics

Lipids were extracted from plasma and tissue of mice by the methyl-tert-butyl ether (MTBE)-based method as described (Matyash et al., 2008; Yan et al., 2020) with minor modifications. Briefly, 20 μL plasma or 10 mg heart tissue spiked with internal standard mixture was blended into 400 μL 75% methanol. MTBE (1 mL) was added and vortexed for 1 h. Phase separation was induced by adding 250 μL Milli-Q water. After 10 min of incubation at room temperature and centrifugation at 14,000 g for another 10 min, the upper (organic) phase was collected and evaporated to dryness.

Targeted profiling of sphingolipids involved the use of a 5500 QTRAP hybrid triple quadrupole linear ion trap mass spectrometer (AB Sciex, Foster City, CA) equipped with a Turbo Ion Spray electrospray ionization source. The mass spectrometer was operated with Analyst software (version 1.6.1, AB Sciex, Foster City, CA). Lipid species were detected by multiple reaction monitoring (MRM) scanning mode. The dwell time used for all MRM experiments was 5 ms. The ion source parameters were CUR = 30 psi, GS1 = 30 psi, GS2 = 30 psi, CAD = MEDIUM, TEMP = 500°C, IS = 5500 V (for positive ions) or -4500 V (for negative ions). Chromatographic separation involved the use of a UPLC BEH C18 column (1.7 μm, 100 × 2.1 mm i.d.) consisting of ethylene-bridged hybrid particles (Waters, Milford, MA). The column was maintained at 25°C and the injection volume was set to 1 μL. Solvent A: 60% acetonitrile. Solvent B: acetonitrile/isopropanol (1/9, v/v). The mobile-phase flow rate was 0.25 mL/min. The gradient started from 25% solvent B and was maintained for

3 min, then 3–15 min solvent B to 99% and maintained for 2 min, and 17-19 min solvent B reduced to 25% and maintained for 1 min.

Multiquant software (version 3.0.2, AB Sciex, Foster City, CA) was used to process raw data. Metaboanalyst 3.0[1] (Xia and Wishart, 2016) was used for data analysis and visualization, including partial least squares discriminant analysis (PLS-DA), the goal of fold change analysis, variable importance in projection (VIP) scores, and hierarchical clustering and presenting as a heatmap.

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted from hearts of mice with QIAzol and purified by the use of the QIAGEN RNeasy Mini Kit (Cat No. 74104), then 1 μg of RNA was reverse transcribed with SuperScript III and random primers (Cat No. 12574035, Thermo Fisher, Grand Island, NY), as defined by the manufacturers' manuals. Real-time PCR involved the Brilliant II SYBR Green qPCR Master Mix (Stratagene, CA, United States) and the StepOnePlus Real-Time PCR System (Applied Biosystems, Waltham, MA). Results were normalized to GAPDH. The following primer sequences for murine tissue were used (gene subsequent sequences of forward and reverse primers): GAPDH (5′-CAT GGC CTT CCG TGT TCC TA-3′, 5′-GCG GCA CGT CAG ATC CA′); Cerk (5′-GAC TGG GAG CAC TGA CAC AA-3′, 5′-GAG GAT GAG GGG AGG CCA TA-3′).

Western Blotting

Lysates of the left ventricle were acquired by direct lysis in a 50 mM Tris buffer (pH 6.7) comprising a complete protease inhibitor cocktail (Cat No. 04693132001, Roche, Indianapolis, IN) and PMSF (Cat No. P0100, Solarbio Life Sciences, Beijing). The protein samples (20μg) were separated by SDS-PAGE, followed by western blotting as described previously (He et al., 2018). Primary antibody against Cerk (Cat No. sc-376730) was from Santa Cruz (Santa Cruz, CA). Primary antibody against GAPDH (Cat No. 60004-1-Ig) and secondary antibody against mouse (Cat No. SA00001-1) were from Proteintech (Rosemont, IL). Membranes were visualized by using an ECL Western blotting detection kit (Cat No. 34580, Thermo Fisher Scientific, Waltham, MA) in a ChemiScope3600 Mini chemiluminescence imaging system (Clinx Science Instruments, Shanghai). The bands were quantified using NIH Image J software.

Immunofluorescence Staining

Seven μm frozen sections of heart were fixed in 4% paraformaldehyde for 15 min, permeabilized with 0.5% Triton X-100 in PBS for 30 min, blocked with 1% BSA for 30 min, and incubated with primary antibodies at 4°C overnight, then with Alexa fluor-conjugated secondary antibodies. Fluoroshield mounting medium with DAPI was used to cover slides. Images were visualized under an Olympus inverted microscope equipped with a charge-coupled camera.

Statistical Analysis

All the data were presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism software v7.0. The number of biological replicates and statistical significance are specified in figure legends. Differences between the two means were tested using unpaired two-tailed Student's *t*-test. $P < 0.05$ was considered statistically significant.

RESULTS

Sphingolipid Profile Altered in Mice After Myocardial Infarction Injury

To study the metabolic status of sphingolipids in the reparative phase of MI injury, we employed an MI model with ligating the left anterior descending (LAD) of mice. Cardiac function was measured with echocardiography at day 7 post-MI (**Figure 1A**). Both ejection fraction (EF) and fractional shortening (FS), the two main indicators to assess the systolic function of left ventricular (LV), were comparable between the sham group and the MI injury group before the surgery. Ligation of LAD induced a significant decrease of EF and FS at the 7 days after the surgery (**Figure 1B**), which is consistent with our previous reports (Ding et al., 2017; Fang et al., 2018). Also, the TTC staining of LV further confirmed the success of the MI model (**Figure 1C**).

Next, to get insight into the potential alteration in plasma sphingolipid profile in MI injury mice, LC-MS analyses with both positive ion mode and negative ion mode were performed. A total number of 193 metabolites were detected including Cer, dihydroceramide (dhCer), Cer1P, dihydroceramide-1-phosphate (dhCer1P), dihexosylceramide (DHC), dihydrodihexosylceramide (dhDHC), monohexosylceramide (MHC), dihydromonohexosylceramide (dhMHC), SM, dihydrosphingomyelin (dhSM), GM3 Ganglioside (GM3), sulfatide, sphingosine (So), sphingosine-1-phosphate (So1P), sphingasine (Sa), and sphingasine-1-phosphate (Sa1P). PLS-DA was used to identify patterns that categorized the two types of samples based on sham and MI. However, the 2-dimensional score plot of PLS-DA proved the classification model could not completely separate the samples by their groups (**Figure 1D**), suggesting the plasma sphingolipid profile might not be largely influenced during the reparative phage post-MI. Consistently, the relative concentrations of the top 50 metabolites shown in the heatmap (**Figure 1E**) were comparable between groups.

Since the plasma might dilute the metabolites and dismissed the potential difference between treatments, we next investigated whether MI injury induced a change of myocardial sphingolipids in heart tissue. Sphingolipids in LV were extracted and analyzed using the metabolomics method with LC-MS in both positive ion mode and negative ion mode. Similarly, a total number of 193 metabolites were detected including Cer, dhCer, Cer1P, dhCer1P and et al. In contrast to the results in plasma, the two-dimensional score plot of sparse PLS-DA proved the classification model could perfectly separate the samples of heart tissue by

[1]http://www.metaboanalyst.ca

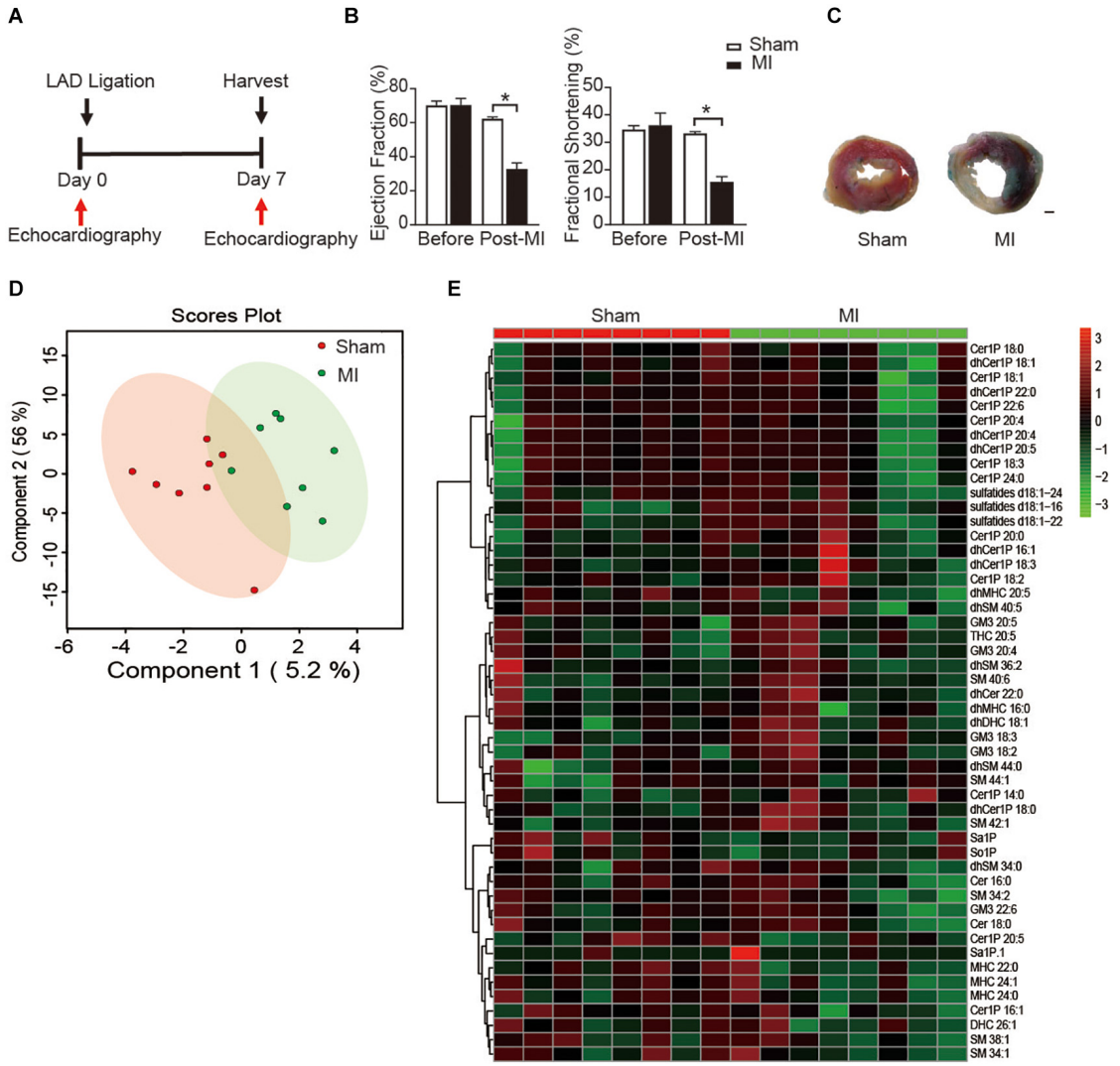


FIGURE 1 | Plasma sphingolipid profile of mice at day 7 post-MI. **(A)** Timeline of the myocardial infarction (MI) experiment. **(B)** Quantification of ejection fraction (EF) and fraction shortening (FS). Data are mean ± SEM, $^*P < 0.05$, $n = 6$. **(C)** Left ventricular (LV) tissue sections stained with Alcian blue and 2,3,5- triphenyltetrazolium chloride (TTC) at day 7 after MI. Scale bar, 500 μm. **(D)** PLS-DA score plot of the sphingolipid profile. **(E)** Heatmap showing the plasma sphingolipid profile of mice at day 7 post-MI. The colors represent increasing (red) or decreasing (green) expression, with the intensity reflecting the corresponding concentration.

their groups (**Figure 2A**), indicating an obvious change of myocardial sphingolipids profile in LV. The concentrations of the top 50 changed metabolites were shown in **Figure 2B**. The magnitude of changes in sphingolipids between the MI and sham group were visualized by fold change (FC) analysis (**Figure 2C**). The threshold of FC was set to 2. Among the 42 upregulated metabolites, the top 5 were dhCer1P 16:0 (log2(FC) = 5.26), Cer1P 22:6 (log2(FC) = 4.90), Cer1P 22:5 (log2(FC) = 4.89), dhCer1P 16:1 (log2(FC) = 4.86) and dhCer1P 22:5 (log2(FC) = 4.72). A total of 34 metabolites, among which the top 5 were dhSM 38:5 (log2(FC) = −3.05), dhSM 44:0 (log2(FC) = −2.85), SM 385 (log2(FC) = −2.78), dhSM 38:4 (log2(FC) = −2.74) and SM 40:6 (log2(FC) = −2.59), were reduced after MI injury (**Figure 2C**). In addition, variable importance for prediction (VIP) scores were calculated, and the top 10 sphingolipids mostly contributing to the classification were shown on the Y-axis (**Figure 2D**). DHC 24:0, Cer1P 18:1, and MHC 24:1 were significantly increased in heart tissue after MI injury and contributed highly to the alteration of sphingolipid profile (**Figure 2D**). Taken together, we found myocardial but not plasma sphingolipid profile was markedly changed in mice at day 7 after MI injury via employing an unbiased LC-MS analysis.

The Variations of the Sphingolipids After MI Injury

We next statistically analyzed the relative level of each metabolite of sphingolipid. Consistent to previous studies (Laaksonen et al., 2016; de Carvalho et al., 2018), all the Cers showed an increasing trend in the MI group compared to the sham group

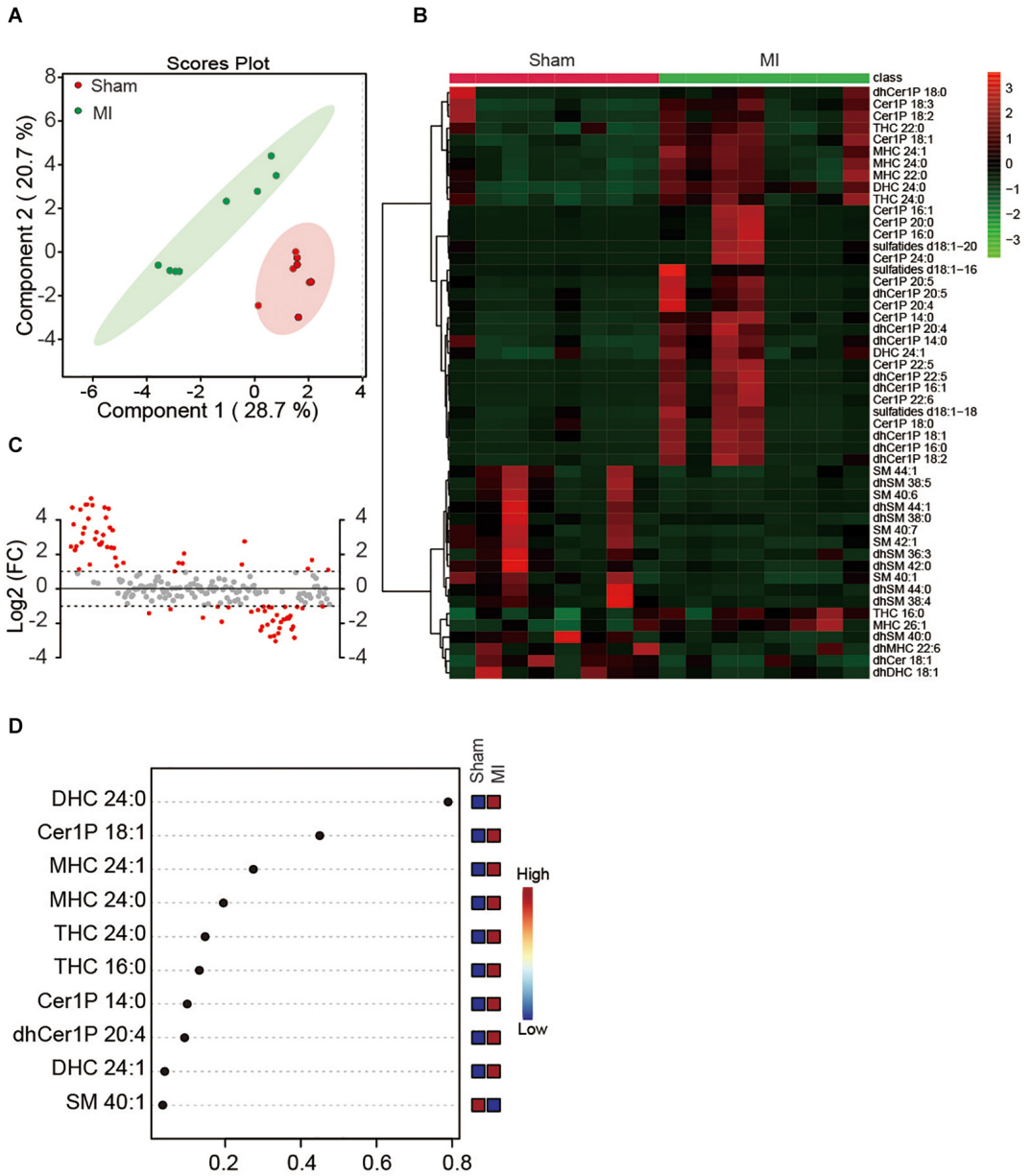


FIGURE 2 | Myocardial sphingolipid profile of mice at day 7 post-MI. **(A)** The sparse PLS-DA score plot of the sphingolipid profile. **(B)** Heatmap showing the myocardial sphingolipid profile of mice at day7 post-MI. The colors represent increasing (red) or decreasing (green) expression, with the intensity reflecting the corresponding concentration. **(C)** The goal of fold change (FC) analysis of the absolute value of change between MI and Sham group means. Metabolites with Log2 (FC) $\geq$ or $\leq$ 1 were labeled red. **(D)** The top 10 most significant metabolites based on variable importance for prediction (VIP) scores from PLS-DA. The X-axis shows the correlation scores and the Y-axis the metabolites. Color bars show the median intensity of variables in the respective groups.

(**Figures 3A,B**). However, only Cer 16:0 and Cer 24:1 showed a statistically significant increase (**Figure 3A**). As metabolites of Cer, all the Cer1P also showed a similar trend as Cer (**Figures 3C,D**). Cer1P 14:0, Cer1P 18:1, Cer1P 18:2, and Cer1P 18:3 were significantly upregulated in MI group (**Figure 3C**). Concentration of the dhCer1Ps including dhCer1P 14:0, dhCer1P 16:0, dhCer1P 18:2, dhCer1P 20:4, and dhCer1P 20:5 was increased with significance (**Figure 3D**). Levels of four MHC (MHC 20:0, MHC 22:0, MHC 24:0, and MHC 24:1), three DHC (DHC 22:0, DHC 24:1, and DHC 24:0), and one GM3 (GM3 22:5) were significantly higher, while the levels of two dhSM (dhSM 38:4 and dhSM 40:0) were significantly lower in MI groups (**Figures 3E,G,J,K**). Although sulfatide d18:1-18:0, sulfatide d18:1-22:0, and sulfatide d18:2-24:0 showed a relatively low level among all the sulfatides detected, they were significantly increased in the MI group (**Figure 3L**). Other metabolites including all the dhCer (**Figure 3B**); dhMHC (**Figure 3F**); dhDHC (**Figure 3H**); SM (**Figure 3I**); So, Sa,

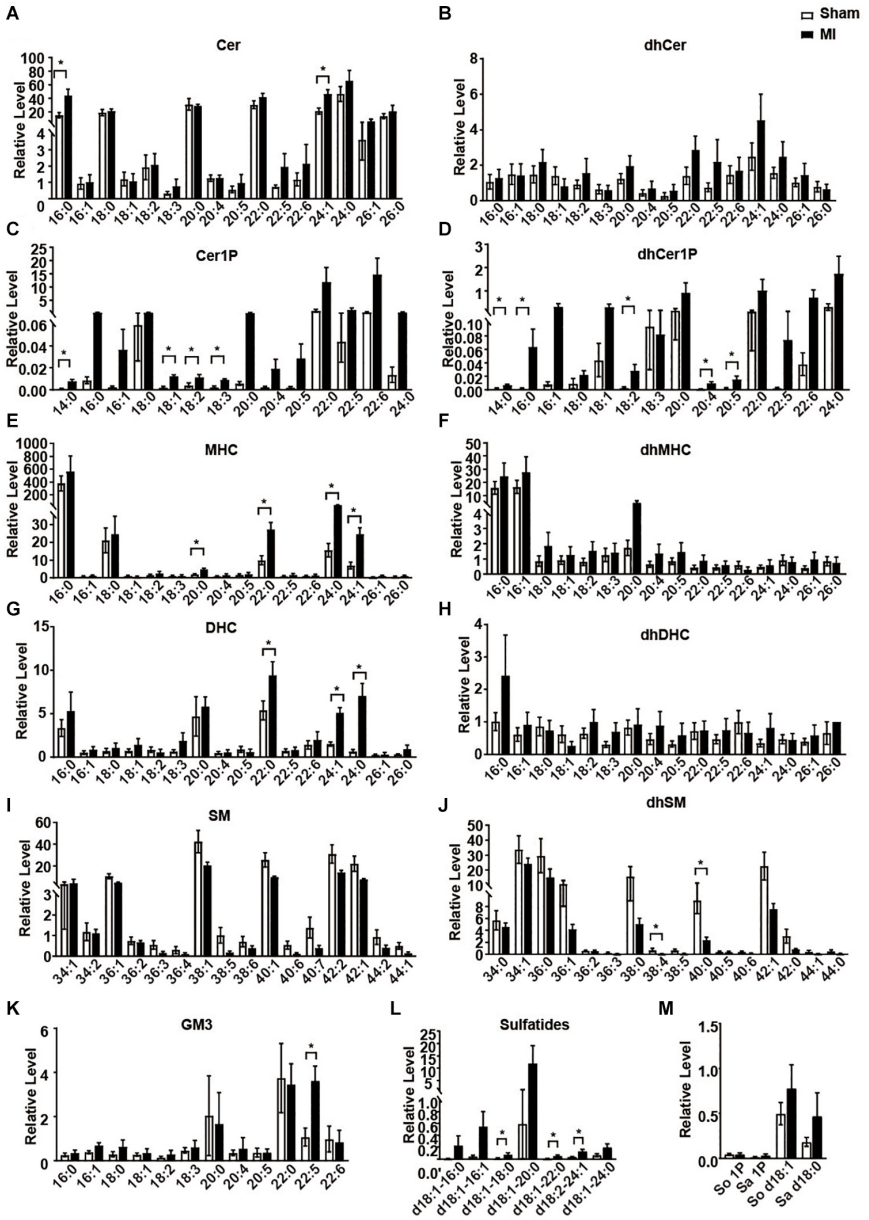


FIGURE 3 | The relative levels of myocardial sphingolipids in the Sham and post-MI mice. **(A–M)** Sham or MI surgery was performed in mice. Liquid chromatography-tandem mass spectrometry detection of sphingolipids-derived metabolites in the heart tissue. Data are mean ± SEM, $n = 8$, $^*P < 0.05$, unpaired two-tailed *t*-test.

So1P, and Sa1P (**Figure 3M**) were not changed in the heart following MI injury.

Expression of Cerk Was Upregulated in the Heart in Post-MI Mice

Emerging evidence suggests that Cer serves as a biomarker of AMI and also contributes to MI injury (Laaksonen et al., 2016; de Carvalho et al., 2018). However, we found among all the Cers, only Cer 16:0 and Cer 24:1 were significantly higher in the MI group (**Figure 3A**). Since Cer might convert to Cer1P and the latter contributed highly to the difference of sphingolipid profile between groups (**Figures 2C,D**), we speculated a conversion of Cer to Cer1P might exist during the reparative phage of MI injury. We next investigated the ratio of Cer1P and Cer. As shown in **Figure 4A**, all the 13 ratios of Cer1P/Cer except Cer1P 18:2/Cer 18:2 were higher in the MI group compared to the sham group, suggesting an increase of conversion from Cer to Cer1P during the reparative phage.

Considering the critical role of Cerk in controlling both Cer levels and production of Cer1P, we further evaluated the

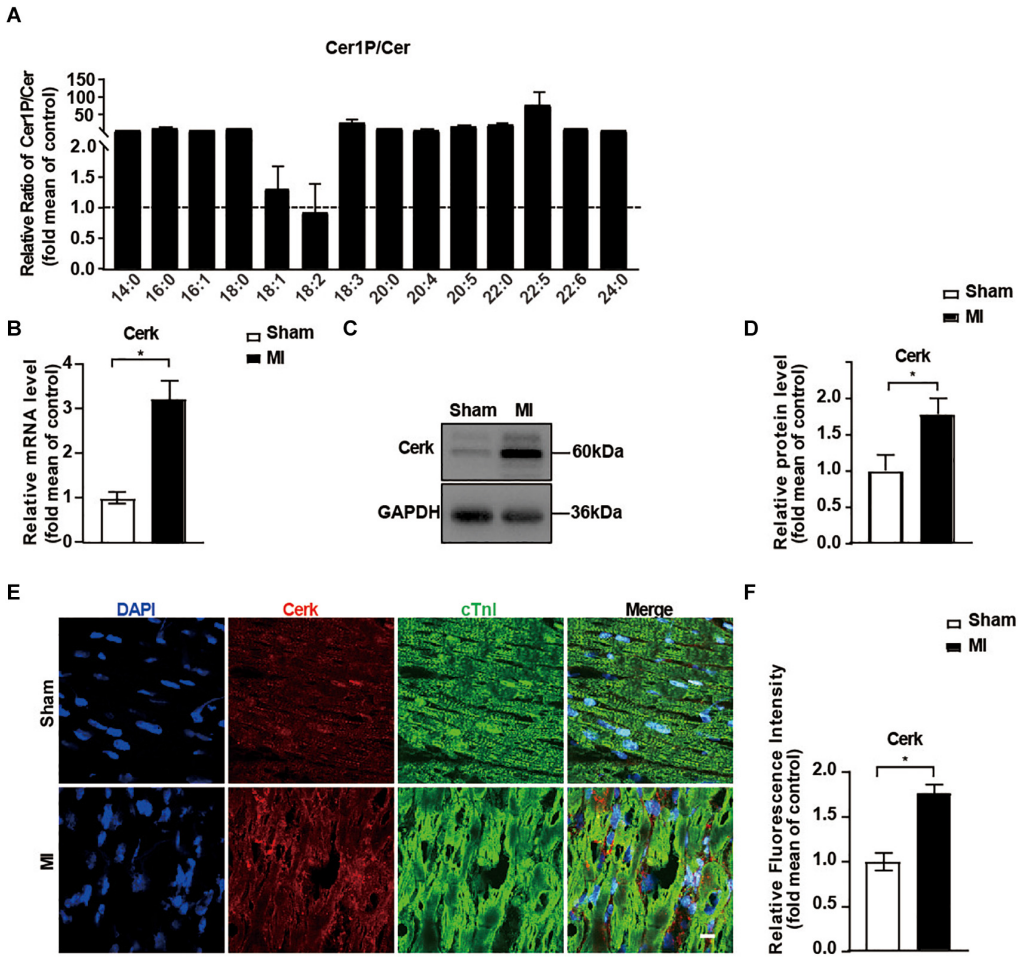


FIGURE 4 | Expression of Cerk was upregulated in the myocardium after MI. **(A)** The relative ratios of Cer1P to Cer detected with LC-MS. Ratios of Cer1P to Cer in Sham group were set to 1. Data are mean ± SEM, $n = 8$. **(B)** RT-PCR analysis of mRNA level of Cerk in heart tissue. Data are mean ± SEM, $n = 5$, $^*P < 0.05$, unpaired two-tailed *t*-test. **(C,D)** Western blot analysis and quantification of Cerk protein levels. Data are mean ± SEM, $n = 5$, $^*P < 0.05$, unpaired two-tailed *t*-test. **(E,F)** Immunofluorescence staining of Cerk in the myocardium. Scale bar, 10 μm. **(F)** Quantification of fluorescence intensity of Cerk. Data are mean ± SEM, $n = 5$, $^*P < 0.05$, unpaired two-tailed *t*-test.

expression of Cerk in heart tissue. As shown in **Figures 4B–D**, both mRNA level and the protein level of Cerk were significantly higher in the MI group compared to the sham group. Similarly, the expression of Cerk in the border area of heart tissue post-MI was also higher compared to non-infarcted area of heart tissue (**Figures 4E,F**). Together, these data suggested that upregulated expression of Cerk in the heart after MI might contribute to the conversion of Cer to Cer1P.

DISCUSSION

Cardiac inflammation and resolution are critical to the severity of cardiac dysfunction post-MI and abnormal lipid metabolism contributes to myocardial injury and remodeling. In the present study, we observed the alternation of the sphingolipid profile during the reparative phage after MI injury. Our results indicated that the sphingolipid profile in heart tissue but not in plasma was largely changed. Meanwhile, we identified several significantly higher or lower levels of metabolites in the heart tissue of the MI group which might serve as biomarkers or contribute to cardiac remodeling. Moreover, we found the ratios of Cer1P to Cer were markedly upregulated in heart after MI, which might be due to the upregulation of the expression of Cerk post-MI.

Abnormal Cer accumulation has been implicated in AMI (de Carvalho et al., 2018; Hadas et al., 2020). Consistently, we also found an increasing trend of the level of myocardial Cer in the reparative phage after MI injury. Because excess Cer was implicated in cardiac lipotoxicity, previous studies focused on the synthesis of Cer and tried to improve heart function by targeting cardiac Cer accumulation. Pharmacologically or genetic inhibition of serine palmitoyltransferase, a rate-limiting enzyme in Cer biosynthesis, reduces fatty acid and prevents cardiac toxicity in a mouse model of dilated cardiomyopathy (Park et al., 2008). Nevertheless, whether the reduction of Cer levels will improve cardiac remodeling is still controversial.

Genetic deletion of the serine-palmitoyl transferase long chain 2 (SPTLC2) gene significantly decreased the level of total and individual Cer in the myocardium of mice but did not alleviate acute cardiac dysfunction at 2 weeks post-MI (Ji et al., 2017). Cer, accumulated in the post-ischemic heart, was thought to be mediated by acid sphingomyelinase activity rather than neutral sphingomyelinase activity or *de novo* sphingolipid synthesis (Klevstig et al., 2016). Heterozygote knockout of acid sphingomyelinase (Smpd1) limited Cer accumulation in the post-MI heart without improving cardiac function or survival (Klevstig et al., 2016). Reducing Cer level in mice via the deletion of ceramide synthase 2 (CERS2) leads to the development of progressive myoclonic epilepsy (Mosbech et al., 2014). These failures might be due to that some important downstream metabolites of Cer were simultaneously limited. However, there has been much less emphasis on the downstream metabolites of Cer and the potential effects of Cer1P on cardiac remodeling.

Cer1P has been well characterized to promote proliferation and inhibit apoptosis. The early studies reported that Cer1P induced DNA synthesis in fibroblasts and promoted cellular proliferation and growth (Gomez-Munoz et al., 1995, 1997). Cer1P could also stimulate the proliferation of macrophages (Gangoiti et al., 2010) and C2C12 myoblasts (Gangoiti et al., 2012). In addition, Cer1P attenuated the activation of caspases and prevented DNA fragmentation induced by serum deprivation in macrophages (Gomez-Munoz et al., 2004). Hence, Cer1P serves as a pro-survival player and antagonizes the proapoptotic effects of Cer (Hoeferlin et al., 2013). Moreover, Cer1P inhibited the production of pro-inflammatory cytokines and leukocyte infiltration in pulmonary tissue induced by cigarette smoke in mice, suggesting anti-inflammatory properties of Cer1P (Baudiss et al., 2015). In addition, Cer1P also has a pro-angiogenic role evidenced by stimulating endothelial cell-mediated capillary-like tubule formation *in vitro* and matrigel implant vascularization (Kim et al., 2013). In the present study, we found myocardial Cer1P, including Cer1P 14:0, Cer1P 18:1, Cer1P 18:2, and Cer1P 18:3, were significantly increased at day 7 in the MI group. Besides, Cer1P 18:1 and Cer1P 14:0 contributed highly to the alternation of sphingolipid profile post-MI. Hence, our data suggested that Cer1P might play a vital role during the reparative phase after MI. Nevertheless, whether Cer1P directly involved and exerted an important role in the repair of MI deserves further investigation.

Besides quantifying the levels of metabolites of sphingolipid, we also studied the ratio of Cer1P and Cer. Cer1P is generated by phosphorylation of Cer (N-acyl sphingosine) and is believed to be mainly mediated by Cerk (Boath et al., 2008). The role of Cerk in heart is largely unknown. Cerk was expressed at high level in the heart tissue (Sugiura et al., 2002) and its expression was upregulated in primary cardiomyocytes by treating with the serum of patient with single ventricle congenital heart disease (Garcia et al., 2018). Cerk deficient mice displayed decreased C1P and enhanced Cer, and lacked the capacity to phosphorylate Cer (Graf et al., 2008). However, the genetic deficiency of Cerk improved diet-induced obesity and insulin resistance in mice (Mitsutake et al., 2012), indicating a complicated role of Cer1P. Since Cer1P has diversified roles in the regulation of various physiological and pathological processes, myocardial-specific overexpression of Cerk might reduce local Cer and be beneficial to the reparative process. In further support of this concept is that the overexpression of acid ceramidase (AC), the only enzyme known to hydrolyze Cer and generates sphingosine, could counteract the negative effects of elevated Cer and provide cardioprotection after MI (Hadas et al., 2020). Since gene therapy with adeno-associated virus system has shown promising translational potentials in HF (Eulalio et al., 2012; Gabisonia and Recchia, 2018; Ding et al., 2020), whether cardiac-specifically targeting Cerk has the therapeutic potential in ischemic heart diseases needs further investigation.

Overall, we characterized the significant changes of myocardial sphingolipid profile in the reparative phage after MI injury and observed activation of Cerk/Cer1P pathway. Further work will help to identify the function of the pathway and will lead to a better understanding of the role of sphingolipids in cardiac remodeling after MI.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The study protocols involving the use of animals were approved by Institutional Animal Care and Use Committee of Tianjin Medical University.

AUTHOR CONTRIBUTIONS

TH, QB, and JH designed the research. TH, QB, XH, and WC performed the research and analyzed the data. TH, QB, and JH wrote the manuscript. All authors read and approved the submitted version.

FUNDING

This work was supported by National Natural Science Foundation of China Grants (82070451, 81870313, and 81800251), Young Elite Scientists Sponsorship Program by CAST (2019QNRC001), and Tianjin Municipal Science and Technology Project (20JCQNJC00540, 18JCZDJC44900, and 17JCJQJC45700).

ACKNOWLEDGMENTS

The authors acknowledge Dr. Xu Zhang at Tianjin Medical University for his kind support on the LC-MS method.

REFERENCES

Barrier, L., Ingrand, S., Fauconneau, B., and Page, G. (2010). Gender-dependent accumulation of ceramides in the cerebral cortex of the APP(SL)/PS1Ki mouse model of *Alzheimer's* disease. *Neurobiol. Aging* 31, 1843–1853. doi: 10.1016/j.neurobiolaging.2008.10.011

Baudiss, K., Ayata, C. K., Lazar, Z., Cicko, S., Beckert, J., Meyer, A., et al. (2015). Ceramide-1-phosphate inhibits cigarette smoke-induced airway inflammation. *Eur. Respir. J. Official J. Eur. Soc. Clin. Respir. Physiol.* 45, 1669–1680. doi: 10.1183/09031936.00080014

Benjamin, E. J., Muntner, P., Alonso, A., Bittencourt, M. S., Callaway, C. W., Carson, A. P., et al. (2019). Heart disease and stroke statistics-2019 update: a report from the american heart association. *Circulation* 139, e56–e528. doi: 10.1161/CIR.0000000000000659

Boath, A., Graf, C., Lidome, E., Ullrich, T., Nussbaumer, P., and Bornancin, F. (2008). Regulation and traffic of ceramide 1-phosphate produced by ceramide kinase: comparative analysis to glucosylceramide and sphingomyelin. *J. Biol. Chem.* 283, 8517–8526. doi: 10.1074/jbc.M707107200

Cahill, T. J., and Kharbanda, R. K. (2017). Heart failure after myocardial infarction in the era of primary percutaneous coronary intervention: mechanisms, incidence and identification of patients at risk. *World J. Cardiol.* 9, 407–415. doi: 10.4330/wjc.v9.i5.407

de Carvalho, L. P., Tan, S. H., Ow, G. S., Tang, Z., Ching, J., Kovalik, J. P., et al. (2018). Plasma Ceramides as prognostic biomarkers and their arterial and myocardial tissue correlates in acute myocardial infarction. *JACC Basic Transl. Sci.* 3, 163–175. doi: 10.1016/j.jacbts.2017.12.005

Ding, L., Lu, S., Zhou, Y., Lyu, D., Ouyang, C., Ma, Z., et al. (2020). The 3' Untranslated region protects the heart from angiotensin II-induced cardiac dysfunction via AGGF1 expression. *Mol. Ther.* 28, 1119–1132. doi: 10.1016/j.ymthe.2020.02.002

Ding, Y., Li, Y., Zhang, X., He, J., Lu, D., Fang, X., et al. (2017). Soluble epoxide hydrolase activation by S-nitrosation contributes to cardiac ischemia-reperfusion injury. *J. Mol. Cell. Cardiol.* 110, 70–79. doi: 10.1016/j.yjmcc.2017.07.006

Eulalio, A., Mano, M., Dal Ferro, M., Zentilin, L., Sinagra, G., Zacchigna, S., et al. (2012). Functional screening identifies miRNAs inducing cardiac regeneration. *Nature* 492, 376–381. doi: 10.1038/nature11739

Fang, X., Cai, W., Cheng, Q., Ai, D., Wang, X., Hammock, B. D., et al. (2018). Omega-3 PUFA attenuate mice myocardial infarction injury by emerging a protective eicosanoid pattern. *Prostaglandins Other Lipid Mediat.* 139, 1–9. doi: 10.1016/j.prostaglandins.2018.09.002

Gabisonia, K., and Recchia, F. A. (2018). Gene therapy for heart failure: new perspectives. *Curr. Heart Fail Rep.* 15, 340–349. doi: 10.1007/s11897-018-0410-z

Gangoiti, P., Bernacchioni, C., Donati, C., Cencetti, F., Ouro, A., Gomez-Munoz, A., et al. (2012). Ceramide 1-phosphate stimulates proliferation of C2C12 myoblasts. *Biochimie* 94, 597–607. doi: 10.1016/j.biochi.2011.09.009

Gangoiti, P., Granado, M. H., Arana, L., Ouro, A., and Gomez-Munoz, A. (2010). Activation of protein kinase C-alpha is essential for stimulation of cell proliferation by ceramide 1-phosphate. *FEBS Lett.* 584, 517–524. doi: 10.1016/j.febslet.2009.11.086

Garcia, A. M., Nakano, S. J., Karimpour-Fard, A., Nunley, K., Blain-Nelson, P., Stafford, N. M., et al. (2018). Phosphodiesterase-5 is elevated in failing single ventricle myocardium and affects cardiomyocyte remodeling *In Vitro*. *Circ. Heart Fail* 11:e004571. doi: 10.1161/CIRCHEARTFAILURE.117.004571

Gomez-Munoz, A., Duffy, P. A., Martin, A., O'Brien, L., Byun, H. S., Bittman, R., et al. (1995). Short-chain ceramide-1-phosphates are novel stimulators of DNA synthesis and cell division: antagonism by cell-permeable ceramides. *Mol. Pharmacol.* 47, 833–839.

Gomez-Munoz, A., Frago, L. M., Alvarez, L., and Varela-Nieto, I. (1997). Stimulation of DNA synthesis by natural ceramide 1-phosphate. *Biochem. J.* 325(Pt 2), 435–440. doi: 10.1042/bj3250435

Gomez-Munoz, A., Kong, J. Y., Salh, B., and Steinbrecher, U. P. (2004). Ceramide-1-phosphate blocks apoptosis through inhibition of acid sphingomyelinase in macrophages. *J. Lipid Res.* 45, 99–105. doi: 10.1194/jlr.M300158-JLR200

Graf, C., Niwa, S., Muller, M., Kinzel, B., and Bornancin, F. (2008). Wild-type levels of ceramide and ceramide-1-phosphate in the retina of ceramide kinase-like-deficient mice. *Biochem. Biophys. Res. Commun.* 373, 159–163. doi: 10.1016/j.bbrc.2008.06.002

Hadas, Y., Vincek, A. S., Youssef, E., Zak, M. M., Chepurko, E., Sultana, N., et al. (2020). Altering Sphingolipid metabolism attenuates cell death and inflammatory response after myocardial infarction. *Circulation* 141, 916–930. doi: 10.1161/CIRCULATIONAHA.119.041882

Hannun, Y. A., and Obeid, L. M. (2018). Sphingolipids and their metabolism in physiology and disease. *Nat. Rev. Mol. Cell Biol.* 19, 175–191. doi: 10.1038/nrm.2017.107

He, J., Bao, Q., Zhang, Y., Liu, M., Lv, H., Liu, Y., et al. (2018). Yes-associated protein promotes angiogenesis via signal transducer and activator of transcription 3 in endothelial cells. *Circ. Res.* 122, 591–605. doi: 10.1161/CIRCRESAHA.117.311950

Hoeferlin, L. A., Wijesinghe, D. S., and Chalfant, C. E. (2013). The role of ceramide-1-phosphate in biological functions. *Handb. Exp. Pharmacol.* 215, 153–166. doi: 10.1007/978-3-7091-1368-4_8

Ji, R., Akashi, H., Drosatos, K., Liao, X., Jiang, H., Kennel, P. J., et al. (2017). Increased de novo ceramide synthesis and accumulation in failing myocardium. *JCI Insight* 2:e82922. doi: 10.1172/jci.insight.82922

Kim, C., Schneider, G., Abdel-Latif, A., Mierzejewska, K., Sunkara, M., Borkowska, S., et al. (2013). Ceramide-1-phosphate regulates migration of multipotent stromal cells and endothelial progenitor cells–implications for tissue regeneration. *Stem Cells* 31, 500–510. doi: 10.1002/stem.1291

Klevstig, M., Stahlman, M., Lundqvist, A., Scharin Tang, M., Fogelstrand, P., Adiels, M., et al. (2016). Targeting acid sphingomyelinase reduces cardiac ceramide accumulation in the post-ischemic heart. *J. Mol. Cell. Cardiol.* 93, 69–72. doi: 10.1016/j.yjmcc.2016.02.019

Kovilakath, A., and Cowart, L. A. (2020). Sphingolipid mediators of myocardial pathology. *J. Lipid Atheroscler.* 9, 23–49. doi: 10.12997/jla.2020.9.1.23

Kovilakath, A., Jamil, M., and Cowart, L. A. (2020). Sphingolipids in the Heart: from cradle to grave. *Front. Endocrinol. (Lausanne)* 11:652. doi: 10.3389/fendo.2020.00652

Laaksonen, R., Ekroos, K., Sysi-Aho, M., Hilvo, M., Vihervaara, T., Kauhanen, D., et al. (2016). Plasma ceramides predict cardiovascular death in patients with stable coronary artery disease and acute coronary syndromes beyond LDL-cholesterol. *Eur. Heart J.* 37, 1967–1976. doi: 10.1093/eurheartj/ehw148

Matyash, V., Liebisch, G., Kurzchalia, T. V., Shevchenko, A., and Schwudke, D. (2008). Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. *J. Lipid Res.* 49, 1137–1146. doi: 10.1194/jlr.D700041-JLR200

Mitsutake, S., Date, T., Yokota, H., Sugiura, M., Kohama, T., and Igarashi, Y. (2012). Ceramide kinase deficiency improves diet-induced obesity and insulin resistance. *FEBS Lett.* 586, 1300–1305. doi: 10.1016/j.febslet.2012.03.032

Mosbech, M. B., Olsen, A. S., Neess, D., Ben-David, O., Klitten, L. L., Larsen, J., et al. (2014). Reduced ceramide synthase 2 activity causes progressive myoclonic epilepsy. *Ann. Clin. Transl. Neurol.* 1, 88–98. doi: 10.1002/acn3.28

Olivetti, G., Capasso, J. M., Sonnenblick, E. H., and Anversa, P. (1990). Side-to-side slippage of myocytes participates in ventricular wall remodeling acutely after myocardial infarction in rats. *Circ. Res.* 67, 23–34. doi: 10.1161/01.res.67.1.23

Park, T. S., Hu, Y., Noh, H. L., Drosatos, K., Okajima, K., Buchanan, J., et al. (2008). Ceramide is a cardiotoxin in lipotoxic cardiomyopathy. *J. Lipid Res.* 49, 2101–2112. doi: 10.1194/jlr.M800147-JLR200

Parveen, F., Bender, D., Law, S. H., Mishra, V. K., Chen, C. C., and Ke, L. Y. (2019). Role of ceramidases in sphingolipid metabolism and human diseases. *Cells* 8:1573. doi: 10.3390/cells8121573

Prabhu, S. D., and Frangogiannis, N. G. (2016). The biological basis for cardiac repair after myocardial infarction: from inflammation to fibrosis. *Circ. Res.* 119, 91–112. doi: 10.1161/CIRCRESAHA.116.303577

Spiegel, S., Cuvillier, O., Edsall, L., Kohama, T., Menzeleev, R., Olivera, A., et al. (1998). Roles of sphingosine-1-phosphate in cell growth, differentiation, and death. *Biochemistry (Mosc)* 63, 69–73.

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

Xia, J., and Wishart, D. S. (2016). Using metaboanalyst 3.0 for comprehensive metabolomics data analysis. *Curr. Protoc. Bioinformatics* 55, 14101–141091. doi: 10.1002/cpbi.11

Yan, M., Cai, W. B., Hua, T., Cheng, Q., Ai, D., Jiang, H. F., et al. (2020). Lipidomics reveals the dynamics of lipid profile altered by omega-3 polyunsaturated fatty acid supplementation in healthy people. *Clin. Exp. Pharmacol. Physiol.* 47, 1134–1144. doi: 10.1111/1440-1681.13285

Yang, F., Liu, Y. H., Yang, X. P., Xu, J., Kapke, A., and Carretero, O. A. (2002). Myocardial infarction and cardiac remodelling in mice. *Exp. Physiol.* 87, 547–555. doi: 10.1113/eph8702385

Yu, J., Pan, W., Shi, R., Yang, T., Li, Y., Yu, G., et al. (2015). Ceramide is upregulated and associated with mortality in patients with chronic heart failure. *Can. J. Cardiol.* 31, 357–363. doi: 10.1016/j.cjca.2014.12.007

Conflict of Interest: 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.

frontiers in Physiology

MINI REVIEW
published: 30 April 2021
doi: 10.3389/fphys.2021.659551

Bioactive Compounds in Edible Oils and Their Role in Oxidative Stress and Inflammation

Alessandra Mazzocchi[1]†, Valentina De Cosmi[1,2]†, Patrizia Risé[3], Gregorio Paolo Milani[1,4], Stefano Turolo[5], Marie-Louise Syrén[1], Angelo Sala[3,6]* and Carlo Agostoni[1,2]*

[1]Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy, [2]Pediatric Intermediate Care Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [3]Department of Pharmaceutical Sciences, University of Milan, Milan, Italy, [4]Pediatric Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [5]Pediatric Nephrology, Dialysis and Transplant Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [6]Istituto per la Ricerca e l'Innovazione Biomedica (IRIB), Consiglio Nazionale delle Ricerche (CNR), Palermo, Italy

Diet and inflammatory response are recognized as strictly related, and interest in exploring the potential of edible fats and oils for health and chronic diseases is emerging worldwide. Polyunsaturated fatty acids (PUFAs) present in fish oil (FO), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), may be partly converted into oxygenated bioactive lipids with anti-inflammatory and/or pro-resolving activities. Moreover, the co-presence of phenolic compounds and vitamins in edible oils may prevent the development of chronic diseases by their anti-inflammatory, antioxidant, neuroprotective, and immunomodulatory activities. Finally, a high content in mono-unsaturated fatty acids may improve the serum lipid profile and decrease the alterations caused by the oxidized low-density lipoproteins and free radicals. The present review aims to highlight the role of lipids and other bioactive compounds contained in edible oils on oxidative stress and inflammation, focusing on critical and controversial issues that recently emerged, and pointing to the opposing role often played by edible oils components and their oxidized metabolites.

Keywords: edible oil, vegetable oil, linoleic acid, alpha-linolenic acid, docosahexaenoic acid, immune response, inflammation resolution, marine oil

OPEN ACCESS

Edited by:
Jun Yang,
University of California, Davis, United States

Reviewed by:
Jetty Chung-Yung Lee,
The University of Hong Kong, Hong Kong
Guodong Zhang,
University of Massachusetts Amherst, United States

***Correspondence:**
Alessandra Mazzocchi
alessandra.mazzocchi@unimi.it
orcid.org/0000-0003-0291-2874
Angelo Sala
angelo.sala@unimi.it
orcid.org/0000-0001-9290-0257

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Lipid and Fatty Acid Research, a section of the journal Frontiers in Physiology

Received: *27 January 2021*
Accepted: *12 April 2021*
Published: *30 April 2021*

Citation:
Mazzocchi A, De Cosmi V, Risé P, Milani GP, Turolo S, Syrén M-L, Sala A and Agostoni C (2021) Bioactive Compounds in Edible Oils and Their Role in Oxidative Stress and Inflammation. Front. Physiol. 12:659551. doi: 10.3389/fphys.2021.659551

INTRODUCTION

Reactive oxygen species (ROS) are radical and non-radical chemical species formed by the partial reduction of oxygen that physiologically accumulate in parallel with cellular aerobic respiration (Angelova and Abramov, 2016). If unchecked, these compounds may result in DNA damages and cellular death. **Figure 1** shows possible endogenous and exogenous sources of ROS, highlighting respiration as the major contributor to endogenous ROS production. Moreover, during stress conditions, the endoplasmic reticulum releases Ca^{2+} that may (a) contribute to the activation of the cytoplasmic protein NLR-Family Pyrin Domain Containing 3 (NLRP3)

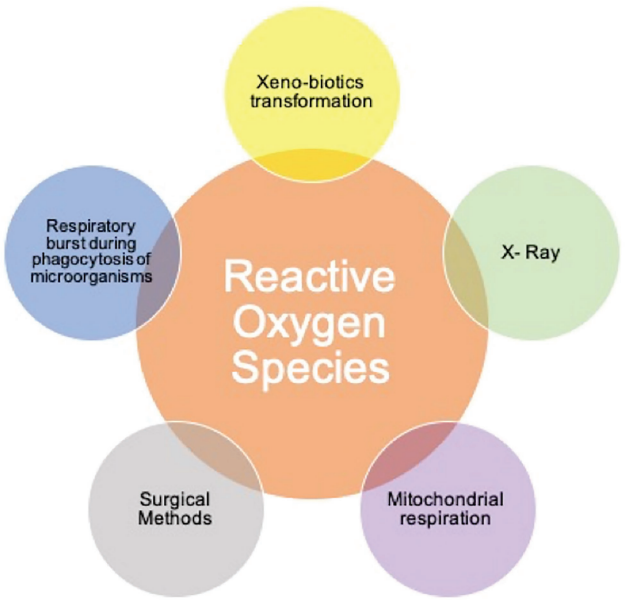


FIGURE 1 | Possible sources of Reactive Oxygen Species (ROS).

and therefore of the inflammasome, and (b) enter the mitochondria with subsequent generation and release of ROS (Zorov et al., 2014). Cytoplasmic ROS can also activate nuclear transcription factor kappa B (NF-κB), that migrates into the cellular nucleus promoting the transcription of inflammatory and oxidative genes, like cyclooxygenase (COX)-2, inducible-nitric oxide synthases (NOS), tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, and IL-1β (Mitchell et al., 2016). Transcription of inflammatory and oxidative genes can also be activated by Activator Protein 1 (AP-1) and Mitogen-Activated Protein Kinase (MAPK) resulting from toll like receptor's (TLR) engagement (He et al., 2009; Kuriakose et al., 2019).

Additional sources of ROS include drug-derived ROS produced as a consequence of their oxidative metabolism, X-rays, and surgical interventions, but ROS formation is also significantly associated with the inflammatory response. Inflammation is the physiological response to damage and is normally temporarily limited and solved by either specific or non-specific immune mechanisms. When an inflammatory response to an insult, whichever the origin, is not contained and then eliminated by the immune response, the self-perpetuation extends beyond the primary *foci* within a generalized hyper-reaction generating ROS that may contribute to the pathogenesis of non-communicable chronic diseases (NCDs), including cardiovascular diseases (CVDs), metabolic diseases, and cancer (Liguori et al., 2018).

The body possesses defense mechanisms against ROS, such as specific enzymes (i.e., superoxide dismutase, catalase, and glutathione peroxidase) and thiolic antioxidant (i.e., glutathione, and albumin), and generates biologically active metabolites playing an important role in the physiological resolution of the inflammatory process. The diet may contribute to these processes by providing micronutrients, such as vitamin C and vitamins A and E, that can neutralize ROS, as well as macronutrients such as omega (ω)3 fatty acids that are substrates for the biosynthesis of resolution mediators.

Besides vitamins, other micronutrients can modulate inflammation, including minerals like Se, Cu, and Zn (Galland, 2010), while focusing on macronutrients (that is, dietary components supplying energy, such as proteins, carbohydrates, and fats), hyper-caloric Western diets based on energy-dense foods, rich in simple sugars, and low in fibers, greatly contribute to an increase of endogenous lipogenesis to store the excess of energy. This process leads to high serum levels of saturated fatty acids (SFAs) which, in turn positively correlate with inflammatory markers such as circulating fibrinogen (Galland, 2010). By contrast, consumption of polyunsaturated fatty acids (PUFAs) and ω3 fatty acids in particular, increases their circulating levels and shows opposite associations (He et al., 2009). Compared with ω3, ω6 fatty acids show variable effects on inflammation, and available data are controversial (Innes and Calder, 2018), but dietary, circulating monounsaturated fatty acids (MUFAs), especially oleic acid, may have anti-inflammatory effects (Mashek and Wu, 2015).

Thus, dietary patterns, according to their specific nutrient and food composition, can either help to preserve a functional health status, or increase the risk of developing NCDs. The Mediterranean Diet (MD) is considered a healthy food pattern, especially considering its potential role in protecting against inflammation (Gotsis et al., 2015). The term "Mediterranean Diet" is usually referred to as a diet characterized by the high consumption of fruits, vegetables, whole grain cereals, seafood, legumes, nuts, and seeds, with a limited intake of meat and fermented beverages (Widmer et al., 2015). Replacing the intake of SFAs with PUFAs is recommended by dietary guidelines focused on cardiovascular health [National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and T. of H. B. C. in A. (Adult T. P. I.), 2002], and consuming dietary oils derived from plants, seeds, or of marine origin is a strategy to increase the intake of PUFAs.

Plant-derived oils contain the two precursors of the ω6 and ω3 families, i.e., linoleic and alpha-linolenic acids (LA, ALA), together with protective micronutrients, such as tocopherols, carotenoids, phytosterol, beta-carotene, nitrogen compounds, minerals (e.g., phosphorous, magnesium, manganese, copper, iron, zinc, and potassium), vitamins, and phenolic compounds (Vergallo, 2020). Marine-derived oils, in particular oils from fatty fish, are an important source of ω3 fatty acids, predominantly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA; Abedi and Sahari, 2014). Freshwater fish also ensures a good supply of ω6 PUFAs, but the possible presence of toxic contaminants or heavy metals and antibiotics may raise concerns in using fish oil (FO) as a source of PUFAs. Furthermore, undesirable flavors and tastes of FO may contribute to limiting their consumption (Abedi and Sahari, 2014).

In the first part of this narrative review, we will focus on the specific families of compounds present in plant- and marine-derived oils and their potential role relative to the formation of ROS and the inflammatory response. Particular attention will be on their specific activity and function, their dietary

sources and the principal evidence related to their association with human health.

The second part of this work will briefly summarize a selection of dietary oils (vegetable or marine-derived) focusing on the results of human trials available in the literature highlighting their effects in different disease conditions.

A specific attention will be paid to controversial or debated issues relative to selected components or edible oils.

LIPIDS, LIPID-DERIVED COMPOUNDS, AND MICRONUTRIENTS FROM EDIBLE OILS: ROLE IN OXIDATIVE STRESS AND INFLAMMATION

Edible oils and their components can play different and opposing roles in oxidative stress and inflammation. During the inflammatory responses and in presence of pathologies characterized by high tissue production of ROS, an increase intake of oils with significant amounts of long-chain PUFAs (LC-PUFAs) may increase PUFA in membrane phospholipids and, because of their high susceptibility to peroxidation (Pamplona, 2008), this could cause increased levels of PUFA peroxidation-derived compounds, such as oxidized phospholipids and isoprostanes, possessing significant pro-inflammatory properties (Takahashi et al., 1992; Marathe et al., 2001; Leitinger, 2003). On the other hand, recent reports identified oxidized phospholipids endowed with potent activities leading to the physiological resolution of the inflammatory process (Friedli and Freigang, 2017), pointing to the balance between these potential effects of LC-PUFA peroxidation as a critical factor in defining the outcome of the increased consumption of LC-PUFAs.

Oxygenase enzymes, such as lipoxygenases and COXs, acting on LC-PUFAs can also contribute to the formation of ROS, both directly, as a by-product of their enzymatic activity (Swindle et al., 2007), and through the formation of ω6 arachidonic acid (AA) metabolites such as leukotriene (LT) B_4, that activate the NADPH oxidase (NOX; Yun et al., 2010). Conversely, lipoxygenase-derived metabolites of ω3 LC-PUFAs may limit the formation of ROS (Chattopadhyay et al., 2017), control the inflammatory response, and promote its resolution (Serhan et al., 2008), suggesting again how a fine balance may define the contribution of LC-PUFAs to the physiological resolution of the inflammatory response rather than its evolution into chronic inflammation and pathology.

Finally, micronutrients from vegetable oils, such as vitamins or polyphenols, may also provide protection against ROS formation and its effects by their anti-oxidant activity, thus preserving membrane integrity (Ayala et al., 2014; Bochkov et al., 2017) and limiting the formation of pro-inflammatory mediators (Santus et al., 2005). Nevertheless, also in this case, the discussion is ongoing about the ability of these compounds to significantly affect human health in consideration of the low concentrations observed in many edible oils and the limited bioavailability of phenolic compounds (Nediani et al., 2019).

LC-PUFAs

Long-chain polyunsaturated fatty acids are synthesized by elongation and desaturation of the carbon chain from the parent essential PUFAs: LA for the ω6 series and ALA for the ω3 series (Agostoni and Bruzzese, 1992). The metabolic pathway consists of successive carbon chain elongation and desaturation steps (by inserting double bonds into the carbon chain), that are controlled by elongase and desaturase enzymes, respectively. It begins with a Δ6-desaturation step, followed by chain elongation and desaturation thereof, to yield EPA when the initial substrate is ALA, or to yield AA when the initial substrate is LA (Zárate et al., 2017).

Arachidonic Acid and Its Metabolites

Arachidonic acid (AA) is the main ω6 product and is present esterified to the 2-position in specific classes of membrane phospholipids (Chilton and Murphy, 1986). Upon release from membrane phospholipids by the activity of the cytosolic phospholipase A2, AA is enzymatically converted by several oxygenases into eicosanoids, a large family of mostly pro-inflammatory molecules, while non-enxymatic peroxidation of esterified AA leads to the formation of isoprostanes (Morrow et al., 1990), that can be released from phospholipids by the activity of platelet-activating factor acetylhydrolases and soluble phospholipases A2 (Kuksis and Pruzanski, 2017).

Cyclooxygenase (COX)-1 and COX-2 drive the synthesis of prostaglandins (PGs), prostacyclin, and thromboxane (TX; Smith et al., 1996); although these bioactive lipids are involved in a number of physiological and homeostatic processes, including hemostasis, vascular function, and gastric cytoprotection (Robert, 1979; Bunting et al., 1983; Weksler, 1984), they are mostly renowned for their ability to initiate and maintain inflammation. PGD_2, PGE_2, PGI_2, and $PGF_{2\alpha}$ represent to date a central subject of study among eicosanoids in inflammation, especially in light of the ability of NSAIDs to block their synthesis by inhibition of COX-1/2, which in turn results in the prevention of inflammation (Vane, 2002). A significant body of evidence is available that COX-2-derived PGE_2 may play a role in tumor angiogenesis by increasing vascular endothelial growth factor (VEGF; Eibl et al., 2003), and recent data strongly suggest a contribution of platelet COX-1-derived thromboxane in colorectal cancer (Patrignani and Patrono, 2018).

Non enzymatic isoprostanes have been widely used as marker of oxidative stress *in vivo*, but biological activities through the interaction with the thromboxane receptor have also been reported for 8-isoprostaglandin $F_{2\alpha}$ (Takahashi et al., 1992), suggesting that isoprostanes could play a role in the control of vascular tone and hemostasis (Capra et al., 2014). Recent reports have nevertheless shown that non enzymatic lipid peroxidation of esterified AA also generates cyclopentenone-containing oxydised phospholipids and related isoprostanes possessing potent pro-resolution activities. These compounds inhibit TLR activation and NF-kB signaling, and activate the Nrf2-pathway leading to the expression of anti-oxidant genes, limiting inflammation and cellular damage (Friedli and Freigang, 2017).

5-, 12-, and 15-lipoxygenases (5/12/15-LOX) generate leukotrienes (LTs), hydroxyeicosatetraenoic acids (HETEs;

Kuhn et al., 2015), and lipoxins (LXs; Romano et al., 2015); Leukotrienes also play a significant role in inflammation (Sala et al., 1998), and their increased expression has been reported in response to Th2 cytokines; neutrophilic tissue infiltration, and activation in response to LTB4, a main metabolite of 5-LO, is a mainstay of the acute inflammatory response (Mashima and Okuyama, 2015). Again, together with the proinflammatory role of lipoxygenase products, evidence have emerged early on that trihydroxy-derivatives of AA such as lipoxin A4 (LXA4) and LXB4, acting through the G-protein coupled receptor ALX/FPR2, inhibit inflammation responses by leukocytes, endothelial and epithelial cells (Romano et al., 2015).

Finally, diHETEs and epoxyeicosatrienoic acids represent the products of P450 epoxygenases (Bellien and Joannides, 2013), while ω and ω-1 monohydroxy metabolites of AA are generated by P450 ω-hydroxylases. Also in this case while the latter compounds can play pathophysiological role in cancer progression by promoting angiogenesis (Johnson et al., 2015), the former compounds have been reported to be endowed of significant anti-inflammatory activities, mediated by the inhibition of NF-kB, and the increase of peroxisome proliferator-activated receptor-gamma (PPAR-γ) transcription activity (Norwood et al., 2010).

ω3 Long Chain PUFAs and Their Metabolites

The main activities of ω3 LC-PUFAs are to directly and indirectly inhibit the inflammatory response: DHA can reduce endoplasmic reticulum stress and ROS production in mitochondria, inhibit TLR activation, and upregulate cytoprotective proteins, intracellular antioxidants, and anti-inflammatory and detoxifying enzymes through the activation of nuclear factor erythroid 2-related factor 2 (NRF2). Activated NRF2 inhibits the activity of AP-1, NF-κB, and MPK and promotes the transcription of anti-inflammatory and anti-oxidative genes like IL-10, IL-4, superoxide dismutase, heme oxidase-1, and glutathione (Yamagata, 2017). DHA and EPA can regulate the expression of oxidized low-density lipoprotein receptor 1, plasminogen activator inhibitor 1, thromboxane A2 receptor, vascular cell adhesion molecule-1, monocyte chemoattractant protein-1, and intercellular adhesion molecule 1, regulating, de facto, the inflammation response (Yamagata, 2017). Moreover, DHA inhibits TLR activation acting as an antagonist of SFA and blocking the inflammation triggered by TLR (Hwang et al., 2016; **Figure 2**).

ω3 LC-PUFAs, such as EPA, DPA, and DHA, are also the precursors of a number of oxygenated lipids, typically resulting from the coordinated activities of multiple lipoxygenases, including the 15/17R-lipoxygenase activity resulting from the aspirin acetylation of COX-2 (Serhan et al., 2002). The resulting compounds have been collectively described as a novel genus of specialized pro-resolving lipid mediators (SPM; Serhan et al., 2020) and are endowed with potent anti-inflammatory, pro-resolution activities within the immune system. SPMs, including D- and E-series resolvins, protectins, maresins (Serhan, 2017), and maresin conjugates in tissue regeneration (MCTRs; Dalli et al., 2016; Chiang et al., 2018), proved effective in limiting inflammation and contributing to speed-up the physiological resolution of the inflammatory response (Serhan and Levy, 2018). Several reports have linked

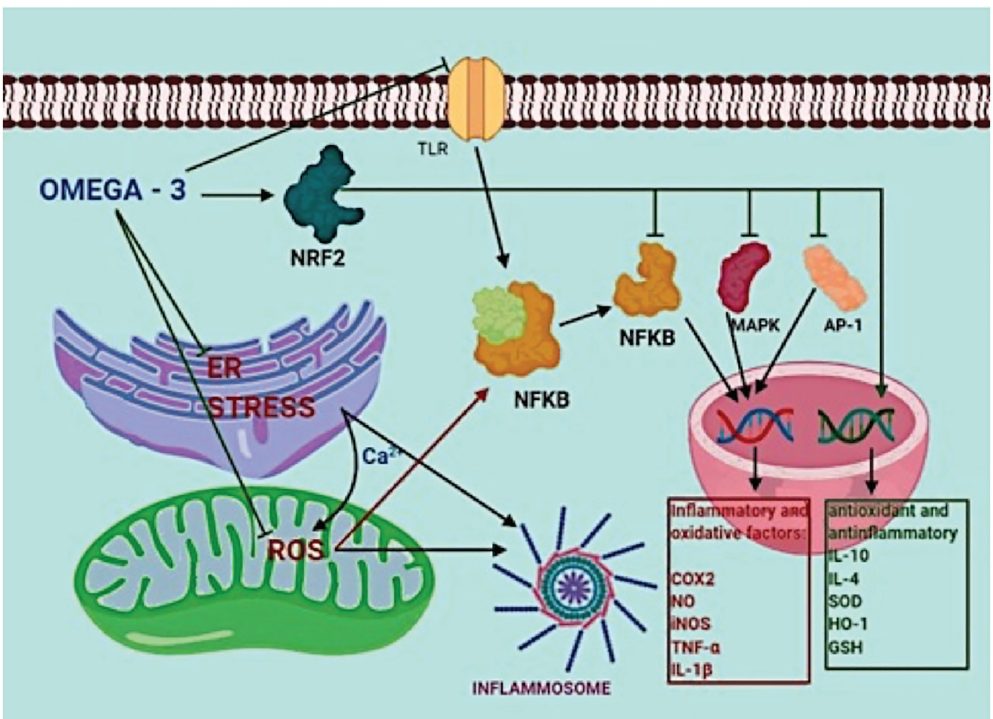


FIGURE 2 | The role of ω-3 fatty acids in inflammation.

supplementation with ω3 LC-PUFAs with increased concentrations of SPM in plasma (Grenon et al., 2015; Mas et al., 2016), but a critical study carried out both using ω3 LC-PUFAs supplementation and LPS challenge *in vivo* in humans raised questions about the effective correlation between plasma SPM and dietary supplementation (Skarke et al., 2015). Recent results using human hepatic cells showed extremely rapid β-oxidation of protectin D1, suggesting that plasma determination of this SPM may not be the appropriate approach to the assessment of its *in vivo* production (Balas et al., 2019). Indeed, *in vivo* production of eicosanoids has always been assessed either at the local site of production (if possible) or through the determination of stable hepatic metabolites in urines (Catella et al., 1986), while plasma concentrations were early on ruled out as unreliable or undetectable. In agreement with these considerations, supplementation with DHA in cystic fibrosis patients resulted in increased concentrations of DHA-derived oxygenated metabolites in sputum (Teopompi et al., 2019), but the relevance of ω3 LC-PUFAs supplementation to the production of SPM remains to be assessed.

Linoleic Acid and Its Metabolites

Linoleic acid, defined as an essential fatty acid in mammals because of their inability to synthesize it (Marangoni et al., 2020), is common in the human diet, being widely distributed in foods. In many vegetable oils, it represents more than 50% of the lipid content; high amounts of LA are also present in nuts, while lower levels are found in cereals (more in whole grains), legumes, some meats, eggs, and dairy products (Marangoni et al., 2020). In animal cells can be converted into AA; however, LA conversion to AA is very low (< 1%), and increasing the intake of dietary LA does not lead to a significant increase of its metabolites tissue levels (Rett and Whelan, 2011; Whelan and Fritsche, 2013). The effect of LA on human health is still controversial: a recent review discussed evidence about the potential benefit of increasing dietary intake of LA (Marangoni et al., 2020), reporting that epidemiological studies indicate that an adequate intake of LA reduces amounts of plasma low-density lipoprotein cholesterol (LDL-C) and dietary intervention studies showed that replacing 5% of the dietary energy derived from SFA with ω6 PUFAs reduces LDL-C by up to 10% (Siri-Tarino et al., 2015). Furthermore, in prospective cohort study circulating concentrations of LA are inversely associated with new cases of type 2 diabetes (Fretts et al., 2019). On the contrary, the reevaluation of data obtained in double-blind randomized controlled trials (RCTs), together with a systematic review and meta-analysis, while reporting a significant reduction of serum cholesterol did not confirm the original hypothesis of a significant effect on the risk of death by coronary heart disease (CHD) or all causes (Ramsden et al., 2013, 2016). The controversial role of linoleic acid is also underlined by several evidence suggesting that high intake of this PUFA is associated to an increase risk of colonic inflammation and colonic cancer. Indeed the risk of colorectal adenoma increased in correlation to plasma concentrations of linoleic acid in an endoscopy-based case–control study (Pot et al., 2008), and dietary linoleic acid was found to potentially contribute to an increased risk of ulcerative colitis in a prospective cohort study (Hart et al., 2009).

Linoleic acid has also been reported to be the substrate for CYP450 enzymes, including CYP2J2, CYP2C8, CYP2C9, and CYP1A1, leading to the formation of linoleic epoxides 9,10-epoxyoctadecenoic acid (9,10-EpOME), and 12,13-epoxyoctadecenoic acid (12,13-EpOME) known as leukotoxin and isoleukotoxin (Hildreth et al., 2020); these epoxides are then metabolized by the soluble epoxide hydrolases (sEH) into the dihydroxyderivatives 9,10-DiHOME and 12,13-DiHOME. The concentration of these compounds is dependent on both the regulation of biosynthetic pathways (CYP450s and s EH) and the dietary intake of their parent fatty acid LA. 9,10-EpOME is a major contributor to pulmonary toxicity in Acute Respiratory Distress Syndrome (ARDS), an effect that is enabled by the conversion into DiHOME (Zheng et al., 2001). DiHOMEs may also play a dual role in inflammation, stimulating neutrophil chemotaxis at low concentrations while inhibiting neutrophil respiratory burst at higher concentrations (Thompson and Hammock, 2007).

Alpha-Linolenic Acid and Its Metabolites

Just like LA, ALA is defined as an essential fatty acid in mammals (Das, 2006), and its principal diet sources are nuts, fish, leafy vegetables, and seed oils. After absorption, it can be catabolized into longer chain and more unsaturated FAs such as EPA and DHA, but similar to LA conversion into AA within the ω6 series, the endogenous production of ALA derivatives is low in humans. Tracer studies observed conversion of ALA to EPA, ω3 docosapentaenoic acid (DPA), and DHA, from birth to adulthood, in male and female, but in infants the conversion of ALA to DHA is about 1% whereas in adults is even lower (Brenna et al., 2009). ALA supplementation (by diet, capsules etc.) generally increases EPA and DPA, but has limited effects on DHA levels in plasma fractions and in circulating blood cells, while DHA supplementation increases the concentrations of this FA in blood and tissues (Arterburn et al., 2006). It should be also underlined that there is a competition between ω3 and ω6 FAs for the same metabolic pathway enzymes (desaturases and elongases), and that an increased LA intake decreases ω3 LC-PUFAs. Indeed, dietary ALA conversion appears to be decreased by high LA/ALA dietary ratios (Brenna et al., 2009).

Inconsistent data have recently been reported from studies examining how fatty acids, and particularly ω3 LC-PUFAs, can prevent or treat food allergy, atopic dermatitis, and asthma (Venter et al., 2019). These results can be partially justified by differences in bioavailability and interindividual variability in response to supplementation. Providing preformed ω3 derivatives or foods rich in ω3 LC-PUFAs seems more effective than supplying ALA because of the reported limited conversion capability in humans.

Non enzymatic oxidation of ALA originates analogs of isoprostanes termed phytoprostanes (Galano et al., 2017), possessing antinflammatory activities (Gilles et al., 2009). ALA-enriched diet in rats was reported to reduce oxidative stress and inflammation during myocardial infarction, while

increasing the formation of phytoprostane, suggesting their contribution to the observed effects (Leung et al., 2021). Recent data have also shown that novel metabolites generated by gut lactic acid bacteria from ALA, namely 13-hydroxy-octadecadienoic acid and 13-oxo-octadecadienoic acid, are capable to induce, both *in vitro* and *in vivo*, the differentiation of macrophage toward the anti-inflammatory phenotype M2 (Ohue-Kitano et al., 2018), through the activation of G protein-coupled receptor 40.

Monounsaturated Fatty Acids

Dietary MUFAs sources are both vegetable (e.g., olive oils, nuts, and seeds) and animal (e.g., meat from poultry and pig). The most abundant MUFA within the MD is oleic acid (about 90% of all MUFAs), followed by palmitoleic and vaccenic acids. A recent meta-analysis focusing on the effects of different dietary sources of MUFAs on CVD provided evidence that only olive oil was associated with a significant risk reduction of all-cause mortality, cardiovascular events, and stroke (Schwingshackl and Hoffmann, 2014), consistent with the fact that virgin olive oil is a supplier of other biologically active components such as polyphenols (Visioli and Bernardini, 2011), in particular oleuropein, in addition to MUFA.

Phenolic Compounds

The beneficial effects of oils have been widely attributed to their content in phenols, and more than 8,000 phenolic compounds have been identified. The structure of polyphenols is characterized by aromatic rings surrounded by hydroxyl groups (Quideau et al., 2011). Among these bioactive oil components there are tocopherols and tocotrienols (corn oil, soybean oil, wheat oil, and others), flavonoids (olive oil, sunflower seed oil), sterols, and phenolic acids (as esterified or free molecules, aldehyde forms, and glycosides). With exception of sterols that have beneficial effect on serum lipids (decreasing LDL-C and increasing HDL-C), the other compounds possess mainly radical scavenging, antioxidant and anti-inflammatory activities (Pandey and Rizvi, 2009), and the ability to modulate the immune response, affecting the multiplication of white blood cells and the production of cytokines (Gorzynik-Debicka et al., 2018). Different components alone have been tested *in vitro*, in cells or cell free assays, and some extra virgin olive oil (EVOO) phenolic compounds, but not all of them, inhibit IL-1β, PGE_2, and INFγ production (Miles et al., 2005). *In vitro* experimental evidence also showed significant anti-neuroinflammatory effects of lignanamides from hemp seeds (Luo et al., 2017; Wang et al., 2019). In *in vivo* experiments, sesamol decreased oxidative stress and inflammation (Kuhad and Chopra, 2008), and sesamine (0.2% in diet) decreased lipid peroxidation in plasma and liver in rats (Yamashita et al., 2000). Sesame lignans were also investigated in human, comparing the effects of a supplement of sesamin/episesamin 1:1 ratio (10 mg) + vitamin E (101 mg) formulated in wheat germ oil with wheat germ oil alone, showing a significant increase of antioxidant capacity evaluated as an increased lag-time in plasma LDL oxidation (Takemoto et al., 2015).

The predominant compounds found in EVOO are represented by oleuropein, hydroxytyrosol, and their derivatives (Pedan et al., 2019). The European Food Safety Authority (EFSA) approved in 2011 a claim that EVOO's polyphenols protect blood lipids against oxidative stress at a minimal dose of 5 mg/kg/day. The 5 mg of hydroxytyrosol should be available by consuming 23 g of EVOO in the context of a balanced diet (EFSA Panel on NDA, 2011).

Lipophenols are an emerging class of molecules that have been studied in these last years; they are characterized by condensation (esterification/acylation) of polyphenols and fatty acids, mainly unsaturated ones. Phenols and PUFAs, as described above, are natural compounds both endowed with biological activities on inflammation, oxidation, cancer, and CVDs, and the combination of these molecules could be of therapeutic advantage. In particular, the conjugation of polyphenols, such as flavonoids, phloroglucinol, and catechol derivatives with ω3 LC-PUFAs generates lipophenols (or phenolipids) of interest in which ω3 LC-PUFAs confer hydrophobicity, cell membrane penetration, and bioavailability to phenols, while the latter protect PUFAs from oxidation, possibly promoting their beneficial effects (Crauste et al., 2016).

Lipophenols can be obtained by enzymatic or chemical synthesis, but also from natural sources such as algae and marine species. The biological activity of lipophenols has been assessed in *in vitro* assays, frequently in non-cellular system, and only few studies have been performed *in vivo* in experimental animals. The antioxidant activity is a radical scavenging activity, even if the conjugation with FA frequently causes a decrease in the antioxidant properties of phenol, depending on the type of FA and on the phenol site of esterification (Moine et al., 2021). N-acyl dopamine and N-acyl-vanillylamines derivatives (containing a phenolic moiety), have also been shown to inhibit NO, IL-1β, IL-6, and TNF-α production, an effect that was dependent on the nature of FA with major effect for FA with a ketone group, then PUFA, MUFA, and saturated FA (Dang et al., 2011), suggesting a minor role for the phenolic moiety.

However, it must be stressed that the contribution to human health of all phenolic compounds depends on several factors including the concentration and whole oil composition, the extent of absorption and metabolism and the bioavailability in target tissues (Santangelo et al., 2017).

Vitamins

Vitamin E (α-tocopherol) and carotenoids are lipophilic antioxidants contained in vegetable oils (e.g., canola, olive, and soybean oil). They are known to decrease serum LDL levels and to prevent their oxidation (Upritchard et al., 2000). A large clinical trial showed a significant increase in the risk of prostate cancer in healthy men upon Vitamin E supplementation (Klein et al., 2011) and there is no specific advice on the intake of vitamin E. Its metabolism is related to vitamin C, vitamin B3, selenium, and glutathione, that all should be included within the diet to reach an optimal effect (Kurutas, 2016).

Vitamin E and carotenoids present in oils play an important role in the protection of PUFAs from oxidation: a pilot study carried out in experimental animals showed that while different

preparations of fish oil resulted in similar changes in plasma lipids, a significant increase in plasma lipid peroxidation was observed in the absence of fish oil stabilization with a natural antioxidant mixture rich in α-tocopherol (Engstrm et al., 2009). Carotenoids have been well-characterized for their antioxidant activity *in vitro* (Sandmann, 2019), and recent data have also shown their ability to specifically limit PUFA peroxidation in lipid membranes (Widomska et al., 2019).

Vitamin A is abundant in FO, in the liver and in dairy foods, and has a role in maintaining the immune system functions (Gilbert, 2013). Most effects of vitamin A are exerted by its metabolite, retinoic acid (RA), which through ligation of nuclear receptors controls the transcriptional expression of RA target genes. Within the immune system, RA has a central role in orchestrating immune responses and dendritic cells (DCs), and macrophages seem responsible for its production (Erkelens and Mebius, 2017).

EDIBLE PLANTS/SEEDS OILS

Edible oils are obtained from seeds, fruits, and pulps of plants, including many herbaceous plants, and comprise major components (such as triacylglycerols) and minor compounds (such as sterols, carotenoids, and tocopherols). They are known to be an essential dietary requirement for humans and may also play a critical role in the economy of several countries (in Tunisia 1.7 million ha are planted with olive tree, producing 4% of the olive oil production worldwide; Jabeur et al., 2014). Given their economic value, adulteration with cheaper oils is a common problem (Salah and Nofal, 2021) sometimes resulting in serious effects on consumers health as in the case of the Spanish oil toxic syndrome (Gelpí et al., 2002). In this section, we will review the results of clinical studies carried out with the different commercially available edible oils.

Hemp (*Cannabis sativa*) Seed Oil

Hemp seed oil is obtained from *Cannabis sativa*, and is characterized by high PUFAs content and low SFAs amounts (for composition see **Table 1**), with significant amounts of antioxidant such as tocopherols and phenolic compounds (Smeriglio et al., 2016). While studies in humans using supplementation with hempseed oil are scarce, there are nonetheless positive reports showing effects on clinical symptoms of dermatological diseases (atopic dermatitis; Callaway et al., 2005), as well as reduction of plasma triglycerides and improvement of the ratio total cholesterol/HDL cholesterol (Schwab et al., 2006). Nevertheless, the effects on plasma lipids could not be confirmed in subsequent studies in normal volunteers (Kaul et al., 2008) and in adolescent with hyperlipidemia (Del Bo et al., 2019).

Flaxseed Oil

Flaxseed oil (for composition see **Table 1**) is a good source of essential FA and contains lignans, cyanogenic glycosides, and cyclic peptides. In spite of the rich content of essential FA, the impact of flaxseed oil on serum lipids is controversial (Pan et al., 2009; Prasad, 2009): a reduction of plasma triglycerides and the improvement of the ratio total cholesterol/HDL cholesterol have been reported (Schwab et al., 2006), but has not been confirmed (Kaul et al., 2008). With respect to potential effects on cardiovascular inflammation, a RCT in healthy abdominally obese adults treated for 8 weeks with flaxseed oil capsules found no modifications in C-reactive protein (CRP), serum amyloid A (SAA), IL-6, and TNF-α (Nelson et al., 2007). On the contrary, 6 weeks of flaxseed oil administration resulted in a significant reduction of CRP (Zhao et al., 2004), an effect confirmed by two additional studies that observed a decrease of CRP, SAA, and IL-6 (Rallidis et al., 2003; Bemelmans et al., 2004).

Olive Oil and Extra-Virgin Olive Oil

Olive oils (for composition see **Table 1**) possess many health properties of higher nutritional quality, in particular the EVOO (Martín-Peláez et al., 2013). The majority of benefits are ascribed to minimal constituents in the unsaponifiable fraction, like phenolic compounds, phytosterols, tocopherols, and pigments (Mazzocchi et al., 2019). The effects on human health have been linked to its efficacy in preventing and treating of chronic disease resulting from anti-inflammatory, antioxidant, neuroprotective, and immunomodulatory activities (Carmen Crespo et al., 2018). The phenolic part accounts from 50 to 800 mg/kg and the predominant compound is made-up by oleuropein and its breakdown derivatives, hydroxytyrosol and tyrosol, but additional components contribute to the anti-inflammatory effects of EVOO as shown by the NUTRAOLEUM study: in this double-blind RCT, patients were supplemented with three different virgin olive oils (standard, high in phenolic compounds, and enriched with triterpenes oleanolic and maslinic acids) in a 3-week intervention. Over this period of time, plasma inflammatory biomarkers (IL-8 and TNF-alpha) and DNA oxidation significantly decreased in the group of subjects receiving the functional EVOO enriched with triterpenes (Sanchez-Rodriguez et al., 2019).

Soybean Oil

Soybean oil is extracted from the seeds of the soybean (for composition see **Table 1**). Phytosterols present in the oil may be responsible of its reported cholesterol-lowering activity (Zhu et al., 2019). The introduction of Δ6 desaturase from primrose (Primula juliae) and Δ15-desaturase from red bread mold (Neurospora crassa) into the soybean resulted in the production of a soybean oil rich in stearidonic acid (SDA; 18:4, n-3). Together, these two enzymes reduce the amount of linoleic acid by converting it into ALA and γ-linolenic acid, which are in turn ultimately converted into SDA, representing up to 20–30% of total fatty acids in the resulting oil. SDA, unlike ALA, may then be able to raise tissue levels of EPA and possibly DHA in humans, making widely used soybean oil a potential dietary source of this "pro-EPA" fatty acid (Harris, 2012). Indeed, two separate clinical trials showed increased EPA and ω3 index in adult overweight subjects (Harris et al., 2008; Lemke et al., 2010), confirming the ability of SDA to raise EPA plasma levels.

TABLE 1 | Components of selected edible oils (unless specified otherwise the amounts reported are per 100 g of oil).

Oil Description	Flaxseed (Pennington, 2002)	Olive (Pennington, 2002)	Canola (Pennington, 2002)	Soybean (Pennington, 2002)	Hempseed (Wang et al., 2019)	Cod liver (Pennington, 2002)	Krill (Xie et al., 2019)
Fatty acids, total saturated (g)	8.976	13.808	7.365	15.65	6.67	22.608	
Fatty acids, total monounsaturated (g)	18.438	72.961	63.276	22.783	13.33	46.711	
Fatty acids, total polyunsaturated (g)	67.849	10.523	28.142	57.74	66.67	22.541	
Choline, total (mg)	0.2	0.3	0.2	0.2			
Vitamin E (alpha-tocopherol) (mg)	0.47	14.35	17.46	8.18			14.74–63.0
Vitamin K (mcg)	9.3	60.2	71.3	183.9			
Calcium (mg)	1	1					1,322
Phosphorus (mg)	1						1,140
Magnesium (mg)							360
Iron (mg)		0.56		0.05			
Zinc (mg)	0.07			0.01			
Potassium (mg)		1					
Sodium (mg)		2					
Vitamin D (μg)						250	
Vitamin A (μg)						30,000	16.4–28.5 mg/100 g
Ω-6/ω-3 ratio					1.71–2.27		
Chlorophylls (μg/g)					0.041–2.64		
Tocopherols (mg/100 g)					100–150		
Carotenoids (μg/g)					0.29–1.73		
10:0 (g)	0.008						
12:0 (g)	0.018						
14:0 (g)	0.077					3.568	
16:0 (g)	5.109	11.29	4.298	10.455		10.63	
18:0 (g)	3.367	1.953	2.087	4.435		2.799	
16:1 (g)	0.06	1.255	0.214	0		8.309	
18:1 (g)	18.316	71.269	61.744	22.55		20.653	
20: 1(g)		0.311	1.317	0.233		10.422	
22:1 (g)	0.031					7.328	
18:2 (g)	14.327	9.762	19.005	50.952		0.935	
18:3 (g)	53.368	0.761	9.137	6.789		0.935	
18:4 (g)						0.935	
20:4 (g)						0.935	
20:5 n-3 (g)						6.898	
22:5 n-3 (g)						0.935	
22:6 n-3 (g)						10.968	
Cholesterol (mg)						570	

Canola Oil

Canola oil is obtained from the seeds of different species of Brassica family (for composition see **Table 1**). *Brassica napus* is known as rapeseed and its oil has a high content of erucic acid, a FA suspected of having pathogenic potential, but as edible oil, canola oil originates from selected Brassica typically showing erucic acid levels below 2%. Lin et al. (2013) reviewed the studies investigating the effects of canola oil, reporting significant reduction in total and LDL cholesterol, increased tocopherol levels, and insulin sensitivity. No effects were observed with respect to lipid peroxidation or susceptibility of LDL to oxidation, but platelets showed decreased ATP secretion and aggregation. Inflammatory markers were not affected by canola oil-based diets while potential linkages between canola oil usage and modifications of cancer risk remain undefined.

Recently, industries manufactured the high-oleic canola oil (HOCO), new canola oil with a modified formulation in fatty acids. HOCO is richer in MUFAs, lower in SFAs, and has a lower ratio of ω6/ω3 fatty acids than standard canola oil. A multicenter RCT provided evidence that a DHA-enriched HOCO improves lipid profiles and lowers CVD risk in abdominal obese subjects (Jones et al., 2014).

EDIBLE MARINE-DERIVED OILS

Marine Animal-Derived Oils

It is widely recognized that regularly eating fish decreases the risk of CVDs and related mortality (Hu et al., 2002; He et al., 2004), and dietary guidelines for ω3 LC-PUFAs and fish intake recommend two portions of fatty fish per week to assume

250–500 mg/die of EPA + DHA and prevent coronary diseases (American Heart Association Nutrition Committee et al., 2006; Mozaffarian and Rimm, 2006). While a food-based approach is preferable (Kris-Etherton et al., 2007), nutritional supplements are suitable substitutes for people who do not eat fish, and up to 3 g/day of fish oil is "generally recognized as safe" by the United States Food and Drug Administration. Cod liver oil (for composition see **Table 1**) is a dietary supplement extracted from Atlantic cod containing saturated, monounsaturated, and various PUFAs, including both EPA and DHA, together with vitamin A and D (Hu et al., 2019). While fish oil supplementation is recommended by the American Heart Association for CHD patients, insufficient evidence was found to grant the use in prevention for patient at high CVD risk only (Siscovick et al., 2017). A large meta-analysis of 10 recent RCT also found no effect of marine ω3 PUFAs supplementation on fatal and non-fatal CHD (Aung et al., 2018) fueling the debate about the health benefit of fish oil supplementation, but a new meta-analysis that included also three recent, large RCTs (VITAL, ASCEND, and REDUCE-IT), finally confirmed that marine ω3 supplementation reduce the risk for myocardial infarction, CHD and CVD death, also defining a clear dose-response relationship between the ω3 dose assumed and the effects (Hu et al., 2019).

In consideration of fish being a limited resource, attention to different sources of ω3 LC-PUFAs is also emerging. Krill oil, for example, is obtained from "*Euphausia superba*" and, along with a wide variety of fatty acid compounds (for composition see **Table 1**), also provides antioxidants, such as the carotenoid astaxanthin, vitamin E, and vitamin A. A RCT in healthy individuals showed a significant increase in EPA and EPA + DHA levels in plasma after consumption of 3 g/die for 4 weeks of krill oil when compared to FO (Ramprasath et al., 2013). Since both FO and krill oils deliver the same amount of total ω3 PUFAs, the results of this study may suggest that the bioavailability of ω3 PUFAs from krill oil is better than that from FO, but a reexamination of the bioavailability studies failed to confirm a difference between FO and krill oil (Salem and Kuratko, 2014). Lipids from different marine sources show a wide variability in ω3 LC-FAs content (Schuchardt and Hahn, 2013), and their bioavailability depends on several factors, including the concomitant intake of food (mainly its fat content) and the co-presence of other components (Kutzner et al., 2017). The marine sources of ω3 LC-FA not only differ in terms of absolute amounts of specific ω3 LC-FA, but also with respect to their chemical structures. In fish and in fish-derived oils, ω3 LC-FA is present primarily as triglycerides and, to a lesser extent, as free fatty acids. In krill oil, besides the two fractions mentioned, a substantial percentage of ω3 LC-FA is bound into phospholipids, raising the possibility that this form of ω3 LC-FA may also affect the bioavailability (Kutzner et al., 2017). It must be noted that in the near future, the capture of krill may also be restricted because of ecological concerns.

Algae

Algae consist of an intricate and non-specialized cluster of organisms characterized by an elementary reproductive structure and of photosynthetic nature. Currently, many species are cited in the literature as sources of bioactive compounds that are suitable as functional food ingredients (Ibañez and Cifuentes, 2013; Rengasamy et al., 2020). Micro and macro-algae represent a more sustainable source of PUFA-rich oils than fish. The PUFA profile varies among algal species: in macroalgae, lipid content is ~2–5% of dry weight, but the PUFA proportion of these lipids can represent up to 70% (Cofrades et al., 2010). Certain species of microalgae are capable of *de novo* production of LC-PUFAs thanks to their specific enzymatic systems, and the LC-PUFA content varies among species, but EPA and DHA are predominant in most species (Ratledge, 2004).

Algal biomass contains significant amounts of lipid-soluble carotenoids, with fucoxhantin and astaxanthin being the most abundant (Rengasamy et al., 2020). Limited evidence about their activites are available (Šimat et al., 2020), and a meta-analysis of the RCT carried out with astaxhantin showed unclear results (Wu et al., 2020) suggesting that additional studies are necessary to establish their potential health benefits.

The use of marine algae-derived antioxidants and PUFAs is a desirable goal, and in the last 2 decades, the potential of microalgae and microbes as sources of fatty acids has been increasingly recognized, leading to the large-scale production of PUFA supplements (Martins et al., 2013). The process of lipid production from microalgae and other microorganisms, i.e., single cell oil (SCO) production, has been recently proposed, and is of current industrial interest for use of these materials as dietary supplements in adults and infant nutrition (Ratledge, 2004).

DISCUSSION

Available evidence indicates that consumption of LC-PUFAs, MUFAs, and polyphenols from edible oils correlates to decreased levels of oxidative stress and inflammation. Dietary lipids act directly and indirectly through the formation of oxygenated metabolites possessing potent biological activities, such as eicosanoids and specialized pro-resolving mediators. In consideration of the different and often opposing biological activities of the families of LC-PUFAs oxygenated derivatives, it is of critical importance to assess the relative abundance of their precursors in cell membranes resulting from specific dietary habits, because LC-PUFAs may compete for the same metabolic pathways, affecting the resulting levels of bioactive metabolites in organs and tissues (Zárate et al., 2017). Furthermore the same metabolite sometimes generates opposing effects at different concentrations, as reported for PGE2 that may differentially activate VEGF at low concentrations and IL-8 at higher concentrations (Bonanno et al., 2016), introducing an additional layer of complexity in predicting the final biological outcome resulting from the activation of specific biosynthetic pathways.

The sensibility of LC-PUFAs to peroxidation may also lead to the formation of a number of biologically active metabolites, that in parallel to what observed for enzymatic metabolites possess often opposing biological activities, enhancing inflammation, oxidative stress, and cellular damage on one side, and promoting the resolution of the inflammatory response on

the other hand. Recently a web-based interactive interface has been made available to search for thousands of interconnected biochemical pathways leading to specific phenotypes of relevance for the inflammation and its resolution process (Serhan et al., 2020).

The Mediterranean Diet, thanks to its high supply of vegetables, seeds, and marine food sources rich in ω3 lipids, may be considered an anti-inflammatory diet, and the beneficial roles of plant, seeds, and marine-derived oils in the human body are of growing interest. Major consumption of these oils in their present form, or as nutraceutical supplements, as is the case of oils from fish and algae, may highly contribute replacing SFAs with PUFAs in dietary patterns. Nevertheless, the health benefits associated to increased PUFAs concentrations in cellular membranes have been the object of significant debate (Tummala et al., 2019), with the most comprehensive meta-analysis to-date still supporting the efficacy of marine ω3 supplementation in reducing cardiovascular risk (Hu et al., 2019).

In conclusion, it must be noted that the high heterogeneity in oil composition, inclusive of both the fat and the non-fat components, even from the same primary sources, as well the heterogeneity of clinical study designs reporting the beneficial effects of edible oils, may play a significant role in the health outcome associated to their consumption, often making it difficult to propose firm recommendations from both a quantitative and a qualitative standpoint.

AUTHOR CONTRIBUTIONS

AM, VC, and PR drafted the manuscript, proofread, and sorted the references. CA and AS critically reviewed and finalized the manuscript, while GM, ST, and M-LS reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

Research during work for this review was supported by a contribution from the Italian Ministry of Health (IRCCS grant).

REFERENCES

Abedi, E., and Sahari, M. A. (2014). Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. *Food Sci. Nutr.* 2, 443–463. doi: 10.1002/fsn3.121

Agostoni, C., and Bruzzese, M. G. (1992). Fatty acids: their biochemical and functional classification. *Med. Surg. Pediatr.* 14, 473–479.

American Heart Association Nutrition Committee, Lichtenstein, A. H., Appel, L. J., Brands, M., Carnethon, M., Daniels, S., et al. (2006). Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. *Circulation* 114, 82–96. doi: 10.1161/CIRCULATIONAHA.106.176158

Angelova, P. R., and Abramov, A. Y. (2016). Functional role of mitochondrial reactive oxygen species in physiology. *Free Radic. Biol. Med.* 100, 81–85. doi: 10.1016/j.freeradbiomed.2016.06.005

Arterburn, L. M., Hall, E. B., and Oken, H. (2006). Distribution, interconversion, and dose response of n-3 fatty acids in humans. *Am. J. Clin. Nutr.* 83, 1467S–1476S. doi: 10.1093/ajcn/83.6.1467S

Aung, T., Halsey, J., Kromhout, D., Gerstein, H. C., Marchioli, R., Tavazzi, L., et al. (2018). Associations of omega-3 fatty acid supplement use with cardiovascular disease risks meta-analysis of 10 trials involving 77 917 individuals. *JAMA Cardiol.* 3, 225–234. doi: 10.1001/jamacardio.2017.5205

Ayala, A., Muñoz, M. F., and Argüelles, S. (2014). Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Med. Cell. Longev.* 2014:360438. doi: 10.1155/2014/360438

Balas, L., Risé, P., Gandrath, D., Rovati, G., Bolego, C., Stellari, F., et al. (2019). Rapid metabolization of protectin D1 by β-oxidation of its polar head chain. *J. Med. Chem.* 62, 9961–9975. doi: 10.1021/acs.jmedchem.9b01463

Bellien, J., and Joannides, R. (2013). Epoxyeicosatrienoic acid pathway in human health and diseases. *J. Cardiovasc. Pharmacol.* 61, 188–196. doi: 10.1097/FJC.0b013e318273b007

Bemelmans, W. J. E., Lefrandt, J. D., Feskens, E. J. M., van Haelst, P. L., Broer, J., Meyboom-de Jong, B., et al. (2004). Increased α-linolenic acid intake lowers C-reactive protein, but has no effect on markers of atherosclerosis. *Eur. J. Clin. Nutr.* 58, 1083–1089. doi: 10.1038/sj.ejcn.1601938

Bochkov, V., Gesslbauer, B., Mauerhofer, C., Philippova, M., Erne, P., and Oskolkova, O. V. (2017). Pleiotropic effects of oxidized phospholipids. *Free Radic. Biol. Med.* 111, 6–24. doi: 10.1016/j.freeradbiomed.2016.12.034

Bonanno, A., Albano, G. D. G. D., Siena, L., Montalbano, A. M. A. M., Riccobono, L., Anzalone, G., et al. (2016). Prostaglandin E2 possesses different potencies in inducing vascular endothelial growth factor and Interleukin-8 production in COPD human lung fibroblasts. *Prostaglandins Leukot. Essent. Fat. Acids* 106, 11–18. doi: 10.1016/j.plefa.2016.01.005

Brenna, J. T., Salem, N., Sinclair, A. J., and Cunnane, S. C. (2009). α-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. *Prostaglandins Leukot. Essent. Fat. Acids* 80, 85–91. doi: 10.1016/j.plefa.2009.01.004

Bunting, S., Moncada, S., and Vane, J. R. (1983). The prostacyclin-thromboxane A2 balance: pathophysiological and therapeutic implications. *Br. Med. Bull.* 39, 271–276. doi: 10.1093/oxfordjournals.bmb.a071832

Callaway, J., Schwab, U., Harvima, I., Halonen, P., Mykkänen, O., Hyvönen, P., et al. (2005). Efficacy of dietary hempseed oil in patients with atopic dermatitis. *J. Dermatolog. Treat.* 16, 87–94. doi: 10.1080/09546630510035832

Capra, V., Bäck, M., Angiolillo, D. J., Cattaneo, M., and Sakariassen, K. S. (2014). Impact of vascular thromboxane prostanoid receptor activation on hemostasis, thrombosis, oxidative stress, and inflammation. *J. Thromb. Haemost.* 12, 126–137. doi: 10.1111/jth.12472

Carmen Crespo, M., Tomé-Carneiro, J., Dávalos, A., and Visioli, F. (2018). Pharma-nutritional properties of olive oil phenols. Transfer of new findings to human nutrition. *Foods* 7:90. doi: 10.3390/foods7060090

Catella, F., Nowak, J., and Fitzgerald, G. A. (1986). Measurement of renal and non-renal eicosanoid synthesis. *Am. J. Med.* 81, 23–29. doi: 10.1016/0002-9343(86)90905-8

Chattopadhyay, R., Raghavan, S., and Rao, G. N. (2017). Resolvin D1 via prevention of ROS-mediated SHP2 inactivation protects endothelial adherens junction integrity and barrier function. *Redox Biol.* 12, 438–455. doi: 10.1016/j.redox.2017.02.023

Chiang, N., Riley, I. R., Dalli, J., Rodriguez, A. R., Spur, B. W., and Serhan, C. N. (2018). New maresin conjugates in tissue regeneration pathway counters leukotriene D 4 –stimulated vascular responses. *FASEB J.* 32, 4043–4052. doi: 10.1096/fj.201701493R

Chilton, F. H., and Murphy, R. C. (1986). Remodeling of arachidonate-containing phosphoglycerides within the human neutrophil. *J. Biol. Chem.* 261, 7771–7777. doi: 10.1016/S0021-9258(19)57467-1

Cofrades, S., López-Lopez, I., Bravo, L., Ruiz-Capillas, C., Bastida, S., Larrea, M. T., et al. (2010). Nutritional and antioxidant properties of different brown and red Spanish edible seaweeds. *Food Sci. Technol. Int.* 16, 361–370. doi: 10.1177/1082013210367049

Crauste, C., Rosell, M., Durand, T., and Vercauteren, J. (2016). Omega-3 polyunsaturated lipophenols, how and why? *Biochimie* 120, 62–74. doi: 10.1016/j.biochi.2015.07.018

Dalli, J., Sanger, J. M., Rodriguez, A. R., Chiang, N., Spur, B. W., and Serhan, C. N. (2016). Identification and actions of a novel third maresin conjugate in

tissue regeneration: MCTR3. *PLoS One* 11:e149319. doi: 10.1371/journal.pone.0149319

Dang, H. T., Kang, G. J., Yoo, E. S., Hong, J., Choi, J. S., Kim, H. S., et al. (2011). Evaluation of endogenous fatty acid amides and their synthetic analogues as potential anti-inflammatory leads. *Bioorg. Med. Chem.* 19, 1520–1527. doi: 10.1016/j.bmc.2010.12.046

Das, U. N. (2006). Essential fatty acids: biochemistry, physiology and pathology. *Biotechnol. J.* 1, 420–439. doi: 10.1002/biot.200600012

Del Bo, C., Deon, V., Abello, F., Massini, G., Porrini, M., Riso, P., et al. (2019). Eight-week hempseed oil intervention improves the fatty acid composition of erythrocyte phospholipids and the omega-3 index, but does not affect the lipid profile in children and adolescents with primary hyperlipidemia. *Food Res. Int.* 119, 469–476. doi: 10.1016/j.foodres.2018.12.045

EFSA Panel on NDA (2011). Scientific opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL cholesterol concentrations (ID 1639), maintenance of normal blood pressure (ID 3781), "anti-inflammatory properties" (ID 1882), "contributes to the upper respiratory tract health" (ID 3468), "can help to maintain a normal function of gastrointestinal tract" (3779), and "contributes to body defences against external agents" (ID 3467) pursuant to article 13(1) of regulation (EC) No 1924/2006. *EFSA J.* 9, 1–25. doi: 10.2903/j.efsa.2011.2033

Eibl, G., Bruemmer, D., Okada, Y., Duffy, J. P., Law, R. E., Reber, H. A., et al. (2003). PGE2 is generated by specific COX-2 activity and increases VEGF production in COX-2-expressing human pancreatic cancer cells. *Biochem. Biophys. Res. Commun.* 306, 887–897. doi: 10.1016/S0006-291X(03)01079-9

Engstrm, K., Saldeen, A. S., Yang, B., Mehta, J. L., and Saldeen, T. (2009). Effect of fish oils containing different amounts of EPA, DHA, and antioxidants on plasma and brain fatty acids and brain nitric oxide synthase activity in rats. *Ups. J. Med. Sci.* 114, 206–213. doi: 10.3109/03009730903268958

Erkelens, M. N., and Mebius, R. E. (2017). Retinoic acid and immune homeostasis: a balancing act. *Trends Immunol.* 38, 168–180. doi: 10.1016/j.it.2016.12.006

Fretts, A. M., Imamura, F., Marklund, M., Micha, R., Wu, J. H. Y., Murphy, R. A., et al. (2019). Associations of circulating very-long-chain saturated fatty acids and incident type 2 diabetes: a pooled analysis of prospective cohort studies. *Am. J. Clin. Nutr.* 109, 1216–1223. doi: 10.1093/ajcn/nqz005

Friedli, O., and Freigang, S. (2017). Cyclopentenone-containing oxidized phospholipids and their isoprostanes as pro-resolving mediators of inflammation. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1862, 382–392. doi: 10.1016/j.bbalip.2016.07.006

Galano, J. M., Lee, Y. Y., Oger, C., Vigor, C., Vercauteren, J., Durand, T., et al. (2017). Isoprostanes, neuroprostanes and phytoprostanes: an overview of 25 years of research in chemistry and biology. *Prog. Lipid Res.* 68, 83–108. doi: 10.1016/j.plipres.2017.09.004

Galland, L. (2010). Diet and inflammation. *Nutr. Clin. Pract.* 25, 634–640. doi: 10.1177/0884533610385703

Gelpí, E., Posada de la Paz, M., Terracini, B., Abaitua, I., Gómez de la Cámara, A., Kilbourne, E. M., et al. (2002). The spanish toxic oil syndrome 20 years after its onset: a multidisciplinary review of scientific knowledge. *Environ. Health Perspect.* 110, 457–464. doi: 10.1289/ehp.110-1240833

Gilbert, C. (2013). What is vitamin A and why do we need it? Community eye Heal. 26, 65. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24782580 (Accessed January 26, 2021).

Gilles, S., Mariani, V., Bryce, M., Mueller, M. J., Ring, J., Jakob, T., et al. (2009). Pollen-derived E 1 -phytoprostanes signal via PPAR-γ and NF-κB-dependent mechanisms. *J. Immunol.* 182, 6653–6658. doi: 10.4049/jimmunol.0802613

Gorzynik-Debicka, M., Przychodzen, P., Cappello, F., Kuban-Jankowska, A., Marino Gammazza, A., Knap, N., et al. (2018). Potential health benefits of olive oil and plant polyphenols. *Int. J. Mol. Sci.* 19:686. doi: 10.3390/ijms19030686

Gotsis, E., Anagnostis, P., Mariolis, A., Vlachou, A., Katsiki, N., and Karagiannis, A. (2015). Health benefits of the mediterranean diet: an update of research over the last 5 years. *Angiology* 66, 304–318. doi: 10.1177/0003319714532169

Grenon, S. M., Owens, C. D., Nosova, E. V., Hughes-Fulford, M., Alley, H. F., Chong, K., et al. (2015). Short-term, high-dose fish oil supplementation increases the production of ω-3 fatty acid-derived mediators in patients with peripheral artery disease (the OMEGA-PAD I trial). *J. Am. Heart Assoc.* 4:e002034. doi: 10.1161/JAHA.115.002034

Harris, W. S. (2012). Stearidonic acid-enhanced soybean oil: a plant-based source of (n-3) fatty acids for foods. *J. Nutr.* 142, 600S–604S. doi: 10.3945/jn.111.146613

Harris, W. S., Lemke, S. L., Hansen, S. N., Goldstein, D. A., DiRienzo, M. A., Su, H., et al. (2008). Stearidonic acid-enriched soybean oil increased the omega-3 index, an emerging cardiovascular risk marker. *Lipids* 43, 805–811. doi: 10.1007/s11745-008-3215-0

Hart, A., Tjonneland, A., Olsen, A., Overvad, K., Bergmann, M. M., Boeing, H., et al. (2009). Linoleic acid, a dietary n-6 polyunsaturated fatty acid, and the aetiology of ulcerative colitis: a nested case-control study within a European prospective cohort study. *Gut* 58, 1606–1611. doi: 10.1136/gut.2008.169078

He, K., Liu, K., Daviglus, M. L., Jenny, N. S., Mayer-Davis, E., Jiang, R., et al. (2009). Associations of dietary long-chain n-3 polyunsaturated fatty acids and fish With biomarkers of inflammation and endothelial activation (from the multi-ethnic study of atherosclerosis [MESA]). *Am. J. Cardiol.* 103, 1238–1243. doi: 10.1016/j.amjcard.2009.01.016

He, K., Song, Y., Daviglus, M. L., Liu, K., Van Horn, L., Dyer, A. R., et al. (2004). Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies. *Circulation* 109, 2705–2711. doi: 10.1161/01.CIR.0000132503.19410.6B

Hildreth, K., Kodani, S. D., Hammock, B. D., and Zhao, L. (2020). Cytochrome P450-derived linoleic acid metabolites EpOMEs and DiHOMEs: a review of recent studies. *J. Nutr. Biochem.* 86:108484. doi: 10.1016/j.jnutbio.2020.108484

Hu, F. B., Bronner, L., Willett, W. C., Stampfer, M. J., Rexrode, K. M., Albert, C. M., et al. (2002). Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. *JAMA* 287, 1815–1821. doi: 10.1001/jama.287.14.1815

Hu, Y., Hu, F. B., and Manson, J. A. E. (2019). Marine Omega-3 supplementation and cardiovascular disease: an updated meta-analysis of 13 randomized controlled trials involving 127 477 participants. *J. Am. Heart Assoc.* 8:e013543. doi: 10.1161/JAHA.119.013543

Hwang, D. H., Kim, J.-A., and Lee, J. Y. (2016). Mechanisms for the activation of toll-like receptor 2/4 by saturated fatty acids and inhibition by docosahexaenoic acid. *Eur. J. Pharmacol.* 785, 24–35. doi: 10.1016/j.ejphar.2016.04.024

Ibañez, E., and Cifuentes, A. (2013). Benefits of using algae as natural sources of functional ingredients. *J. Sci. Food Agric.* 93, 703–709. doi: 10.1002/jsfa.6023

Innes, J. K., and Calder, P. C. (2018). Omega-6 fatty acids and inflammation. *Prostaglandins Leukot. Essent. Fat. Acids* 132, 41–48. doi: 10.1016/j.plefa.2018.03.004

Jabeur, H., Zribi, A., Makni, J., Rebai, A., Abdelhedi, R., and Bouaziz, M. (2014). Detection of Chemlali extra-virgin olive oil adulteration mixed with soybean oil, corn oil, and sunflower oil by using GC and HPLC. *J. Agric. Food Chem.* 62, 4893–4904. doi: 10.1021/jf500571n

Johnson, A. L., Edson, K. Z., Totah, R. A., and Rettie, A. E. (2015). Cytochrome P450 ω-hydroxylases in inflammation and cancer. *Adv. Pharmacol.* 74, 223–262. doi: 10.1016/bs.apha.2015.05.002

Jones, P. J. H., Senanayake, V. K., Pu, S., Jenkins, D. J. A., Connelly, P. W., Lamarche, B., et al. (2014). Dha-enriched high-oleic acid canola oil improves lipid profile and lowers predicted cardiovascular disease risk in the canola oil multicenter randomized controlled trial. *Am. J. Clin. Nutr.* 100, 88–97. doi: 10.3945/ajcn.113.081133

Kaul, N., Kreml, R., Austria, J. A., Richard, M. N., Edel, A. L., Dibrov, E., et al. (2008). A comparison of fish oil, flaxseed oil and hempseed oil supplementation on selected parameters of cardiovascular health in healthy volunteers. *J. Am. Coll. Nutr.* 27, 51–58. doi: 10.1080/07315724.2008.10719674

Klein, E. A., Thompson, I. M., Tangen, C. M., Crowley, J. J., Lucia, M. S., Goodman, P. J., et al. (2011). Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E cancer Prevention Trial (SELECT). *JAMA* 306, 1549–1556. doi: 10.1001/jama.2011.1437

Kris-Etherton, P. M., and Innis, S., Ammerican Dietetic Assocition, Dietitians of Canada (2007). Position of the American dietetic association and Dietitians of Canada: dietary fatty acids. *J. Am. Diet. Assoc.* 107, 1599.e1–1599.e15. doi: 10.1016/j.jada.2007.07.024

Kuhad, A., and Chopra, K. (2008). Effect of sesamol on diabetes-associated cognitive decline in rats. *Exp. Brain Res.* 185, 411–420. doi: 10.1007/s00221-007-1166-y

Kuhn, H., Banthiya, S., and van Leyen, K. (2015). Mammalian lipoxygenases and their biological relevance. *Biochim. Biophys. Acta* 1851, 308–330. doi: 10.1016/j.bbalip.2014.10.002

Kuksis, A., and Pruzanski, W. (2017). Hydrolysis of Phosphatidylcholine-Isoprostanes (PtdCho-IP) by peripheral human group IIA, V and X secretory phospholipases A2 (sPLA2). *Lipids* 52, 477–488. doi: 10.1007/s11745-017-4264-z

Kuriakose, S., Onyilagha, C., Singh, R., Olayinka-Adefemi, F., Jia, P., and Uzonna, J. E. (2019). TLR-2 and MyD88-dependent activation of MAPK and STAT proteins regulates proinflammatory cytokine response and immunity to experimental Trypanosoma congolense infection. *Front. Immunol.* 10:2673. doi: 10.3389/fimmu.2019.02673

Kurutas, E. B. (2016). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutr. J.* 15:71. doi: 10.1186/s12937-016-0186-5

Kutzner, L., Ostermann, A. I., Konrad, T., Riegel, D., Hellhake, S., Schuchardt, J. P., et al. (2017). Lipid class specific quantitative analysis of n-3 polyunsaturated fatty acids in food supplements. *J. Agric. Food Chem.* 65, 139–147. doi: 10.1021/acs.jafc.6b03745

Leitinger, N. (2003). Oxidized phospholipids as modulators of inflammation in atherosclerosis. *Curr. Opin. Lipidol.* 14, 421–430. doi: 10.1097/00041433-200310000-00002

Lemke, S. L., Vicini, J. L., Su, H., Goldstein, D. A., Nemeth, M. A., Krul, E. S., et al. (2010). Dietary intake of stearidonic acid-enriched soybean oil increases the omega-3 index: randomized, double-blind clinical study of efficacy and safety. *Am. J. Clin. Nutr.* 92, 766–775. doi: 10.3945/ajcn.2009.29072

Leung, K. S., Galano, J.-M., Oger, C., Durand, T., and Lee, C.-Y. (2021). Enrichment of alpha-linolenic acid in rodent diet reduced oxidative stress and inflammation during myocardial infarction. *Free Radic. Biol. Med.* 162, 53–64. doi: 10.1016/j.freeradbiomed.2020.11.025

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., et al. (2018). Oxidative stress, aging, and diseases. *Clin. Interv. Aging* 13, 757–772. doi: 10.2147/CIA.S158513

Lin, L., Allemekinders, H., Dansby, A., Campbell, L., Durance-Tod, S., Berger, A., et al. (2013). Evidence of health benefits of canola oil. *Nutr. Rev.* 71, 370–385. doi: 10.1111/nure.12033

Luo, Q., Yan, X., Bobrovskaya, L., Ji, M., Yuan, H., Lou, H., et al. (2017). Anti-neuroinflammatory effects of grossamide from hemp seed via suppression of TLR-4-mediated NF-κB signaling pathways in lipopolysaccharide-stimulated BV2 microglia cells. *Mol. Cell. Biochem.* 428, 129–137. doi: 10.1007/s11010-016-2923-7

Marangoni, F., Agostoni, C., Borghi, C., Catapano, A. L., Cena, H., Ghiselli, A., et al. (2020). Dietary linoleic acid and human health: focus on cardiovascular and cardiometabolic effects. *Atherosclerosis* 292, 90–98. doi: 10.1016/j.atherosclerosis.2019.11.018

Marathe, G. K., Prescott, S. M., Zimmerman, G. A., and McIntyre, T. M. (2001). Oxidized LDL contains inflammatory PAF-like phospholipids. *Trends Cardiovasc. Med.* 11, 139–142. doi: 10.1016/S1050-1738(01)00100-1

Martín-Peláez, S., Covas, M. I., Fitó, M., Kušar, A., and Pravst, I. (2013). Health effects of olive oil polyphenols: recent advances and possibilities for the use of health claims. *Mol. Nutr. Food Res.* 57, 760–771. doi: 10.1002/mnfr.201200421

Martins, D. A., Custódio, L., Barreira, L., Pereira, H., Ben-Hamadou, R., Varela, J., et al. (2013). Alternative sources of n-3 long-chain polyunsaturated fatty acids in marine microalgae. *Mar. Drugs* 11, 2259–2281. doi: 10.3390/md11072259

Mas, E., Barden, A., Burke, V., Beilin, L. J., Watts, G. F., Huang, R. C., et al. (2016). A randomized controlled trial of the effects of n-3 fatty acids on resolvins in chronic kidney disease. *Clin. Nutr.* 35, 331–336. doi: 10.1016/j.clnu.2015.04.004

Mashek, D. G., and Wu, C. (2015). MUFAs. *Adv. Nutr.* 6, 276–277. doi: 10.3945/an.114.005926

Mashima, R., and Okuyama, T. (2015). The role of lipoxygenases in pathophysiology; new insights and future perspectives. *Redox Biol.* 6, 297–310. doi: 10.1016/j.redox.2015.08.006

Mazzocchi, A., Leone, L., Agostoni, C., and Pali-Schöll, I. (2019). The secrets of the Mediterranean diet. Does [only] olive oil matter? *Nutrients* 11:2941. doi: 10.3390/nu11122941

Miles, E. A., Zoubouli, P., and Calder, P. C. (2005). Differential anti-inflammatory effects of phenolic compounds from extra virgin olive oil identified in human whole blood cultures. *Nutrition* 21, 389–394. doi: 10.1016/j.nut.2004.06.031

Mitchell, S., Vargas, J., and Hoffmann, A. (2016). Signaling via the NFκB system. *Wiley Interdiscip. Rev. Syst. Biol. Med.* 8, 227–241. doi: 10.1002/wsbm.1331

Moine, E., Boukhallat, M., Cia, D., Jacquemot, N., Guillou, L., Durand, T., et al. (2021). New lipophenols prevent carbonyl and oxidative stresses involved in macular degeneration. *Free Radic. Biol. Med.* 162, 367–382. doi: 10.1016/j.freeradbiomed.2020.10.316

Morrow, J. D., Hill, K. E., Burk, R. F., Nammour, T. M., Badr, K. F., and Roberts, L. J. (1990). A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. *Proc. Natl. Acad. Sci. U. S. A.* 87, 9383–9387. doi: 10.1073/pnas.87.23.9383

Mozaffarian, D., and Rimm, E. B. (2006). Fish intake, contaminants, and human health evaluating the risks and the benefits. *J. Am. Med. Assoc.* 296, 1885–1899. doi: 10.1001/jama.296.15.1885

National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and T. of H. B. C. in A. (Adult T. P. I.) (2002). Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III) final report. *Circulation* 106, 3143–3421. doi: 10.1161/circ.106.25.3143

Nediani, C., Ruzzolini, J., Romani, A., and Calorini, L. (2019). Oleuropein, a bioactive compound from *Olea europaea* L., as a potential preventive and therapeutic agent in non-communicable diseases. *Antioxidants* 8:578. doi: 10.3390/antiox8120578

Nelson, T. L., Stevens, J. R., and Hickey, M. S. (2007). Inflammatory markers are not altered by an eight week dietary α-linolenic acid intervention in healthy abdominally obese adult males and females. *Cytokine* 38, 101–106. doi: 10.1016/j.cyto.2007.05.010

Norwood, S., Liao, J., Hammock, B. D., and Yang, G. Y. (2010). Epoxyeicosatrienoic acids and soluble epoxide hydrolase: potential therapeutic targets for inflammation and its induced carcinogenesis. *Am. J. Transl. Res.* 2, 447–457.

Ohue-Kitano, R., Yasuoka, Y., Goto, T., Kitamura, N., Park, S. B., Kishino, S., et al. (2018). A-linolenic acid–derived metabolites from gut lactic acid bacteria induce differentiation of anti-inflammatory M2 macrophages through G protein-coupled receptor 40. *FASEB J.* 32, 304–318. doi: 10.1096/fj.201700273R

Pamplona, R. (2008). Membrane phospholipids, lipoxidative damage and molecular integrity: a causal role in aging and longevity. *Biochim. Biophys. Acta-Bioenerg.* 1777, 1249–1262. doi: 10.1016/j.bbabio.2008.07.003

Pan, A., Yu, D., Demark-Wahnefried, W., Franco, O. H., and Lin, X. (2009). Meta-analysis of the effects of flaxseed interventions on blood lipids. *Am. J. Clin. Nutr.* 90, 288–297. doi: 10.3945/ajcn.2009.27469

Pandey, K. B., and Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Med. Cell. Longev.* 2, 270–278. doi: 10.4161/oxim.2.5.9498

Patrignani, P., and Patrono, C. (2018). Aspirin, platelet inhibition and cancer prevention. *Platelets* 29, 779–785. doi: 10.1080/09537104.2018.1492105

Pedan, V., Popp, M., Rohn, S., Nyfeler, M., and Bongartz, A. (2019). Characterization of phenolic compounds and their contribution to sensory properties of olive oil. *Molecules* 24:2041. doi: 10.3390/molecules24112041

Pennington, J. A. T. (2002). Food composition databases for bioactive food components. *J. Food Compos. Anal.* 15, 419–434. doi: 10.1006/jfca.2002.1073

Pot, G. K., Geelen, A., van Heijningen, E.-M. B., Siezen, C. L. E., van Kranen, H. J., and Kampman, E. (2008). Opposing associations of serum n-3 and n-6 polyunsaturated fatty acids with colorectal adenoma risk: an endoscopy-based case-control study. *Int. J. Cancer* 123, 1974–1977. doi: 10.1002/ijc.23729

Prasad, K. (2009). Flaxseed and cardiovascular health. *J. Cardiovasc. Pharmacol.* 54, 369–377. doi: 10.1097/FJC.0b013e3181af04e5

Quideau, S., Deffieux, D., Douat-Casassus, C., and Pouységu, L. (2011). Plant polyphenols: chemical properties, biological activities, and synthesis. *Angew. Chem. Int. Ed. Eng.* 50, 586–621. doi: 10.1002/anie.201000044

Rallidis, L. S., Paschos, G., Liakos, G. K., Velissaridou, A. H., Anastasiadis, G., and Zampelas, A. (2003). Dietary α-linolenic acid decreases C-reactive protein, serum amyloid A and interleukin-6 in dyslipidaemic patients. *Atherosclerosis* 167, 237–242. doi: 10.1016/S0021-9150(02)00427-6

Ramprasath, V. R., Eyal, I., Zchut, S., and Jones, P. J. (2013). Enhanced increase of omega-3 index in healthy individuals with response to 4-week n-3 fatty acid supplementation from krill oil versus fish oil. *Lipids Health Dis.* 12:178. doi: 10.1186/1476-511X-12-178

Ramsden, C. E., Zamora, D., Leelarthaepin, B., Majchrzak-Hong, S. F., Faurot, K. R., Suchindran, C. M., et al. (2013). Use of dietary linoleic acid for secondary

prevention of coronary heart disease and death: evaluation of recovered data from the Sydney Diet Heart Study and updated meta-analysis. *BMJ* 346:e8707. doi: 10.1136/bmj.e8707

Ramsden, C. E., Zamora, D., Majchrzak-Hong, S., Faurot, K. R., Broste, S. K., Frantz, R. P., et al. (2016). Re-evaluation of the traditional diet-heart hypothesis: analysis of recovered data from Minnesota Coronary Experiment (1968–73). *BMJ* 353:i1246. doi: 10.1136/bmj.i1246

Ratledge, C. (2004). Fatty acid biosynthesis in microorganisms being used for Single Cell Oil production. *Biochimie* 86, 807–815. doi: 10.1016/j.biochi.2004.09.017

Rengasamy, K. R., Mahomoodally, M. F., Aumeeruddy, M. Z., Zengin, G., Xiao, J., and Kim, D. H. (2020). Bioactive compounds in seaweeds: an overview of their biological properties and safety. *Food Chem. Toxicol.* 135:111013. doi: 10.1016/j.fct.2019.111013

Rett, B. S., and Whelan, J. (2011). Increasing dietary linoleic acid does not increase tissue arachidonic acid content in adults consuming Western-type diets: a systematic review. *Nutr. Metab.* 8:36. doi: 10.1186/1743-7075-8-36

Robert, A. (1979). Cytoprotection by prostaglandins. *Gastroenterology* 77, 761–767. doi: 10.1016/0016-5085(79)90235-X

Romano, M., Cianci, E., Simiele, F., and Recchiuti, A. (2015). Lipoxins and aspirin-triggered lipoxins in resolution of inflammation. *Eur. J. Pharmacol.* 760, 49–63. doi: 10.1016/j.ejphar.2015.03.083

Sala, A., Zarini, S., and Bolla, M. (1998). Leukotrienes: lipid bioeffectors of inflammatory reactions. *Biochemistry* 63, 84–92.

Salah, W. A., and Nofal, M. (2021). Review of some adulteration detection techniques of edible oils. *J. Sci. Food Agric.* 101, 811–819. doi: 10.1002/jsfa.10750

Salem, N., and Kuratko, C. N. (2014). A reexamination of krill oil bioavailability studies. *Lipids Health Dis.* 13:137. doi: 10.1186/1476-511X-13-137

Sanchez-Rodriguez, E., Biel-Glesson, S., Fernandez-Navarro, J. R., Calleja, M. A., Espejo-Calvo, J. A., Gil-Extremera, B., et al. (2019). Effects of virgin olive oils differing in their bioactive compound contents on biomarkers of oxidative stress and inflammation in healthy adults: a randomized double-blind controlled trial. *Nutrients* 11:561. doi: 10.3390/nu11030561

Sandmann, G. (2019). Antioxidant protection from UV-and light-stress related to carotenoid structures. *Antioxidants* 8, 1–13. doi: 10.3390/antiox8070219

Santangelo, C., Varì, R., Scazzocchio, B., De Sancti, P., Giovannini, C., and D'Archivio, M., et al., (2017). Anti-inflammatory activity of extra virgin olive oil polyphenols: which role in the prevention and treatment of immune-mediated inflammatory diseases? *Endocr. Metab. Immune Disord. Drug Targets* 18, 36–50. doi: 10.2174/1871530317666171114114321

Santus, P., Sola, A., Carlucci, P., Fumagalli, F., Di Gennaro, A., Mondoni, M., et al. (2005). Lipid peroxidation and 5-lipoxygenase activity in chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 171, 838–843. doi: 10.1164/rccm.200404-558OC

Schuchardt, J. P., and Hahn, A. (2013). Bioavailability of long-chain omega-3 fatty acids. *Prostaglandins Leukot. Essent. Fat. Acids* 89, 1–8. doi: 10.1016/j.plefa.2013.03.010

Schwab, U. S., Callaway, J. C., Erkkilä, A. T., Gynther, J., Uusitupa, M. I. J., and Järvinen, T. (2006). Effects of hempseed and flaxseed oils on the profile of serum lipids, serum total and lipoprotein lipid concentrations and haemostatic factors. *Eur. J. Nutr.* 45, 470–477. doi: 10.1007/s00394-006-0621-z

Schwingshackl, L., and Hoffmann, G. (2014). Monounsaturated fatty acids, olive oil and health status: a systematic review and meta-analysis of cohort studies. *Lipids Health Dis.* 13:154. doi: 10.1186/1476-511X-13-154

Serhan, C. N. (2017). Treating inflammation and infection in the 21st century: new hints from decoding resolution mediators and mechanisms. *FASEB J.* 31, 1273–1288. doi: 10.1096/fj.201601222R

Serhan, C. N., Gupta, S. K., Perretti, M., Godson, C., Brennan, E., Li, Y., et al. (2020). The atlas of inflammation resolution (AIR). *Mol. Asp. Med.* 74:100894. doi: 10.1016/j.mam.2020.100894

Serhan, C. N., Hong, S., Gronert, K., Colgan, S. P., Devchand, P. R., Mirick, G., et al. (2002). Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *J. Exp. Med.* 196, 1025–1037. doi: 10.1084/jem.20020760

Serhan, C. N., and Levy, B. D. (2018). Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. *J. Clin. Invest.* 128, 2657–2669. doi: 10.1172/JCI97943

Serhan, C. N., Yacoubian, S., and Yang, R. (2008). Anti-inflammatory and proresolving lipid mediators. *Annu. Rev. Pathol.* 3, 279–312. doi: 10.1146/annurev.pathmechdis.3.121806.151409

Šimat, V., Elabed, N., Kulawik, P., Ceylan, Z., Jamroz, E., Yazgan, H., et al. (2020). Recent advances in marine-based nutraceuticals and their health benefits. *Mar. Drugs* 18, 1–40. doi: 10.3390/md18120627

Siri-Tarino, P. W., Chiu, S., Bergeron, N., and Krauss, R. M. (2015). Saturated fats versus polyunsaturated fats versus carbohydrates for cardiovascular disease prevention and treatment. *Annu. Rev. Nutr.* 35, 517–543. doi: 10.1146/annurev-nutr-071714-034449

Siscovick, D. S., Barringer, T. A., Fretts, A. M., Wu, J. H. Y., Lichtenstein, A. H., Costello, R. B., et al. (2017). Omega-3 polyunsaturated fatty acid (fish oil) supplementation and the prevention of clinical cardiovascular disease: a science advisory from the American Heart Association. *Circulation* 135, e867–e884. doi: 10.1161/CIR.0000000000000482

Skarke, C., Alamuddin, N., Lawson, J. A., Li, X., Ferguson, J. F., Reilly, M. P., et al. (2015). Bioactive products formed in humans from fish oils. *J. Lipid Res.* 56, 1808–1820. doi: 10.1194/jlr.M060392

Smeriglio, A., Galati, E. M., Monforte, M. T., Lanuzza, F., D'Angelo, V., and Circosta, C. (2016). Polyphenolic compounds and antioxidant activity of cold-pressed seed oil from Finola cultivar of *Cannabis sativa* L. *Phyther. Res.* 30, 1298–1307. doi: 10.1002/ptr.5623

Smith, W. L., Garavito, R. M., and DeWitt, D. L. (1996). Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. *J. Biol. Chem.* 271, 33157–33160. doi: 10.1074/jbc.271.52.33157

Swindle, E. J., Coleman, J. W., DeLeo, F. R., and Metcalfe, D. D. (2007). FcεRI- and Fcγ receptor-mediated production of reactive oxygen species by mast cells is lipoxygenase- and cyclooxygenase-dependent and NADPH oxidase-independent. *J. Immunol.* 179, 7059–7071. doi: 10.4049/jimmunol.179.10.7059

Takahashi, K., Nammour, T. M., Fukunaga, M., Ebert, J., Morrow, J. D., Roberts, L. J., et al. (1992). Glomerular actions of a free radical-generated novel prostaglandin, 8-epi- prostaglandin F(2α), in the rat. Evidence for interaction with thromboxane A2 receptors. *J. Clin. Invest.* 90, 136–141. doi: 10.1172/JCI115826

Takemoto, D., Yasutake, Y., Tomimori, N., Ono, Y., Shibata, H., and Hayashi, J. (2015). Sesame lignans and vitamin E supplementation improve subjective statuses and anti-oxidative capacity in healthy humans with feelings of daily fatigue. *Global J. Health Sci.* 7, 1–10. doi: 10.5539/gjhs.v7n6p1

Teopompi, E., Risé, P., Pisi, R., Buccellati, C., Aiello, M., Pisi, G., et al. (2019). Arachidonic acid and docosahexaenoic acid metabolites in the airways of adults with cystic fibrosis: effect of docosahexaenoic acid supplementation. *Front. Pharmacol.* 10:938. doi: 10.3389/fphar.2019.00938

Thompson, D. A., and Hammock, B. D. (2007). Dihydroxyoctadecamonoenoate esters inhibit the neutrophil respiratory burst. *J. Biosci.* 32, 279–291. doi: 10.1007/s12038-007-0028-x

Tummala, R., Ghosh, R. K., Jain, V., Devanabanda, A. R., Bandyopadhyay, D., Deedwania, P., et al. (2019). Fish oil and cardiometabolic diseases: recent updates and controversies. *Am. J. Med.* 132, 1153–1159. doi: 10.1016/j.amjmed.2019.04.027

Upritchard, J. E., Sutherland, W. H. F., and Mann, J. I. (2000). Effect of supplementation with tomato juice, vitamin E, and vitamin C on LDL oxidation and products of inflammatory activity in type 2 diabetes. *Diabetes Care* 23, 733–738. doi: 10.2337/diacare.23.6.733

Vane, J. R. (2002). Biomedicine: Back to an aspirin a day? *Science* 296, 474–475. doi: 10.1126/science.1071702

Venter, C., Meyer, R. W., Nwaru, B. I., Roduit, C., Untersmayr, E., Adel-Patient, K., et al. (2019). EAACI position paper: influence of dietary fatty acids on asthma, food allergy, and atopic dermatitis. *Allergy* 74, 1429–1444. doi: 10.1111/all.13764

Vergallo, C. (2020). Nutraceutical vegetable oil nanoformulations for prevention and management of diseases. *Nanomaterials* 10, 1–30. doi: 10.3390/nano10061232

Visioli, F., and Bernardini, E. (2011). Extra virgin olive oil's polyphenols: biological activities. *Curr. Pharm. Des.* 17, 786–804. doi: 10.2174/138161211795428885

Wang, S., Luo, Q., and Fan, P. (2019). Cannabisin F from hemp (*Cannabis sativa*) seed suppresses lipopolysaccharide-induced inflammatory responses in BV2 microglia as SIRT1 modulator. *Int. J. Mol. Sci.* 20:507. doi: 10.3390/ijms20030507

Weksler, B. B. (1984). Prostaglandins and vascular function. Circulation 70, III63-71. Available at: http://www.ncbi.nlm.nih.gov/pubmed/6386223 (Accessed March 24, 2021).

Whelan, J., and Fritsche, K. (2013). Linoleic acid. *Adv. Nutr.* 4, 311–312. doi: 10.3945/an.113.003772

Widmer, R. J., Flammer, A. J., Lerman, L. O., and Lerman, A. (2015). The Mediterranean diet, its components, and cardiovascular disease. *Am. J. Med.* 128, 229–238. doi: 10.1016/j.amjmed.2014.10.014

Widomska, J., Welc, R., and Gruszecki, W. I. (2019). The effect of carotenoids on the concentration of singlet oxygen in lipid membranes. *Biochim. Biophys. Acta-Biomembr.* 1861, 845–851. doi: 10.1016/j.bbamem.2019.01.012

Wu, D., Xu, H., Chen, J., and Zhang, L. (2020). Effects of astaxanthin supplementation on oxidative stress. *Int. J. Vitam. Nutr. Res.* 90, 179–194. doi: 10.1024/0300-9831/a000497

Xie, D., Gong, M., Wei, W., Jin, J., Wang, X. X., Wang, X. X., et al. (2019). Antarctic krill (*Euphausia superba*) oil: a comprehensive review of chemical composition, extraction technologies, health benefits, and current applications. *Compr. Rev. Food Sci. Food Saf.* 18, 514–534. doi: 10.1111/1541-4337.12427

Yamagata, K. (2017). Docosahexaenoic acid regulates vascular endothelial cell function and prevents cardiovascular disease. *Lipids Health Dis.* 16:118. doi: 10.1186/s12944-017-0514-6

Yamashita, K., Kagaya, M., Higuti, N., and Kiso, Y. (2000). Sesamin and α-tocopherol synergistically suppress lipid-peroxide in rats fed a high docosahexaenoic acid diet. *Biofactors* 11, 11–13. doi: 10.1002/biof.5520110104

Yun, M. R., Park, H. M., Seo, K. W., Lee, S. J., Im, D. S., and Kim, C. D. (2010). 5-Lipoxygenase plays an essential role in 4-HNE-enhanced ROS production in murine macrophages via activation of NADPH oxidase. *Free Radic. Res.* 44, 742–750. doi: 10.3109/10715761003758122

Zárate, R., Jaber-Vazdekis, N., Tejera, N., Pérez, J. A., and Rodríguez, C. (2017). Significance of long chain polyunsaturated fatty acids in human health. *Clin. Transl. Med.* 6:25. doi: 10.1186/s40169-017-0153-6

Zhao, G., Etherton, T. D., Martin, K. R., West, S. G., Gillies, P. J., and Kris-Etherton, P. M. (2004). Dietary α-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. *J. Nutr.* 134, 2991–2997. doi: 10.1093/jn/134.11.2991

Zheng, J., Plopper, C. G., Lakritz, J., Storms, D. H., and Hammock, B. D. (2001). Leukotoxin-diol: a putative toxic mediator involved in acute respiratory distress syndrome. *Am. J. Respir. Cell Mol. Biol.* 25, 434–438. doi: 10.1165/ajrcmb.25.4.4104

Zhu, H., Chen, J., He, Z., Hao, W., Liu, J., Kwek, E., et al. (2019). Plasma cholesterol-lowering activity of soybean germ phytosterols. *Nutrients* 11:2784. doi: 10.3390/nu11112784

Zorov, D. B., Juhaszova, M., and Sollott, S. J. (2014). Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol. Rev.* 94, 909–950. doi: 10.1152/physrev.00026.2013

Conflict of Interest: 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.

frontiers in Physiology

REVIEW
published: 25 May 2021
doi: 10.3389/fphys.2021.646491

Effect of ω-3 Polyunsaturated Fatty Acids-Derived Bioactive Lipids on Metabolic Disorders

Jinjie Duan[1,2†], Yayue Song[1,2†], Xu Zhang[1] and Chunjiong Wang[1,2]*

[1] Department of Physiology and Pathophysiology, The Province and Ministry Co-Sponsored Collaborative Innovation Center for Medical Epigenetics, Tianjin Medical University, Tianjin, China, [2] Tianjin Key Laboratory of Medical Epigenetics, Tianjin Medical University, Tianjin, China

OPEN ACCESS

Edited by:
Naim Akhtar Khan,
Université de Bourgogne, France

Reviewed by:
Undurti Narasimha Das,
UND Life Sciences LLC,
United States
Jetty Chung-Yung Lee,
The University of Hong Kong,
Hong Kong

***Correspondence:**
Chunjiong Wang
wangchunjiong@tmu.edu.cn

[†] These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Lipid and Fatty Acid Research, a section of the journal Frontiers in Physiology

Received: 27 December 2020
Accepted: 26 April 2021
Published: 25 May 2021

Citation:
Duan J, Song Y, Zhang X and Wang C (2021) Effect of ω-3 Polyunsaturated Fatty Acids-Derived Bioactive Lipids on Metabolic Disorders. Front. Physiol. 12:646491. doi: 10.3389/fphys.2021.646491

Arachidonic acid (ARA) is an important ω-6 polyunsaturated fatty acid (PUFA), and docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and n-3 docosapentaenoic acid (n-3 DPA) are three well-known ω-3 PUFAs. These fatty acids can be metabolized into a number of bioactive lipids. Eicosanoids derived from ARA have drawn great attention because of their important and complex biofunctions. Although EPA, DHA and n-3 DPA have also shown powerful biofunctions, we have fewer studies of metabolites derived from them than those from ARA. Recently, growing research has focused on the bioaction of ω-3 PUFA-derived metabolites, which indicates their great potential for treating metabolic disorders. Most of the functional studies of these bioactive lipids focused on their anti-inflammatory effects. However, several studies elucidated their direct effects on pancreatic β cells, hepatocytes, adipocytes, skeletal muscle cells, and endothelial cells. These researches revealed the importance of studying the functions of metabolites derived from ω-3 polyunsaturated fatty acids other than themselves. The current review summarizes research into the effects of ω-3 PUFA-derived oxylipins on metabolic disorders, including diabetes, non-alcoholic fatty liver disease, adipose tissue dysfunction, and atherosclerosis.

Keywords: ω-3 PUFA, eicosanoids, metabolic disorders, diabetes, NAFLD, adipose tissue, atherosclerosis

Abbreviations: ALA, α-linolenic acid; ARA, arachidonic acid; AMPK, AMP-activated protein kinase; BLT, leukotriene B4 receptor; cAMP, cyclic AMP; CCL, C-C motif chemokine ligand; COX, cyclooxygenase; CYP, cytochrome P450; DHA, docosahexaenoic acid; DHEA, docosahexaenoyl ethanolamine; DiHDPA, dihydroxydocosapentaenoic acid; DiHETE, dihydroxyeicosatetraenoic acid; DPA, docosapentaenoic acid; EDP, epoxydocosapentaenoic acid; EEQ, epoxyeicosatetraenoic acid; EPA, eicosapentaenoic acid; EPEA, *N*-eicosapentaenoyl ethanolamine; ERV-1, Resolvin E1 Receptor; GPCR, G protein-coupled receptors; HDoHE, hydroxydocosahexaenoic acid; HEPE, hydroxyeicosapentaenoic acid; HFD, high-fat-diet; IFN-γ, interferon γ; IL, interleukin; LGR6, leucine-rich repeat containing G protein-coupled receptor 6; LOX, lipoxygenase; LXA4, lipoxin A4; MaR, maresin; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PD, Protectins; PDX, Protectin DX; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; RvD, D-series resolvin; RvE, E-series resolvin; sEH, soluble epoxide hydrolase; TNF-α, tumor necrosis factor α; 7,17-DHDPA, 7,17dihydro-dipicolinic acid; 9-HOTRE, 9-hydroxy-octadecatrienoic acid; 13-(S)-HOTRE, 13-(*S*)-hydroxyoctadecatrienoic acid; 13-(S)-HPOTRE, 13-(*S*)-hydroperoxyoctadecatrienoic acid; 13-oxo-OTA, 13-Oxo-9(Z),11(E),15(Z)-octadecatrienoic acid; 14,15-DIHETRE,14,15-dihydroxy-5,8,11-eicosatrienoic acid.

INTRODUCTION

Polyunsaturated fatty acids (PUFAs) refer to fatty acids with two or more double bonds in their backbone. Arachidonic acid (ARA) is an important ω-6 PUFA, which can be metabolized from linoleic acid (Schmitz and Ecker, 2008). Docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and n-3 docosapentaenoic acid (n-3 DPA) are three well-known ω-3 PUFAs and they can be derived from α-linolenic acid (ALA). The estimated conversion rate of ALA to EPA was 8–20% in human, while that to DHA was 0.5–9%, even lesser (Stark et al., 2008). Those PUFAs are precursors of a series of bioactive lipids metabolized by cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450s (CYPs) and autoxidized non-enzymatically (Zhang et al., 2015).

Eicosanoids derived from ARA have drawn great attention because of their important and complex biofunctions. Many studies have examined the functions of ARA metabolites, including prostaglandins, thromboxanes, leukotrienes, lipoxins hydroxyeicosatetraenoic acids, and epoxyeicosatrienoic acid. These metabolites play vital roles in many physiological and pathophysiological processes. The effects of dietary supplement of ω-3 PUFAs are mediated not only by the precursor *per se* and their metabolites but also by competing the enzymes with ARA in the eicosanoid-producing process (Calder, 2020b). The effects of ARA and ARA-derived eicosanoids are well documented by several reviews (Sonnweber et al., 2018; Calder, 2020b). However, although ω-3 PUFAs also showed powerful biofunctions, we have fewer studies of their derived metabolites than those of ARA. Thus, we focused on the ω-3 PUFA derived bioactive lipids in the current review.

Metabolic disorders, such as obesity, diabetes, non-alcoholic fatty liver disease (NAFLD), and cardiovascular disease greatly threaten human health, and the prevalence of the diseases is increasing worldwide (Lavie et al., 2009; Younossi et al., 2016, 2018; Glovaci et al., 2019). In metabolic diseases, the profile of metabolites derived from ω-3 PUFAs is changed because of disturbed PUFA metabolism (Wang et al., 2017; Laguna-Fernandez et al., 2018; Garcia-Jaramillo et al., 2019). In the current review, we summarize the growing research into the effect of ω-3 PUFA-derived bioactive lipids on metabolic disorders, including diabetes, NAFLD, adipose tissue dysfunction and atherosclerosis.

THE METABOLIC PATHWAYS OF ALA, EPA, DHA, AND n-3 DPA

The metabolic pathways of ALA, EPA, DHA, and n-3 DPA were profoundly described by several reviews (Gabbs et al., 2015; Kuda, 2017; Drouin et al., 2019) and we briefly summarized as below:

ALA can be metabolized into hydroxy fatty acids by the COX and LOX pathway and epoxygenated fatty acids by the CYP pathway (Gabbs et al., 2015). In addition, ALA is the precursor of EPA, n-3 DPA and DHA. The rate limiting step is addition of a fourth double bond by Δ-6 desaturase. Next by elongation and desaturation, EPA is produced (Stark et al., 2008). EPA can be metabolized into 3-series prostaglandins and thromboxanes by the COX pathway; hydroxyeicosapentaenoic acids (HEPEs), E-series resolvins (RvE; RvE1-E3), 5-series leukotrienes and lipoxins by the LOX pathway; and epoxyeicosatetraenoic acids (EEQs) and dihydroxyeicosatetraenoic acids (diHETEs) by the CYP pathway (Zhang et al., 2015). Of note, 18-HEPE is derived from EPA by the CYP pathways or by aspirin-acetylated COX2 and then metabolized into RvEs by the LOX pathway (**Figure 1**; Gabbs et al., 2015).

Docosahexaenoic acid can be metabolized into hydroxydocosahexaenoic acids (HDoHEs), D-series resolvins (RvD; RvD1-D6), maresins (MaR; maresin 1 and 2), protectins (PD; PD1 and PDX) by the LOX pathway and epoxydocosapentaenoic acids (EDPs) and dihydroxydocosapentaenoic acids (DiHDPAs) by the CYP pathway (Zhang et al., 2015). 17-hydroperoxydocosahexaenoic acid (17-H(p)DHA) is the precursor of DHA-derived specialized pro-resolving mediators. 17S-H(p)DHA can be metabolized from DHA by the LOX pathway and then metabolized into 17(S)-Hydroxy docosahexaenoic acid (17S-HDHA) and PD1. 17S-HDHA is further metabolized into RvDs and PDX. 17R-H(p)DHA is produced from DHA by aspirin-acetylated COX2 and then metabolized into 17R-HDHA and AT-PD1. 17R-HDHA can be further metabolized to AT-RvDs (**Figure 1**; Gabbs et al., 2015; Kuda, 2017).

n-3 DPA can be formed from EPA by elongase and converts to DHA by Δ6 or Δ4/-desaturase (Park et al., 2015; Drouin et al., 2019) thus it is an important intermediate in the conversion pathway of EPA and DHA (**Figure 2**). In addition, it can metabolized into $PD_{n\text{-}3DPA}$ ($PD1_{n\text{-}3DPA}$ and $PD2_{n\text{-}3DPA}$), $RvD_{n\text{-}3DPA}$ ($RvD1_{n\text{-}3DPA}$, $RvD2_{n\text{-}3DPA}$, and $RvD5_{n\text{-}3DPA}$), $MaR_{n\text{-}3DPA}$ ($MaR1_{n\text{-}3DPA}$, $MaR2_{n\text{-}3DPA}$, and $MaR3_{n\text{-}3DPA}$) and hydroxy-DPA through LOX pathway; 13-series Rvs though COX pathway and 13-oxo derivatives by COX pathway when aspirin is existed (**Figure 2**; Drouin et al., 2019).

As ARA, ω-3 PUFA can also generate oxylipins non-enzymatically, which is mediated by uncontrolled oxidation (Galano et al., 2015; Hajeyah et al., 2020). ALA generates phytoprostanes, EPA generates F3-isoprostanes and DHA generates F4-neuroprostanes and neurofurans non-enzymatically (Galano et al., 2015).

In addition to ω-3 PUFA-derived oxylipins, conjugates of ω-3 PUFA with ethanolamine form acylethanolamides, which belong to fatty acid amides. Ethanolamine conjugates of DHA and EPA termed docosahexaenoyl ethanolamine (DHEA) and *N*-eicosapentaenoyl ethanolamine (EPEA), respectively (Meijerink et al., 2013). DHEA and/or EPEA can also be further metabolized by COX, LOX and CYP pathway (de Bus et al., 2019). DHEA and EPEA showed anti-inflammatory effects (de Bus et al., 2019), which indicates they may have bioactive effects on metabolic disorders. Besides, ω-3 PUFA intake was reported to reduced endocannabinoid levels in plasma and various tissues (Saleh-Ghadimi et al., 2020).

In the present review, we focus on the oxylipins enzymatically derived from ω-3 PUFA.

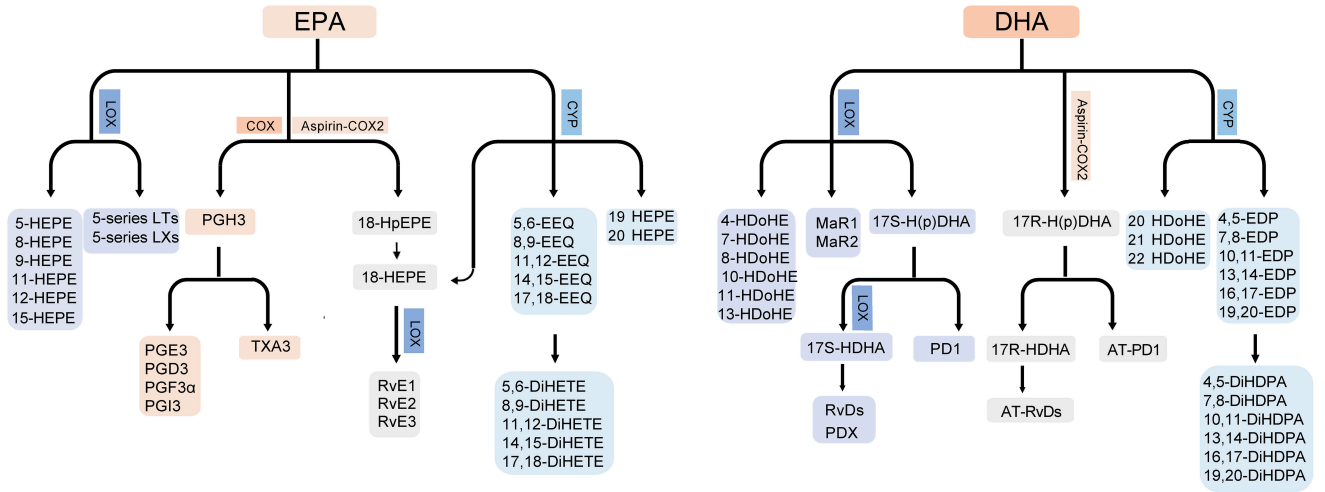


FIGURE 1 | Biosynthesis of EPA/DHA-derived bioactive lipids. LT, leukotrienes; LX, lipoxins.

THE IDENTIFIED RECEPTORS OF ω-3 PUFA-DERIVED OXYLIPINS

Identifying the receptors of these lipid mediators is vital to investigate their functions and the underlying mechanisms. Several studies have revealed that the effects of metabolites derived from ω-3 PUFA are mediated by G protein-coupled receptors (GPCRs) or nuclear receptors (**Table 1**). Krishnamoorthy et al. (2010) reported that RvD1 can directly bind to two GPCRs, ALX, and GPR32. ALX was first identified as an LXA4 receptor and GPR32 was considered an orphan receptor. The authors further revealed that RvD1-stimulated phagocytosis in macrophages was mediated by ALX and GPR32 (Krishnamoorthy et al., 2010). GPR18 is identified as a RvD2 receptor (Chiang et al., 2015). The protective effects of PDX on oxidative stress in vascular endothelial cells were mediated by GPR120, thus GPR120 may be a putative receptor of PDX (Hwang et al., 2019). MaR1 derived from DHA specifically binds to and activates human leucine-rich repeat containing G protein-coupled receptor 6 (LGR6) (Chiang et al., 2019). RvE1 binds to leukotriene B4 receptor 1 (BLT-1) and ERV-1 (also known as ChemR23) (Freire et al., 2017). 5-HEPE is an agonist of GPR119, a GPCR that regulates insulin secretion in pancreatic β cells (Kogure et al., 2011).

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that can sense fatty acid and regulate lipid and glucose metabolism (Xu et al., 2018). The PPAR family includes three members, PPARα, PPARβ/δ, and PPARγ. HEPEs derived from EPA can activate PPARs (Yamada et al., 2014). 8-HEPE and 9-HEPE show higher ligand activities for PPARs than do 5-HEPE, 12-HEPE, 18-HEPE and EPA. Besides PPARs, MaR1 is an endogenous ligand of retinoic acid-related orphan receptor α (RORα) (Han et al., 2019). However, whether other ω-3 PUFA-derived metabolites are ligands of GPCRs or nuclear receptors is still unknown.

EFFECT OF ω-3 PUFA-DERIVED OXYLIPINS ON DIABETES

Type 1 diabetes is described as immune-mediated destruction of pancreatic β cells, and the characteristics of type 2 diabetes are insulin resistance and progressive β-cell failure (Yang et al., 2018). Diabetes is a major metabolic disorder with high prevalence and is a risk factor for relevant public health issues such as cardiovascular disease, retinopathy, microangiopathy, and impaired wound healing (Yang et al., 2018).

EPA and DHA have shown beneficial effects for both type 1 and type 2 diabetes in rodents (Krishna Mohan and Das, 2001; Suresh and Das, 2003; Bi et al., 2017; Lepretti et al., 2018) and there is increasing evidence that the metabolites of EPA and DHA regulate these procedures. However, clinical trials showed conflicting results of dietary supplement of EPA/DHA on metabolic parameters in diabetic patients. A 6-month EPA treatment decreased postprandial glucose level of newly diagnosed impaired glucose metabolism patients (Sawada et al., 2016). Another clinical research also revealed the beneficial effects of ω-3 PUFA supplement on metabolic parameters including glucose and glycosylated hemoglobin in type 2 diabetic patients (Jacobo-Cejudo et al., 2017). However, several clinical studies revealed neutral effects of ω-3 PUFAs on metabolic profiles in type 2 diabetic patients (Wong et al., 2010; Poreba et al., 2017). The disagreement of these studies may be related with different sample sizes, baseline characteristics of patients, different doses and purities of these fatty acid, different time courses of the treatments and different basic medicine of these patients. Moreover, Poreba et al. (2017) also demonstrated high-dose ω-3 PUFAs did not increase RvD1 level in patients with atherosclerosis and type 2 diabetes and this is an important clue that the production of bioactive metabolites of ω-3 PUFAs is related to their therapeutic effects (Poreba et al., 2017). Thus, to study the effects and mechanism of ω-3 PUFA-derived

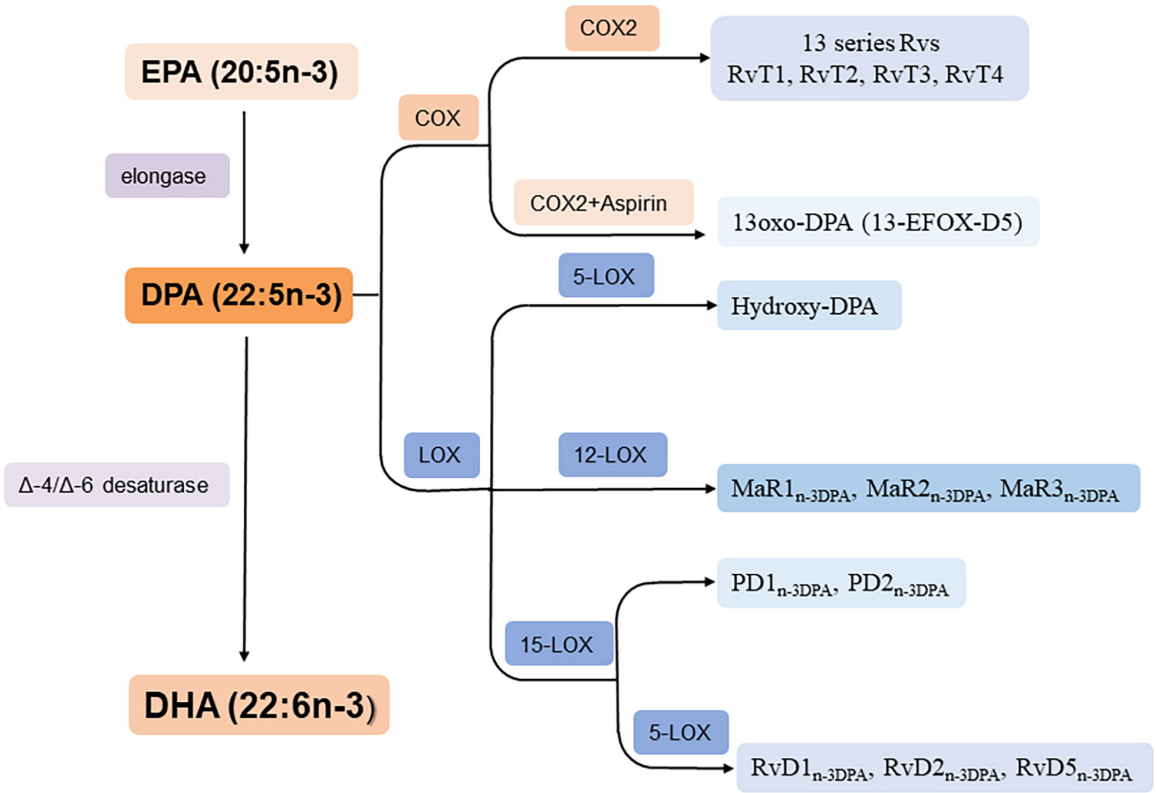


FIGURE 2 | Biosynthesis of n-3 DPA-derived bioactive lipids. RvT1: 7,13R, 20-tri hydroxy-n-3 DPA; RvT2: 7,12,13R-tri hydroxy-n-3 DPA; RvT3: 7,8,13R-trihydroxy-n-3 DPA; RvT4: 7,13R-dihydroxy-n-3 DPA.

TABLE 1 | The receptors of ω-3 PUFA-derived bioactive lipids.

Metabolites	Precursors	Putative receptors	
		GPCR	NR
RvD1	DHA	ALX	
		GPR32	
RvD2	DHA	GPR18	
MaR1	DHA	LGR6	RORα
PDX	DHA	GPR120	
RvE1	EPA	BLT-1	
		ERV-1	
5-HEPE	EPA	GPR119	PPARs
8-HEPE	EPA		PPARs
9-HEPE	EPA		PPARs
12-HEPE	EPA		PPARs
18-HEPE	EPA		PPARs

GPCR, G protein-coupled receptor; NR, nuclear receptor.

metabolites is important to develop new strategies to confront diabetes.

Recently, the bioactive lipids derived from EPA or DHA, including RvD1, RvD2, PDX, RvE1, and 5-HEPE, were reported to affect insulin resistance or pancreatic β-cell function (**Table 2**). Moreover, ω-3 PUFA metabolites can be involved in diabetic complications, including impaired wound healing and diabetic retinopathy (**Table 2**).

Effect of DHA-Derived Oxylipins on Diabetes

The levels of RvD1 and 17-HDHA were decreased in adipose tissue of genetic as well as diet-induced obese mice (Neuhofer et al., 2013). 17-HDHA treatment was further found to improve adipose tissue inflammation and insulin sensitivity in high-fat-diet (HFD)-fed mice (Neuhofer et al., 2013). Also, RvD1 has beneficial effects on insulin resistance. Hellmann et al. (2011) demonstrated that RvD1 improved glucose tolerance and increased insulin-stimulated pAkt level in liver, adipose tissue and skeletal muscle in db/db mice. The authors further found that RvD1 increased the ratio of M2 and M1 adipose-tissue macrophages (Bathina et al., 2020) and ameliorated adipose tissue inflammation (Hellmann et al., 2011). RvD1 was also reported to improve insulin resistance through the PI3K-Akt-mTOR axis in brain tissue (Bathina et al., 2020). *In vitro* study also indicated that RvD1 could attenuate interferon γ (IFN-γ)/lipopolysaccharide-induced pro-inflammatory cytokine expression in macrophages (Titos et al., 2011). Collectively, RvD1 improves insulin sensitivity by inhibiting tissue inflammation. Moreover, RvD1 ameliorated streptozotocin induced type1 diabetes in mice (Bathina and Das, 2021). In addition, local RvD1 delivery can accelerate

TABLE 2 | The functions of ω-3 PUFA-derived bioactive lipids on metabolic disorders.

Metabolites	Function	Diseases	*In vivo*	*In vitro*	References
RvD1	– Insulin resistance; – Adipose tissue Inflammation	Type 2 diabetes	√	√	Hellmann et al., 2011; Bathina et al., 2020
	– Oxidative stress – Inflammation	Type 1 diabetes	√		Bathina and Das, 2021
	+ Healing of diabetic wounds	Diabetic complications	√		Bathina and Das, 2021
	– Pro-angiogenic potential of retinal photoreceptors	Diabetic complications		√	Maisto et al., 2020
	– NASH	NASH	√	√	Rius et al., 2014; Li et al., 2020
	– Macrophage inflammation	Obesity		√	Titos et al., 2011
	– Advanced atherosclerosis	Atherosclerosis	√		Fredman et al., 2016
17-HDHA, RvD1 precursor	– Adipose tissue Inflammation	Obesity;Type 2 diabetes	√		Neuhofer et al., 2013
	– NAFLD; – Liver inflammation	NAFLD	√		Rodriguez-Echevarria et al., 2018
RvD2	– Adiposity; + Glucose tolerance	Obesity	√		Pascoal et al., 2017
Protectin DX	– Insulin resistance; + skeletal muscle IL-6 secretion	Type 2 diabetes	√	√	White et al., 2014
	– Skeletal muscle cell Insulin resistance	Type 2 diabetes	√	√	Jung et al., 2017
	– Hepatocyte insulin resistance; – Fetuin-A and selenoprotein	Type 2 diabetes		√	Jung et al., 2019
	– Adipocyte Inflammation; – Adipocyte Insulin resistance	Insulin resistance		√	Jung et al., 2018a
	– Hepatic steatosis	NAFLD	√	√	Jung et al., 2018c
MaR1	– TNFα induced lipolysis	Obesity		√	Laiglesia et al., 2018a
	– Insulin resistance; – Adipose tissue Inflammation; + Adiponectin secretion	Obesity;Type 2 diabetes	√	√	Martinez-Fernandez et al., 2017, 2020
	– Hepatic steatosis;	NAFLD	√	√	Rius et al., 2017; Jung et al., 2018b; Laiglesia et al., 2018b
	+ M2 polarity of liver macrophages	NASH	√	√	Han et al., 2019
MaR1 + RvD2	– Atherosclerosis; – Macrophage inflammatory	Atherosclerosis	√	√	Viola et al., 2016
PD1	+ Adiponectin secretion	Obesity		√	Gonzalez-Periz et al., 2009
19,20-DiHDPA	+ Diabetic retinopathy	Diabetic complications	√	√	Hu et al., 2017
19,20-EDP	+ Autophagy (hepatocyte); – Insulin resistance (adipocyte)	NAFLD;Obesity		√	Lopez-Vicario et al., 2015
RvE1	– Hepatic steatosis – Liver inflammation	NAFLD	√		Gonzalez-Periz et al., 2009
	– Atherosclerosis	Atherosclerosis		√	Salic et al., 2016
RvE1 (RvE1 receptor overexpression)	– Insulin resistance; – Inflammation	Obesity;Type 2 diabetes	√		Sima et al., 2017; Pal et al., 2020
18-HEPE/Resolvin E1 (RvE1 receptor deletion)	– Macrophage oxLDL uptake; – Atherosclerosis	Atherosclerosis	√	√	Laguna-Fernandez et al., 2018
18-HEPE	– NAFLD; – Liver inflammation	NAFLD	√		Rodriguez-Echevarria et al., 2018
	– Endothelial activation	Atherosclerosis		√	Liu et al., 2018
8-HEPE	– Dyslipidemia – Liver steatosis	NAFLD	√		Saito et al., 2020
17,18-EEQ	– Insulin resistance (adipocyte)	Obesity		√	Lopez-Vicario et al., 2015
	– Endothelial activation	Atherosclerosis		√	Liu et al., 2018
17,18-EEQ 9-HEPE 5-HEPE	– Liver steatosis; – Adipose tissue inflammation – Macrophage inflammation	NAFLD	√	√	Wang et al., 2017
5-HEPE	+ T-reg in adipose tissue	Obesity		√	Onodera et al., 2017
	+ Insulin secretion	Diabetes		√	Kogure et al., 2011

(Continued)

TABLE 2 | Continued

Metabolites	Function	Diseases	*In vivo*	*In vitro*	References
12-HEPE	+ Cold adaptation; +Glucose uptake (adipocyte and skeletal muscle)	Diabetes	√	√	Leiria et al., 2019
$RvD5_{n-3DPA}$	– Leukocyte and platelet activation – Aortic lesions	Atherosclerosis	√	√	Colas et al., 2018
13-oxo-OTA	+ Glucose uptake (adipocyte)	Diabetes		√	Takahashi et al., 2015

–, inhibit; +, promote; Ref., reference.

wound closure in diabetic mice by stimulating macrophage phagocytosis to enhance clearance of apoptotic cells (Tang et al., 2013). *In vitro* study demonstrated RvD1 reduced the pro-angiogenic potential of retinal photoreceptors treated by high glucose by increasing anti-angiogenic miRNAs and decreasing VEGF content in exosomes (Maisto et al., 2020).

PDX-treated mice showed protection from lipid-induced insulin resistance. Along with this effect, PDX inhibited lipid-induced secretion of C-C motif chemokine ligand (CCL) 2, CCL5, tumor necrosis factor α (TNF-α), IFN-γ, interleukin 1β (IL-1β), IL-2, and IL-17. However, IL-6 level was significantly increased with PDX treatment, which was from skeletal muscle and suppressed gluconeogenic gene expression in liver (White et al., 2014). In addition, PDX can activate AMPK independent of IL-6 (White et al., 2014). Consistent with this finding, PDX improved HFD-induced insulin resistance in mouse skeletal muscle and palmitate-induced insulin resistance in skeletal muscle cells by activating AMPK and increasing PPARα expression (Jung et al., 2017). In hepatocyte, PDX ameliorated palmitate-induced insulin resistance by downregulating the expression of fetuin-A and selenoprotein P (Jung et al., 2019). Fetuin-A and selenoprotein P were hepatokines and their levels were increased in the plasma of obesity patients (Jung et al., 2019). PDX also improved lipopolysaccharide-induced insulin resistance in adipocytes (Jung et al., 2018a).

MaR1 treatment ameliorated insulin resistance in db/db mice and HFD-fed mice by suppressing inflammation and improving insulin sensitivity in adipose tissue (Martinez-Fernandez et al., 2017). The effects of MaR1 on insulin sensitivity were also confirmed in human adipocytes which were mediated by improving Akt activation (Martinez-Fernandez et al., 2020).

Soluble epoxide hydrolase (sEH) is a member of the epoxide hydrolase family in the CYP pathway (He et al., 2016). It hydrolyses EDPs into DiHDPAs. sEH expression and activity was found increased in retinas of diabetic mice, and the level of its product 19,20-DiHDPA was elevated in eyes. However, levels of other sEH substrates and products were comparable between control and diabetic mice. 19,20-DiHDPA was further found to increase endothelial cell permeability and induce the migration of pericytes into the extravascular space (Hu et al., 2017). Of note, the expression of sEH was increased in retinas of patients with non-proliferative diabetic retinopathy as compared with non-diabetic individuals (Hu et al., 2017), so sEH has potential as a therapeutic target of diabetic retinopathy.

Effect of EPA-Derived Oxylipins on Diabetes

BLT-1 and ERV-1 are two receptors for RvE1 (Freire et al., 2017). In type 2 diabetic patients' neutrophils, ERV-1 expression was significantly upregulated and BLT-1 expression was decreased. In addition, the serum level of RvE1 was decreased in type 2 diabetic patients versus healthy controls. RvE1 was further found to facilitate neutrophil phagocytosis from healthy individuals, and a higher dose was needed to achieve a similar response in neutrophils of diabetic patients (Freire et al., 2017). These data indicate that repressed RvE1 signaling is involved in neutrophil phagocytosis dysfunction in type 2 diabetes. In addition, overexpression of the RvE1 receptor ERV-1 in myeloid cells attenuated diet-induced obesity, hepatic steatosis and glucose intolerance in mice. A mechanism study revealed that ERV-1 overexpression maintained peripheral blood monocyte and adipose-tissue macrophage skewing to an M2 phenotype in mice with an HFD (Sima et al., 2017). Besides, RvE1 was reported to improve hyperinsulinemia and hyperglycemia in HFD fed mice by activating ERV-1. The authors further demonstrated genetic diversity and variability defined the therapeutic effects of RvE1 by using the diversity outbred mice. This research highlights the genetic variants in the RvE1 response need to be considered when exploring the therapeutic effects of EPA clinically (Pal et al., 2020).

Eicosapentaenoic acid could increase glucose-stimulated insulin secretion from ob/ob mice (Neuman et al., 2017) 5-HEPE derived from EPA could increase glucose-stimulated insulin secretion in MIN6 cells by activating the GPR119/cAMP pathway (Kogure et al., 2011). Thus, the effect of EPA on insulin secretion may be mediated by its metabolites, which needs further investigation.

Effect of ω-3 PUFA-Derived Oxylipins on Non-alcoholic Fatty Liver Disease

Non-alcoholic fatty liver disease is defined as the accumulation of excess fat in the liver in the absence of excessive alcohol drinking and any secondary cause and thus a hepatic manifestation of metabolic syndrome (Ahmed, 2015). In NAFLD, simple steatosis can progress into non-alcoholic steatohepatitis (NASH), estimated to be the major reason for liver transplantation in the United States by 2020 (Diehl and Day, 2017). EPA and DHA showed protective effects on NAFLD (Scorletti and Byrne, 2018;

Yan et al., 2018; Jordao Candido et al., 2019). Moreover, to better understand the underlying mechanisms, increasing studies have focused on the functions of their derived metabolites in NAFLD.

Effect of ω-3 PUFA-Derived Oxylipins on Hepatic Steatosis

Hepatic steatosis is considered the first hit in the current "multiple-hit" theory proposed for the pathogenesis of NAFLD (Ahmed, 2015). PDX, MaR1, 19,20-EDP, and 17-HDHA derived from DHA and 17,18-EEQ, 18-HEPE, and RvE1 derived from EPA showed potential to ameliorate hepatic steatosis (**Table 2**).

PDX and MaR1 suppress palmitate-induced lipid accumulation in hepatocytes by attenuating endoplasmic reticulum stress (Rius et al., 2017; Jung et al., 2018b,c). For the mechanism, MaR1 activated AMPK and then induced sarcoendoplasmic reticulum Ca^{2+}-ATPase 2b expression, which alleviated the palmitate-induced endoplasmic reticulum stress (Jung et al., 2018b). Consistent with the *in vitro* study, in HFD-fed mice and ob/ob mice, MaR1 alleviated hepatic steatosis (Jung et al., 2018b; Laiglesia et al., 2018b). 18-HEPE and 17-HDHA could improve HFD-induced hepatic steatosis. Also, 18-HEPE and 17-HDHA increased adiponectin level in HFD mouse (Rodriguez-Echevarria et al., 2018). However, whether the beneficial effects of 18-HEPE and 17-HDHA depend on adiponectin need further studies. 18-HEPE is the precursor of RvE1. Moreover, intraperitoneal injection of RvE1 significantly ameliorated the hepatic steatosis and inflammation of ob/ob mice (Gonzalez-Periz et al., 2009). Our recent study found that 17,18-EEQ, 5-HEPE and 9-HEPE derived from EPA ameliorated short-term HFD-induced liver steatosis by attenuating adipose tissue inflammation. In the study, we also found the anti-inflammatory effect of HEPEs and EEQs was more pronounced than the same dose of EPA (1 μM), although EPA at 50 μM showed a significant anti-inflammatory effects (Wang et al., 2017). In addition, 8-HEPE improved dyslipidemia and liver steatosis in low-density lipoprotein (LDL) receptor deficient mice fed with high cholesterol diet (Saito et al., 2020).

sEH can decrease EEQ and EDP level by hydrolyzing them into less active diols (He et al., 2016). Inhibition of sEH reinforced the protective role of *fat-1* transgenic mice in HFD-induced liver inflammation and steatosis by increasing 17,18-EEQ and 19,20-EDP production (Lopez-Vicario et al., 2015). For the mechanism, 19,20-EDP and 17,18-EEQ ameliorated insulin signaling in palmitate-treated adipocytes; 19,20-EDP restored autophagy in palmitate-treated hepatocytes (Lopez-Vicario et al., 2015).

Effect of ω-3 PUFA-Derived Derived Oxylipins on Non-alcoholic Steatohepatitis

Non-alcoholic steatohepatitis is characterized by liver steatosis, inflammation, hepatocellular injury and different degrees of fibrosis and is the progressive form of NAFLD (Schuster et al., 2018). A recent study found RvD1 treatment mitigated lipid accumulation, inflammation and hepatic fibrosis in MCD-diet induced NASH mice. For the mechanism, RvD1 suppressed oxidative stress by activating nuclear factor E2-related factor 2 and ameliorated inflammation by inhibiting NF-κB and MAPK signaling pathways (Li et al., 2020). In addition, RvD1 had additional protective effects on calorie restrictive-improved NASH, as evidenced by decreased macrophage infiltration with decreased expression of M1 macrophage markers and increased expression of M2 macrophage markers (Rius et al., 2014). Also, Han et al. (2019) demonstrated that MaR1 derived from DHA increased the M2 polarity of liver macrophages and then ameliorated NASH by activating RORα. RORα, as a nuclear receptor, in turn increased MaR1 production by transcriptional induction of 12-lipoxygenase expression (Han et al., 2019). These studies suggest that these specialized pro-resolving lipid mediators derived from ω-3 PUFAs have therapeutic potential for NASH by promoting M2 polarization of liver macrophages.

EFFECT OF ω-3 PUFA-DERIVED OXYLIPINS ON ADIPOSE TISSUE FUNCTION

Depending on the adipocyte, adipose tissue can be divided into white and brown adipose tissue. Also, inducible cells within white adipose tissue, called "beige" adipocytes, can generate heat under cold exposure (Rosen and Spiegelman, 2014; Ye et al., 2020). Adipose tissue functions, including adipose tissue inflammation, lipolysis, adipogenesis, endocrine function, and browning, are closely related to obesity-related diseases. The studies of the effects of ω-3 PUFA derivatives on adipose tissue function mainly focused on the immune response of adipose tissue. Their influence on macrophage function contributing to adipose tissue inflammation was discussed in the previous section (**Table 2**). In addition, Onodera et al. (2017) demonstrated that EPA increased the number and proportion of T regulatory cells in epididymal adipose tissue of db/db mice. This result was mediated by 5-HEPE, which is derived from EPA by 5-LOX (Onodera et al., 2017).

In addition to the immune response, other adipose tissue functions are regulated by ω-3 PUFA-derived bioactive metabolites.

Effect of ω-3 PUFA-Derived Oxylipins on Lipogenesis and Lipolysis

The imbalance of lipogenesis and lipolysis of adipose tissue can increase the risk of obesity-induced disease (Lafontan, 2014). MaR1 inhibited TNF-α-induced lipolysis in 3T3-L1 adipocytes (Laiglesia et al., 2018a). Increased adipocyte lipolysis may increase plasma free fatty acid level and lead to insulin resistance and fatty liver disease (Matsuzaka and Shimano, 2011).

Nevertheless, PDX treatment inhibited lipid accumulation in 3T3-L1 cells during differentiation (Jung et al., 2018a). GPR120, also called free fatty acid receptor 4, is a free fatty acid receptor. Recently, DHA is found to promote adipogenesis by activating GPR120 in the cilia of preadipocytes. For the mechanism, GPR120 activation induced a rapid increase in ciliary cyclic AMP (cAMP) level, which in turn promoted adipogenesis by activating exchange factor directly

activated by cAMP (EPAC) (Hilgendorf et al., 2019). Because GPR120 can be activated by PDX, this research implies the complicated effects of ω-3 PUFA-derived bioactive lipids on adipogenesis. In addition, more studies are needed to demonstrate whether ω-3 derived bioactive lipids can affect the lipid storage and release function of adipose tissue *in vivo*.

Effect of ω-3 PUFA-Derived Oxylipins on Endocrine Function of Adipose Tissue

Adipose tissue, as an endocrine tissue, can affect other tissue functions by secreting cytokines. MaR1, 18-HEPE, 17-HDHA, RvD1, and PD1 could increase adiponectin level (Gonzalez-Periz et al., 2009; Hellmann et al., 2011; Rius et al., 2014; Martinez-Fernandez et al., 2017; Rodriguez-Echevarria et al., 2018). Adiponectin is an adipose-derived cytokine, one of the most abundant proteins in circulation (Wang et al., 2010). Because adiponectin is beneficial for diabetes, inflammation, and atherosclerosis (Achari and Jain, 2017), these bioactive lipids may affect metabolic disorders indirectly by promoting adiponectin secretion, which needs to be further explored.

Effect of ω-3 PUFA-Derived Oxylipins on Brown and Beige Adipose Tissue

Brown and beige adipocytes, as heat-producing cells, are considered to counteract metabolic diseases, including obesity and type 2 diabetes. Leiria et al. (2019) found that the 12-LOX biosynthetic pathway was activated in brown adipose tissue under cold exposure, which promoted the generation and release of 12-HEPE. Then, 12-HEPE exerted a glucose-shuttling effect on tissues to support thermogenesis (Leiria et al., 2019).

GPR120 is highly expressed in brown adipose tissue and significantly upregulated in beige adipose tissue induced by cold exposure. It was further found to mediate ω-3 PUFA-induced thermogenic gene expression in beige adipocytes by upregulating fibroblast growth factor 21 expression (Quesada-Lopez et al., 2016). However, the role of ω-3 PUFA metabolites in white adipose tissue browning remains unknown. GPR120 can be activated by PDX, but whether these ω-3 PUFA-derived bioactive lipids could regulate this process is worth studying.

Besides the direct effects on adipose tissue, ω-3 PUFA metabolites are reported to indirectly regulate adipose tissue function. GPR18, the receptor for RvD2, is widely expressed in hypothalamus and was decreased in level by HFD feeding in mice. In addition, the production of hypothalamic RvD2 was decreased in HFD-fed mice. When obese mice were treated with intra-cerebroventricular injection of RvD2, visceral fat was reduced, and hypothalamic leptin resistance was reversed (Pascoal et al., 2017).

EFFECT OF ω-3 PUFA-DERIVED OXYLIPINS ON ATHEROSCLEROSIS

Atherosclerosis causes ischemic heart disease, strokes, and peripheral vascular disease (Kobiyama and Ley, 2018). Metabolic syndrome is responsible for the initial disease and disease progression (Varghese et al., 2018). Endothelial-cell dysfunction is the initial step of atherosclerosis. Plaque is chronically built up with the assistance of macrophages differentiated from monocytes, smooth muscle cells and multiple chemokines and growth factors (Gimbrone and Garcia-Cardena, 2016). The metabolites derived from EPA or DHA, including RvE1, RvD2, MaR1, 18-HEPE, and 17,18-EEQ, have shown positive effects on anti-atherosclerosis (**Table 2**).

Systematic plasma lipidomic research has identified 18-HEPE as a central molecule derived from EPA. 18-HEPE is an RvE1 precursor, and knockout of the RvE1 receptor ERV-1 enhanced atherosclerosis and promoted changes in plaque composition in ApoE–/– mice. The mechanism study showed that ERV-1/ChemR23–/– macrophages enhanced oxidized low-density lipoprotein uptake and decreased phagocytosis (Laguna-Fernandez et al., 2018). RvE1 can ameliorate atherosclerosis (Salic et al., 2016). In addition, 18-HEPE and 17,18-EEQ ameliorated endothelial-cell activation and monocyte adhesion by inhibiting the TNFα-induced NF-κB pathway (Liu et al., 2018).

In the ApoE–/– mouse aorta, RvD2 and MaR1 levels are correlated negatively with vulnerability plaque index, which is decreased by HFD treatment. In addition, RvD2 and MaR1 administration suppressed atheroprogression. The protective effects of RvD2 and MaR1 on atherosclerosis were mediated by preventing the macrophage inflammatory response (Viola et al., 2016). RvD1 was decreased in vulnerable regions as compared with stable regions in human carotid atherosclerotic plaques. Additionally, its level was decreased in advanced versus early atherosclerotic lesion in western diet-fed mice deficient in low-density-lipoprotein receptor (Fredman et al., 2016). These studies suggest that several metabolites of EPA and DHA are beneficial for atherosclerosis. However, more *in vivo* and mechanistic studies are needed to better understand their effects on atherosclerosis.

EFFECT OF n-3 DPA AND ITS DERIVATIVES ON METABOLIC DISORDERS

n-3 DPA, an important ω-3 PUFA, is also a precursor of various docosanoids. Besides, it is an important intermediate in the conversion pathway of EPA and DHA (**Figure 2**; Drouin et al., 2019). n-3 DPA supplement significantly improved homeostasis model assessment of insulin resistance (HOMA-IR) in HFD fed mice, while DHA and EPA showed a minor effect (Guo et al., 2018). In human, n-3 DPA and its pro-resolving mediators have beneficial effects on cardiometabolic disease (Li et al., 2018). Moreover, It has been proved to be more potent than EPA in inducing the differentiation process in preadipocytes, and inhibits the pro-inflammatory signaling pathways (Murali et al., 2014). Although, it showed more beneficial effects on those metabolic disorders mentioned above than EPA and DHA, the functions of its metabolites are poorly studied.

n-3 DPA can be metabolized into $PD_{n\text{-}3DPA}$, $RvD_{n\text{-}3DPA}$, $MaR_{n\text{-}3DPA}$, hydroxylated derivatives from n-3 DPA, 13-serie Rvs etc. Several functional studies about n-3 DPA derivatives indicates their anti-inflammation function. A recent study found significant decreases in plasma $RvD_{n\text{-}3DPA}$ concentrations in CVD patients and $RvD_{n\text{-}3DPA}$ reduce leukocyte and platelet activation in peripheral blood from healthy volunteers as well as CVD patients. In addition, $RvD5_{n\text{-}3DPA}$ reduced aortic lesions in western diet-fed ApoE−/− mice (Colas et al., 2018). $PD_{n\text{-}3DPA}$ also found to play an important role in regulating macrophage resolution responses (Pistorius et al., 2018). $PD1_{n\text{-}3DPA}$ and $RvD5_{n\text{-}3DPA}$ were reported to decrease leukocyte–endothelial interaction and attenuate intestinal inflammation (Gobbetti et al., 2017). Although these n-3 DPA derivatives are identified as novel specialized proresolving lipid mediators, their effects on metabolic disorders, such as diabetes, NAFLD, obesity and atherosclerosis are still largely unknown.

EFFECT OF ALA AND ITS DERIVATIVES ON METABOLIC DISORDERS

In addition to partially converted into EPA, n-3 DPA and DHA (with low conversion rate to DHA in human) (de Lorgeril and Salen, 2004; Stark et al., 2008, 2016), the effects of oxylipins derived from ALA by LOX and CYP have also gained attention. Recently, a clinical research showed that 9-hydroxy-octadecatrienoic acid (9-HOTRE) combined with 7,17dihydro-dipicolinic acid (7,17-DHDPA), 14,15-dihydroxy-5,8,11-eicosatrienoic acid (14,15-DIHETRE) and free adrenic acid is a biomarker to predict improvement in hepatic collagen content in NASH patients (Caussy et al., 2020). Besides, in obese rats, 9-HOTRE showed a negative correlation with mean glomerular volume (Caligiuri et al., 2013). 13-Oxo-9(Z),11(E),15(Z)-octadecatrienoic acid (13-oxo-OTA), a product from ALA catalyzed by LOX, was reported to promote glucose uptake in 3T3-L1 cells by activating PPARγ (Takahashi et al., 2015). Moreover, 13-(*S*)-hydroperoxyoctadecatrienoic acid [13-(S)-HPOTRE] and 13-(S)-hydroxyoctadecatrienoic acid [13-(S)-HOTRE] showed anti-inflammatory effects by inactivating NLRP3 inflammasome complex in macrophages, which indicates that they may play protective roles in metabolic disorders (Kumar et al., 2016). However, the studies about the effects of ALA derivatives on metabolic are limited, especially the *in vivo* study.

CONCLUSION

Although we have fewer studies of the biofunctions of ω-3 PUFA-derived bioactive lipids than ARA metabolites, the former have been increasingly emphasized recently, especially for metabolic disorders (**Table 2**). Most of the functional studies focused on their anti-inflammatory effects. These metabolites can be more effective against inflammation than the precursors *per se*. Because PUFAs are vulnerable to lipid peroxidation, ω-3 PUFA supplement can lead to increased lipid peroxidation products, which may limits their clinical applications (Zaloga, 2021). It is important to increase their anti-inflammatory efficiency and decrease the dosage. Therefore, studying the function ω-3 PUFA metabolites may help us to find novel lipid mediators to treat metabolic disorders better than dietary supplement of EPA and DHA. Moreover, the anti-inflammatory efficiency of these metabolites should be further compared to provide more information for the future clinical applications.

In addition, several studies revealed the direct effects of ω-3 PUFA-derived oxylipins on pancreatic β cells, hepatocytes, adipocytes, skeletal muscle cells and endothelial cells. These bioactive lipids may have potential effects other than anti-inflammatory effects, which needs more exploration. Metabolites derived from ω-3 PUFAs are numerous, with attention to RvEs, RvDs, and PDs. Other metabolites such as EEQs, EDPs, HEPEs, and n-3 DPA derivatives need more mechanistic studies. In addition, the explorations of the biofunctions of ω-3 PUFA-derived bioactive metabolites, including their effects on cellular function, tissue micro-environment and interactions among metabolic tissues, are important for understanding their roles in energy metabolic disorders and related diseases. We also need more studies to identify their receptors and elucidate the downstream signaling pathway, which may provide potential therapeutic strategies for metabolic disorders.

In animal studies, the age, sex, and background of animals are well controlled. However, plasma and tissue levels of EPA and DHA and their metabolites in human can be altered by age, sex and disease status (Calder, 2020a), which indicates the complexity of clinical application of EPA and DHA. The genetic variants in the specialized pro-resolving mediator response also need to be considered when exploring the therapeutic effects of EPA and DHA clinically. Thus, the individualized treatment regimens of clinical applications of ω-3 PUFAs may achieve better effects on metabolic disorders. Moreover, according to a recent clinical trial, high-dose ω-3 PUFA supplement failed to increased RvD1 levels in diabetic patients, indicating the importance to study the disturbance of ω-3 PUFA metabolism in some disease status.

AUTHOR CONTRIBUTIONS

JD and YS contributed to the drafting, figure composition, subsequent edits, and final composition of the manuscript. XZ provided the comments and corrections. CW contributed to the concept and design, drafting of the manuscript, and guarantor of the manuscript. All authors have read and approved the final manuscript.

FUNDING

This work was supported by the National Natural Science Foundation of China (81822006 and 81770836) and the Natural Science Foundation of Tianjin (20JCYBJC01120).

REFERENCES

Achari, A. E., and Jain, S. K. (2017). Adiponectin, a therapeutic target for obesity, diabetes, and endothelial dysfunction. *Int. J. Mol. Sci.* 18:1321. doi: 10.3390/ijms18061321

Ahmed, M. (2015). Non-alcoholic fatty liver disease in 2015. *World J. Hepatol.* 7, 1450–1459. doi: 10.4254/wjh.v7.i11.1450

Bathina, S., and Das, U. N. (2021). Resolvin D1 decreases severity of streptozotocin-induced Type 1 diabetes mellitus by enhancing BDNF levels, reducing oxidative stress, and suppressing inflammation. *Int. J. Mol. Sci.* 22:1516. doi: 10.3390/ijms22041516

Bathina, S., Gundala, N. K. V., Rhenghachar, P., Polavarapu, S., Hari, A. D., Sadananda, M., et al. (2020). Resolvin D1 Ameliorates Nicotinamide-streptozotocin-induced Type 2 Diabetes mellitus by its anti-inflammatory action and modulating PI3K/Akt/mTOR pathway in the brain. *Arch. Med. Res.* 51, 492–503. doi: 10.1016/j.arcmed.2020.05.002

Bi, X., Li, F., Liu, S., Jin, Y., Zhang, X., Yang, T., et al. (2017). omega-3 polyunsaturated fatty acids ameliorate type 1 diabetes and autoimmunity. *J. Clin. Invest.* 127, 1757–1771. doi: 10.1172/JCI87388

Calder, P. C. (2020a). Eicosapentaenoic and docosahexaenoic acid derived specialised pro-resolving mediators: concentrations in humans and the effects of age, sex, disease and increased omega-3 fatty acid intake. *Biochimie* 178, 105–123. doi: 10.1016/j.biochi.2020.08.015

Calder, P. C. (2020b). Eicosanoids. *Essays Biochem.* 64, 423–441. doi: 10.1042/EBC20190083

Caligiuri, S. P., Love, K., Winter, T., Gauthier, J., Taylor, C. G., Blydt-Hansen, T., et al. (2013). Dietary linoleic acid and alpha-linolenic acid differentially affect renal oxylipins and phospholipid fatty acids in diet-induced obese rats. *J. Nutr.* 143, 1421–1431. doi: 10.3945/jn.113.177360

Caussy, C., Chuang, J. C., Billin, A., Hu, T., Wang, Y., Subramanian, G. M., et al. (2020). Plasma eicosanoids as noninvasive biomarkers of liver fibrosis in patients with nonalcoholic steatohepatitis. *Ther. Adv. Gastroenterol.* 13:1756284820923904. doi: 10.1177/1756284820923904

Chiang, N., Dalli, J., Colas, R. A., and Serhan, C. N. (2015). Identification of resolvin D2 receptor mediating resolution of infections and organ protection. *J. Exp. Med.* 212, 1203–1217. doi: 10.1084/jem.20150225

Chiang, N., Libreros, S., Norris, P. C., de la Rosa, X., and Serhan, C. N. (2019). Maresin 1 activates LGR6 receptor promoting phagocyte immunoresolvent functions. *J. Clin. Invest.* 129, 5294–5311. doi: 10.1172/JCI129448

Colas, R. A., Souza, P. R., Walker, M. E., Burton, M., Zaslona, Z., Curtis, A. M., et al. (2018). impaired production and diurnal regulation of vascular RvDn-3 DPA increase systemic inflammation and cardiovascular disease. *Circ. Res.* 122, 855–863. doi: 10.1161/CIRCRESAHA.117.312472

de Bus, I., Witkamp, R., Zuilhof, H., Albada, B., and Balvers, M. (2019). The role of n-3 PUFA-derived fatty acid derivatives and their oxygenated metabolites in the modulation of inflammation. *Prostaglandins Lipid Mediat.* 144:106351. doi: 10.1016/j.prostaglandins.2019.106351

de Lorgeril, M., and Salen, P. (2004). Alpha-linolenic acid and coronary heart disease. *Nutr. Metab. Cardiovasc. Dis.* 14, 162–169. doi: 10.1016/s0939-4753(04)80037-1

Diehl, A. M., and Day, C. (2017). Cause, pathogenesis, and treatment of nonalcoholic steatohepatitis. *N. Engl. J. Med.* 377, 2063–2072. doi: 10.1056/NEJMra1503519

Drouin, G., Rioux, V., and Legrand, P. (2019). The n-3 docosapentaenoic acid (DPA): a new player in the n-3 long chain polyunsaturated fatty acid family. *Biochimie* 159, 36–48. doi: 10.1016/j.biochi.2019.01.022

Fredman, G., Hellmann, J., Proto, J. D., Kuriakose, G., Colas, R. A., Dorweiler, B., et al. (2016). An imbalance between specialized pro-resolving lipid mediators and pro-inflammatory leukotrienes promotes instability of atherosclerotic plaques. *Nat. Commun.* 7:12859. doi: 10.1038/ncomms12859

Freire, M. O., Dalli, J., Serhan, C. N., and Van Dyke, T. E. (2017). Neutrophil resolvin E1 receptor expression and function in Type 2 diabetes. *J. Immunol.* 198, 718–728. doi: 10.4049/jimmunol.1601543

Gabbs, M., Leng, S., Devassy, J. G., Monirujjaman, M., and Aukema, H. M. (2015). Advances in our understanding of oxylipins derived from dietary PUFAs. *Adv. Nutr.* 6, 513–540. doi: 10.3945/an.114.007732

Galano, J. M., Lee, J. C., Gladine, C., Comte, B., Le Guennec, J. Y., Oger, C., et al. (2015). Non-enzymatic cyclic oxygenated metabolites of adrenic, docosahexaenoic, eicosapentaenoic and alpha-linolenic acids; bioactivities and potential use as biomarkers. *Biochim. Biophys. Acta* 1851, 446–455. doi: 10.1016/j.bbalip.2014.11.004

Garcia-Jaramillo, M., Lytle, K. A., Spooner, M. H., and Jump, D. B. (2019). A lipidomic analysis of docosahexaenoic acid (22:6, omega3) mediated attenuation of western diet induced nonalcoholic steatohepatitis in male Ldlr(-/-) Mice. *Metabolites* 9:252. doi: 10.3390/metabo9110252

Gimbrone, M. A. Jr., and Garcia-Cardena, G. (2016). Endothelial cell dysfunction and the pathobiology of atherosclerosis. *Circ. Res.* 118, 620–636. doi: 10.1161/CIRCRESAHA.115.306301

Glovaci, D., Fan, W., and Wong, N. D. (2019). Epidemiology of diabetes mellitus and cardiovascular disease. *Curr. Cardiol. Rep.* 21:21. doi: 10.1007/s11886-019-1107-y

Gobbetti, T., Dalli, J., Colas, R. A., Federici Canova, D., Aursnes, M., Bonnet, D., et al. (2017). Protectin D1n-3 DPA and resolvin D5n-3 DPA are effectors of intestinal protection. *Proc. Natl. Acad. Sci. U.S.A.* 114, 3963–3968. doi: 10.1073/pnas.1617290114

Gonzalez-Periz, A., Horrillo, R., Ferre, N., Gronert, K., Dong, B., Moran-Salvador, E., et al. (2009). Obesity-induced insulin resistance and hepatic steatosis are alleviated by omega-3 fatty acids: a role for resolvins and protectins. *FASEB J.* 23, 1946–1957. doi: 10.1096/fj.08-125674

Guo, X. F., Sinclair, A. J., Kaur, G., and Li, D. (2018). Differential effects of EPA. DPA and DHA on cardio-metabolic risk factors in high-fat diet fed mice. *Prostaglandins Leukot Essent Fatty Acids* 136, 47–55. doi: 10.1016/j.plefa.2017.09.011

Hajeyah, A. A., Griffiths, W. J., Wang, Y., Finch, A. J., and O'Donnell, V. B. (2020). The biosynthesis of enzymatically oxidized lipids. *Front. Endocrinol.* 11:591819. doi: 10.3389/fendo.2020.591819

Han, Y. H., Shin, K. O., Kim, J. Y., Khadka, D. B., Kim, H. J., Lee, Y. M., et al. (2019). A maresin 1/RORalpha/12-lipoxygenase autoregulatory circuit prevents inflammation and progression of nonalcoholic steatohepatitis. *J. Clin. Invest.* 129, 1684–1698. doi: 10.1172/JCI124219

He, J., Wang, C., Zhu, Y., and Ai, D. (2016). Soluble epoxide hydrolase: a potential target for metabolic diseases. *J. Diabetes* 8, 305–313. doi: 10.1111/1753-0407.12358

Hellmann, J., Tang, Y., Kosuri, M., Bhatnagar, A., and Spite, M. (2011). Resolvin D1 decreases adipose tissue macrophage accumulation and improves insulin sensitivity in obese-diabetic mice. *FASEB J.* 25, 2399–2407. doi: 10.1096/fj.10-178657

Hilgendorf, K. I., Johnson, C. T., Mezger, A., Rice, S. L., Norris, A. M., Demeter, J., et al. (2019). Omega-3 fatty acids activate ciliary FFAR4 to control adipogenesis. *Cell* 179, 1289.e21–1305.e21. doi: 10.1016/j.cell.2019.11.005

Hu, J., Dziumbla, S., Lin, J., Bibli, S. I., Zukunft, S., de Mos, J., et al. (2017). Inhibition of soluble epoxide hydrolase prevents diabetic retinopathy. *Nature* 552, 248–252. doi: 10.1038/nature25013

Hwang, H. J., Jung, T. W., Kim, J. W., Kim, J. A., Lee, Y. B., Hong, S. H., et al. (2019). Protectin DX prevents H2O2-mediated oxidative stress in vascular endothelial cells via an AMPK-dependent mechanism. *Cell. Signal.* 53, 14–21. doi: 10.1016/j.cellsig.2018.09.011

Jacobo-Cejudo, M. G., Valdes-Ramos, R., Guadarrama-Lopez, A. L., Pardo-Morales, R. V., Martinez-Carrillo, B. E., and Harbige, L. S. (2017). Effect of n-3 polyunsaturated fatty acid supplementation on metabolic and inflammatory biomarkers in Type 2 diabetes mellitus patients. *Nutrients* 9:573. doi: 10.3390/nu9060573

Jordao Candido, C., Silva Figueiredo, P., Del Ciampo Silva, R., Candeloro Portugal, L., Augusto Dos Santos Jaques , J., Alves de Almeida, J., et al. (2019). Protective effect of alpha-linolenic acid on non-alcoholic hepatic steatosis and interleukin-6 and -10 in wistar rats. *Nutrients* 12:9. doi: 10.3390/nu12010009

Jung, T. W., Ahn, S. H., Shin, J. W., Kim, H. C., Park, E. S., Abd El-Aty, A. M., et al. (2019). Protectin DX ameliorates palmitate-induced hepatic insulin resistance through AMPK/SIRT1-mediated modulation of fetuin-A and SeP expression. *Clin. Exp. Pharmacol. Physiol.* 46, 898–909. doi: 10.1111/1440-1681.13131

Jung, T. W., Chung, Y. H., Kim, H. C., Abd El-Aty, A. M., and Jeong, J. H. (2018a). Protectin DX attenuates LPS-induced inflammation and insulin resistance in adipocytes via AMPK-mediated suppression of the NF-kappaB pathway. *Am. J. Physiol. Endocrinol. Metab.* 315, E543–E551. doi: 10.1152/ajpendo.00408.2017

Jung, T. W., Kim, H. C., Abd El-Aty, A. M., and Jeong, J. H. (2018b). Maresin 1 attenuates NAFLD by suppression of endoplasmic reticulum stress via AMPK-SERCA2b pathway. *J. Biol.Chem.* 293, 3981–3988. doi: 10.1074/jbc.RA117.000885

Jung, T. W., Kyung, E. J., Kim, H. C., Shin, Y. K., Lee, S. H., Park, E. S., et al. (2018c). Protectin DX ameliorates hepatic steatosis by suppression of endoplasmic reticulum stress via AMPK-Induced ORP150 Expression. *J. Pharmacol.Exp. Ther.* 365, 485–493. doi: 10.1124/jpet.117.246686

Jung, T. W., Kim, H. C., Abd El-Aty, A. M., and Jeong, J. H. (2017). Protectin DX ameliorates palmitate- or high-fat diet-induced insulin resistance and inflammation through an AMPK-PPARalpha-dependent pathway in mice. *Sci. Rep.* 7:1397. doi: 10.1038/s41598-017-01603-9

Kobiyama, K., and Ley, K. (2018). Atherosclerosis. *Circ. Res.* 123, 1118–1120. doi: 10.1161/CIRCRESAHA.118.313816

Kogure, R., Toyama, K., Hiyamuta, S., Kojima, I., and Takeda, S. (2011). 5-Hydroxy-eicosapentaenoic acid is an endogenous GPR119 agonist and enhances glucose-dependent insulin secretion. *Biochem. Biophys. Res. Commun.* 416, 58–63. doi: 10.1016/j.bbrc.2011.10.141

Krishna Mohan, I., and Das, U. N. (2001). Prevention of chemically induced diabetes mellitus in experimental animals by polyunsaturated fatty acids. *Nutrition* 17, 126–151. doi: 10.1016/s0899-9007(00)00468-8

Krishnamoorthy, S., Recchiuti, A., Chiang, N., Yacoubian, S., Lee, C. H., Yang, R., et al. (2010). Resolvin D1 binds human phagocytes with evidence for proresolving receptors. *Proc. Natl. Acad. Sci. U.S.A.* 107, 1660–1665. doi: 10.1073/pnas.0907342107

Kuda, O. (2017). Bioactive metabolites of docosahexaenoic acid. *Biochimie* 136, 12–20. doi: 10.1016/j.biochi.2017.01.002

Kumar, N., Gupta, G., Anilkumar, K., Fatima, N., Karnati, R., Reddy, G. V., et al. (2016). 15-Lipoxygenase metabolites of alpha-linolenic acid, [13-(S)-HPOTrE and 13-(S)-HOTrE], mediate anti-inflammatory effects by inactivating NLRP3 inflammasome. *Sci. Rep.* 6:31649. doi: 10.1038/srep31649

Lafontan, M. (2014). Adipose tissue and adipocyte dysregulation. *Diabetes Metab.* 40, 16–28. doi: 10.1016/j.diabet.2013.08.002

Laguna-Fernandez, A., Checa, A., Carracedo, M., Artiach, G., Petri, M. H., Baumgartner, R., et al. (2018). ERV1/ChemR23 signaling protects against atherosclerosis by modifying oxidized low-density lipoprotein uptake and phagocytosis in macrophages. *Circulation* 138, 1693–1705. doi: 10.1161/CIRCULATIONAHA.117.032801

Laiglesia, L. M., Lorente-Cebrian, S., Lopez-Yoldi, M., Lanas, R., Sainz, N., Martinez, J. A., et al. (2018a). Maresin 1 inhibits TNF-alpha-induced lipolysis and autophagy in 3T3-L1 adipocytes. *J. Cell. Physiol.* 233, 2238–2246. doi: 10.1002/jcp.26096

Laiglesia, L. M., Lorente-Cebrian, S., Martinez-Fernandez, L., Sainz, N., Prieto-Hontoria, P. L., Burrell, M. A., et al. (2018b). Maresin 1 mitigates liver steatosis in ob/ob and diet-induced obese mice. *Int. J. Obes.* 42, 572–579. doi: 10.1038/ijo.2017.226

Lavie, C. J., Milani, R. V., and Ventura, H. O. (2009). Obesity and cardiovascular disease: risk factor, paradox, and impact of weight loss. *J. Am. Coll Cardiol.* 53, 1925–1932. doi: 10.1016/j.jacc.2008.12.068

Leiria, L. O., Wang, C. H., Lynes, M. D., Yang, K., Shamsi, F., Sato, M., et al. (2019). 12-Lipoxygenase regulates cold adaptation and glucose metabolism by producing the Omega-3 Lipid 12-HEPE from Brown Fat. *Cell Metab.* 30, 768.e7–783.e7. doi: 10.1016/j.cmet.2019.07.001

Lepretti, M., Martucciello, S., Burgos Aceves, M. A., Putti, R., and Lionetti, L. (2018). Omega-3 fatty acids and insulin resistance: focus on the regulation of mitochondria and endoplasmic reticulum stress. *Nutrients* 10:350. doi: 10.3390/nu10030350

Li, J., Deng, X., Bai, T., Wang, S., Jiang, Q., and Xu, K. (2020). Resolvin D1 mitigates non-alcoholic steatohepatitis by suppressing the TLR4-MyD88-mediated NF-kappaB and MAPK pathways and activating the Nrf2 pathway in mice. *Int, Immunopharmacol.* 88:106961. doi: 10.1016/j.intimp.2020.106961

Li, K., Sinclair, A. J., Zhao, F., and Li, D. (2018). Uncommon fatty acids and cardiometabolic health. *Nutrients* 10:1559. doi: 10.3390/nu10101559

Liu, Y., Fang, X., Zhang, X., Huang, J., He, J., Peng, L., et al. (2018). Metabolic profiling of murine plasma reveals eicosapentaenoic acid metabolites protecting against endothelial activation and atherosclerosis. *Br. J. Pharmacol.* 175, 1190–1204. doi: 10.1111/bph.13971

Lopez-Vicario, C., Alcaraz-Quiles, J., Garcia-Alonso, V., Rius, B., Hwang, S. H., Titos, E., et al. (2015). Inhibition of soluble epoxide hydrolase modulates inflammation and autophagy in obese adipose tissue and liver: role for omega-3 epoxides. *Proc. Natl. Acad. Sci. U.S.A.* 112, 536–541. doi: 10.1073/pnas.1422590112

Maisto, R., Trotta, M. C., Petrillo, F., Izzo, S., Cuomo, G., Alfano, R., et al. (2020). Resolvin D1 modulates the intracellular VEGF-Related miRNAs of retinal photoreceptors challenged with high glucose. *Front. Pharmacol.* 11:235. doi: 10.3389/fphar.2020.00235

Martinez-Fernandez, L., Gonzalez-Muniesa, P., Laiglesia, L. M., Sainz, N., Prieto-Hontoria, P. L., Escote, X., et al. (2017). Maresin 1 improves insulin sensitivity and attenuates adipose tissue inflammation in ob/ob and diet-induced obese mice. *FASEB J.* 31, 2135–2145. doi: 10.1096/fj.201600859R

Martinez-Fernandez, L., Gonzalez-Muniesa, P., Sainz, N., Escote, X., Martinez, J. A., Arbones-Mainar, J. M., et al. (2020). Maresin 1 regulates insulin signaling in human adipocytes as well as in adipose tissue and muscle of lean and obese mice. *J. Physiol. Biochem.* 77, 167–173. doi: 10.1007/s13105-020-00775-9

Matsuzaka, T., and Shimano, H. (2011). Molecular mechanisms involved in hepatic steatosis and insulin resistance. *J. Diabetes Invest.* 2, 170–175. doi: 10.1111/j.2040-1124.2011.00111.x

Meijerink, J., Balvers, M., and Witkamp, R. N. - (2013). Acyl amines of docosahexaenoic acid and other n-3 polyunsatured fatty acids - from fishy endocannabinoids to potential leads. *Br. J. Pharmacol.* 169, 772–783. doi: 10.1111/bph.12030

Murali, G., Desouza, C. V., Clevenger, M. E., Ramalingam, R., and Saraswathi, V. (2014). Differential effects of eicosapentaenoic acid and docosahexaenoic acid in promoting the differentiation of 3T3-L1 preadipocytes. *Prostaglandins Leukot Essent Fatty Acids* 90, 13–21. doi: 10.1016/j.plefa.2013.10.002

Neuhofer, A., Zeyda, M., Mascher, D., Itariu, B. K., Murano, I., Leitner, L., et al. (2013). Impaired local production of proresolving lipid mediators in obesity and 17-HDHA as a potential treatment for obesity-associated inflammation. *Diabetes* 62, 1945–1956. doi: 10.2337/db12-0828

Neuman, J. C., Schaid, M. D., Brill, A. L., Fenske, R. J., Kibbe, C. R., Fontaine, D. A., et al. (2017). Enriching islet phospholipids with eicosapentaenoic acid reduces Prostaglandin E2 signaling and enhances diabetic beta-cell function. *Diabetes* 66, 1572–1585. doi: 10.2337/db16-1362

Onodera, T., Fukuhara, A., Shin, J., Hayakawa, T., Otsuki, M., and Shimomura, I. (2017). Eicosapentaenoic acid and 5-HEPE enhance macrophage-mediated Treg induction in mice. *Sci. Rep.* 7:4560. doi: 10.1038/s41598-017-04474-2

Pal, A., Al-Shaer, A. E., Guesdon, W., Torres, M. J., Armstrong, M., Quinn, K., et al. (2020). Resolvin E1 derived from eicosapentaenoic acid prevents hyperinsulinemia and hyperglycemia in a host genetic manner. *FASEB J.* 34, 10640–10656. doi: 10.1096/fj.202000830R

Park, H. G., Park, W. J., Kothapalli, K. S., and Brenna, J. T. (2015). The fatty acid desaturase 2 (FADS2) gene product catalyzes Δ4 desaturation to yield n-3 docosahexaenoic acid and n-6 docosapentaenoic acid in human cells. *FASEB J.* 29, 3911–3919. doi: 10.1096/fj.15-271783

Pascoal, L. B., Bombassaro, B., Ramalho, A. F., Coope, A., Moura, R. F., Correa-da-Silva, F., et al. (2017). Resolvin RvD2 reduces hypothalamic inflammation and rescues mice from diet-induced obesity. *J. Neuroinflammation* 14:5. doi: 10.1186/s12974-016-0777-2

Pistorius, K., Souza, P. R., De Matteis, R., Austin-Williams, S., Primdahl, K. G., Vik, A., et al. (2018). PD(n-3 DPA) pathway regulates human monocyte differentiation and macrophage function. *Cell Chem. Biol.* 25, 749.e9–760.e9. doi: 10.1016/j.chembiol.2018.04.017

Poreba, M., Mostowik, M., Siniarski, A., Golebiowska-Wiatrak, R., Malinowski, K. P., Haberka, M., et al. (2017). Treatment with high-dose n-3 PUFAs has no effect on platelet function, coagulation, metabolic status or inflammation in patients with atherosclerosis and type 2 diabetes. *Cardiovasc. Diabetol.* 16:50. doi: 10.1186/s12933-017-0523-9

Quesada-Lopez, T., Cereijo, R., Turatsinze, J. V., Planavila, A., Cairo, M., Gavalda-Navarro, A., et al. (2016). The lipid sensor GPR120 promotes brown fat activation and FGF21 release from adipocytes. *Nat. Commun.* 7:13479. doi: 10.1038/ncomms13479

Rius, B., Duran-Guell, M., Flores-Costa, R., Lopez-Vicario, C., Lopategi, A., Alcaraz-Quiles, J., et al. (2017). The specialized proresolving lipid mediator maresin 1 protects hepatocytes from lipotoxic and hypoxia-induced

endoplasmic reticulum stress. *FASEB J.* 31, 5384–5398. doi: 10.1096/fj.201700394R

Rius, B., Titos, E., Moran-Salvador, E., Lopez-Vicario, C., Garcia-Alonso, V., Gonzalez-Periz, A., et al. (2014). Resolvin D1 primes the resolution process initiated by calorie restriction in obesity-induced steatohepatitis. *FASEB J.* 28, 836–848. doi: 10.1096/fj.13-235614

Rodriguez-Echevarria, R., Macias-Barragan, J., Parra-Vargas, M., Davila-Rodriguez, J. R., Amezcua-Galvez, E., and Armendariz-Borunda, J. (2018). Diet switch and omega-3 hydroxy-fatty acids display differential hepatoprotective effects in an obesity/nonalcoholic fatty liver disease model in mice. *World J. Gastroenterol.* 24, 461–474. doi: 10.3748/wjg.v24.i4.461

Rosen, E. D., and Spiegelman, B. M. (2014). What we talk about when we talk about fat. *Cell* 156, 20–44. doi: 10.1016/j.cell.2013.12.012

Saito, M., Ishida, N., Yamada, H., Ibi, M., and Hirose, M. (2020). 8-HEPE-concentrated materials from pacific krill improve plasma cholesterol levels and hepatic steatosis in high cholesterol diet-fed low-density lipoprotein (LDL) receptor-deficient mice. *Biol. Pharm. Bull.* 43, 919–924. doi: 10.1248/bpb.b20-00162

Saleh-Ghadimi, S., Kheirouri, S., Maleki, V., Jafari-Vayghan, H., and Alizadeh, M. (2020). Endocannabinoid system and cardiometabolic risk factors: a comprehensive systematic review insight into the mechanistic effects of omega-3 fatty acids. *Life Sci.* 250:117556. doi: 10.1016/j.lfs.2020.117556

Salic, K., Morrison, M. C., Verschuren, L., Wielinga, P. Y., Wu, L., Kleemann, R., et al. (2016). Resolvin E1 attenuates atherosclerosis in absence of cholesterol-lowering effects and on top of atorvastatin. *Atherosclerosis* 250, 158–165. doi: 10.1016/j.atherosclerosis.2016.05.001

Sawada, T., Tsubata, H., Hashimoto, N., Takabe, M., Miyata, T., Aoki, K., et al. (2016). Effects of 6-month eicosapentaenoic acid treatment on postprandial hyperglycemia, hyperlipidemia, insulin secretion ability, and concomitant endothelial dysfunction among newly-diagnosed impaired glucose metabolism patients with coronary artery disease. An open label, single blinded, prospective randomized controlled trial. *Cardiovasc. Diabetol.* 15:121. doi: 10.1186/s12933-016-0437-y

Schmitz, G., and Ecker, J. (2008). The opposing effects of n-3 and n-6 fatty acids. *Prog. Lipid Res.* 47, 147–155. doi: 10.1016/j.plipres.2007.12.004

Schuster, S., Cabrera, D., Arrese, M., and Feldstein, A. E. (2018). Triggering and resolution of inflammation in NASH. *Nat. Rev. Gastroenterol Hepatol.* 15, 349–364. doi: 10.1038/s41575-018-0009-6

Scorletti, E., and Byrne, C. D. (2018). Omega-3 fatty acids and non-alcoholic fatty liver disease: evidence of efficacy and mechanism of action. *Mol. Aspects Med.* 64, 135–146. doi: 10.1016/j.mam.2018.03.001

Sima, C., Montero, E., Nguyen, D., Freire, M., Norris, P., Serhan, C. N., et al. (2017). ERV1 overexpression in myeloid cells protects against high fat diet induced obesity and glucose intolerance. *Sci. Rep.* 7:12848. doi: 10.1038/s41598-017-13185-7

Sonnweber, T., Pizzini, A., Nairz, M., Weiss, G., and Tancevski, I. (2018). Arachidonic acid metabolites in cardiovascular and metabolic diseases. *Int. J. Mol. Sci.* 19:3285. doi: 10.3390/ijms19113285

Stark, A. H., Crawford, M. A., and Reifen, R. (2008). Update on alpha-linolenic acid. *Nutr. Rev.* 66, 326–332. doi: 10.1111/j.1753-4887.2008.00040.x

Stark, A. H., Reifen, R., and Crawford, M. A. (2016). Past and present insights on alpha-linolenic acid and the Omega-3 fatty acid family. *Crit. Rev. Food Sci. Nutr.* 56, 2261–2267. doi: 10.1080/10408398.2013.828678

Suresh, Y., and Das, U. N. (2003). Long-chain polyunsaturated fatty acids and chemically induced diabetes mellitus. Effect of omega-3 fatty acids. *Nutrition* 19, 213–228. doi: 10.1016/s0899-9007(02)00855-9

Takahashi, H., Hara, H., Goto, T., Kamakari, K., Wataru, N., Mohri, S., et al. (2015). 13-Oxo-9(Z),11(E),15(Z)-octadecatrienoic acid activates peroxisome proliferator-activated receptor gamma in adipocytes. *Lipids* 50, 3–12. doi: 10.1007/s11745-014-3972-x

Tang, Y., Zhang, M. J., Hellmann, J., Kosuri, M., Bhatnagar, A., and Spite, M. (2013). Proresolution therapy for the treatment of delayed healing of diabetic wounds. *Diabetes* 62, 618–627. doi: 10.2337/db12-0684

Titos, E., Rius, B., Gonzalez-Periz, A., Lopez-Vicario, C., Moran-Salvador, E., Martinez-Clemente, M., et al. (2011). Resolvin D1 and its precursor docosahexaenoic acid promote resolution of adipose tissue inflammation by eliciting macrophage polarization toward an M2-like phenotype. *J. Immunol.* 187, 5408–5418. doi: 10.4049/jimmunol.1100225

Varghese, J. F., Patel, R., and Yadav, U. C. S. (2018). Novel insights in the metabolic syndrome-induced oxidative stress and inflammation-mediated atherosclerosis. *Curr. Cardiol. Rev.* 14, 4–14. doi: 10.2174/1573403X13666171009112250

Viola, J. R., Lemnitzer, P., Jansen, Y., Csaba, G., Winter, C., Neideck, C., et al. (2016). Resolving lipid mediators Maresin 1 and Resolvin D2 prevent atheroprogression in mice. *Circ. Res.* 119, 1030–1038. doi: 10.1161/CIRCRESAHA.116.309492

Wang, C., Guan, Y., and Yang, J. (2010). Cytokines in the progression of pancreatic beta-cell dysfunction. *Int. J. Endocrinol.* 2010:515136. doi: 10.1155/2010/515136

Wang, C., Liu, W., Yao, L., Zhang, X., Ye, C., Jiang, H., et al. (2017). Hydroxyeicosapentaenoic acids and epoxyeicosatetraenoic acids attenuate early occurrence of nonalcoholic fatty liver disease. *Br. J. Pharmacol.* 174, 2358–2372. doi: 10.1111/bph.13844

White, P. J., St-Pierre, P., Charbonneau, A., Mitchell, P. L., St-Amand, E., Marcotte, B., et al. (2014). Protectin DX alleviates insulin resistance by activating a myokine-liver glucoregulatory axis. *Nat. Med.* 20, 664–669. doi: 10.1038/nm.3549

Wong, C. Y., Yiu, K. H., Li, S. W., Lee, S., Tam, S., Lau, C. P., et al. (2010). Fish-oil supplement has neutral effects on vascular and metabolic function but improves renal function in patients with Type 2 diabetes mellitus. *Diabet Med.* 27, 54–60. doi: 10.1111/j.1464-5491.2009.02869.x

Xu, P., Zhai, Y., and Wang, J. (2018). The role of PPAR and its cross-talk with CAR and LXR in obesity and atherosclerosis. *Int. J. Mol. Sci.* 19:1260. doi: 10.3390/ijms19041260

Yamada, H., Oshiro, E., Kikuchi, S., Hakozaki, M., Takahashi, H., and Kimura, K. (2014). Hydroxyeicosapentaenoic acids from the Pacific krill show high ligand activities for PPARs. *J. Lipid Res.* 55, 895–904. doi: 10.1194/jlr.M047514

Yan, J. H., Guan, B. J., Gao, H. Y., and Peng, X. E. (2018). Omega-3 polyunsaturated fatty acid supplementation and non-alcoholic fatty liver disease: a meta-analysis of randomized controlled trials. *Medicine* 97:e12271. doi: 10.1097/MD.0000000000012271

Yang, J., Zhang, L. J., Wang, F., Hong, T., and Liu, Z. (2018). Molecular imaging of diabetes and diabetic complications: beyond pancreatic beta-cell targeting. *Adv. Drug Deliv. Rev.* 139, 32–50. doi: 10.1016/j.addr.2018.11.007

Ye, C., Duan, J., Zhang, X., Yao, L., Song, Y., Wang, G., et al. (2020). Cold-induced Yes-associated-protein expression through miR-429 mediates the browning of white adipose tissue. *Sci. China Life Sci.* doi: 10.1007/s11427-020-1779-2 [Epub ahead of print].

Younossi, Z., Anstee, Q. M., Marietti, M., Hardy, T., Henry, L., Eslam, M., et al. (2018). Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat. Rev. Gastroenterol. Hepatol.* 15, 11–20. doi: 10.1038/nrgastro.2017.109

Younossi, Z. M., Koenig, A. B., Abdelatif, D., Fazel, Y., Henry, L., and Wymer, M. (2016). Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 64, 73–84. doi: 10.1002/hep.28431

Zaloga, G. P. (2021). Narrative Review of n-3 polyunsaturated fatty acid supplementation upon immune functions, resolution molecules and lipid peroxidation. *Nutrients* 13:662. doi: 10.3390/nu13020662

Zhang, X., Yang, N., Ai, D., and Zhu, Y. (2015). Systematic metabolomic analysis of eicosanoids after omega-3 polyunsaturated fatty acid supplementation by a highly specific liquid chromatography-tandem mass spectrometry-based method. *J. Proteome Res.* 14, 1843–1853. doi: 10.1021/pr501200u

Conflict of Interest: 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.

frontiers in Physiology

ORIGINAL RESEARCH
published: 31 May 2021
doi: 10.3389/fphys.2021.688259

Cytochrome P450 Omega-Hydroxylase 4a14 Attenuates Cholestatic Liver Fibrosis

Sha Li[1,2,3†], Chenghai Wang[2†], Xiaxia Zhang[4] and Wen Su[3,5]*

[1] Medical College, Hebei University of Engineering, Handan, China, [2] Hebei Key Laboratory of Applied Basic Research of Blood Purification, Affiliated Hospital of Hebei Engineering University, Handan, China, [3] Health Science Center, Shenzhen University, Shenzhen, China, [4] Department of Gastroenterology and Hepatology, Handan Central Hospital, Handan, China, [5] Shenzhen Key Laboratory of Metabolism and Cardiovascular Homeostasis, Shenzhen, China

Background: Cholestasis is a pathological condition involving obstruction of bile secretion and excretion that results in hepatotoxicity, inflammation, fibrosis, cirrhosis, and eventually liver failure. Common bile duct ligation (BDL) model is a well-established murine model to mimic cholestatic liver fibrosis. We previously reported that cytochrome P450 omega-hydroxylase 4a14 (Cyp4a14) plays an important role in the pathogenesis of non-alcoholic fatty liver disease (NAFLD)-related fibrosis. The goal of this study was to determine the role of Cyp4a14 in cholestatic-induced liver fibrosis.

Methods: C57BL/6 mice were subjected to BDL for 14 days, and Cyp4a14 mRNA and protein levels were examined and compared with those of the sham group. Cyp4a14 knockout mice and adeno-associated virus (AAV)-mediated overexpression of Cyp4a14 in C57BL/6 mice underwent BDL and liver histology, and key fibrosis markers were examined.

Results: Both hepatic Cyp4a14 mRNA and protein levels were markedly reduced in BDL liver compared with the time-matched sham group. Cyp4a14 gene-deficient mice aggravates whereas its overexpression alleviates BDL-induced hepatic fibrosis, which were determined by liver function, liver histology, and levels of key fibrotic markers including α-smooth muscle actin (α-SMA), transforming growth factor-β1 (TGF-β1), and collagen 1a2 (Col1a2).

Conclusion: Cyp4a14 exerts a contrasting role in different hepatic fibrosis models. Strategies that enhance Cyp4a14 activity may be potential strategies to cholestatic related liver fibrosis.

Keywords: Cyp4a14, bile duct ligation, liver injury, liver fibrosis, cholestasis

OPEN ACCESS

Edited by:
Xu Zhang,
Tianjin Medical University, China

Reviewed by:
Ying Sun,
Xuzhou Medical University, China
Liming Yu,
University of Texas Southwestern Medical Center, United States

****Correspondence:***
Wen Su
casufa@163.com

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Lipid and Fatty Acid Research, a section of the journal Frontiers in Physiology

Received: *30 March 2021*
Accepted: *22 April 2021*
Published: *31 May 2021*

Citation:
Li S, Wang C, Zhang X and Su W (2021) Cytochrome P450 Omega-Hydroxylase 4a14 Attenuates Cholestatic Liver Fibrosis. Front. Physiol. 12:688259. doi: 10.3389/fphys.2021.688259

INTRODUCTION

Liver fibrosis is a progressive pathological process of most chronic liver diseases with a global prevalence of more than 2%. It is characterized by excess accumulation of extracellular matrix (ECM; Aydin and Akcali, 2018). If left untreated, liver fibrosis will lead to cirrhosis and ultimately end-stage liver failure and death. Almost all the chronic liver diseases can cause liver fibrosis, such as alcoholism, chronic viral hepatitis [hepatitis B (HBV) and hepatitis C (HCV)], non-alcoholic

steatohepatitis, obesity, autoimmunity hepatitis, parasitic diseases (e.g., schistosomiasis), metabolic disorders, biliary tract diseases, long-term exposure to toxicity and chemical substances, and drug-induced chronic liver disease (Toosi, 2015). Because of the complicated mechanisms in the progression of liver fibrosis (Parola and Pinzani, 2019; Seki and Brenner, 2015), even though we have made considerable progress in the prevention and treatment of liver fibrosis, treatment of liver fibrosis remains limited (Altamirano-Barrera et al., 2017; Roehlen et al., 2020).

Cholestatic liver fibrosis is caused by the gradual destruction of the bile duct, the blockade of bile acid outflow, and the activation of the pro-inflammatory process, leading to the damage of bile duct cells and liver cells (Santiago et al., 2018). Primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) are the two most common chronic cholestatic liver fibrosis (Gulamhusein and Hirschfield, 2020; Vesterhus and Karlsen, 2020). However, the underlying mechanism remains obscure, the mechanistic basis of which the fibrotic response is an area of current investigation.

Cytochrome P450 omega-hydroxylase 4a14 (Cyp4a14) belongs to the enzyme family of Cyp450, which are NADPH monooxygenases responsible for catalyzing a variety of substrates. Cyp4a14 was firstly cloned from mice in 1997 and mainly exists in the liver, kidney, and blood vessel (Heng et al., 1997). The main function of Cyp4a14 is to catalyze ω-hydroxylation of medium-chain fatty acids and arachidonic acid (AA; Xu et al., 2011). Peroxisome proliferator-activated receptor (PPAR) α serves as a biological lipid sensor, and Cyp4a14 is an established target gene of PPARα, always thought to be involved in lipid metabolism (Hsu et al., 2007; Kroetz et al., 1998). Our previous data and others revealed a critical role of Cyp4a14 in the pathogenesis of non-alcoholic fatty liver disease (NAFLD) and related fibrosis (Gilani et al., 2018; Zhang et al., 2017).

In the current study, we checked the expression level of Cyp4a14 in cholestatic related liver fibrosis and systematically studied the role of Cyp4a14 in bile duct ligation (BDL) model by using knockout and adeno-associated virus (AAV)-mediated overexpression technology. Notably, we observed an opposite effect of Cyp4a14 in BDL model. Our results indicate a different but also important role of Cyp4a14 in the pathogenesis of cholestatic liver fibrosis.

MATERIALS AND METHODS

Animals and Treatments

Wild-type (WT) and Cyp4a14 gene knockout ($Cyp4a14^{-/-}$) mice on a 129/SvJ background originally generated by Dr. J. Capdevila, Vanderbilt University, were kindly provided by Pro. YF Guan (Dalian Medical University, China). Male C57Bl/6 mice (6 weeks old) were purchased from the Institute of Medical Laboratory Animal Center, Guangdong, China. Animals were housed under a 12:12-h light/dark cycle and permitted *ad libitum* consumption of water and diet. All experimental procedures were approved by the Animal Experimentation Committee of Shenzhen University Health Science Center. For BDL model, C57BL/6 mice were randomly divided into two groups (sham operation and surgery group), and all surgeries were performed between 9:00 AM and 11:00 AM to minimize the variation. Then we performed BDL in $Cyp4a14^{-/-}$ mice ($Cyp4a14^{-/-}$ + BDL, n = 8) and its WT (WT + Sham, n = 7; WT + BDL, n = 8). For overexpression experiments, C57BL/6J mice were randomly divided into three groups: sham operation (Sham, n = 7), BDL operation in combination with injection of AAV-control (AAV-control + BDL, n = 8), and BDL operation in combination with injection of AAV-pGFAP-Cyp4a14 (AAV-Cyp4a14 + BDL, n = 8). The AAV was injected via the tail vein 4 weeks before the surgery (1×10^{11} pfu per mouse). Two weeks after BDL, all mice were sacrificed under isoflurane anesthesia. Blood were collected from the vena cava to isolate serum. The left lateral liver lobe was fixed in 4% paraformaldehyde, then changed in 20% sucrose solution, and embedded in paraffin. The remaining livers were snap frozen in liquid nitrogen.

Immunohistochemistry

Mouse livers were fixed, dehydrated, and embedded in paraffin wax. Fixed liver sections (5 μm) were incubated with an anti-α-smooth muscle actin (anti-α-SMA) antibody (1:200), an anti-Col1a2 antibody (1:500), or an anti-smad3 antibody (1:100) overnight at 4°C and then with a polyperoxidase-conjugated goat anti-rabbit IgG (Zhongshan Golden Bridge, Beijing, China) for 30 min at 37°C. The slides were counterstained with hematoxylin. ImageJ was used to semi-quantify the H-score of immunohistochemistry (IHC) staining.

Reagents and Chemicals

Kits for determining serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Total collagen content was tested by measuring the amount of hydroxyproline (Hyp) in liver tissue using Hyp assay kit obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The liver sections imbedded in paraffin were cut (5 μm) and stained with hematoxylin–eosin (H&E), Sirius Red, and Masson's trichrome. Image J was used to determine the Sirius Red-positive area. The macroscopic examination was blindly carried out by two independent observers. AAV (type9)-control and AAV-promoter of glial fibrillary acidic protein (pGFAP)-Cyp4a14 (type9, contract number HH20181228LQJ-AAV01) were purchased from Hanbio, Shanghai, China.

Quantitative RT-PCR Analysis

The analysis method was performed as described previously (Zhang et al., 2017). Total RNA was extracted from the frozen liver tissues by using a High Purity Total RNA Extraction Kit (BioTeke, Beijing, China). Five micrograms of total RNA from each sample was reverse-transcribed into complementary DNAs (cDNAs). Each cDNA sample was diluted 1:100, and 4 μl was used as a template per PCR (Thermo Fisher Scientific, Massachusetts, CA, United States). The quantitative PCR was performed on an Agilent Mx3000P PCR System (Agilent Technologies, Santa Clara, CA) using the TransStart Top Green qPCR SuperMix (TransGen, Beijing, China). Expression levels of the target genes were normalized against an endogenous

TABLE 1 | Sequences of primers used for real-time quantitative PCR.

Gene	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)	Reaction temperature (°C)
Cyp4a14	TGAATTGCTGCCAGATCCCAC	GTTCAGTGGCTGGTCAGAGTT	59
Atca2	CTGACAGAGGCACCACTGAA	CATCTCCAGAGTCCAGCACA	59
Tgfb1	TGACGTCACTGGAGTTGTACGG	GGTTCATGTCATGGATGGTGC	59
Col1a1	CAATGCAATGAAGAACTGGACTGT	TCCTACATCTTCTGAGTTTGGTGA	59
Col1a2	GCAGGGTTCCAACGATGTTG	GCAGCCATCGACTAGGACAGA	59
Mmp2	CAAGTTCCCCGGCGATGTC	TTCTGGTCAAGGTCACCTGTC	59
Mmp9	CTGGACAGCCAGACACTAAAG	CTCGCGGCAAGTCTTCAGAG	59
Timp1	GCAACTCGGACCTGGTCATAA	CGGCCCGTGATGAGAAACT	59
Tgfbr1	GGCGAAGGCATTACAGTGTT	TGCACATACAAATGGCCTGT	59
Smad3	CTGGGCCTACTGTCCAATGT	GCAGCAAATTCCTGGTTGTT	59
Pparα	ATGCCAGTACTGCCGTTTTC	GGCCTTGACCTTGTTCATGT	59
GAPDH	AGAACATCATCCCTGCATCC	TTGTCATTGAGAGCAATGCC	59

reference gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH). For each sample and each gene, PCR was carried out in duplicate and repeated for a few times. The specific primer sequences are listed in **Table 1**.

Western Blot Analysis of Hepatic Proteins

The analysis method was performed as described previously (Zhang et al., 2017). To determine the expression levels of selected proteins, 80 μg of liver protein was separated by 10% sodium dodecyl sulfate (SDS) gel. Western blot analysis was performed as described using the antibodies including anti-GAPDH (1:3,000, abs830030, Absin), anti-Cyp4a14 (1:3,000, E10821, ABclonal), anti-Col1a2 (1:1,000, BS-1530), and anti-α-SMA (1:1,000, SIGMA-A2547, Sigma). Immunoblot was performed, and the membrane was developed with enhanced chemiluminescence.

Statistical Analysis

Statistical evaluation was performed by Student's *t* test when only two value sets were compared and one-way ANOVA followed by Bonferroni's test when the data involved multiple groups. $p < 0.05$ was considered statistically significant.

RESULTS

Hepatic Cyp4a14 Expression Is Reduced in Cholestatic Liver

To explore the biological relevance between Cyp4a14 and cholestatic liver fibrosis, we first examined Cyp4a14 expression levels in both sham and BDL mice liver. As shown in **Figure 1**, 14 days after BDL, hepatic Cyp4a14 mRNA levels were downregulated markedly by 75% compared with the sham-operated mice (**Figure 1A**). In accordance with that, Cyp4a14 protein expression was reduced to 15% in the sham-operated mice (**Figure 1B**). The decrease of Cyp4a14 in BDL model is opposite with NAFLD murine model such as high fat diet (HFD)-treated mice, db/db mice, and methionine and choline-deficient (MCD) diet-treated mice (Zhang et al., 2017). The above findings suggest that Cyp4a14 may play a different role in the pathogenesis of cholestatic liver fibrosis.

Ablation of Cyp4a14 Gene Aggravates Bile Duct Ligation-Induced Liver Fibrosis

On the basis of the decrease of Cyp4a14 expression in fibrotic liver, we next examined the effects of Cyp4a14 ablation on BDL-induced liver fibrosis. BDL was performed in Cyp4a14$^{-/-}$ mice and respective WT controls. At day 14 after surgery, the body weight was significantly decreased, and liver weight was significantly increased in the WT + BDL group compared with the WT + Sham group (**Figure 2A**), which was consistent with previous research (Lee et al., 2021). There was no difference between the WT + BDL group and the Cyp4a14$^{-/-}$ + BDL group (**Figure 2A**). We used Masson and Sirius Red staining to determine the formation of fibrillar collagen as a measure of liver fibrosis. As **Figure 2B** shows, BDL leads to the proliferation of the biliary duct with matrix deposition in the periductal and lobular areas. Next, the effects of Cyp4a14 in BDL-induced fibrosis were compared in liver fibrosis between WT and Cyp4a14$^{-/-}$ mice. Compared with the WT + BDL group, the Cyp4a14$^{-/-}$ + BDL group showed a marked increase in fibrotic area as evidenced by the Masson and Sirius Red staining (**Figure 2B**). We also tested the liver total collagen content by measuring the amount of Hyp in liver tissue. In line with the histological analysis, Hyp level is also increased in the Cyp4a14$^{-/-}$ + BDL group compared with WT + BDL group (**Figure 2C**). Moreover, serum ALT and AST were significantly higher in the Cyp4a14$^{-/-}$ + BDL group compared with the WT + BDL group, which revealed an increased liver injury (**Figures 2D,E**). These data indicate increased liver damage after BDL in the Cyp4a14$^{-/-}$ + BDL group.

Cyp4a14 Ablation Enhanced α-Smooth Muscle Actin, Col1a2, and smad3 Expression in Bile Duct Ligation Mice

We next examined a series of pro-fibrotic factors. Hepatic stellate cells (HSCs) play pivotal roles in the pathological development of liver fibrosis. α-SMA acts as a marker of activated HSCs.

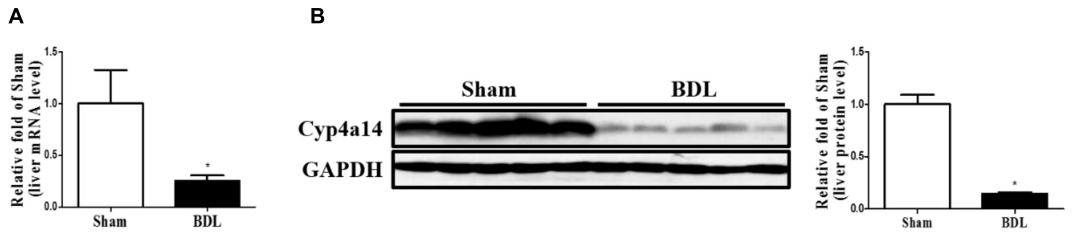


FIGURE 1 | The expression of Cyp4a14 was decreased in the bile duct ligation (BDL)-induced fibrotic liver. **(A)** Real-time PCR assay demonstrating that Cyp4a14 mRNA expression was significantly decreased in BDL mice. **(B)** Western blot analysis showing a significant decrease in hepatic Cyp4a14 protein expression in BDL mice. $n = 5$, $^{*}p < 0.05$, Sham and BDL.

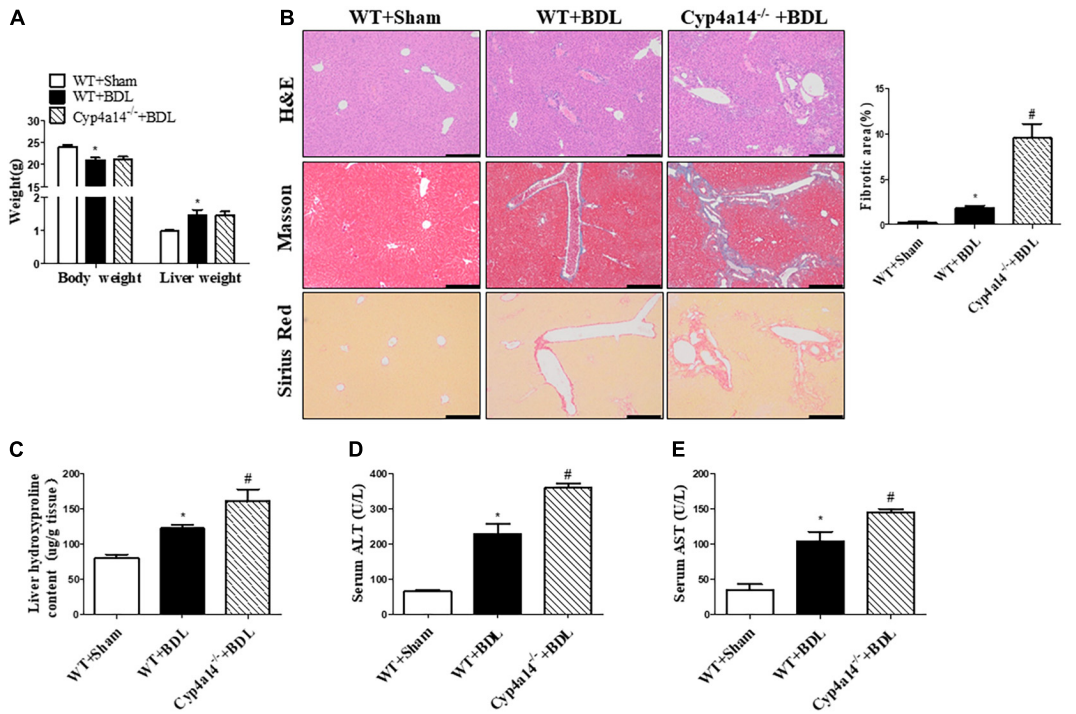


FIGURE 2 | Cyp4a14 deficiency aggravated bile duct ligation (BDL)-induced hepatic fibrosis. Wild-type (WT) and Cyp4a14$^{-/-}$ mice were subjected to BDL for 14 days. **(A)** Body weight and liver weight. **(B)** H&E, Masson, and Sirius Red staining of the mouse liver tissues. **(C)** Hepatic collagen content was measured by biochemical determination of hydroxyproline (Hyp). Serum alanine aminotransferase (ALT) **(D)** and aspartate aminotransferase (AST) **(E)** levels of WT and Cyp4a14$^{-/-}$ mice. $n = 6$–7. $^{*}p < 0.05$, WT + Sham and WT + BDL. $^{\#}p < 0.05$, WT + BDL and Cyp4a14$^{-/-}$ + BDL. Scale bar = 100 μm.

To evaluate the involvement of Cyp4a14 in HSC activation in cholestatic liver fibrosis, expression of α-SMA was examined via both IHC assay and western blot analysis. As expected, α-SMA staining and protein level were markedly increased in the WT-BDL group mice compared with the sham group, and about two-fold higher expression was observed in the Cyp4a14$^{-/-}$ + BDL group mice compared with the WT-BDL group (**Figures 3A,B**). In accordance with that, other key pro-fibrotic factors including TGF-β1, Col1a2, and smad3 were also increased in the Cyp4a14$^{-/-}$ + BDL group compared with the WT + BDL group (**Figures 3A,B**). In addition, BDL-induced gene expression of pro-fibrotic and fibrosis-related genes (*Acta2*, *Tgfb1*, *Col1a1*, *Col1a2*, *Mmp2*, *Mmp9*, *Timp1*, *TgfbR1*, *Smad3*, and *Pparα*) was markedly worsened in the Cyp4a14$^{-/-}$ + BDL group compared with the WT + BDL group (**Figure 3C**). Together, the above data showed that liver injury and fibrosis were more prominent in Cyp4a14$^{-/-}$ livers after BDL.

Hepatic Cyp4a14 Overexpression Attenuates Bile Duct Ligation-Induced Liver Fibrosis

After showing that Cyp4a14 was an important player in liver fibrosis after BDL, we then addressed the question whether overexpression could rescue this effect. HSC activation is the center process in BDL-induced fibrosis. We hypothesized that Cyp4a14 may play an important role in the HSC activation. To explore the exact role of Cyp4a14 in HSC activation,

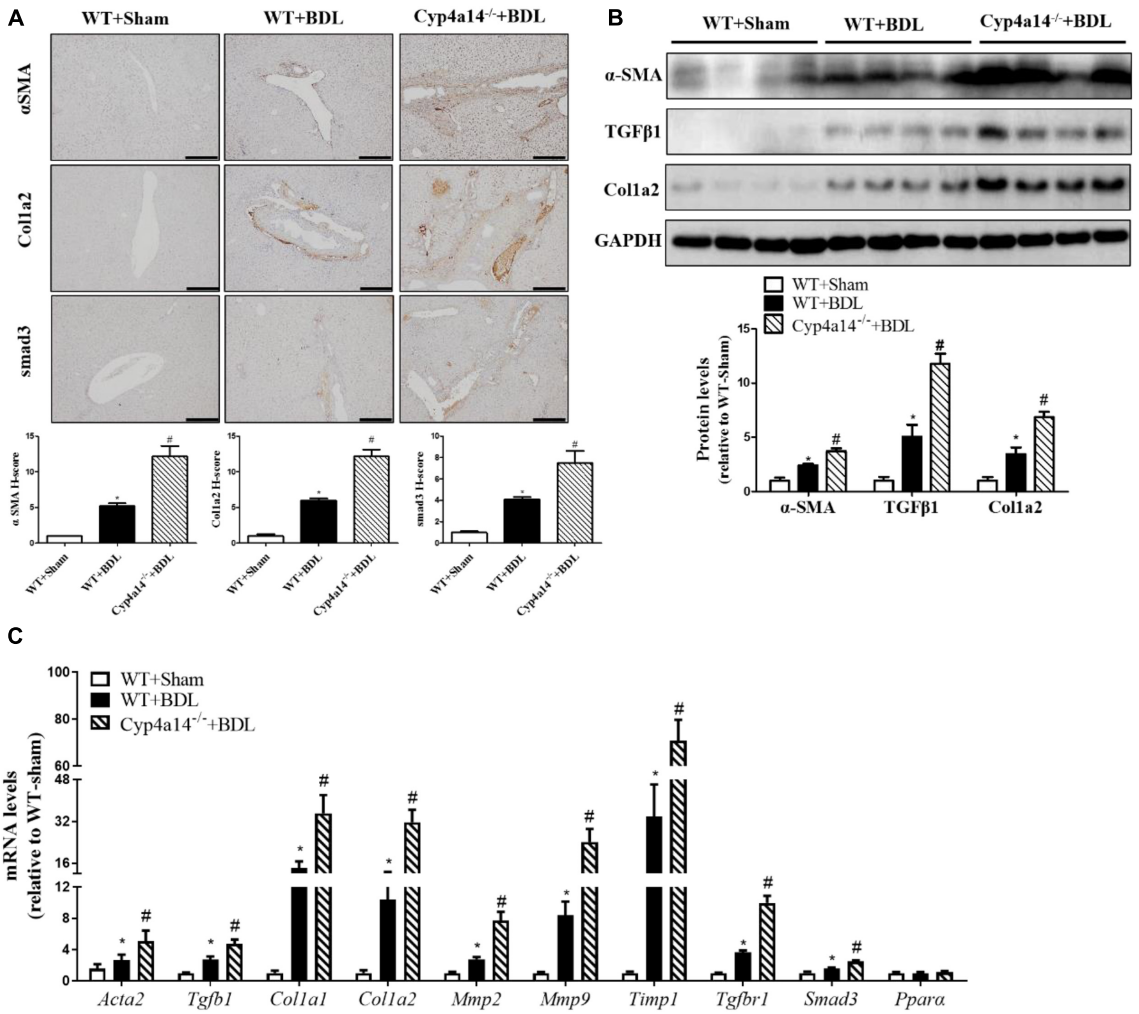


FIGURE 3 | Cyp4a14 ablation enhanced α-smooth muscle actin (α-SMA), Col1a2, and smad3 expression in bile duct ligation (BDL) mice. **(A)** Cyp4a14 gene ablation markedly exacerbated BDL-induced α-SMA, Col1a2, and smad3 protein expression in the livers as assessed by an immunostaining analysis. **(B)** Western blot assay showing the protein levels of α-SMA, TGF-β1, and Col1a2. **(C)** Quantitative RT-PCR analysis showing the mRNA levels of *Acta2*, *Tgfb1*, *Col1a1*, *Col1a2*, *Mmp2*, *Mmp9*, *Timp1*, *TgfbR1*, *Smad3*, and *Pparα*. $n = 6$–7. $^{*}p < 0.05$, WT + Sham and WT + BDL. $^{\#}p < 0.05$, WT + BDL and Cyp4a14$^{-/-}$ + BDL. Scale bar = 100 μm.

we constructed a GFAP-promoter driven AAV9-Cyp4a14. We overexpressed Cyp4a14 in the HSCs of WT mice via tail vein injection 2 weeks before BDL surgery. Cyp4a14 overexpression was succeeded and validated by mRNA and protein levels by four- to seven-fold (**Figures 4A,B**). The body weight was also obviously decreased while liver weight was increased in the AAV-control + BDL group compared with the sham group (**Figure 4C**). There was no difference between the AAV-control + BDL group and the AAV-Cyp4a14 group (**Figure 4C**). Masson and Sirius Red staining revealed a significant decrease in fibrotic area in the AAV-Cyp4a14 + BDL group compared with the AAV-control + BDL group (**Figure 4D**). In line with the histological analysis, liver Hyp content is also improved in the AAV-Cyp4a14 + BDL group compared with the AAV-control + BDL group (**Figure 4E**). In addition, serum ALT and AST were significantly lower in the AAV-Cyp4a14 + BDL group compared with the AAV-control + BDL group (**Figures 4F,G**). These together confirmed that BDL-induced liver damage was alleviated after HSC Cypa14 overexpression.

Hepatic Cyp4a14 Overexpression Inhibited α-Smooth Muscle Actin, Col1a2, and smad3 Expression in Bile Duct Ligation Mice

We also examined the protein expression of α-SMA, TGF-β1, Col1a2, and smad3. In accordance with the reduction of collagen deposition seen by the morphological changes, all the pro-fibrotic factors were significantly decreased in the AAV-Cyp4a14 + BDL group compared with the AAV-control + BDL group (**Figures 5A,B**). And the hepatic expression of pro-fibrotic and fibrosis-related gene (*Acta2*, *Tgfb1*, *Col1a1*, *Col1a2*, *Mmp2*, *Mmp9*, *Timp1*, *TgfbR1*, *Smad3*, and *Pparα*) mRNAs were also decreased in the AAV-Cyp4a14 + BDL group compared with the

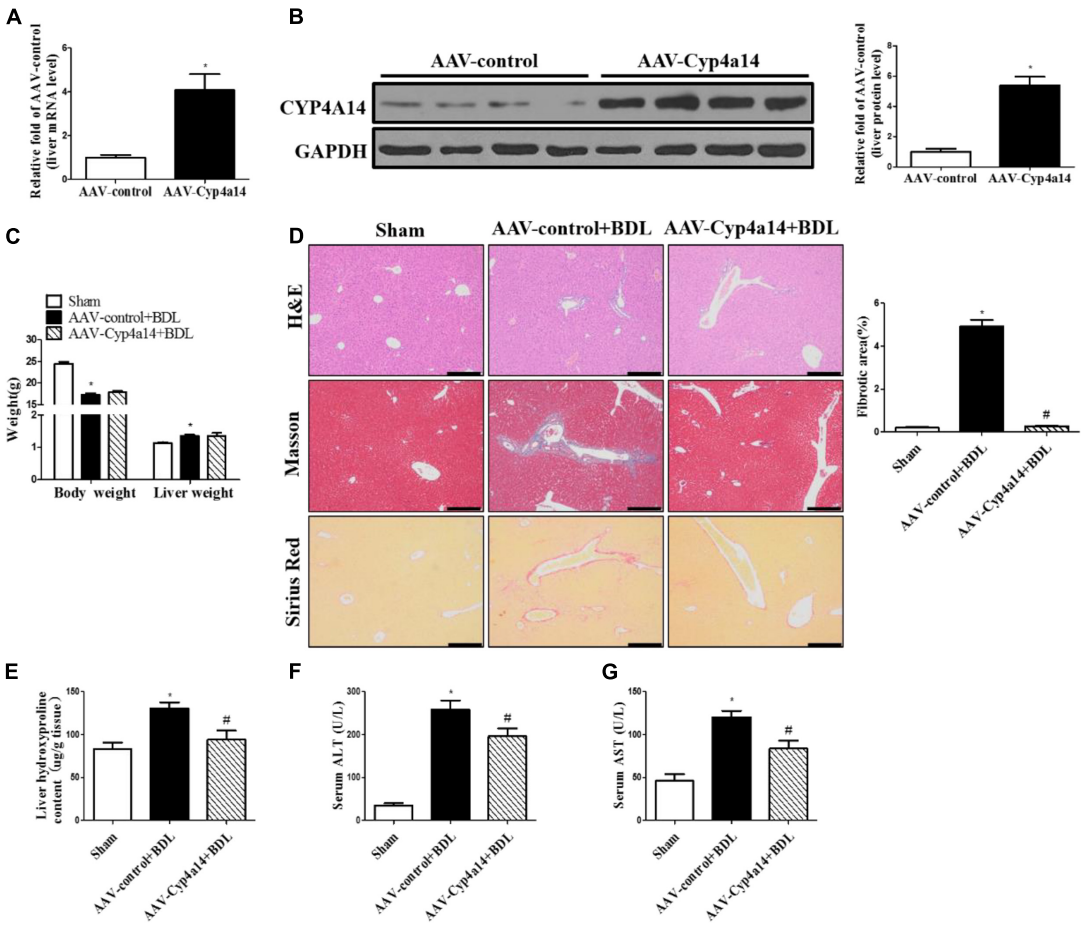


FIGURE 4 | Hepatic Cyp4a14 overexpression ameliorated bile duct ligation (BDL)-induced hepatic fibrosis. Adeno-associated virus (AAV)-control and AAV-Cyp4a14 mice were subjected to BDL for 14 days. **(A)** Body weight and liver weight. **(B)** H&E, Masson, and Sirius Red staining of the mouse liver tissues. **(C)** Hepatic collagen content was measured by biochemical determination of hydroxyproline (Hyp). Serum alanine aminotransferase (ALT) **(D)** and aspartate aminotransferase (AST) **(E)** levels of AAV-control and AAV-Cyp4a14 mice. $n = 6$–7. $^{*}p < 0.05$, Sham and AAV-control + BDL. $^{\#}p < 0.05$, AAV-control + BDL and AAV-Cyp4a14 + BDL. Scale bar = 100 μm.

AAV-control + BDL group (**Figure 5C**). These results suggest that the increase of Cyp4a14 in HSCs is critical for protection of BDL-induced liver fibrosis.

DISCUSSION

Hepatic fibrosis is a dynamic process in which the production and dissolution of ECM are unbalanced (Aydin and Akcali, 2018). The development of liver fibrosis is coordinated by a variety of cell types, including parenchymal and non-parenchymal cells.

We had previously demonstrated that Cyp4a14 is involved in the pathogenesis of liver fibrosis and kidney fibrosis (Zhang et al., 2017; Zhou et al., 2018). Our previous study showed that Cyp4a14 is activated in mice suffering from MCD-induced liver fibrosis, and Cyp4a14$^{-/-}$ mice could attenuate the MCD-induced liver fibrosis, accompanied by reduced hepatic FAT/CD36 expression (Zhang et al., 2017). In the present study, we aim to explore the role of Cyp4a14 in BDL-induced cholestatic liver fibrosis. To our surprise, hepatic Cyp4a14 expression was significantly decreased in BDL liver, which is opposite with NAFLD-related fibrosis. Cyp4a14 ablation mice suffering from BDL exhibited a markedly aggravated HSC activation (shown by α-SMA mRNA and protein level) and collagen deposition (shown by Masson and Sirius Red staining). The evidence revealed that Cyp4a14 might regulate activation of HSCs to play a role in cholestatic liver fibrosis. Thus, we propose a critical role of Cyp4a14 in HSCs. We overexpressed AAV9-Cyp4a14 driven by an HSC-specific promoter GFAP to overexpress Cyp4a14 in HSCs. We found that overexpression of Cyp4a14 in the HSCs markedly rescued BDL-induced liver fibrosis. HSC activation was significantly inhibited (shown by α-SMA mRNA and protein level) and collagen deposition ameliorated (shown by Masson and Sirius Red staining). TGF-β1 plays an important role in HSC activation (Dewidar et al., 2019). Our study shows an obviously reduction of liver TGF-β1 upon Cyp4a14 overexpression mice, which is increased in Cyp4a14$^{-/-}$ mouse livers. Additionally, the expression levels of *Acta2*, *Tgfb1*, *Col1a1*, *Col1a2*, *Mmp2*, *Mmp9*, *Timp1*, *TgfbR1*,

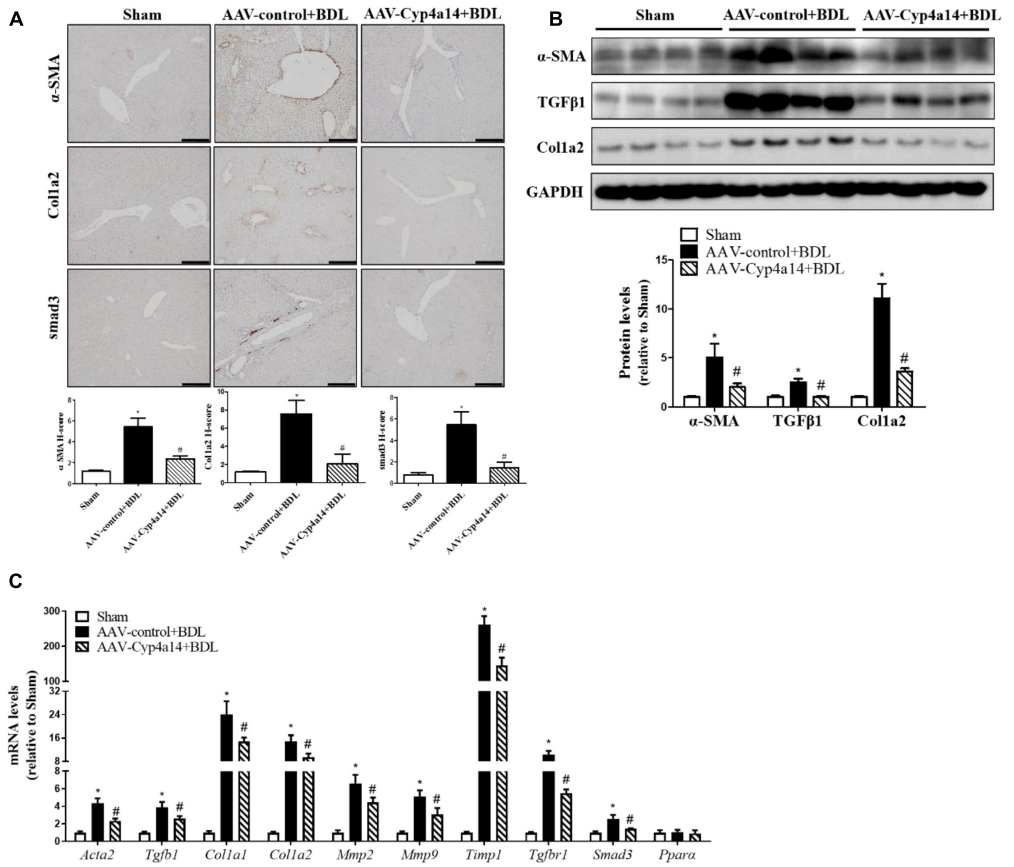


FIGURE 5 | Hepatic Cyp4a14 overexpression inhibited α-smooth muscle actin (α-SMA), Col1a2, and smad3 expression in bile duct ligation (BDL) mice. **(A)** Cyp4a14 gene overexpression obviously attenuated BDL-induced α-SMA, Col1a2, and smad3 protein expression in the livers as assessed by an immunostaining analysis. **(B)** Western blot assay showing the protein levels of α-SMA, TGF-β1, and Col1a2. **(C)** Quantitative RT-PCR analysis showing the mRNA levels of *Acta2*, *Tgfb1*, *Col1a1*, *Col1a2*, *Mmp2*, *Mmp9*, *Timp1*, *TgfbR1*, *Smad3*, and *Pparα*. $n = 6–7$. $^{*}p < 0.05$, Sham and AAV-control + BDL. $^{\#}p < 0.05$, AAV-control + BDL and AAV-Cyp4a14 + BDL. Scale bar = 100 μm.

Smad3, and *Pparα* in BDL-mouse livers were downregulated by Cyp4a14 overexpression and upregulated by Cyp4a14 ablation. These results together indicate that Cyp4a14 might have a different role in hepatocyte and stellate cell during fibrosis.

Bile duct ligation-induced cholestatic liver fibrosis model is commonly used to mimic human hepatic fibrosis, and many researchers are seeking anti-fibrotic therapeutics by studying the etiology and pathology (Liedtke et al., 2013; Tag et al., 2015). BDL leads to the stasis of bile acids in the liver. Excess accumulation of toxic bile salts in hepatocytes results in inflammatory reactions, hepatocyte necrosis, and peri-ductular fibrosis (Frissen et al., 2020). HSC activation plays a pivotal role in the development of liver fibrosis. Farnesoid X Receptor (FXR) is a promising target in clinical trials for the treatment of liver fibrosis. FXR agonists such as obeticholic acid (OCA) have been approved by the Food and Drug Administration (FDA) and are currently widely used for treatment of PBC (Chapman and Lynch, 2020). However, the response rate of OCA treatment to NASH-induced liver fibrosis is only 23%, and common adverse effects such as pruritus and unfavorable changes in lipid profile led OCA to be declined by the FDA for treatment of NASH (Younossi et al., 2019). The discrepancy of FXR in PBC and NASH might reflect the complexity of the HSC activation process (Dewidar et al., 2019; Khomich et al., 2019). The discrepancy of Cyp4a14 in NASH and PBC animal model is very similar to FXR. FXR activation by OCA or GW4064 increased expression of Pparα, Cpt1α, and Cyp4a14 in HFD mice (Gai et al., 2016). The nuclear receptor SHP mediates inhibition of HSCs by FXR and protects against liver fibrosis (Fiorucci et al., 2004). It is reported that SHP upregulates Cyp4a14 through inhibition of Rev-erbα, a transcriptional repressor the Cyp4a14 (Zhang et al., 2018). Thus, it is possible that Cyp4a14 might mediate FXR activation benefits indirectly in OCA-treated PBC. In addition, different causes lead to the different pathogenesis of liver fibrosis and may provide a basis for the new treatment methods (Gressner et al., 2007; Roehlen et al., 2020).

There are some limitations to our study. First, primary hepatocytes and stellate cell experiment with silencing or overexpressing Cyp4a14 would be helpful to elucidate how exactly Cyp4a14 affect BDL-induced fibrosis. Second, animal experiments including hepatocyte specific Cyp4a14 overexpression and HSC specific Cyp4a14 deletion in BDL

model should be checked to rule out the contribution and role of Cyp4a14. Furthermore, whether Cyp4a14 affects bile acid pool or FXR signaling might give some clues to explain our current data. More work needs to performed to elucidate the mechanisms of how Cyp4a14 is involved in the pathogenesis of cholestatic liver fibrosis.

In conclusion, this study shows a protective effect of Cyp4a14 in cholestatic liver fibrosis through inhibiting HSC activation. The study is the first to elucidate that Cyp4a14 plays a different role in different liver fibrosis model. At present, the reason for the different roles of Cyp4a14 in MCD mice and BDL mice remains obscure. Further study of Cyp4a14 in different fibrosis process might help us better distinguish the different mechanisms behind NASH-related fibrosis and cholestatic-induced liver fibrosis and provide a potential strategy to target and treat clinical liver fibrosis.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Experimentation Committee of Shenzhen University Health Science Center.

AUTHOR CONTRIBUTIONS

SL designed, performed, analyzed, and interpreted the majority of animal experiments and drafted the manuscript. CW and XZ supported the animal experiments. WS designed, planned, and interpreted the study. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Natural Science Foundation of China (Nos. 81800549 and 81870405), the Natural Science Foundation of Hebei Province, China (No. H2020402002), SZU Top Ranking Project of China (No. 86000000210), and SZU Medical Young Scientists Program of China (No. 71201-000001).

REFERENCES

Altamirano-Barrera, A., Barranco-Fragoso, B., and Mendez-Sanchez, N. (2017). Management strategies for liver fibrosis. *Ann. Hepatol.* 16, 48–56. doi: 10.5604/16652681.1226814

Aydin, M. M., and Akcali, K. C. (2018). Liver fibrosis. *Turk. J. Gastroenterol.* 29, 14–21. doi: 10.5152/tjg.2018.17330

Chapman, R. W., and Lynch, K. D. (2020). Obeticholic acid-a new therapy in PBC and NASH. *Br. Med. Bull.* 133, 95–104. doi: 10.1093/bmb/ldaa006

Dewidar, B., Meyer, C., Dooley, S., and Meindl-Beinker, A. N. (2019). TGF-beta in hepatic stellate cell activation and liver fibrogenesis-Updated 2019. *Cells* 8:1419. doi: 10.3390/cells8111419

Fiorucci, S., Antonelli, E., Rizzo, G., Renga, B., Mencarelli, A., Riccardi, L., et al. (2004). The nuclear receptor SHP mediates inhibition of hepatic stellate cells by FXR and protects against liver fibrosis. *Gastroenterology* 127, 1497–1512. doi: 10.1053/j.gastro.2004.08.001

Frissen, M., Liao, L., Schneider, K. M., Djudjaj, S., Haybaeck, J., Wree, A., et al. (2020). Bidirectional role of NLRP3 during acute and chronic cholestatic liver injury. *Hepatology* 73, 1836–1854. doi: 10.1002/hep.31494

Gai, Z., Gui, T., Hiller, C., and Kullak-Ublick, G. (2016). Farnesoid X receptor protects against kidney injury in uninephrectomized obese mice. *J. Biol. Chem.* 291, 2397–2411. doi: 10.1074/jbc.M115.694323

Gilani, A., Pandey, V. G., García, V., Agostinucci, K., Singh, S., Schragenheim, J., et al. (2018). High-fat diet-induced obesity and insulin resistance in CYP4a14(-/-) mice is mediated by 20-HETE. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 315, R934–R944. doi: 10.1152/ajpregu.00125.2018

Gressner, O. A., Weiskirchen, R., and Gressner, A. M. (2007). Evolving concepts of liver fibrogenesis provide new diagnostic and therapeutic options. *Comp. Hepatol.* 6:7. doi: 10.1186/1476-5926-6-7

Gulamhusein, A. F., and Hirschfield, G. M. (2020). Primary biliary cholangitis: pathogenesis and therapeutic opportunities. *Nat. Rev. Gastroenterol. Hepatol.* 17, 93–110. doi: 10.1038/s41575-019-0226-7

Heng, Y. M., Kuo, C. S., Jones, P. S., Savory, R., Schulz, R. M., Tomlinson, S. R., et al. (1997). A novel murine P-450 gene, Cyp4a14, is part of a cluster of Cyp4a and Cyp4b, but not of CYP4F, genes in mouse and humans. *Biochem. J.* 325(Pt 3), 741–749. doi: 10.1042/bj3250741

Hsu, M. H., Savas, U., Griffin, K. J., and Johnson, E. F. (2007). Human cytochrome p450 family 4 enzymes: function, genetic variation and regulation. *Drug Metab. Rev.* 39, 515–538. doi: 10.1080/03602530701468573

Khomich, O., Ivanov, A. V., and Bartosch, B. (2019). Metabolic hallmarks of hepatic stellate cells in liver fibrosis. *Cells* 9:24. doi: 10.3390/cells9010024

Kroetz, D. L., Yook, P., Costet, P., Bianchi, P., and Pineau, T. (1998). Peroxisome proliferator-activated receptor alpha controls the hepatic CYP4A induction adaptive response to starvation and diabetes. *J. Biol. Chem.* 273, 31581–31589. doi: 10.1074/jbc.273.47.31581

Lee, C. H., Choi, Y., Cho, H., Bang, I. H., Hao, L., Lee, S., et al. (2021). Histone deacetylase 8 inhibition alleviates cholestatic liver injury and fibrosis. *Biochem. Pharmacol.* 183:114312. doi: 10.1016/j.bcp.2020.114312

Liedtke, C., Luedde, T., Sauerbruch, T., Scholten, D., Streetz, K., Tacke, F., et al. (2013). Experimental liver fibrosis research: update on animal models, legal issues and translational aspects. *Fibrogenesis Tissue Repair* 6:19. doi: 10.1186/1755-1536-6-19

Parola, M., and Pinzani, M. (2019). Liver fibrosis: pathophysiology, pathogenetic targets and clinical issues. *Mol. Aspects Med.* 65, 37–55. doi: 10.1016/j.mam.2018.09.002

Roehlen, N., Crouchet, E., and Baumert, T. F. (2020). Liver fibrosis: mechanistic concepts and therapeutic perspectives. *Cells* 9:875. doi: 10.3390/cells9040875

Santiago, P., Scheinberg, A. R., and Levy, C. (2018). Cholestatic liver diseases: new targets, new therapies. *Therap. Adv. Gastroenterol.* 11:1756284818787400. doi: 10.1177/1756284818787400

Seki, E., and Brenner, D. A. (2015). Recent advancement of molecular mechanisms of liver fibrosis. *J. Hepatobiliary Pancreat. Sci.* 22, 512–518. doi: 10.1002/jhbp.245

Tag, C. G., Weiskirchen, S., Hittatiya, K., Tacke, F., Tolba, R. H., and Weiskirchen, R. (2015). Induction of experimental obstructive cholestasis in mice. *Lab. Anim.* 49(Suppl. 1), 70–80. doi: 10.1177/0023677214567748

Toosi, A. E. (2015). Liver fibrosis: causes and methods of assessment, a review. *Rom. J. Intern. Med.* 53, 304–314. doi: 10.1515/rjim-2015-0039

Vesterhus, M., and Karlsen, T. H. (2020). Emerging therapies in primary sclerosing cholangitis: pathophysiological basis and clinical opportunities. *J. Gastroenterol.* 55, 588–614. doi: 10.1007/s00535-020-01681-z

Xu, X., Zhang, X. A., and Wang, D. W. (2011). The roles of CYP450 epoxygenases and metabolites, epoxyeicosatrienoic acids, in cardiovascular and malignant diseases. *Adv. Drug Deliv. Rev.* 63, 597–609. doi: 10.1016/j.addr.2011.03.006

Younossi, Z. M., Ratziu, V., Loomba, R., Rinella, M., Anstee, Q. M., Goodman, Z., et al. (2019). Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet* 394, 2184–2196. doi: 10.1016/S0140-6736(19)33041-7

Zhang, T., Yu, F., Guo, L., Chen, M., Yuan, X., and Wu, B. (2018). Small heterodimer partner regulates circadian cytochromes p450 and drug-induced hepatotoxicity. *Theranostics* 8, 5246–5258. doi: 10.7150/thno.28676

Zhang, X., Li, S., Zhou, Y., Su, W., Ruan, X., Wang, B., et al. (2017). Ablation of cytochrome P450 omega-hydroxylase 4A14 gene attenuates hepatic steatosis and fibrosis. *Proc. Natl. Acad. Sci. U.S.A.* 114, 3181–3185. doi: 10.1073/pnas.1700172114

Zhou, Y., Yu, J., Liu, J., Cao, R., Su, W., Li, S., et al. (2018). Induction of cytochrome P450 4A14 contributes to angiotensin II-induced renal fibrosis in mice. *Biochim. Biophys. Acta Mol. Basis Dis.* 1864, 860–870. doi: 10.1016/j.bbadis.2017.12.028

Conflict of Interest: 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.

frontiers in Physiology

REVIEW
published: 15 July 2021
doi: 10.3389/fphys.2021.640374

Cyclopentenone Prostaglandins: Biologically Active Lipid Mediators Targeting Inflammation

Bohae Rachel Lee†, May Hnin Paing† and Neelam Sharma-Walia†*

H. M. Bligh Cancer Research Laboratories, Department of Microbiology and Immunology, Chicago Medical School, Rosalind Franklin University of Medicine and Science, North Chicago, IL, United States

Cyclopentenone prostaglandins (cyPGs) are biologically active lipid mediators, including PGA_2, PGA_1, PGJ_2, and its metabolites. cyPGs are essential regulators of inflammation, cell proliferation, apoptosis, angiogenesis, cell migration, and stem cell activity. cyPGs biologically act on multiple cellular targets, including transcription factors and signal transduction pathways. cyPGs regulate the inflammatory response by interfering with NF-κB, AP-1, MAPK, and JAK/STAT signaling pathways via both a group of nuclear receptor peroxisome proliferator-activated receptor-gamma (PPAR-γ) dependent and PPAR-γ independent mechanisms. cyPGs promote the resolution of chronic inflammation associated with cancers and pathogen (bacterial, viral, and parasitic) infection. cyPGs exhibit potent effects on viral infections by repressing viral protein synthesis, altering viral protein glycosylation, inhibiting virus transmission, and reducing virus-induced inflammation. We summarize their anti-proliferative, pro-apoptotic, cytoprotective, antioxidant, anti-angiogenic, anti-inflammatory, pro-resolution, and anti-metastatic potential. These properties render them unique therapeutic value, especially in resolving inflammation and could be used in adjunct with other existing therapies. We also discuss other α, β -unsaturated carbonyl lipids and cyPGs like isoprostanes (IsoPs) compounds.

Keywords: prostaglandins, PPAR-γ, viral (or virus), inflammation, antiviral

OPEN ACCESS

Edited by:
Jun Yang,
University of California, Davis, United States

Reviewed by:
Cecil Robert Pace-Asciak, The Hospital for Sick Children, Canada
Ginger Milne, Vanderbilt University, United States
Ondrej Kuda, Institute of Physiology, Academy of Sciences of the Czech Republic (ASCR), Czechia

***Correspondence:**
Neelam Sharma-Walia
neelam.sharma-walia@rosalindfranklin.edu

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Lipid and Fatty Acid Research, a section of the journal Frontiers in Physiology

Received: *11 December 2020*
Accepted: *10 May 2021*
Published: *15 July 2021*

Citation:
Lee BR, Paing MH and Sharma-Walia N (2021) Cyclopentenone Prostaglandins: Biologically Active Lipid Mediators Targeting Inflammation. Front. Physiol. 12:640374. doi: 10.3389/fphys.2021.640374

Abbreviations: AD, Alzheimer's disease; AP-1, activating protein-1; ALS, amyotrophic lateral sclerosis; AGMK, African green monkey kidney; COX, cyclooxygenase; CCR, chemokine receptors; CTL, cytotoxic T lymphocytes; CREB, cyclic AMP-responsive element-binding; cyPGs, cyclopentenone PGs; COPD, chronic obstructive pulmonary disease; DCs, dendritic cells; DGLA, dihomo g-linolenic acid; EAE, experimental allergic encephalomyelitis; EBV, Epstein–Barr virus; ERK, extracellular signal-regulated kinases; EMT, epithelial to mesenchymal transition; FRK, c-Fos-regulating kinases; GMCSF, granulocyte-macrophage colony-stimulating factor; GR, glutathione reductase; GPx, glutathione peroxidase 1; GCS, c-glutamylcysteine synthetase; HCMV, human cytomegalovirus; HDACs, histone deacetylases; HO-1, heme oxygenase-1; HSV, herpes simplex virus; Hep-2, human epithelial type 2; HSP70, heat shock protein70; HTLV-1, human T-cell leukemia virus type-I; hTERT, human telomerase reverse transcriptase; ICAM-1, intercellular adhesion molecule 1; IBD, inflammatory bowel disease; IsoPs, isoprostanes; IKK, IκB kinase; JAK, Janus kinase; Keap1, Kelch-like ECH-associated protein 1; KSHV, Kaposi's sarcoma herpesvirus; LBD, ligand-binding domain; mTOR, mammalian target of rapamycin; MMP-9, matrix metalloproteinase; Nrf2, NF-E2-related nuclear factor erythroid-2; NSCLC, non-small cell lung carcinoma; NE, nanoemulsion; NGS, next-generation sequencing; NLS, nuclear localization sequence; NSAIDs, non-steroidal anti-inflammatory drugs; NQO1, NAD(P)H dehydrogenase quinone 1; PAI-1, plasminogen activator inhibitor-1; PD-1, programmed cell death protein-1; PDL-1, programmed cell death ligand-1; PG, prostaglandin; PUFA, polyunsaturated fatty acid; 15-PGDH, 15−hydroxyprostaglandin dehydrogenase; PLA_2, phospholipase A_2; PPAR-γ, peroxisome proliferator-activated receptor-gamma; ROS, reactive oxygen species; RTT, Rett syndrome; SOCS, suppressor of cytokine signaling; SLN, solid lipid nanoparticles; SOD, superoxide dismutase; SLOS, Smith–Lemli–Opitz syndrome; TAR, transactivation response element; TGF-β, transforming growth factor-β; TGZ, troglitazone; TXA_2, thromboxane A_2; uPA, urokinase plasminogen activator; VEGF, vascular endothelial growth factor; VSV, vesicular stomatitis virus; VZV, varicella zoster virus.

INTRODUCTION

Prostaglandins (PGs) are a group of lipids or oxygenated derivatives of arachidonic acid (AA) that sustain homeostatic functions and mediate the inflammatory response (Aoki and Narumiya, 2012). There are two types of PGs: conventional or classic PGs and cyclopentenone PGs (cyPGs). Examples of traditional PGs are PGD_2, PGE_2, prostacyclin (PGI_2), $PGF_{2\alpha}$, and thromboxane A_2 (TXA_2), while the members of cyPGs include PGA_1, PGA_2, PGJ_2, and metabolites of PGJ_2, such as 15-Deoxy-Δ-^{12,14}-Prostaglandin J_2 (15d-PGJ_2) and Δ^{12}-PGJ_2. As the name implies, cyPGs contain a cyclopentenone ring structure with a highly reactive α, β-unsaturated carbonyl group, which can alter many proteins and their functional properties covalent attachments with thiol groups of the proteins (Straus and Glass, 2001). cyPGs are potent bioactive molecules and have a wide range of functions (Burstein, 2020). cyPGs can repress inflammatory responses, inhibit cell growth, angiogenesis, and increase apoptosis. cyPGs can interfere with virus infections and cancer development, indicating their potential to serve as therapeutic agents. This review discusses cyPGs biosynthesis, mechanism of action, functions, and their effects on virus infection and cancer development. Despite the existing knowledge, the resolving, antiviral, anti-inflammatory, and anticancer potential of cyPGs have been minimally explored and warrant further attention.

BIOSYNTHESIS OF CYCLOPENTENONE PROSTAGLANDINS (PGA_1, PGA_2, AND PGJ_2 AND ITS METABOLITES)

AA is liberated from membrane phospholipids by the enzyme phospholipase A_2 (PLA_2) (Vane and Botting, 1990). Myosin, an actin-binding protein, is phosphorylated when there is an increase in intracellular calcium levels, causing PLA_2 to translocate from the cytoplasm to the intracellular membrane to access the phospholipids. Arachidonate is metabolized to PGG_2 by cyclooxygenase (COX) 1 and 2 (COX-1 and COX-2), which are contained in the endoplasmic reticulum (ER) and nuclear membranes (Vane and Botting, 1990; Hanna and Hafez, 2018) (**Figure 1**). PGG_2 is converted into PGH_2 by hydroxyperoxidase. Unstable PGH_2 diffuses from the ER lumen to the cytoplasm through the ER membrane. Due to its unstable nature, PGH_2 is enzymatically converted into different PGs, including PGI_2, PGF_2, and TXA_2, through the action of specific PG synthases (**Figure 1**). When PGH_2 is acted upon by PGD_2 synthase, PGD_2 is created. PGD_2 is unstable and spontaneously undergoes non-enzymatic dehydration to yield either 15d-PGD_2 or PGJ_2 (**Figure 1**). Further dehydration and a 13, 14 double bond rearrangement of PGJ_2 yield 15-Deoxy-Δ-^{12,14}-prostaglandin J_2 (15d-PGJ_2) in an albumin-independent manner, while PGJ_2 dependent on serum albumin results in Δ12-PGJ_2 (Figueiredo-Pereira et al., 2014). PGs of the J series are synthesized *in vivo* as Δ12-PGJ_2 is a natural component of human body fluids. Its synthesis is inhibited by treatment with COX inhibitors (Hirata et al., 1988). When PGH_2 is acted upon by PGE_2 synthase, PGE_2 is formed. Dehydration of PGE_2 leads to PGA_2 (Hamberg and Samuelsson, 1966; Nugteren et al., 1966) (**Figure 1**). 15d-PGJ_2 could function in both an autocrine and paracrine manner and can be produced intracellularly and extracellularly via non-enzymatic conversion of PGD_2 (Shibata et al., 2002).

The formation of the cyclopentenone PGA_1 has a different genesis pathway compared to the other members of its family (PGA_2 and PGJ_2). The formation of PGA_1 begins with linoleic acid (LA). In the human diet, linoleic acid is the most consumed polyunsaturated fatty acid (PUFA) (Whelan and Fritsche, 2013). Linoleic acid, an essential omega 6 ($n = 6$) fatty acid, is converted to γ-linoleic acid (GLA; GLA, 18:3-6) through the membrane-bound enzyme 6-desaturase (Δ-6-desaturase). GLA is then metabolized to dihomo γ-linolenic acid (DGLA, 20:3-6) by a Δ6 elongase. From this point, DGLA can be converted into AA by the enzyme 5-Desaturase, or PGE_1 by the enzyme COX. PGE_1 undergoes dehydration to become PGA_1 (Kapoor and Huang, 2006; Kapoor et al., 2007).

15d-PGJ_2 acts via G-protein-coupled seven-transmembrane PGD_2 receptors (D prostanoid; DP_1 and DP_2) and through interaction with intracellular targets (Kato et al., 1986; Kim et al., 1993; Negishi and Katoh, 2002). DP_2 (chemoattractant receptor-homologous molecule or GPR44 or CD294) is expressed on Th2 cells, eosinophils, activated mast cells, and basophils (Negishi and Katoh, 2002; Nagata et al., 2017). PGE_1/PGA_1 is native/endogenous ligands of orphan nuclear receptor-related 1 protein (Nurr1; NR4A2) and activates its transcriptional function (Negishi and Katoh, 2002; Pearen and Muscat, 2010; Kurakula et al., 2014).

CYCLOPENTENONE PROSTAGLANDINS AND INFLAMMATION

Cyclopentenone Prostaglandins in Various Diseases

15d-PGJ_2 is an immune regulator to modulate human autoimmune diseases as multiple sclerosis (MS), experimental allergic encephalomyelitis (EAE), polymyositis, Bechet's diseases, rheumatoid arthritis (RA), atopic dermatitis, systemic lupus erythematosus (SLE) (Li and Pauza, 2009), and age-related neurodegenerative diseases, including Alzheimer's (AD) and Parkinson's disease (PD) (Koharudin et al., 2011). γΔT cells have been studied in context with autoimmune diseases in humans. γΔT cells possess the cytotoxic activity and produce IFN-γ, tumor necrosis factor-alpha; TNF-α, and chemokines involved in recruiting monocytes and macrophages. The induction of cytokines and secretion of interleukin-17 (IL-17) contributes to inflammatory processes and promotes autoimmunity. 15d-PGJ_2, along with rosiglitazone (Avandia), suppressed γΔT cell proliferation in response and downregulated cytokine production (Li and Pauza, 2009). 15d-PGJ_2 also plays an essential regulatory role in osteosarcoma, bone

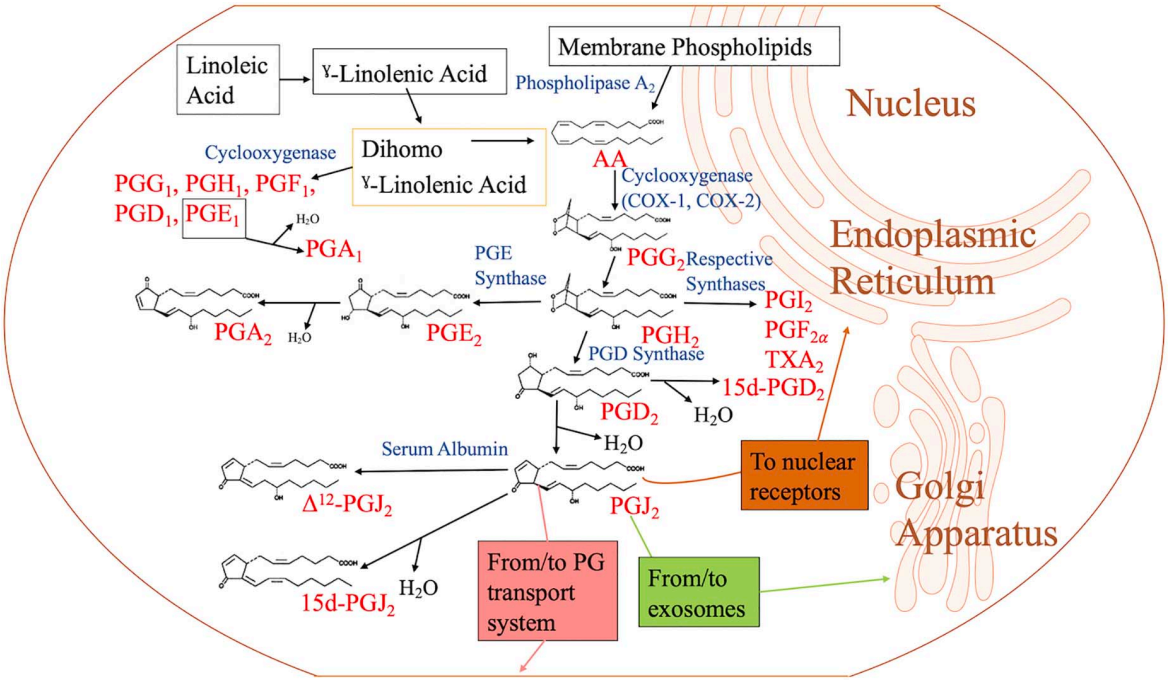


FIGURE 1 | Biosynthesis of cyclopentenone prostaglandins. When the cell is activated by stressful stimuli, such as mechanical trauma, interferon, interleukin, or growth factors, the enzyme phospholipase A_2 moves from the cytoplasm to intracellular membranes to liberate arachidonic acid (AA) from the nuclear envelope or endoplasmic reticulum. AA is converted by cyclooxygenase-1 (COX-1) or cyclooxygenase-2 (COX-2) to prostaglandin G_2 (PGG_2), followed by hydroperoxidation of PGG_2 to PGH_2. PGH_2 is converted to other PGH_2 metabolites such as PGD_2, PGE_2, PGF_2, PGI_2, and thromboxane A_2 (TXA_2) by their respective synthases. Of the metabolites, PGD_2 is dehydrated to form J_2 PGs. PGJ_2 may be located in exosomes, transport systems, or nuclear receptors to execute its function. PGE_2 is dehydrated to form PGA_2. PGA_1 is a metabolite of linoleic acid, which is obtained through diet.

metastases, and bone metabolism (Kitz et al., 2011; Kim et al., 2015).

Cyclopentenone Prostaglandins Elicit Anti-inflammatory Responses via Regulating Transcription Factors Crucial for Inflammatory Response

$15d\text{-}PGJ_2$ directly inhibits multiple steps in the NF-κB signaling pathway and NF-κB-dependent gene expression (Straus et al., 2000). NF-κB represents a family of structurally related inducible transcription factors (NF-κB1; p50, NF-κB2; p52, RelA; p65, RelB, and c-Rel) located in the cytoplasm, which activates genes responsible for inflammation and innate and adaptive immunity (Senftleben et al., 2001). The NF-κB proteins are typically sequestered in the cytoplasm by a family of inhibitory proteins, including IκB family members, which sterically block the nuclear localization sequence (NLS) of NF-κB (Senftleben et al., 2001; Sun, 2017). The IκB kinase (IKK) complex is crucial for the activation of NF-κB, as it can degrade the NF-κB inhibitor IκB through phosphorylation, subsequently freeing NF-κB (Senftleben et al., 2001). NF-κB is involved in the pathogenesis of inflammatory diseases, including RA, inflammatory bowel disease (IBD), MS, atherosclerosis, SLE, type 1 diabetes, chronic obstructive pulmonary disease (COPD), and asthma (Pai and Thomas, 2008). NF-κB activation induces proinflammatory cytokines (IL-1β, IL-1, IL-2, IL-6, IL-8, and TNF-α) (Lawrence, 2009; Wang et al., 2014) and regulates inflammasome function (Guo et al., 2015) in both innate and adaptive immune cells. PGA_1, another cyPG, is also a potent inhibitor of NF-κB activation in human cells by inhibiting phosphorylation and preventing degradation of the NF-κB inhibitor IκB-α (Rossi et al., 1997). The α, β-unsaturated carbonyl group in the cyPGs, when reactive, can undergo a Michael reaction with the cysteine nucleophile at position 179 on the IKKβ subunit of the IKK complex. This cysteine is located in the activation loop of the enzyme, and the alkylation of the cysteine inhibits the phosphorylation of the activation loop. Therefore, cyPGs inhibit IKK complex activity by directly modifying the IKKβ subunit (Rossi et al., 2000). By doing so, the degradation IκB is inhibited, and NF-κB is unable to enter the nucleus.

$15d\text{-}PGJ_2$ inhibits transcription factor activity of activating protein-1 (AP-1) (Perez-Sala et al., 2003). AP-1 is composed of dimeric complexes, which included members of four families of DNA-binding proteins such as Jun, Fos, ATF/cyclic AMP-responsive element-binding (CREB), and musculoaponeurotic fibrosarcoma (Maf) (Milde-Langosch, 2005; Hernandez et al., 2008). $15d\text{-}PGJ_2$ covalently modifies c-Jun and directly inhibits the DNA binding activity of AP-1 (Perez-Sala et al., 2003). AP-1 plays critical roles in inflammation, proliferation, innate immune response and stimulates growth factors and proinflammatory cytokines mediated by serine/threonine kinases as c-Jun

NH2-terminal kinases (JNK), p38, extracellular signal-regulated kinases (ERK), and c-Fos-regulating kinases (FRK) MAP kinase pathways (Lin et al., 1995; Minden et al., 1995).

15d-PGJ_2 non-specifically inhibits Signal transducer and activator of transcription (STAT) (Ji et al., 2005) and Janus kinase (JAK)-STAT signaling pathway in lymphocytes (Kim et al., 2005). STAT1 can be activated upon tyrosine phosphorylation by JAK1 tyrosine kinase (Mowen and David, 2000). Upon activation, STAT/STAT interactions occur immediately, and dimerized STATs can then enter the nucleus and regulate the transcription of inflammatory genes of cytokine and interferon signaling (Seif et al., 2017).

Anti-inflammatory, Anti-tumorigenic, Anti-angiogenic, Anti-metastatic, Anti-fibrotic, Resolving, and Antioxidant Modes of Action of Cyclopentenone Prostaglandins

cyPGs, such as 15d-PGJ_2, PGJ_2, PGA_1, and PGA_2, can activate peroxisome proliferator-activated receptor-gamma (PPAR-γ), and many of their biological functions are either PPAR-γ dependent or independent (Mukherjee et al., 1994; Ricote et al., 1998b; Yagami et al., 2018). PPAR-γ is one of the members (PPAR-α, PPAR-δ, and PPAR-γ) of the nuclear receptor superfamily and is a ligand-dependent transcription factor. The ligand 15d-PGJ_2 activates PPAR-γ, and PPAR-γ then forms a heterodimer with retinoid X receptor (RXR) in the cytoplasm. Complex enters the nucleus (Scher and Pillinger, 2005; Li et al., 2019). This complex binds to specific PPAR response element (PPRE) regions in the DNA to activate different target genes (Forman et al., 1996).

Anti-inflammatory Actions

Peroxisome proliferator-activated receptor-gamma inhibits TNF-α, IL-6, inducible NO synthase (iNOS), gelatinase B, and COX-2 by acting as an antagonist to AP-1 and NF-κB (Welch et al., 2003). This inhibition mode was observed in activated macrophages expressing high levels of PPAR-γ (Ricote et al., 1998a,b; Straus et al., 2000). In general, when IFN-γ stimulated peritoneal macrophages were treated with 15d-PGJ_2, instead of observing activated macrophages, morphological features classic of resting cells were seen (Ricote et al., 1998a,b). 15d-PGJ_2 treatment inhibited the induction of iNOS transcription by inhibiting the binding of AP-1 and NF-κB on iNOS promoter (Ricote et al., 1998a,b). Usually, iNOS is upregulated in activated macrophages accompanied by the overproduction of nitric oxide (NO), which causes inflammation (Sharma and Staels, 2007). Excess NO also induces *s*-nitrosylation of Sirt1, an inhibitor of p65 NF-κB, which inactivates Sirt1 and enhances pro-inflammatory response (Nakazawa et al., 2017). 15d-PGJ_2 treatment inhibits matrix metalloproteinase (MMP-9) or also called Gelatinase B in activated macrophages (Ricote et al., 1998a,b) at the transcription level. Inhibition by 15d-PGJ_2 is mediated at the level of AP-1 binding as MMP-9 transcriptional activation is dependent on AP-1 (Saarialho-Kere et al., 1993). 15d-PGJ_2 and TZDs reduced dendritic cells (DCs) stimulation with toll-like receptor (TLR) ligands via the MAP kinase and NF-κB pathways (Appel et al., 2005). In RAW264.7 cells, monocyte/macrophage-like cell lineage stimulated with LPS, a similar outcome to that of Jurkat cells was observed when treated with cyPGs (Straus et al., 2000). A different result was observed in HeLa cells, strengthening the fact that cyPGs' effect is cell type specific. Instead of inhibiting IKK complex activity, cyPGs impede the binding of NF-κB to DNA since p50 and p65 have cysteine residues at C62 and C38, respectively. Alkylation of these cysteines via the Michael reaction results in the inhibition of the binding of NF-κB to DNA (Straus et al., 2000).

In human astrocytes treated with 15d-PGJ_2, NF-κB was inhibited from binding to the COX-2 promoter on DNA (Janabi, 2002). In glial cells, 15d-PGJ_2 induces the transcription of suppressor of cytokine signaling 1 and 3 (SOCS1 and SOCS3) can inhibit JAK, eventually inhibiting the transcription of inflammatory genes (Park E. J. et al., 2003; Park S. H. et al., 2003). 15d-PGJ_2 inhibited the JAK/STAT1 mediated interferon regulatory factor-1 (IRF-1) expression, thereby decreasing the IFN-γ-induced costimulatory molecule B7-H1 expression needed by tumors to evade the host immune response (Seo et al., 2014). 15d-PGJ_2 inhibits lethal anthrax toxin (LT) activation of the NLRP1 and nigericin-mediated activation of the NLRP3 inflammasome and associated IL-1β release (Maier et al., 2015). 15d-PGJ_2 mitigates the macrophage hyperinflammatory response (Monroy et al., 2007).

PGD_2 and the J_2-series PGJ_2 and Δ12-PGJ_2 are critical components of the inflammatory response within adipose tissue during obesity thus producing inflammation-related adipokines implicated in insulin sensitivity (Peeraully et al., 2006). 15d-PGJ_2 is the most potent inducer of fat cell (adipocyte) differentiation *in vitro* (Forman et al., 1995; Bell-Parikh et al., 2003). PGD_2, PGJ_2, and Δ12-PGJ_2 treatment strongly down-regulates the production of leptin, a hormone secreted by adipocytes (Peeraully et al., 2006).

Anti-tumorigenic Actions

15d-PGJ_2 exerts antitumor activity by regulating the Myc/Mad/max transcription factors to promote cell apoptosis, tubulin binding activity, inhibiting the expression of human telomerase reverse transcriptase (hTERT), enhancing TRAIL-induced apoptosis by downregulating AKT phosphorylation, reactive oxygen species (ROS)-dependent cell death pathway, ROS-dependent AKT activation, inhibition of COX-2, STAT-3, cell cycle (G2/M or G1) blockade, inhibition of vascular endothelial factor (VEGF), growth and expansion of tumor stem cells in gastric cancer (Inoue et al., 2000; Sato et al., 2000; Takashima et al., 2001; Yuan et al., 2005; Chearwae and Bright, 2008; Dionne et al., 2010; Li et al., 2017), oral squamous cell carcinoma (Nikitakis et al., 2002), leukemia (Han et al., 2007), lymphoma (Inoue et al., 2000; Sato et al., 2000; Takashima et al., 2001; Yuan et al., 2005; Chearwae and Bright, 2008; Dionne et al., 2010; Li et al., 2017), oesophageal cancer (Takashima et al., 2001), endometrial cancer (Li and Narahara, 2013), breast cancer (Cocca et al., 2009), osteosarcoma (Yen et al., 2014), and brain tumors (Inoue et al., 2000; Sato et al., 2000; Takashima et al., 2001; Yuan et al., 2005;

TABLE 1 | Biological effects of cyclopentenone prostaglandins.

Anti-inflammatory	Specific function	Site of action	References
15d-PGJ_2	Inhibition of iNOS promoter containing binding sites for AP-1 and NF-κB	Macrophages	Ricote et al., 1998a,b
15d-PGJ_2	Gelatinase B or MMP-9	Macrophages	Ricote et al., 1998a,b
15d-PGJ_2 and TZDs	MAPK and NF-κB signaling	Dendritic cells (DCs)	Appel et al., 2005
15d-PGJ_2, other cyPGs	Inhibition of NF-κB binding to DNA	RAW264.7 cells, monocyte/macrophage-like cell lineage	Straus et al., 2000
15d-PGJ_2	Inhibition of NF-κB binding to the COX-2 promoter STAT-1 and c-Jun expression	Human astrocytes, microglia	Janabi, 2002
15d-PGJ_2	Transcription of SOCS1 and SOCS3	Brain inflammation	Park E. J. et al., 2003; Park S. H. et al., 2003
15d-PGJ_2	Inhibition of the JAK/STAT1 mediated IRF-1 expression decreasing cytokine production	B16F10 melanoma cells	Seo et al., 2014
15d-PGJ_2	Inhibition of caspase-1 activation by NLRP1 and NLRP3 inflammasomes prevents the autoproteolytic activation of caspase-1 and the maturation of IL-1β	NLRP3-dependent peritonitis model	Maier et al., 2015
15d-PGJ_2	Mitigates the macrophage hyperinflammatory response and inflammatory cytokines	Macrophages	Monroy et al., 2007
PGD_2, PGJ_2, and Δ12-PGJ_2	Down-regulate the production of leptin	3T3-L1 adipocytes	Peeraully et al., 2006
15d-PGJ_2	Inhibition of NF-κB signaling and at PI3K/Akt pathway	Primary astrocytes	Giri et al., 2004
PGA_1, PGJ_2, PGD and 15d-PGJ_2	Direct inhibition, and modification of the IKKβ subunit, improve the utility of COX2 inhibitors.	Jurkat cells (immortalized line of human T lymphocyte cells)	Rossi et al., 2000
Anti-tumorigenic			
15d-PGJ_2	Myc/Mad/max transcription factors	Gastric cancer, Oral Squamous cell carcinoma, Leukemia, Lymphoma, Oesophageal cancer, Endometrial cancer, Breast cancer, and Brain tumors	Inoue et al., 2000; Sato et al., 2000; Takashima et al., 2001; Nikitakis et al., 2002; Yuan et al., 2005; Han et al., 2007; Chearwae and Bright, 2008; Cocca et al., 2009; Dionne et al., 2010; Li and Narahara, 2013; Li et al., 2017
15d-PGJ_2	Enhancing TRAIL-induced apoptosis by downregulating AKT expression and phosphorylation	Leukemia	Han et al., 2007
15d-PGJ_2	ROS-dependent AKT activation, cell cycle inhibition	Osteosarcoma	Yen et al., 2014
15d-PGJ_2	A tubulin-binding agent that destabilizes microtubules and induces mitotic arrest	Breast cancer	Cocca et al., 2009
15d-PGJ_2	Cell cycle blockade	Oesophageal cancer	Takashima et al., 2001
15d-PGJ_2 and TZDs	Tumor cell growth, migration, and invasion	Hepatocellular carcinoma (HCC)	Hsu and Chi, 2014
15d-PGJ_2 and its derivatives (J11-C1)	Expression of genes associated with cell cycle arrest, apoptosis, and autophagy, decreased expression of the anti-apoptotic Bcl-2	Ovarian cancer SKOV3 cells	Tae et al., 2018
15d-PGJ_2	Inhibition of STAT-3	Oral Squamous cell carcinoma	Nikitakis et al., 2002
15d-PGJ_2	Apoptosis rate, Apoptosis-promoting protein, and reduced apoptosis-inhibiting protein expression	Hepatitis B virus (HBV) × protein (HBx)-positive HL7702-HBx and HL7702 liver cells	Chen et al., 2014
Anti-angiogenic Anti-metastatic			
15d-PGJ_2 Pioglitazone	Inhibiting VEGF	Renal cell carcinoma (RCC)	Yuan et al., 2005
15d-PGJ_2	Inhibiting angiopoietin-1 (Ang-1)	Gastric cancer	Fu et al., 2006
15d-PGJ_2	Reduced VEGF receptor 1 (Flt-1) and 2 (Flk/KDR), urokinase plasminogen activator (uPA), and increased plasminogen activator inhibitor-1 (PAI-1) mRNA	Human umbilical vein endothelial cells (HUVEC)	Xin et al., 1999; Funovics et al., 2006
15d-PGJ_2 (PPAR-γ dependent), BRL49653, Ciglitizone	Block angiogenesis	Rat cornea	Xin et al., 1999

(Continued)

TABLE 1 | Continued

Anti-inflammatory	Specific function	Site of action	References
15d-PGJ_2 HO-1-dependent mechanism	NF-κB and AP-1 mediated MMP-9 expression and invasion	MCF-7 breast cancer cells	Jang et al., 2020
15d-PGJ_2	Disassembled focal adhesions, downregulation of FAK signaling	Renal cell carcinoma (RCC) metastasis	Yamamoto et al., 2020
Antioxidant			
15d-PGJ_2	Nrf2-Keap1 signaling pathway	Atherosclerosis	Itoh et al., 2004; Levonen et al., 2004; Mochizuki et al., 2005
15d-PGJ_2	HO-1, SOD, catalase, NAD(P)H dehydrogenase quinone 1 (NQO1), c-glutamylcysteine synthetase (GCS), glutathione reductase (GR), glutathione peroxidase 1 (GPx)	Pleurisy, atherosclerosis	Diers et al., 2010; Itoh et al., 2004; Kansanen et al., 2009; Magesh et al., 2012
15d-PGJ_2	15-PGDH gene expression, protein level, and its activity, AP-1 and HO-1	Human colon cancer cell line HCT-116	Park and Na, 2019a,b; Tauber and Parker, 2019
15d-PGJ_2	eIF2α phosphorylation, Activation of Integrated stress response (ISR)	Neurodegenerative diseases	Park and Na, 2019a,b; Tauber and Parker, 2019
Resolving inflammation			
15d-PGJ_2	Cytoprotective, Shifting PG production from PGE_2 to PGD_2 and 15d-PGJ_2	Dextran sodium sulfate-induced colitis in the rat and TNF-α-induced activation of PG production and PG synthase expression in cultured human peripheral blood monocytes (hPBMC)	Niro et al., 2010
15d-PGJ_2	DP1 receptor activation checkpoint controller of cytokine/chemokine synthesis as well as leukocyte influx and efflux	Self-resolving peritonitis	Rajakariar et al., 2007
15d-PGJ_2	PPAR-γ and CD36 expression	Enhance hematoma resolution	Flores et al., 2016
15d-PGJ_2	Inhibition of pro-inflammatory cytokines, such as IL-5, IL-13, IL-17, TNF-α Inhibition of NF-κB phosphorylation	Peribronchial accumulation of eosinophils and neutrophils, subepithelial fibrosis, and also mucus exacerbation	Coutinho et al., 2017
Prostanylation and protein modification			
PGE_1 and PGA_1	Interact with the ligand-binding domain (LBD) of orphan nuclear receptor Nurr1, neuroprotective, enhanced expression of Nurr1 target genes in midbrain dopaminergic (mDA) neurons and improved motor deficits	Mouse models of Parkinson's disease	Rajan et al., 2020
15d-PGJ_2 and PGA_1	IKKα and β, NF-κB P65 and P50 subunits cysteine modification at various positions	Inhibition of NF-κB pathway	Castrillo et al., 2000; Rossi et al., 2000; Cernuda-Morollon et al., 2001
15d-PGJ_2 and PGA_1	H-Ras modification at various cysteines	Activation of H-Ras	Oliva et al., 2003
15d-PGJ_2	c-Jun and c-Fos modification at various cysteines	Inhibition	Perez-Sala et al., 2003
PGA_1	Thioredoxin, thioredoxin reductase, and Keap1	Inhibition	Levonen et al., 2001, 2004; Shibata et al., 2003a; Itoh et al., 2004
15d-PGJ_2	Proteasome	Inhibition	Shibata et al., 2003b

Chearwae and Bright, 2008; Dionne et al., 2010; Li et al., 2017) (**Table 1**). Transforming growth factor-β (TGF-β) induces cell growth, cell migration, and epithelial to mesenchymal transition (EMT) and promotes HCC progression (Giannelli et al., 2014). Interestingly, TZDs and 15d-PGJ_2 display antitumor effects on HCC (Hsu and Chi, 2014). PPAR-γ activation inhibits TGF-β expression via dephosphorylation of zinc finger transcription factor-9 (Zf9) (Lee et al., 2006). Zf9 is crucial for TGFβ1 gene regulation, and a phosphorylated form of Zf9 transactivates the TGFβ1 promoter (Kim et al., 1998).

15d-PGJ_2 and its derivatives exert antitumor activity by selectively modulating the expression of genes associated with cell cycle arrest, apoptosis, and autophagy (Inoue et al., 2000; Sato et al., 2000; Takashima et al., 2001; Yuan et al., 2005; Chearwae and Bright, 2008; Dionne et al., 2010; Li et al., 2017). Notably, J11-C1 is a novel candidate of class III histone deacetylases (HDACs) called Sirtuin SIRT1 inhibitor with anticancer activity. SIRTs are involved in biological functions, including aging, energy mobilization, and stress responses. SIRTs regulate cancer cell apoptosis and are potential targets for

novel anticancer drugs that regulate the levels of deacetylated histone proteins, p53, and several transcriptional factors (**Table 1**) (Tae et al., 2018). 15d-PGJ_2 treatment significantly induced apoptosis rate, apoptosis-promoting protein expression, and reduced apoptosis-inhibiting protein expression in the hepatitis B virus (HBV) × protein (HBx)-positive HL7702-HBx and HL7702 liver cells (Chen et al., 2014).

Anti-angiogenic/Anti-metastatic Actions

15d-PGJ_2 exerts anti-angiogenic activity by inhibiting VEGF and angiopoietin-1 (Ang-1) in renal cancer (Yuan et al., 2005) and gastric cancer (Fu et al., 2006), respectively. Treatment of human umbilical vein endothelial cells (HUVEC) with 15d-PGJ_2 reduced mRNA levels of VEGF receptors 1 (Flt-1) and 2 (Flk/KDR) and urokinase plasminogen activator (uPA) and increased plasminogen activator inhibitor-1 (PAI-1) mRNA (Funovics et al., 2006). Administration of 15d-PGJ_2 could inhibit VEGF-induced angiogenesis in the rat cornea in a PPAR-γ dependent manner (Xin et al., 1999) (**Table 1**). Rosiglitazone (Avandia) and troglitazone (TGZ) inhibit cell migration via the upregulation of E-cadherin expression in HepG2 cells (Lee et al., 2009). 15d-PGJ_2 inhibits NF-κB and AP-1-mediated MMP-9 expression and invasion of MCF-7 breast cancer cells employing a heme oxygenase-1 (HO-1)-dependent mechanism (Jang et al., 2020). Treatment with a low concentration of 15d-PGJ_2 disassembled focal adhesions, reduced focal adhesion kinase (FAK) phosphorylation, and caused extensive filamentous actin reorganization (Yamamoto et al., 2020). PPAR-γ did not mediate the inhibitory effect of 15d-PGJ_2 on the migration of Caki-2 cells and did not affect RCC metastasis (Yamamoto et al., 2020).

Antioxidant and Resolving Actions

Inflammation is accompanied by the production of ROS, and 15d-PGJ_2 has antioxidant properties (Itoh et al., 2004; Levonen et al., 2004; Mochizuki et al., 2005) (**Table 1**). 15d-PGJ_2 and structurally related isoprostanoids alkylate Kelch-like ECH-associated protein 1 (Keap1) to induce the NF-E2-related nuclear factor erythroid-2 (Nrf2-) dependent antioxidant bioactivity (Levonen et al., 2004; Kansanen et al., 2009; Diers et al., 2010; Mills et al., 2018). 15d-PGJ_2 activates Nrf2-Keap1 signaling and induces gene transcription of antioxidant enzymes including HO-1, superoxide dismutase (SOD), catalase, NAD(P)H dehydrogenase quinone 1 (NQO1), c-glutamylcysteine synthetase (GCS), glutathione reductase (GR), and glutathione peroxidase 1 (GPx) (Itoh et al., 2004; Kansanen et al., 2009; Diers et al., 2010; Magesh et al., 2012). 15d-PGJ_2 upregulates 15−hydroxyprostaglandin dehydrogenase (15-PGDH) gene expression, protein level, and its activity in human colon cancer cell line HCT-116 through AP-1 activation (Park and Na, 2019a,b). 15d-PGJ_2 treatment-induces eIF2α phosphorylation and activation of the integrated stress response (ISR), also leading to bulk translation repression and preferential translation of stress response mRNAs (Tauber and Parker, 2019). 15d-PGJ_2 is pro-resolving signaling and a neuroprotective (Rajan et al., 2020) molecule (**Table 1**) (Rajakariar et al., 2007; Niro et al., 2010; Flores et al., 2016; Coutinho et al., 2017).

Pro-metastatic Properties of the Cyclopenenone Prostaglandins

cyPGs also exhibit pro-metastatic properties such as 15d-PGJ_2 significantly enhanced the rate of formation, the size, and the vascularization of papillomas in a murine carcinogenesis model (Millan et al., 2006). 15d-PGJ_2 and PGJ_2 induced the proliferation of COX-2 depleted colorectal cancer (HCA-7) cells at a nanomolar concentration (Chinery et al., 1999). However, the precise mechanisms responsible for tumor proliferative effects of 15d-PGJ_2 remain incompletely clarified. VEGF is well known as a master regulator of angiogenic switch (Bussolati and Mason, 2006). Interestingly, VEGF upregulates HO-1 in vascular endothelial cells, while HO-1 may also regulate the synthesis and activity of VEGF, thus constituting a positive feedback loop (Bussolati and Mason, 2006). 15d-PGJ_2 could stimulate VEGF expression in endothelial cells, human androgen-independent PC3 prostate cancer cells, and the 5,637 urinary bladder carcinoma cell line (Yamakawa et al., 2000; Haslmayer et al., 2002). The upregulation of VEGF by 15d-PGJ_2 was accompanied by activation of PPAR-γ (Jozkowicz et al., 2002). However, the VEGF promoter does not harbor PPRE (Inoue et al., 2001; Jozkowicz et al., 2004). Interestingly, VEGF upregulation by 15d-PGJ_2 could be mimicked by the induction of HO-1 expression (Jozkowicz et al., 2004). 15d-PGJ_2 induced HO-1 expression in MCF-7 human breast cancer cells (Kim et al., 2004).

Nrf2, a transcription factor is responsible for maintenance of cellular redox balance (Loboda et al., 2016). HO-1 is a prototypic Nrf2 target gene, and the aberrant hyperactivation of Nrf2/HO-1 axis contributes to tumor progression, aggressiveness, chemoresistance, and poor prognosis (Zimta et al., 2019). 15d-PGJ_2 induces VEGF expression and angiogenesis in human breast cancer cells through upregulation of HO-1 (Kim et al., 2006; Kweider et al., 2011).

ROLE OF CYCLOPENTENONE PROSTAGLANDINS DURING VIRAL INFECTIONS

Cyclopentenone Prostaglandins as Inhibitor of Viral Replication

cyPGs are potent inhibitors of viral replication (**Table 2**) and are effective against a wide range of viruses. These include negative-strand RNA viruses such as influenza A (Pica et al., 1993, 2000; Conti et al., 2001), Sendai virus (Amici and Santoro, 1991; Amici et al., 2001), and vesicular stomatitis virus (VSV) (Santoro et al., 1987; Pica et al., 1993); positive-strand RNA viruses such as Sindbis virus (Mastromarino et al., 1993), Poliovirus (Conti et al., 1996), and Human immunodeficiency virus-1 (Rozera et al., 1996) and DNA viruses such as herpes simplex virus (HSV) type 1 and 2 (Yamamoto et al., 1987; Amici et al., 2001). The ability of cyPGs to suppress virus production is very dramatic. In the African green monkey kidney (AGMK) cell line, replication of the Sendai virus is almost completely inhibited by 4 mg/ml of PGA_1 (Santoro et al., 1987) and by 4 mg/ml of PGJ_2 (Santoro et al., 1987) without being toxic to uninfected

TABLE 2 | Effects of cyclopentenone prostaglandins in viral infections.

Anti-viral Activity	Virus	CyPGs	Mechanism	References
Inhibition of virus replication by altering viral gene/protein expression (transcription/translation level alteration)	Influenza A	Δ^{12}-PGJ_2	Decrease synthesis of hemagglutinin (HA), nucleoprotein (NP), and membrane protein M1; induction of 70 kDa host HSP70	Pica et al., 1993
		PGA_1	Delayed synthesis of HA, membrane protein M1, structural protein M2, and non-structural protein NS2; induction of 70 kDa host HSP70	Conti et al., 2001
	Vesicular Stomatitis Virus (VSV)	Δ^{12}-PGJ_2, PGA_1	Inhibit VSV RNA polymerase	Bader and Ankel, 1990; Pica et al., 1993; Parker, 1995
	Herpes Simplex Virus Type 1 (HSV-1)	PGA_1	Suppress NF-κB activation by inhibiting IKK complex (independent of the PPAR-γ pathway)	Amici et al., 2001
	Herpes Simplex Virus Type 2 (HSV-2)	Δ7-PGA_1, Δ^{12}-PGJ_2	Inhibited the primary transcription of HSV-2	Yamamoto et al., 1987
	Human Immunodeficiency Virus-1 (HIV-1)	PGJ_2	Suppress NF-κB activation by inhibiting IKK complex (independent of the PPAR-γ pathway)	Rozera et al., 1996; Boisvert et al., 2008
		15d-PGJ_2	Covalently modify HIV-1 transactivating protein, Tat to inhibit virus transcriptional elongation	Kalantari et al., 2009
Inhibition of virus replication by altering viral glycoprotein glycosylation (post-translational level alteration)	Vesicular Stomatitis Virus (VSV)	Δ^{12}-PGJ_2	Inhibit glycosylation of virus glycoprotein G	Pica et al., 1993
	Sendai Virus	PGA_1, Δ^{12}-PGJ_2	Inhibit glycosylation of virus glycoproteins hemagglutinin-neuraminidase (HN) and fusion protein (F)	Santoro et al., 1987; Amici et al., 2001
Inhibition of virus cell-to-cell transmission	Human T-cell Leukemia Virus Type I (HTLV-1)	PGA_1, PGJ_2	Inhibit host cell proliferation by inducing cell arrest at the G1/S interface	D'Onofrio et al., 1992; Lacal et al., 1994a,b
Inhibition of virus-induced inflammation	Influenza	15d-PGJ_2	Decrease virus-induced release of proinflammatory cytokines (IL-6, TNF-α) and chemokines (CCL2, CCL3, CCL4, and CXCL10) via PPAR-γ pathway	Cloutier et al., 2012
	Respiratory Syncytial Virus (RSV)	15d-PGJ_2	Decrease virus-induced release of cytokines (TNF-α, GMCSF, IL-1α, IL-6), and the chemokines (CXCL8 (IL-8) and CCL5) via PPAR-γ pathway. Reduce immune cells adhesion by inhibiting virus-induced up-regulation of intercellular adhesion molecule-1 (ICAM1). Reduce activity of inflammatory pathway, NF-κB.	Arnold et al., 2007
	Human Immunodeficiency Virus-1 (HIV-1)	15d-PGJ_2	Suppress NF-κB activation by inhibiting IKK complex	Boisvert et al., 2008
	Zika virus (ZIKV)	15d-PGJ_2	Control brain inflammation by downregulating microglial activation and by inducing apoptosis of activated microglia	Bernardo and Minghetti, 2006

AGMK cells. Treatment of 6 mg/ml of Δ^{12}-PGJ_2 in Madin–Darby canine kidney cells (MDCK) infected with influenza A H1N1 (PR8) virus drastically suppressed the viral production by 95%. Simultaneously, a higher dose of Δ^{12}-PGJ_2 produced an undetectable virus yield (Pica et al., 1993). PGA_1 treatment also strongly inhibits the viral production of Ulster 73 (H7N1 influenza A) in LLC-monkey kidney epithelial cells (LLC-MK2), African green monkey kidney-37RC cells (AGMK-37RC), and MDCK cells (Conti et al., 2001), suggesting that cyPGs are effective against various subtypes of influenza A virus in multiple host cells. Similarly, *in vivo* studies have shown that PGA_1 and 16, 16-dimethyl-PGA_2 ($dmPGA_2$), a long-acting synthetic analog of PGA, in mice infected with a lethal dose of PR8 virus significantly decreases the virus titers in the lung and increases the survival rate (Santoro et al., 1987; Pica et al., 1993). In another study, the antiviral activity of the synthetic $dmPGA_1$ in HSV-1 and human immunodeficiency virus (HIV)- infected cells was investigated (Hughes-Fulford et al., 1992). $dmPGA_1$ affected HIV-1 replication in acutely infected T cells and chronically infected macrophages as assessed by a quantitative decrease in HIV-1 antigen p24 concentration (Hughes-Fulford et al., 1992). This study highlighted the unusual broad-spectrum antiviral activity of $dmPGA_1$ against HSV and HIV-1 and its therapeutic potential for *in vivo* use (Hughes-Fulford et al., 1992).

Depending on the virus, cyPGs utilize various mechanisms and act on different viral cycle events to interfere with virus production. In HIV-1 infection and avian influenza, A virus infection, cyPGs prevent very early virus infection phases such

as viral adsorption and penetration into target cells (Rozera et al., 1996; Carta et al., 2014). Even though antiviral action mechanisms differ between various viruses and host cell systems, the inhibition of virus replication by cyPGs is often associated with (1) alteration in viral protein synthesis and (2) alteration in viral glycoprotein glycosylation (**Table 2**). PGA_1 treatment inhibited replication of Mayaro virus (MAYV) (an arbovirus endemic to certain humid forests of tropical South America) by 95% at 24 h post-infection in human epithelial type 2 (Hep-2) cells (Caldas et al., 2018). PGA_1 treatment inhibited viral structural protein synthesis by 15%, possibly via heat shock protein70 (HSP70) induction (Caldas et al., 2018).

Cyclopentenone Prostaglandins Alter Viral Protein Synthesis

Inhibition of individual virus replication by cyPGs is marked by dysregulation of viral protein synthesis (**Table 2**). In influenza, A PR8 virus (a mouse-adapted H1N1 influenza virus causing severe infection in mice)-infected cells, treatment of Δ^{12}-PGJ_2 substantially decreased the synthesis of PR8 proteins such as hemagglutinin (HA), nucleoprotein (NP), and membrane protein M1 (Pica et al., 1993). PGA_1 could cause a significant delay in the synthesis of late viral polypeptides: HA, membrane protein M1, structural protein M2, and non-structural protein NS2 (Conti et al., 2001). Furthermore, both studies showed that inhibition or delay of viral protein synthesis is accompanied by induction of a 70 kDa host polypeptide identified as HSP70 by immunoblot analysis (Pica et al., 1993; Conti et al., 2001). Because viral protein synthesis is repressed as long as HSP70 is present in the host cell, HSP70 seems to play an essential role in cyPGs antiviral activity.

In VSV infection, Δ^{12}-PGJ_2 can affect two distinct stages (an early stage and a late-stage) of the virus replication cycle in epithelial monkey cell lines (Pica et al., 1993). The inhibition of the virus at the initial stage is associated with altered viral protein synthesis. When the cells are treated with 8 mg/ml of Δ^{12}-PGJ_2 soon after virus infection, there is a dramatic decrease in VSV protein synthesis. Similar to the effect on influenza A virus replication, inhibition of VSV protein synthesis by Δ^{12}-PGJ_2 is also associated with the induction of a 74 kDa polypeptide belonging to the group of heat shock protein HSP70 (Pica et al., 1993). In another study, PGA_1 treatment decreased VSV proteins' production and the amount of respective viral mRNA (Bader and Ankel, 1990). This study found that PGA_1 exerts its antiviral activity at the VSV genes' primary transcription level, which leads to a reduction in viral mRNA synthesis, viral protein synthesis, and, ultimately, viral replication. To further investigate the antiviral activity of cyPGs, another study performed an RNA polymerase assay and reported that cyPGs potently inhibit VSV RNA polymerase (Parker, 1995). This inhibition correlates with the decrease in VSV replication in infected cells, indicating that cyPGs antiviral activity is due to VSV RNA polymerase inhibition.

In addition to VSV, cyPGs also exert a transcriptional block in the replication of herpes simplex virus type 1 (HSV-1) (Amici et al., 2001), HSV-2 (Yamamoto et al., 1987), and HIV-1 (Rozera et al., 1996). In HSV-1 infected human laryngeal carcinoma cells and neuroblastoma cells and HIV-1 infected colonic epithelial cells (caco-2 cells), cyPGs inhibit viral gene expression by suppressing NF-κB activation, independent of the PPAR-γ pathway (Amici et al., 2001; Boisvert et al., 2008). NF-κB is essential for many processes, including viral gene expression and, consequently, replication of viruses that contain NF-κB binding sites in their genomes. In its inactivated cytosolic form, NF-κB is bound to inhibitory IκB proteins such as IκBα. Stimuli like bacterial and viral infections increase the activity of the IKK complex, which phosphorylates IκBα, leading to ubiquitination and degradation of IκBα by proteasomes. Once NF-κB is free from IκBα, it translocates into the cell nucleus, activating the transcription of many genes, including the viral genes of HSV-1 and HIV-1 (Amici et al., 2001; Boisvert et al., 2008). Amici et al. (2001) showed that PGA_1 significantly decreases the NF-κB induction in HSV-1 infected cells by inhibiting the IKK complex.

Similarly, another study reported that the administration of PGJ_2 reduces IKK activity in HIV-1 infected cells (Boisvert et al., 2008). In both cases, suppression of IKK activity by cyPGs prevents IκBα degradation and NF-κB translocation to the nucleus. As a result, viral gene transcription and protein synthesis were repressed, leading to a significant reduction in virus production. In addition to interfering with NF-κB induction, cyPGs also target another pathway independent of NF-κB to inhibit HIV-1 replication. Kalantari et al. (2009) reported that 15d-PGJ_2 represses HIV-1 transcription by inhibiting HIV-1 transactivating protein, Tat. While the host transcriptional factor NF-κB binds to the 5′ long terminal repeat (LTR) of HIV-1 to initiate transcription, viral Tat protein is recruited to an RNA stem-loop structure called transactivation response element (TAR) and is necessary for transcriptional elongation. Tat then recruits transcription elongation factor p-TEFb, which transactivates HIV LTR and allows the RNA polymerase II to continue the transcription with high processivity. 15d-PGJ_2 interferes with Tat-dependent transcriptional elongation by covalently modifying the thiol groups of Tat's cysteine residues (Kalantari et al., 2009). The resulting altered Tat protein is unable to transactivate HIV LTR in U937 human macrophages, inhibiting the transcription and replication of the virus.

Cyclopentenone Prostaglandins Alter Viral Glycoprotein Glycosylation

cyPGs can also inhibit viral replication at the post-translational level by altering the glycosylation of viral glycoproteins. This is seen in the VSV and Sendai virus (**Table 2**). As mentioned earlier, Δ^{12}-PGJ_2 inhibits the VSV replication in the epithelial monkey cell line at two stages of the virus replication cycle. The inhibition at the early stage is due to a block in viral protein synthesis. Administration of Δ^{12}-PGJ_2 at a later stage (6–8 h post-infection) also leads to a decrease in virus production even though viral protein synthesis should have been completed by that time (Pica et al., 1993). Δ^{12}-PGJ_2 treatment started at a later stage does not affect viral protein synthesis, but it drastically decreases the glucosamine incorporation into the virus glycoprotein G without altering most cellular proteins.

Similarly, PGA_1 treatment in AGMK cells infected with the Sendai virus results in inhibition of glycosylation of viral glycoproteins hemagglutinin-neuraminidase (HN) and fusion protein (F), as indicated by the decrease in glucosamine incorporation (Santoro et al., 1987). The synthesis of non-glycosylated viral polypeptides of RNA transcriptase complex, including proteins P, NP, and matrix protein (M), are not affected by PGA_1 treatment. Likewise, Δ^{12}-PGJ_2 also markedly reduces the incorporation of glucosamine into HN and F viral glycoproteins without inhibiting the synthesis of cellular or viral proteins (Amici et al., 2001). The altered HN glycoprotein cannot insert into the cell membrane, which leads to an inhibition of virus maturation and production.

The Effect of Cyclopentenone Prostaglandins on Viral Transmission

cyPGs can interfere with virus transmission via their antiproliferative activity. When PGA_1 and PGJ_2 are given to human T-cell leukemia virus type-I (HTLV-1) producing MT-2 cell line, they inhibit the growth of the cells in a dose-dependent manner (D'Onofrio et al., 1992). These cyPGs cause the cells to be arrested at the G1/S interface without detectable cellular toxicity. Another study showed that PGA_1 and PGJ_2 inhibit the proliferation of myeloid cells (K562 pluripotent stem cells, HL60 promyelocytic cells, and U937 monoblastoid cells) during early infection of HTLV-1, also in a dose-dependent manner (Lacal et al., 1994a,b). Furthermore, out of the three myeloid cell lines used in the study, the effect of growth inhibition is highest in U937 monoblastoid cells, followed by HL60 promyelocytic cells, and then K562 pluripotent stem cells. This suggests that cyPGs have a more significant antiproliferative effect on differentiated cells.

The primary mode of infection of HTLV-1 is cell-to-cell transmission (Yoshida and Seiki, 1987). Furthermore, for retrovirus-like HTLV-1, integration of proviral DNA occurs after the initiation of cellular DNA synthesis in dividing cells (Varmus et al., 1979). Thus, alterations in cell proliferation and cell cycle can affect the permissiveness of recipient cells to HTLV-1. Indeed, in U937 monoblastoid cells co-cultured with virus-donor cells, PGA_1 and PGJ_2 treatments reduce the transmission of HTLV-1 (Lacal et al., 1994a,b). However, in less differentiated K562 pluripotent stem cells and HL60 promyelocytic cells, infection of recipient cells increased after cyPGs treatment antiproliferative activity is observed in these cells. This suggests that the effect of cyPGs on virus transmission is affected by cell differentiation.

The Effect of Cyclopentenone Prostaglandins on Viral Infection Induced Inflammation

Viral infections such as influenza virus, HIV-1, and respiratory syncytial virus (RSV) are characterized by excessive inflammation with the upregulation of proinflammatory cytokines and chemokines. The amount of these proinflammatory molecules correlates with the severity of illness (Griffin et al., 1994; Wesselingh et al., 1994; Hornsleth et al., 2001; Welliver et al., 2002). Given the anti-inflammatory effects of cyPGs, studies have been done to explore the possibility of utilizing cyPGs as a therapeutic agent for viral infections. In mice infected with lethal influenza infection, administration of 15d-PGJ_2 1 day after infection resulted in reduced influenza morbidity and mortality, accompanied by substantially decreased gene expression of proinflammatory cytokines (IL-6 and TNF-α) and chemokines (CCL2, CCL3, CCL4, and CXCL10) via activation of PPAR-γ pathway (Cloutier et al., 2012). Similarly, 15d-PGJ_2 and other PPAR-γ agonists (ciglitazone and TGZ) can inhibit the RSV-induced release of cytokines TNF-α, GMCSF, IL-1α, IL-6, and the chemokines CXCL8 (IL-8) and CCL5 (Arnold et al., 2007). Moreover, RSV infection of the human airway epithelial cells causes an increase in expression of intercellular adhesion molecule-1 (ICAM1) on the cell surface, which enhances the adhesion of recruited immune effector cells, contributing to an intense inflammatory response and increased cytotoxicity (Wang et al., 2000; Arnold et al., 2007). Treatment of 15d-PGJ_2 and other PPAR-γ agonists results in inhibition of the up-regulation of ICAM1, with the reduced cellular amount of ICAM1 mRNA (Arnold et al., 2007). This leads to a significant reduction in the adhesion of immune cells to RSV-infected cells. Also, the 15d-PGJ_2 treatment in RSV-infected cells is associated with reduced activity of NF-κB, a transcription factor essential for inflammatory responses. In HIV-infected intestinal epithelial cells, 15d-PGJ_2 also reduces the nuclear translocation of NF-κB and represses HIV-1 transcription by decreasing the activity of IKK (Boisvert et al., 2008). Overall, cyPGs can reduce the exaggerated inflammatory response associated with viral infections and great therapeutic value. PGD_2/DP1 axis and 15d-PGJ_2 signaling contributes to the regulation of the CNS-specific response to pathogens such as neurotropic coronavirus (CoV) (Vijay et al., 2017) and acute encephalitis (Rosenberger et al., 2004), chronic demyelinating encephalomyelitis causing neurotropic virus called "MHV" (mouse hepatitis virus strain JHM) (Zheng et al., 2020).

Zika virus (ZIKV), one of the most medically relevant viral infections, affects the developing brain during pregnancy, and its connection with congenital malformations/microcephaly is well documented (de Oliveira et al., 2019). Neuroinflammation is one of the critical factors contributing to ZIKV-related microcephaly, inflammatory processes mediated by glial cells (Wen et al., 2017; Huan et al., 2018). PGD_2, PGE_1, PGE_2, and PGI_2 have been correlated with neuroinflammation, protecting the CNS, and physiological responses to minimize further damage to neural tissue. Their anti-inflammatory reaction has been demonstrated in neuronal injuries (Shi et al., 2010) and neuroprotection during acute brain injury (Liang et al., 2005; An et al., 2014) 15d-PGJ_2 activates PPAR-γ by downregulating microglial activation despite the proinflammatory environment because of the neural damage (Bernardo and Minghetti, 2006).

15d-PGJ_2 has demonstrated beneficial effects in the severe diseases arising from bacterial infections of *Staphylococcus aureus* (Phulwani et al., 2006), *Salmonella enterica* Typhimurium (Buckner et al., 2013), leading to brain abscess, typhoid fever, gastroenteritis, and protozoan hemoflagellate *Trypanosoma*

brucei infection-causing sleeping sickness in humans (Figarella et al., 2006).

OTHER ALPHA, BETA-UNSATURATED CARBONYL LIPIDS AND CYCLOPENTENONE ISOPROSTANES

There is another category of highly reactive electrophilic molecules, which react and modify both proteins and DNA resulting in toxicity, protein dysfunction (Sayre et al., 2006) or tissue damage and disease progression (Lee and Park, 2013). These are α, β-unsaturated aldehydes such as acrolein (ACR), 4-hydroxy-2-non-enal (4-HNE), and crotonaldehyde (CRA) are the most reactive and toxic α, β-unsaturated aldehydes (Lee and Park, 2013).These induce toxicity because of depletion of cellular GSH and inactivation of antioxidant enzymes (GPx and thioredoxin; TRx) subsequently leading to ROS production, reactive nitrogen species (RNS), and free radicals (Stocker and Keaney, 2004; Lee and Park, 2013). Lipid peroxidation (LPO)-derived α, β-unsaturated aldehydes play an important pathophysiological role in vascular diseases by inducing the production of various atherogenic factors, inflammatory mediators, activation of NF-κB signaling pathway, redox signaling mediators leading to cellular and tissue injury (Lee and Park, 2013).

Isoprostanes (IsoPs) are PG-like compounds that are produced *in vivo* independently of COX enzymes, primarily by ROS-mediated or free radical-induced peroxidation of arachidonic acid (Stamatakis and Perez-Sala, 2006). IsoPs along with cyPGs are reactive electrophilic eicosanoids that can form covalent adducts with thiol-containing molecules, cysteine residues in proteins through Michael addition (Stamatakis and Perez-Sala, 2006). Oxidation of DHA in the central nervous system, results in the formation of IsoP-like compounds, termed neuroprostanes and are uniquely valuable to understanding the clinical pharmacology of antioxidants (Montuschi et al., 2007). Cyclopentenone IsoPs are formed abundantly in brain tissue under conditions of oxidative stress (glutathione depletion, ROS generation, activation of redox-sensitive signaling pathways) and may contribute to neuronal death causing neurodegeneration and should be addressed when designing neuroprotective therapies (Musiek et al., 2006, 2007; Porta et al., 2013). IsoPs are measured in the plasma, urine, or cerebral spinal fluid (CSF) and their increase has been observed in obese adults (Morrow, 2005; Basu, 2008), ischemia-reperfusion (Sakamoto et al., 2002; Rossi et al., 2004), Alzheimer's disease (AD) (Montine et al., 1998, 1999a; Pratico et al., 1998, 2000), Huntington's disease (Montine et al., 1999b), Parkinson's disease (Fessel et al., 2003; Seet et al., 2010), and amyotrophic lateral sclerosis (ALS) (D'Amico et al., 2013). Few studies have investigated the associations between levels of F2-IsoPs and risk of breast cancer (Rossner et al., 2006), hepatocellular carcinoma (Wu et al., 2008), prostate cancer (Barocas et al., 2011; Brys et al., 2013) gastric cancer (Asombang et al., 2013). IsoPs are increased in patients with genetic disorders such as autism-spectrum disorders (Ming et al., 2005; Gorrindo et al., 2013), Smith–Lemli–Opitz Syndrome (SLOS) (Korade et al., 2013), sickle cell anemia (Akohoue et al., 2007), cystic fibrosis (Collins et al., 1999; Ciabattoni et al., 2000; Montuschi et al., 2000), Rett syndrome (RTT) (De Felice et al., 2009, 2011; Signorini et al., 2011; Durand et al., 2013), and in various inborn errors of metabolism (Mc Guire et al., 2009).

SUMMARY AND FUTURE DIRECTIONS

There is significant evidence that cyPGs (PGA_1, PGA_2, and PGJ_2), and metabolites of PGJ_2 (15d-PGJ_2 and Δ^{12}- PGJ_2) can induce anti-inflammatory and antiviral effects through covalent modification reactions with their α, β-unsaturated carbonyl group. cyPGs can exert anti-inflammatory and antiviral effects in various ways depending on the host cell and pathogen type. Cell type is not the only influencer on the anti-inflammatory effects of cyPGs. The concentration of cyPGs and the length/time of exposure to cyPGs have varying anti-inflammatory and antiviral effects. Based on these factors, cyPGs can show biphasic targeting of inflammation (Garzon et al., 2011). At high doses, 15d-PGJ_2 has a dual action of stimulating anti-inflammation and anti-proliferation. Still, it can be toxic and induce both inflammation and cell proliferation at lower doses, and the biphasic pharmacodynamics has to be controlled carefully (Abbasi et al., 2016). Dose-related efficacy and safety of oral DP_2 receptor antagonists fevipiprant (QAW039), timapriprant (OC000459), and BI 671800 have been tested in patients with allergic asthma and COPD, and PGD_2 has shown anticancer effects in NSCLC (non-small cell lung carcinoma), kidney and lung fibrosis, and gastric cancer (Bateman Guerreros et al., 2017; Jandl and Heinemann, 2017; Pearson et al., 2017; Sandham et al., 2017a,b; Murillo et al., 2018; Brightling et al., 2020). Further research on outcomes based on specific concentrations is warranted. PPAR-γ antagonist (GW9662) and PPAR-γ ligands are new therapeutic targets in sepsis, hemorrhagic shock, and inflammation (Kaplan et al., 2005, 2010; Zingarelli and Cook, 2005; Chima et al., 2011). Synthetic PPAR-γ ligands rosiglitazone (Avandia) and pioglitazone have exhibited anti-inflammatory and antiviral effects in an EcoHIV mouse model that could decrease neurodegeneration. These drugs prove promising in treating HIV-1 associated neurocognitive disorders (Omeragic et al., 2020). This knowledge could significantly impact how viruses and inflammation can be treated.

The outcome of the 15d-PGJ_2 treatment depends upon its exogenously administered dose as it stimulates anti-inflammation and anti-proliferation at high doses while can have toxic effects at a lower dose (Abbasi et al., 2016). Many strategies have been developed to deal with the biphasic pharmacodynamics of 15d-PGJ_2 and one of them is using a nanoemulsion (NE) composed of triolein/distearoyl phosphatidylcholine/Tween 80 at a high encapsulation ratio (>83%) allowing slow-release kinetics (Abbasi et al., 2016). NE retained a high proportion of 15d-PGJ_2 and directly delivered it to the cytosol, where proapoptotic targets are located, and could bypass cell membrane-associated targets involved in cell proliferation (Abbasi et al., 2016). NE could deliver 15d-PGJ_2 to its desired site of action, excluding undesired sites, on a subcellular level (Abbasi et al., 2016) and could be used as

one of the strategies for treatment. Since the use of solid lipid nanoparticles (SLN) can improve therapeutic properties by increasing drug efficiency and availability, 15d-PGJ_2-SLN was developed and tested for its immunomodulatory potential. The 15d-PGJ_2-SLN formulation showed good colloidal parameters, encapsulation efficiency (96%), and stability (up to 120 days) with low hemolytic effects as compared to unloaded SLN in *in vivo* experiments. The 15d-PGJ_2-SLN formulation using low concentrations reduced neutrophil migration in three inflammation models tested. 15d-PGJ_2-SLN increased IL-10 levels and reduced IL-1β as well as IL-17 in peritoneal fluid thus highlighting the perspectives of a potent anti-inflammatory system (de Melo et al., 2016). cyPGs have a wide spectrum of intracellular targets ranging from nuclear factors to mitochondria. Introduction of cyclopentenone moiety into molecules (jasmonates and chalcones) boosts their anticancer potential (Conti, 2006). Despite advancements made in the pharmacodynamics of cyPGs, a significant effort is needed to explore their unique therapeutic properties and tailor them to be used as leading anti-inflammatory, anticancer, and antiviral drugs.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

We are grateful for funding support from the Center for Cancer Cell Biology, Immunology and Infection and NIH-funded grant R01CA 192970 to NS-W. The funders had no role in the design, decision to publish, or preparation of the manuscript.

ACKNOWLEDGMENTS

All authors contributed equally to the article and approved the submitted version. NS-W apologizes to all the colleagues whose work could not be cited in this manuscript.

REFERENCES

Abbasi, S., Kajimoto, K., and Harashima, H. (2016). Elimination of the biphasic pharmacodynamics of 15d-PGJ2 by controlling its release from a nanoemulsion. *Int. J. Nanomed.* 11, 2685–2694. doi: 10.2147/ijn.s106297

Akohoue, S. A., Shankar, S., Milne, G. L., Morrow, J., Chen, K. Y., Ajayi, W. U., et al. (2007). Energy expenditure, inflammation, and oxidative stress in steady-state adolescents with sickle cell anemia. *Pediatr. Res.* 61, 233–238. doi: 10.1203/pdr.0b013e31802d7754

Amici, C., and Santoro, M. G. (1991). Suppression of virus replication by prostaglandin A is associated with heat shock protein synthesis. *J. Gen. Virol.* 72(Pt 8), 1877–1885. doi: 10.1099/0022-1317-72-8-1877

Amici, C., Belardo, G., Rossi, A., and Santoro, M. G. (2001). Activation of I kappa b kinase by herpes simplex virus type 1. A novel target for anti-herpetic therapy. *J. Biol. Chem.* 276, 28759–28766.

An, Y., Belevych, N., Wang, Y., Zhang, H., Nasse, J. S., Herschman, H., et al. (2014). Prostacyclin mediates endothelial COX-2-dependent neuroprotective effects during excitotoxic brain injury. *J. Inflamm. Res.* 7, 57–67. doi: 10.2147/jir.s63205

Aoki, T., and Narumiya, S. (2012). Prostaglandins and chronic inflammation. *Trends Pharmacol. Sci.* 33, 304–311.

Appel, S., Mirakaj, V., Bringmann, A., Weck, M. M., Grunebach, F., and Brossart, P. P. P. A. R. - (2005). gamma agonists inhibit toll-like receptor-mediated activation of dendritic cells via the MAP kinase and NF-kappaB pathways. *Blood* 106, 3888–3894. doi: 10.1182/blood-2004-12-4709

Arnold, R., Neumann, M., and Konig, W. (2007). Peroxisome proliferator-activated receptor-gamma agonists inhibit respiratory syncytial virus-induced expression of intercellular adhesion molecule-1 in human lung epithelial cells. *Immunology* 121, 71–81. doi: 10.1111/j.1365-2567.2006.02539.x

Asombang, A. W., Kayamba, V., Mwanza-Lisulo, M., Colditz, G., Mudenda, V., Yarasheski, K., et al. (2013). Gastric cancer in Zambian adults: a prospective case-control study that assessed dietary intake and antioxidant status by using urinary isoprostane excretion. *Am. J. Clin. Nutr.* 97, 1029–1035. doi: 10.3945/ajcn.112.051284

Bader, T., and Ankel, H. (1990). Inhibition of primary transcription of vesicular stomatitis virus by prostaglandin A1. *J. Gen. Virol.* 71(Pt 12), 2823–2832. doi: 10.1099/0022-1317-71-12-2823

Barocas, D. A., Motley, S., Cookson, M. S., Chang, S. S., Penson, D. F., Dai, Q., et al. (2011). Oxidative stress measured by urine F2-isoprostane level is associated with prostate cancer. *J. Urol.* 185, 2102–2107. doi: 10.1016/j.juro.2011.02.020

Basu, S. (2008). F2-isoprostanes in human health and diseases: from molecular mechanisms to clinical implications. *Antioxid Redox Signal.* 10, 1405–1434. doi: 10.1089/ars.2007.1956

Bateman Guerreros, A. G., Brockhaus, F., Holzhauer, B., Pethe, A., Kay, R. A.. (2017). Fevipiprant, an oral prostaglandin DP2 receptor (CRTh2) antagonist, in allergic asthma uncontrolled on low-dose inhaled corticosteroids. *Eur. Respir. J.* 50:2.

Bell-Parikh, L. C., Ide, T., Lawson, J. A., McNamara, P., Reilly, M., and FitzGerald, G. A. (2003). Biosynthesis of 15-deoxy-delta12,14-PGJ2 and the ligation of PPARgamma. *J. Clin. Invest.* 112, 945–955. doi: 10.1172/jci200318012

Bernardo, A., and Minghetti, L. P. P. A. R. - (2006). gamma agonists as regulators of microglial activation and brain inflammation. *Curr. Pharm. Des.* 12, 93–109. doi: 10.2174/138161206780574579

Boisvert, M., Cote, S., Vargas, A., Pasvanis, S., Bounou, S., Barbeau, B., et al. (2008). PGJ2 antagonizes NF-kappaB-induced HIV-1 LTR activation in colonic epithelial cells. *Virology* 380, 1–11. doi: 10.1016/j.virol.2008.07.023

Brightling, C. E., Brusselle, G., and Altman, P. (2020). The impact of the prostaglandin D2 receptor 2 and its downstream effects on the pathophysiology of asthma. *Allergy* 75, 761–768. doi: 10.1111/all.14001

Brys, M., Morel, A., Forma, E., Krzeslak, A., Wilkosz, J., Rozanski, W., et al. (2013). Relationship of urinary isoprostanes to prostate cancer occurrence. *Mol. Cell Biochem.* 372, 149–153. doi: 10.1007/s11010-012-1455-z

Buckner, M. M., Antunes, L. C., Gill, N., Russell, S. L., Shames, S. R., and Finlay, B. B. (2013). 15-Deoxy-Delta12,14-prostaglandin J2 inhibits macrophage colonization by *Salmonella enterica* serovar Typhimurium. *PLoS One* 8:e69759. doi: 10.1371/journal.pone.0069759

Burstein, S. H. (2020). The chemistry, biology and pharmacology of the cyclopentenone prostaglandins. *Prostaglandins. Lipid. Mediat.* 148:106408. doi: 10.1016/j.prostaglandins.2020.106408

Bussolati, B., and Mason, J. C. (2006). Dual role of VEGF-induced heme-oxygenase-1 in angiogenesis. *Antioxid. Redox Signal.* 8, 1153–1163. doi: 10.1089/ars.2006.8.1153

Caldas, L. A., Ferreira, D. F., and Freitas, T. R. P. (2018). Prostaglandin A1 triggers Mayaro virus inhibition and heat shock protein 70 expression in an epithelial cell model. *Rev. Soc. Bras. Med. Trop.* 51, 584–590. doi: 10.1590/0037-8682-0235-2018

Carta, S., La Frazia, S., Donatelli, I., Puzelli, S., Rossi, A., and Santoro, M. G. (2014). Prostaglandin A1 inhibits avian influenza virus replication at a postentry level: Effect on virus protein synthesis and NF-kappaB activity. *Prostaglandins Leukot. Essent Fatty Acids.* 91, 311–323. doi: 10.1016/j.plefa.2014.07.009

Castrillo, A., Diaz-Guerra, M. J., Hortelano, S., Martin-Sanz, P., and Bosca, L. (2000). Inhibition of IkappaB kinase and IkappaB phosphorylation by 15-deoxy-Delta(12,14)-prostaglandin J(2) in activated murine macrophages. *Mol. Cell Biol.* 20, 1692–1698. doi: 10.1128/mcb.20.5.1692-1698.2000

Cernuda-Morollon, E., Pineda-Molina, E., Canada, F. J., and Perez-Sala, D. (2001). 15-Deoxy-Delta 12,14-prostaglandin J2 inhibition of NF-kappaB-DNA binding through covalent modification of the p50 subunit. *J. Biol. Chem.* 276, 35530–35536. doi: 10.1074/jbc.m104518200

Chearwae, W., and Bright, J. J. (2008). PPARgamma agonists inhibit growth and expansion of CD133+ brain tumour stem cells. *Br. J. Cancer* 99, 2044–2053. doi: 10.1038/sj.bjc.6604786

Chen, S., Liu, C., Wang, X., Li, X., Chen, Y., and Tang, N. (2014). 15-Deoxy-Delta(12,14)-prostaglandin J2 (15d-PGJ2) promotes apoptosis of HBx-positive liver cells. *Chem. Biol. Interact.* 214, 26–32. doi: 10.1016/j.cbi.2014.02.009

Chima, R. S., LaMontagne, T., Piraino, G., Hake, P. W., Denenberg, A., and Zingarelli, B. (2011). C-peptide, a novel inhibitor of lung inflammation following hemorrhagic shock. *Am. J. Physiol. Lung Cell. Mole. Physiol.* 300, L730–L739.

Chinery, R., Coffey, R. J., Graves-Deal, R., Kirkland, S. C., Sanchez, S. C., Zackert, W. E., et al. (1999). Prostaglandin J2 and 15-deoxy-delta12,14-prostaglandin J2 induce proliferation of cyclooxygenase-depleted colorectal cancer cells. *Cancer Res.* 59, 2739–2746.

Ciabattoni, G., Davi, G., Collura, M., Iapichino, L., Pardo, F., Ganci, A., et al. (2000). In vivo lipid peroxidation and platelet activation in cystic fibrosis. *Am. J. Respir. Crit. Care Med.* 162(4 Pt 1), 1195–1201.

Cloutier, A., Marois, I., Cloutier, D., Verreault, C., Cantin, A. M., and Richter, M. V. (2012). The prostanoid 15-deoxy-Delta12,14-prostaglandin-j2 reduces lung inflammation and protects mice against lethal influenza infection. *J. Infect. Dis.* 205, 621–630. doi: 10.1093/infdis/jir804

Cocca, C., Dorado, J., Calvo, E., Lopez, J. A., Santos, A., and Perez-Castillo, A. (2009). 15-Deoxi-Delta(12,14)-prostaglandin J2 is a tubulin-binding agent that destabilizes microtubules and induces mitotic arrest. *Biochem. Pharmacol.* 78, 1330–1339. doi: 10.1016/j.bcp.2009.06.100

Collins, C. E., Quaggiotto, P., Wood, L., O'Loughlin, E. V., Henry, R. L., and Garg, M. L. (1999). Elevated plasma levels of F2 alpha isoprostane in cystic fibrosis. *Lipids* 34, 551–556. doi: 10.1007/s11745-999-0397-1

Conti, C., Mastromarino, P., Tomao, P., De Marco, A., Pica, F., and Santoro, M. G. (1996). Inhibition of poliovirus replication by prostaglandins A and J in human cells. *Antimicrob. Agents Chemother.* 40, 367–372. doi: 10.1128/aac.40.2.367

Conti, G., Portincasa, P., Visalli, S., and Chezzi, C. (2001). Inhibition by prostaglandin PGA_1 on the multiplication of influenza virus is a dose-dependent effect. *Virus Res.* 75, 43–57. doi: 10.1016/s0168-1702(01)00221-0

Conti, M. (2006). Cyclopentenone: a special moiety for anticancer drug design. *Anticancer Drugs* 17, 1017–1022. doi: 10.1097/01.cad.0000231471.54288.00

Coutinho, D. S., Anjos-Valotta, E. A., do Nascimento, C., Pires, A. L. A., Napimoga, M. H., Carvalho, V. F., et al. (2017). 15-Deoxy-Delta-12,14-Prostaglandin J2 Inhibits Lung Inflammation and Remodeling in Distinct Murine Models of Asthma. *Front. Immunol.* 8:740.

D'Amico, E., Factor-Litvak, P., Santella, R. M., and Mitsumoto, H. (2013). Clinical perspective on oxidative stress in sporadic amyotrophic lateral sclerosis. *Free Radic. Biol. Med.* 65, 509–527. doi: 10.1016/j.freeradbiomed.2013.06.029

De Felice, C., Ciccoli, L., Leoncini, S., Signorini, C., Rossi, M., Vannuccini, L., et al. (2009). Systemic oxidative stress in classic Rett syndrome. *Free Radic. Biol. Med.* 47, 440–448. doi: 10.1016/j.freeradbiomed.2009.05.016

De Felice, C., Signorini, C., Durand, T., Oger, C., Guy, A., Bultel-Ponce, V., et al. (2011). F2-dihomo-isoprostanes as potential early biomarkers of lipid oxidative damage in Rett syndrome. *J. Lipid. Res.* 52, 2287–2297. doi: 10.1194/jlr.p017798

de Melo, N. F., de Macedo, C. G., Bonfante, R., Abdalla, H. B., da Silva, C. M., Pasquoto, T., et al. (2016). 15d-PGJ2-Loaded Solid Lipid Nanoparticles: Physicochemical Characterization and Evaluation of Pharmacological Effects on Inflammation. *PLoS One* 11:e0161796. doi: 10.1371/journal.pone.0161796

de Oliveira, D. N., Lima, E. O., Melo, C., Delafiori, J., Guerreiro, T. M., Rodrigues, R. G. M., et al. (2019). Inflammation markers in the saliva of infants born from Zika-infected mothers: exploring potential mechanisms of microcephaly during fetal development. *Sci. Rep.* 9:13606.

Diers, A. R., Higdon, A. N., Ricart, K. C., Johnson, M. S., Agarwal, A., Kalyanaraman, B., et al. (2010). Mitochondrial targeting of the electrophilic lipid 15-deoxy-Delta12,14-prostaglandin J2 increases apoptotic efficacy via redox cell signalling mechanisms. *Biochem. J.* 426, 31–41. doi: 10.1042/bj20091293

Dionne, S., Levy, E., Levesque, D., and Seidman, E. G. (2010). PPARgamma ligand 15-deoxy-delta 12,14-prostaglandin J2 sensitizes human colon carcinoma cells to TWEAK-induced apoptosis. *Anticancer Res.* 30, 157–166.

D'Onofrio, C., Amici, C., Puglianiello, A., Faraoni, I., Lanzilli, G., Santoro, M. G., et al. (1992). Comparative anti-viral and anti-proliferative activity of PGA_1 and PGJ2 against HTLV-I-infected MT-2 cells. *Int. J. Cancer.* 51, 481–488. doi: 10.1002/ijc.2910510324

Durand, T., De Felice, C., Signorini, C., Oger, C., Bultel-Ponce, V., Guy, A., et al. (2013). F(2)-Dihomo-isoprostanes and brain white matter damage in stage 1 Rett syndrome. *Biochimie* 95, 86–90. doi: 10.1016/j.biochi.2012.09.017

Fessel, J. P., Hulette, C., Powell, S., Roberts, L. J. II, and Zhang, J. (2003). Isofurans, but not F2-isoprostanes, are increased in the substantia nigra of patients with Parkinson's disease and with dementia with Lewy body disease. *J. Neurochem.* 85, 645–650. doi: 10.1046/j.1471-4159.2003.01709.x

Figarella, K., Uzcategui, N. L., Beck, A., Schoenfeld, C., Kubata, B. K., Lang, F., et al. (2006). Prostaglandin-induced programmed cell death in Trypanosoma brucei involves oxidative stress. *Cell Death Differ.* 13, 1802–1814. doi: 10.1038/sj.cdd.4401862

Figueiredo-Pereira, M. E., Rockwell, P., Schmidt-Glenewinkel, T., and Serrano, P. (2014). Neuroinflammation and J2 prostaglandins: linking impairment of the ubiquitin-proteasome pathway and mitochondria to neurodegeneration. *Front. Mol. Neurosci.* 7:104.

Flores, J. J., Klebe, D., Rolland, W. B., Lekic, T., Krafft, P. R., and Zhang, J. H. (2016). PPARgamma-induced upregulation of CD36 enhances hematoma resolution and attenuates long-term neurological deficits after germinal matrix hemorrhage in neonatal rats. *Neurobiol. Dis.* 87, 124–133. doi: 10.1016/j.nbd.2015.12.015

Forman, B. M., Chen, J., and Evans, R. M. (1996). The peroxisome proliferator-activated receptors: ligands and activators. *Ann. N. Y. Acad. Sci.* 804, 266–275. doi: 10.1111/j.1749-6632.1996.tb18621.x

Forman, B. M., Tontonoz, P., Chen, J., Brun, R. P., Spiegelman, B. M., and Evans, R. M. (1995). 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. *Cell* 83, 803–812. doi: 10.1016/0092-8674(95)90193-0

Fu, Y. G., Sung, J. J., Wu, K. C., Bai, A. H., Chan, M. C., Yu, J., et al. (2006). Inhibition of gastric cancer cells associated angiogenesis by 15d-prostaglandin J2 through the downregulation of angiopoietin-1. *Cancer Lett.* 243, 246–254. doi: 10.1016/j.canlet.2005.11.039

Funovics, P., Brostjan, C., Nigisch, A., Fila, A., Grochot, A., Mleczko, K., et al. (2006). Effects of 15d-PGJ(2) on VEGF-induced angiogenic activities and expression of VEGF receptors in endothelial cells. *Prostaglandins Lipid. Mediat.* 79, 230–244. doi: 10.1016/j.prostaglandins.2006.02.002

Garzon, B., Oeste, C. L., Diez-Dacal, B., and Perez-Sala, D. (2011). Proteomic studies on protein modification by cyclopentenone prostaglandins: expanding our view on electrophile actions. *J. Proteomics.* 74, 2243–2263. doi: 10.1016/j.jprot.2011.03.028

Giannelli, G., Villa, E., and Lahn, M. (2014). Transforming growth factor-beta as a therapeutic target in hepatocellular carcinoma. *Cancer Res.* 74, 1890–1894. doi: 10.1158/0008-5472.can-14-0243

Giri, S., Rattan, R., Singh, A. K., and Singh, I. (2004). The 15-deoxy-delta12,14-prostaglandin J2 inhibits the inflammatory response in primary rat astrocytes via down-regulating multiple steps in phosphatidylinositol 3-kinase-Akt-NF-kappaB-p300 pathway independent of peroxisome proliferator-activated receptor gamma. *J. Immunol.* 173, 5196–5208. doi: 10.4049/jimmunol.173.8.5196

Gorrindo, P., Lane, C. J., Lee, E. B., McLaughlin, B., and Levitt, P. (2013). Enrichment of elevated plasma F2t-isoprostane levels in individuals with autism who are stratified by presence of gastrointestinal dysfunction. *PLoS One* 8:e68444. doi: 10.1371/journal.pone.0068444

Griffin, D. E., Wesselingh, S. L., and McArthur, J. C. (1994). Elevated central nervous system prostaglandins in human immunodeficiency virus-associated dementia. *Ann. Neurol.* 35, 592–597. doi: 10.1002/ana.410350513

Guo, H., Callaway, J. B., and Ting, J. P. (2015). Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nat. Med.* 21, 677–687. doi: 10.1038/nm.3893

Hamberg, M., and Samuelsson, B. (1966). Prostaglandins in human seminal plasma. Prostaglandins and related factors 46. *J. Biol. Chem.* 241, 257–263.

Han, H., Shin, S. W., Seo, C. Y., Kwon, H. C., Han, J. Y., Kim, I. H., et al. (2007). 15-Deoxy-delta 12,14-prostaglandin J2 (15d-PGJ 2) sensitizes human leukemic HL-60 cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis through Akt downregulation. *Apoptosis* 12, 2101–2114. doi: 10.1007/s10495-007-0124-2

Hanna, V. S., and Hafez, E. A. A. (2018). Synopsis of arachidonic acid metabolism: A review. *J. Adv. Res.* 11, 23–32. doi: 10.1016/j.jare.2018.03.005

Haslmayer, P., Thalhammer, T., Jager, W., Aust, S., Steiner, G., Ensinger, C., et al. (2002). The peroxisome proliferator-activated receptor gamma ligand 15-deoxy-Delta12,14-prostaglandin J2 induces vascular endothelial growth factor in the hormone-independent prostate cancer cell line PC 3 and the urinary bladder carcinoma cell line 5637. *Int. J. Oncol.* 21, 915–920.

Hernandez, J. M., Floyd, D. H., Weilbaecher, K. N., Green, P. L., and Boris-Lawrie, K. (2008). Multiple facets of junD gene expression are atypical among AP-1 family members. *Oncogene* 27, 4757–4767. doi: 10.1038/onc.2008.120

Hirata, Y., Hayashi, H., Ito, S., Kikawa, Y., Ishibashi, M., Sudo, M., et al. (1988). Occurrence of 9-deoxy-delta 9,delta 12-13,14-dihydroprostaglandin D2 in human urine. *J. Biol. Chem.* 263, 16619–16625. doi: 10.1016/s0021-9258(18)37435-0

Hornsleth, A., Loland, L., and Larsen, L. B. (2001). Cytokines and chemokines in respiratory secretion and severity of disease in infants with respiratory syncytial virus (RSV) infection. *J. Clin. Virol.* 21, 163–170. doi: 10.1016/s1386-6532(01)00159-7

Hsu, H. T., and Chi, C. W. (2014). Emerging role of the peroxisome proliferator-activated receptor-gamma in hepatocellular carcinoma. *J. Hepatocell Carcinoma.* 1, 127–135. doi: 10.2147/jhc.s48512

Huan, T., Tran, T., Zheng, J., Sapkota, S., MacDonald, S. W., Camicioli, R., et al. (2018). Metabolomics Analyses of Saliva Detect Novel Biomarkers of Alzheimer's Disease. *J. Alzheimers Dis.* 65, 1401–1416. doi: 10.3233/jad-180711

Hughes-Fulford, M., McGrath, M. S., Hanks, D., Erickson, S., and Pulliam, L. (1992). Effects of dimethyl prostaglandin A1 on herpes simplex virus and human immunodeficiency virus replication. *Antimicrob. Agents Chemother.* 36, 2253–2258. doi: 10.1128/aac.36.10.2253

Inoue, H., Tanabe, T., and Umesono, K. (2000). Feedback control of cyclooxygenase-2 expression through PPARgamma. *J. Biol. Chem.* 275, 28028–28032. doi: 10.1074/jbc.m001387200

Inoue, M., Itoh, H., Tanaka, T., Chun, T. H., Doi, K., Fukunaga, Y., et al. (2001). Oxidized LDL regulates vascular endothelial growth factor expression in human macrophages and endothelial cells through activation of peroxisome proliferator-activated receptor-gamma. *Arterioscler. Thromb. Vasc. Biol.* 21, 560–566. doi: 10.1161/01.atv.21.4.560

Itoh, K., Mochizuki, M., Ishii, Y., Ishii, T., Shibata, T., Kawamoto, Y., et al. (2004). Transcription factor Nrf2 regulates inflammation by mediating the effect of 15-deoxy-Delta(12,14)-prostaglandin j(2). *Mol. Cell Biol.* 24, 36–45. doi: 10.1128/mcb.24.1.36-45.2004

Janabi, N. (2002). Selective inhibition of cyclooxygenase-2 expression by 15-deoxy-Delta(12,14)(12,14)-prostaglandin J(2) in activated human astrocytes, but not in human brain macrophages. *J. Immunol.* 168, 4747–4755. doi: 10.4049/jimmunol.168.9.4747

Jandl, K., and Heinemann, A. (2017). The therapeutic potential of CRTH2/DP2 beyond allergy and asthma. *Prostaglandins Lipid. Mediat.* 133, 42–48. doi: 10.1016/j.prostaglandins.2017.08.006

Jang, H. Y., Hong, O. Y., Youn, H. J., Kim, M. G., Kim, C. H., Jung, S. H., et al. (2020). 15d-PGJ2 inhibits NF-kappaB and AP-1-mediated MMP-9 expression and invasion of breast cancer cell by means of a heme oxygenase-1-dependent mechanism. *BMB Rep.* 53, 212–217. doi: 10.5483/bmbrep.2020.53.4.164

Ji, J. D., Kim, H. J., Rho, Y. H., Choi, S. J., Lee, Y. H., Cheon, H. J., et al. (2005). Inhibition of IL-10-induced STAT3 activation by 15-deoxy-Delta12,14-prostaglandin J2. *Rheumatology* 44, 983–988. doi: 10.1093/rheumatology/keh657

Jozkowicz, A., Huk, I., Nigisch, A., Weigel, G., Weidinger, F., and Dulak, J. (2002). Effect of prostaglandin-J(2) on VEGF synthesis depends on the induction of heme oxygenase-1. *Antioxid Redox Signal.* 4, 577–585. doi: 10.1089/15230860260220076

Jozkowicz, A., Nigisch, A., Wegrzyn, J., Weigel, G., Huk, I., and Dulak, J. (2004). Opposite effects of prostaglandin-J2 on VEGF in normoxia and hypoxia: role of HIF-1. *Biochem. Biophys. Res. Commun.* 314, 31–38. doi: 10.1016/j.bbrc.2003.12.059

Kalantari, P., Narayan, V., Henderson, A. J., and Prabhu, K. S. (2009). 15-Deoxy-Delta12,14-prostaglandin J2 inhibits HIV-1 transactivating protein, Tat, through covalent modification. *FASEB J.* 23, 2366–2373. doi: 10.1096/fj.08-124982

Kansanen, E., Kivela, A. M., and Levonen, A. L. (2009). Regulation of Nrf2-dependent gene expression by 15-deoxy-Delta12,14-prostaglandin J2. *Free Radic. Biol. Med.* 47, 1310–1317. doi: 10.1016/j.freeradbiomed.2009.06.030

Kaplan, J. M., Cook, J. A., Hake, P. W., O'Connor, M., Burroughs, T. J., and Zingarelli, B. (2005). 15-Deoxy-delta(12,14)-prostaglandin J(2) (15D-PGJ(2)), a peroxisome proliferator activated receptor gamma ligand, reduces tissue leukosequestration and mortality in endotoxic shock. *Shock* 24, 59–65. doi: 10.1097/01.shk.0000167108.88376.f2

Kaplan, J. M., Denenberg, A., Monaco, M., Nowell, M., Wong, H., and Zingarelli, B. (2010). Changes in peroxisome proliferator-activated receptor-gamma activity in children with septic shock. *Intens. Care Med.* 36, 123–130. doi: 10.1007/s00134-009-1654-6

Kapoor, M., Kojima, F., Qian, M., Yang, L., and Crofford, L. J. (2007). Microsomal prostaglandin E synthase-1 deficiency is associated with elevated peroxisome proliferator-activated receptor gamma: regulation by prostaglandin E2 via the phosphatidylinositol 3-kinase and Akt pathway. *J. Biol. Chem.* 282, 5356–5366. doi: 10.1074/jbc.m610153200

Kapoor, R., and Huang, Y. S. (2006). Gamma linolenic acid: an antiinflammatory omega-6 fatty acid. *Curr. Pharm. Biotechnol.* 7, 531–534. doi: 10.2174/138920106779116874

Kato, T., Fukushima, M., Kurozumi, S., and Noyori, R. (1986). Antitumor activity of delta 7-prostaglandin A1 and delta 12-prostaglandin J2 in vitro and in vivo. *Cancer Res.* 46, 3538–3542.

Kim, E. H., Kim, D. H., Na, H. K., and Surh, Y. J. (2004). Effects of cyclopentenone prostaglandins on the expression of heme oxygenase-1 in MCF-7 cells. *Ann. N. Y. Acad. Sci.* 1030, 493–500. doi: 10.1196/annals.1329.061

Kim, E. H., Na, H. K., and Surh, Y. J. (2006). Upregulation of VEGF by 15-deoxy-Delta12,14-prostaglandin J2 via heme oxygenase-1 and ERK1/2 signaling in MCF-7 cells. *Ann. N. Y. Acad. Sci.* 1090, 375–384. doi: 10.1196/annals.1378.041

Kim, H. J., Rho, Y. H., Choi, S. J., Lee, Y. H., Cheon, H., Um, J. W., et al. (2005). 15-Deoxy-delta12,14-PGJ2 inhibits IL-6-induced Stat3 phosphorylation in lymphocytes. *Exp. Mol. Med.* 37, 179–185. doi: 10.1038/emm.2005.24

Kim, I. K., Lee, J. H., Sohn, H. W., Kim, H. S., and Kim, S. H. (1993). Prostaglandin A2 and delta 12-prostaglandin J2 induce apoptosis in L1210 cells. *FEBS Lett.* 321, 209–214. doi: 10.1016/0014-5793(93)80110-g

Kim, K. R., Kim, H. J., Lee, S. K., Ma, G. T., Park, K. K., and Chung, W. Y. (2015). 15-deoxy-delta12,14-prostaglandin j2 inhibits osteolytic breast cancer bone metastasis and estrogen deficiency-induced bone loss. *PLoS One* 10:e0122764. doi: 10.1371/journal.pone.0122764

Kim, Y., Ratziu, V., Choi, S. G., Lalazar, A., Theiss, G., Dang, Q., et al. (1998). Transcriptional activation of transforming growth factor beta1 and its receptors by the Kruppel-like factor Zf9/core promoter-binding protein and Sp1. Potential mechanisms for autocrine fibrogenesis in response to injury. *J. Biol. Chem.* 273, 33750–33758. doi: 10.1074/jbc.273.50.33750

Kitz, K., Windischhofer, W., Leis, H. J., Huber, E., Kollroser, M., and Malle, E. (2011). 15-Deoxy-Delta12,14-prostaglandin J2 induces Cox-2 expression in human osteosarcoma cells through MAPK and EGFR activation involving reactive oxygen species. *Free Radic Biol. Med.* 50, 854–865. doi: 10.1016/j.freeradbiomed.2010.12.039

Koharudin, L. M., Furey, W., and Gronenborn, A. M. (2011). Novel fold and carbohydrate specificity of the potent anti-HIV cyanobacterial lectin from Oscillatoria agardhii. *J. Biol. Chem.* 286, 1588–1597. doi: 10.1074/jbc.m110.173278

Korade, Z., Xu, L., Mirnics, K., and Porter, N. A. (2013). Lipid biomarkers of oxidative stress in a genetic mouse model of Smith-Lemli-Opitz syndrome. *J. Inherit. Metab. Dis.* 36, 113–122. doi: 10.1007/s10545-012-9504-z

Kurakula, K., Koenis, D. S., van Tiel, C. M., and de Vries, C. J. (2014). NR4A nuclear receptors are orphans but not lonesome. *Biochim. Biophys. Acta* 1843, 2543–2555. doi: 10.1016/j.bbamcr.2014.06.010

Kweider, N., Fragoulis, A., Rosen, C., Pecks, U., Rath, W., Pufe, T., et al. (2011). Interplay between vascular endothelial growth factor (VEGF) and nuclear factor

erythroid 2-related factor-2 (Nrf2): implications for preeclampsia. *J. Biol. Chem.* 286, 42863–42872. doi: 10.1074/jbc.m111.286880

Lacal, P. M., Amici, C., Bonmassar, E., and D'Onofrio, C. (1994a). Effects of cyclopentenone prostaglandins on myeloid cells during early infection with HTLV-I. II. Regulation of synthesis of inducible p72 heat shock protein. *J. Pharmacol. Exp. Ther.* 271, 1096–1102.

Lacal, P. M., Puglianiello, A., Bonmassar, E., and D'Onofrio, C. (1994b). Effects of cyclopentenone prostaglandins on myeloid cells during early infection with HTLV-I. I. Cell differentiation determines sensitivity to prostaglandins and virus infection. *J. Pharmacol. Exp. Ther.* 271, 1086–1095.

Lawrence, T. (2009). The nuclear factor NF-kappaB pathway in inflammation. *Cold Spr. Harb. Perspect. Biol.* 1:a001651.

Lee, H. J., Su, Y., Yin, P. H., Lee, H. C., and Chi, C. W. (2009). PPAR(gamma)/PGC-1(alpha) pathway in E-cadherin expression and motility of HepG2 cells. *Anticancer Res.* 29, 5057–5063.

Lee, S. E., and Park, Y. S. (2013). Role of lipid peroxidation-derived alpha, beta-unsaturated aldehydes in vascular dysfunction. *Oxid. Med. Cell Longev.* 2013:629028.

Lee, S. J., Yang, E. K., and Kim, S. G. (2006). Peroxisome proliferator-activated receptor-gamma and retinoic acid X receptor alpha represses the TGFbeta1 gene via PTEN-mediated p70 ribosomal S6 kinase-1 inhibition: role for Zf9 dephosphorylation. *Mol. Pharmacol.* 70, 415–425. doi: 10.1124/mol.106.022954

Levonen, A. L., Dickinson, D. A., Moellering, D. R., Mulcahy, R. T., Forman, H. J., and Darley-Usmar, V. M. (2001). Biphasic effects of 15-deoxy-delta(12,14)-prostaglandin J(2) on glutathione induction and apoptosis in human endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* 21, 1846–1851. doi: 10.1161/hq1101.098488

Levonen, A. L., Landar, A., Ramachandran, A., Ceaser, E. K., Dickinson, D. A., Zanoni, G., et al. (2004). Cellular mechanisms of redox cell signalling: role of cysteine modification in controlling antioxidant defences in response to electrophilic lipid oxidation products. *Biochem. J.* 378(Pt 2), 373–382. doi: 10.1042/bj20031049

Li, H., and Narahara, H. (2013). 15-Deoxy-Delta(12,14)-prostaglandin J(2) induces growth inhibition, cell cycle arrest and apoptosis in human endometrial cancer cell lines. *Int. J. Mol. Med.* 31, 778–788. doi: 10.3892/ijmm.2013.1268

Li, H., and Pauza, C. D. (2009). Effects of 15-deoxy-delta12,14-prostaglandin J2 (15d-PGJ2) and rosiglitazone on human gammadelta2 T cells. *PLoS One* 4:e7726. doi: 10.1371/journal.pone.0007726

Li, J., Guo, C., and Wu, J. (2019). 15-Deoxy–(12,14)-Prostaglandin J2 (15d-PGJ2), an Endogenous Ligand of PPAR-gamma: Function and Mechanism. *PPAR Res.* 2019:7242030.

Li, Y., Atkinson, K., and Zhang, T. (2017). Combination of chemotherapy and cancer stem cell targeting agents: Preclinical and clinical studies. *Cancer Lett.* 396, 103–109. doi: 10.1016/j.canlet.2017.03.008

Liang, X., Wu, L., Hand, T., and Andreasson, K. (2005). Prostaglandin D2 mediates neuronal protection via the DP1 receptor. *J. Neurochem.* 92, 477–486. doi: 10.1111/j.1471-4159.2004.02870.x

Lin, A., Minden, A., Martinetto, H., Claret, F. X., Lange-Carter, C., Mercurio, F., et al. (1995). Identification of a dual specificity kinase that activates the Jun kinases and p38-Mpk2. *Science* 268, 286–290. doi: 10.1126/science.7716521

Loboda, A., Damulewicz, M., Pyza, E., Jozkowicz, A., and Dulak, J. (2016). Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. *Cell Mol. Life Sci.* 73, 3221–3247. doi: 10.1007/s00018-016-2223-0

Magesh, S., Chen, Y., and Hu, L. (2012). Small molecule modulators of Keap1-Nrf2-ARE pathway as potential preventive and therapeutic agents. *Med. Res. Rev.* 32, 687–726. doi: 10.1002/med.21257

Maier, N. K., Leppla, S. H., and Moayeri, M. (2015). The cyclopentenone prostaglandin 15d-PGJ2 inhibits the NLRP1 and NLRP3 inflammasomes. *J. Immunol.* 194, 2776–2785. doi: 10.4049/jimmunol.1401611

Mastromarino, P., Conti, C., Petruzziello, R., De Marco, A., Pica, F., and Santoro, M. G. (1993). Inhibition of Sindbis virus replication by cyclopentenone prostaglandins: a cell-mediated event associated with heat-shock protein synthesis. *Antiviral. Res.* 20, 209–222. doi: 10.1016/0166-3542(93)90021-a

Mc Guire, P. J., Parikh, A., and Diaz, G. A. (2009). Profiling of oxidative stress in patients with inborn errors of metabolism. *Mol. Genet. Metab.* 98, 173–180. doi: 10.1016/j.ymgme.2009.06.007

Milde-Langosch, K. (2005). The Fos family of transcription factors and their role in tumourigenesis. *Eur. J. Cancer* 41, 2449–2461. doi: 10.1016/j.ejca.2005.08.008

Millan, O., Rico, D., Peinado, H., Zarich, N., Stamatakis, K., Perez-Sala, D., et al. (2006). Potentiation of tumor formation by topical administration of 15-deoxy-delta12,14-prostaglandin J2 in a model of skin carcinogenesis. *Carcinogenesis* 27, 328–336. doi: 10.1093/carcin/bgi213

Mills, E. L., Ryan, D. G., Prag, H. A., Dikovskaya, D., Menon, D., Zaslona, Z., et al. (2018). Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1. *Nature* 556, 113–117.

Minden, A., Lin, A., Claret, F. X., Abo, A., and Karin, M. (1995). Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. *Cell* 81, 1147–1157. doi: 10.1016/s0092-8674(05)80019-4

Ming, X., Stein, T. P., Brimacombe, M., Johnson, W. G., Lambert, G. H., and Wagner, G. C. (2005). Increased excretion of a lipid peroxidation biomarker in autism. *Prostaglandins Leukot. Essent Fatty Acids* 73, 379–384. doi: 10.1016/j.plefa.2005.06.002

Mochizuki, M., Ishii, Y., Itoh, K., Iizuka, T., Morishima, Y., Kimura, T., et al. (2005). Role of 15-deoxy delta(12,14) prostaglandin J2 and Nrf2 pathways in protection against acute lung injury. *Am. J. Respir. Crit. Care Med.* 171, 1260–1266.

Monroy, M. A., Opperman, K. K., Pucciarelli, M., Yerrum, S., Berg, D. A., and Daly, J. M. T. H. E. (2007). PPARgamma ligand 15d-PGJ2 modulates macrophage activation after injury in a murine trauma model. *Shock* 28, 186–191. doi: 10.1097/shk.0b013e3180310982

Montine, T. J., Beal, M. F., Cudkowicz, M. E., O'Donnell, H., Margolin, R. A., McFarland, L., et al. (1999a). Increased CSF F2-isoprostane concentration in probable AD. *Neurology* 52, 562–565. doi: 10.1212/wnl.52.3.562

Montine, T. J., Beal, M. F., Robertson, D., Cudkowicz, M. E., Biaggioni, I., O'Donnell, H., et al. (1999b). Cerebrospinal fluid F2-isoprostanes are elevated in Huntington's disease. *Neurology* 52, 1104–1105. doi: 10.1212/wnl.52.5.1104

Montine, T. J., Markesbery, W. R., Morrow, J. D., and Roberts, L. J. II (1998). Cerebrospinal fluid F2-isoprostane levels are increased in Alzheimer's disease. *Ann. Neurol.* 44, 410–413. doi: 10.1002/ana.410440322

Montuschi, P., Barnes, P., and Roberts, L. J. II (2007). Insights into oxidative stress: the isoprostanes. *Curr. Med. Chem.* 14, 703–717. doi: 10.2174/092986707780059607

Montuschi, P., Kharitonov, S. A., Ciabattoni, G., Corradi, M., van Rensen, L., Geddes, D. M., et al. (2000). Exhaled 8-isoprostane as a new non-invasive biomarker of oxidative stress in cystic fibrosis. *Thorax* 55, 205–209. doi: 10.1136/thorax.55.3.205

Morrow, J. D. (2005). Quantification of isoprostanes as indices of oxidant stress and the risk of atherosclerosis in humans. *Arterioscler. Thromb. Vasc. Biol.* 25, 279–286. doi: 10.1161/01.atv.0000152605.64964.c0

Mowen, K., and David, M. (2000). Regulation of STAT1 nuclear export by Jak1. *Mol. Cell Biol.* 20, 7273–7281. doi: 10.1128/mcb.20.19.7273-7281.2000

Mukherjee, R., Jow, L., Noonan, D., and McDonnell, D. P. (1994). Human and rat peroxisome proliferator activated receptors (PPARs) demonstrate similar tissue distribution but different responsiveness to PPAR activators. *J. Steroid Biochem. Mol. Biol.* 51, 157–166. doi: 10.1016/0960-0760(94)90089-2

Murillo, J. C., Dimov, V., and Gonzalez-Estrada, A. (2018). An evaluation of fevipiprant for the treatment of asthma: a promising new therapy? *Exp. Opin. Pharmacother.* 19, 2087–2093. doi: 10.1080/14656566.2018.1540589

Musiek, E. S., Breeding, R. S., Milne, G. L., Zanoni, G., Morrow, J. D., and McLaughlin, B. (2006). Cyclopentenone isoprostanes are novel bioactive products of lipid oxidation which enhance neurodegeneration. *J. Neurochem.* 97, 1301–1313. doi: 10.1111/j.1471-4159.2006.03797.x

Musiek, E. S., McLaughlin, B., and Morrow, J. D. (2007). Electrophilic cyclopentenone isoprostanes in neurodegeneration. *J. Mol. Neurosci.* 33, 80–86. doi: 10.1007/s12031-007-0042-3

Nagata, N., Iwanari, H., Kumagai, H., Kusano-Arai, O., Ikeda, Y., Aritake, K., et al. (2017). Generation and characterization of an antagonistic monoclonal antibody against an extracellular domain of mouse DP2 (CRTH2/GPR44) receptors for prostaglandin D2. *PLoS One* 12:e0175452. doi: 10.1371/journal.pone.0175452

Nakazawa, H., Chang, K., Shinozaki, S., Yasukawa, T., Ishimaru, K., Yasuhara, S., et al. (2017). iNOS as a Driver of Inflammation and Apoptosis in Mouse Skeletal

Muscle after Burn Injury: Possible Involvement of Sirt1 S-Nitrosylation-Mediated Acetylation of p65 NF-kappaB and p53. *PLoS One* 12:e0170391. doi: 10.1371/journal.pone.0170391

Negishi, M., and Katoh, H. (2002). Cyclopentenone prostaglandin receptors. *Prostaglandins. Lipid. Mediat.* 6, 611–617. doi: 10.1016/s0090-6980(02)00059-x

Nikitakis, N. G., Siavash, H., Hebert, C., Reynolds, M. A., Hamburger, A. W., and Sauk, J. J. (2002). 15-PGJ2, but not thiazolidinediones, inhibits cell growth, induces apoptosis, and causes downregulation of Stat3 in human oral SCCa cells. *Br. J. Cancer.* 87, 1396–1403. doi: 10.1038/sj.bjc.6600618

Niro, S., Hennebert, O., and Morfin, R. A. (2010). native steroid hormone derivative triggers the resolution of inflammation. *Horm Mol. Biol. Clin. Investig.* 1, 11–19.

Nugteren, D. H., Van Dorp, D. A., Bergstrom, S., Hamberg, M., and Samuelsson, B. (1966). Absolute configuration of the prostaglandins. *Nature* 212, 38–39.

Oliva, J. L., Perez-Sala, D., Castrillo, A., Martinez, N., Canada, F. J., Bosca, L., et al. (2003). The cyclopentenone 15-deoxy-delta 12,14-prostaglandin J2 binds to and activates H-Ras. *Proc. Natl. Acad. Sci. U S A.* 100, 4772–4777. doi: 10.1073/pnas.0735842100

Omeragic, A., Saikali, M. F., Currier, S., Volsky, D. J., Cummins, C. L., and Bendayan, R. (2020). Selective peroxisome proliferator-activated receptor-gamma modulator, INT131 exhibits anti-inflammatory effects in an EcoHIV mouse model. *FASEB J.* 34, 1996–2010. doi: 10.1096/fj.201901874r

Pai, S., and Thomas, R. (2008). Immune deficiency or hyperactivity-Nf-kappab illuminates autoimmunity. *J. Autoimmun.* 31, 245–251. doi: 10.1016/j.jaut.2008.04.012

Park, E. J., Park, S. Y., Joe, E. H., and Jou, I. (2003). 15d-PGJ2 and rosiglitazone suppress Janus kinase-STAT inflammatory signaling through induction of suppressor of cytokine signaling 1 (SOCS1) and SOCS3 in glia. *J. Biol. Chem.* 278, 14747–14752. doi: 10.1074/jbc.m210819200

Park, J. M., and Na, H. K. (2019a). 15-Deoxy-Delta(12,14)-prostaglandin J2 Upregulates the Expression of 15-Hydroxyprostaglandin Dehydrogenase by Inducing AP-1 Activation and Heme Oxygenase-1 Expression in Human Colon Cancer Cells. *J. Cancer Prev.* 24, 183–191. doi: 10.15430/jcp.2019.24.3.183

Park, J. M., and Na, H. K. (2019b). Erratum: 15-Deoxy-Delta(12,14)-prostaglandin J2 Upregulates the Expression of 15-Hydroxyprostaglandin Dehydrogenase by Inducing AP-1 Activation and Heme Oxygenase-1 Expression in Human Colon Cancer Cells. *J. Cancer Prev.* 24:245. doi: 10.15430/jcp.2019.24.4.245

Park, S. H., Kim, K. E., Hwang, H. Y., and Kim, T. Y. (2003). Regulatory effect of SOCS on NF-kappaB activity in murine monocytes/macrophages. *DNA Cell Biol.* 22, 131–139. doi: 10.1089/104454903321515931

Parker, J. (1995). Prostaglandin A2 protein interactions and inhibition of cellular proliferation. *Prostaglandins* 50, 359–375. doi: 10.1016/0090-6980(95)00136-0

Pearen, M. A., and Muscat, G. E. (2010). Minireview: Nuclear hormone receptor 4A signaling: implications for metabolic disease. *Mol. Endocrinol.* 24, 1891–1903. doi: 10.1210/me.2010-0015

Pearson, D., Weiss, H. M., Jin, Y., Jaap van Lier, J., Erpenbeck, V. J., Glaenzel, U., et al. (2017). Absorption, Distribution, Metabolism, and Excretion of the Oral Prostaglandin D2 Receptor 2 Antagonist Fevipiprant (QAW039) in Healthy Volunteers and In Vitro. *Drug Metab. Dispos.* 45, 817–825. doi: 10.1124/dmd.117.075358

Peeraully, M. R., Sievert, H., Bullo, M., Wang, B., and Trayhurn, P. (2006). Prostaglandin D2 and J2-series (PGJ2, Delta12-PGJ2) prostaglandins stimulate IL-6 and MCP-1, but inhibit leptin, expression and secretion by 3T3-L1 adipocytes. *Pflugers Arch.* 453, 177–187. doi: 10.1007/s00424-006-0118-x

Perez-Sala, D., Cernuda-Morollon, E., and Canada, F. J. (2003). Molecular basis for the direct inhibition of AP-1 DNA binding by 15-deoxy-Delta 12,14-prostaglandin J2. *J. Biol. Chem.* 278, 51251–51260. doi: 10.1074/jbc.m309409200

Phulwani, N. K., Feinstein, D. L., Gavrilyuk, V., Akar, C., and Kielian, T. (2006). 15-deoxy-Delta12,14-prostaglandin J2 (15d-PGJ2) and ciglitazone modulate Staphylococcus aureus-dependent astrocyte activation primarily through a PPAR-gamma-independent pathway. *J. Neurochem.* 99, 1389–1402. doi: 10.1111/j.1471-4159.2006.04183.x

Pica, F., De Marco, A., De Cesare, F., and Santoro, M. G. (1993). Inhibition of vesicular stomatitis virus replication by delta 12-prostaglandin J2 is regulated at two separate levels and is associated with induction of stress protein synthesis. *Antiviral. Res.* 20, 193–208. doi: 10.1016/0166-3542(93)90020-j

Pica, F., Palamara, A. T., Rossi, A., De Marco, A., Amici, C., and Santoro, M. G. (2000). Delta(12)-prostaglandin J(2) is a potent inhibitor of influenza A virus replication. *Antimicrob. Agents Chemother.* 44, 200–204. doi: 10.1128/aac.44.1.200-204.2000

Porta, A., Pasi, M., Brunoldi, E., Zanoni, G., and Vidari, G. (2013). Biology and chemistry of neuroprostanes. First total synthesis of 17-A4-NeuroP: validation of a convergent strategy to a number of cyclopentenone neuroprostanes. *Chem. Phys. Lipids.* 174, 64–74. doi: 10.1016/j.chemphyslip.2013.07.002

Pratico, D., Clark, C. M., Lee, V. M., Trojanowski, J. Q., Rokach, J., and FitzGerald, G. A. (2000). Increased 8,12-iso-iPF2alpha-VI in Alzheimer's disease: correlation of a noninvasive index of lipid peroxidation with disease severity. *Ann. Neurol.* 48, 809–812. doi: 10.1002/1531-8249(200011)48:5<809::aid-ana19>3.0.co;2-9

Pratico, D., Myl, V., Trojanowski, J. Q., Rokach, J., and Fitzgerald, G. A. (1998). Increased F2-isoprostanes in Alzheimer's disease: evidence for enhanced lipid peroxidation in vivo. *FASEB J.* 12, 1777–1783. doi: 10.1096/fasebj.12.15.1777

Rajakariar, R., Hilliard, M., Lawrence, T., Trivedi, S., Colville-Nash, P., Bellingan, G., et al. (2007). Hematopoietic prostaglandin D2 synthase controls the onset and resolution of acute inflammation through PGD2 and 15-deoxyDelta12 14 PGJ2. *Proc. Natl. Acad. Sci. U S A.* 104, 20979–20984. doi: 10.1073/pnas.0707394104

Rajan, S., Jang, Y., Kim, C. H., Kim, W., Toh, H. T., Jeon, J., et al. (2020). PGE1 and PGA_1 bind to Nurr1 and activate its transcriptional function. *Nat. Chem. Biol.* 16, 876–886. doi: 10.1038/s41589-020-0553-6

Ricote, M., Huang, J., Fajas, L., Li, A., Welch, J., Najib, J., et al. (1998a). Expression of the peroxisome proliferator-activated receptor gamma (PPARgamma) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein. *Proc. Natl. Acad. Sci. U S A.* 95, 7614–7619. doi: 10.1073/pnas.95.13.7614

Ricote, M., Li, A. C., Willson, T. M., Kelly, C. J., and Glass, C. K. (1998b). The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* 391, 79–82. doi: 10.1038/34178

Rosenberger, T. A., Villacreses, N. E., Hovda, J. T., Bosetti, F., Weerasinghe, G., Wine, R. N., et al. (2004). Rat brain arachidonic acid metabolism is increased by a 6-day intracerebral ventricular infusion of bacterial lipopolysaccharide. *J. Neurochem.* 88, 1168–1178. doi: 10.1046/j.1471-4159.2003.02246.x

Rossi, A., Elia, G., and Santoro, M. G. (1997). Inhibition of nuclear factor kappa B by prostaglandin A1: an effect associated with heat shock transcription factor activation. *Proc. Natl. Acad. Sci. U S A.* 94, 746–750. doi: 10.1073/pnas.94.2.746

Rossi, A., Kapahi, P., Natoli, G., Takahashi, T., Chen, Y., Karin, M., et al. (2000). Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IkappaB kinase. *Nature* 403, 103–108. doi: 10.1038/47520

Rossi, P., Riutta, A., Kuukasjarvi, P., Vehmas, T., Mucha, I., and Salenius, J. P. (2004). Revascularization decreases 8-isoprostaglandin F2alpha excretion in chronic lower limb ischemia. *Prostaglandins Leukot. Essent Fatty Acids* 71, 97–101. doi: 10.1016/j.plefa.2004.01.003

Rossner, P. Jr., Gammon, M. D., Terry, M. B., Agrawal, M., Zhang, F. F., Teitelbaum, S. L., et al. (2006). Relationship between urinary 15-F2t-isoprostane and 8-oxodeoxyguanosine levels and breast cancer risk. *Cancer Epidemiol. Biomarkers Prev.* 15, 639–644. doi: 10.1158/1055-9965.epi-05-0554

Rozera, C., Carattoli, A., De Marco, A., Amici, C., Giorgi, C., and Santoro, M. G. (1996). Inhibition of HIV-1 replication by cyclopentenone prostaglandins in acutely infected human cells. Evidence for a transcriptional block. *J. Clin. Invest.* 97, 1795–1803. doi: 10.1172/jci118609

Saarialho-Kere, U. K., Chang, E. S., Welgus, H. G., and Parks, W. C. (1993). Expression of interstitial collagenase, 92-kDa gelatinase, and tissue inhibitor of metalloproteinases-1 in granuloma annulare and necrobiosis lipoidica diabeticorum. *J. Invest. Dermatol.* 100, 335–342. doi: 10.1111/1523-1747.ep12470032

Sakamoto, H., Corcoran, T. B., Laffey, J. G., and Shorten, G. D. (2002). Isoprostanes–markers of ischaemia reperfusion injury. *Eur. J. Anaesthesiol.* 19, 550–559. doi: 10.1017/s0265021502000893

Sandham, D. A., Barker, L., Brown, L., Brown, Z., Budd, D., Charlton, S. J., et al. (2017a). Correction to "Discovery of Fevipiprant (NVP-QAW039), a Potent and Selective DP2 Receptor Antagonist for Treatment of Asthma". *ACS Med. Chem. Lett.* 8:987. doi: 10.1021/acsmedchemlett.7b00353

Sandham, D. A., Barker, L., Brown, L., Brown, Z., Budd, D., Charlton, S. J., et al. (2017b). Discovery of Fevipiprant (NVP-QAW039), a Potent and Selective

DP2 Receptor Antagonist for Treatment of Asthma. *ACS Med. Chem. Lett.* 8, 582–586. doi: 10.1021/acsmedchemlett.7b00157

Santoro, M. G., Fukushima, M., Benedetto, A., and Amici, C. (1987). PGJ2, a new antiviral prostaglandin: inhibition of Sendai virus replication and alteration of virus protein synthesis. *J. Gen. Virol.* 68(Pt 4), 1153–1158. doi: 10.1099/0022-1317-68-4-1153

Sato, H., Ishihara, S., Kawashima, K., Moriyama, N., Suetsugu, H., Kazumori, H., et al. (2000). Expression of peroxisome proliferator-activated receptor (PPAR)gamma in gastric cancer and inhibitory effects of PPARgamma agonists. *Br. J. Cancer* 83, 1394–1400. doi: 10.1054/bjoc.2000.1457

Sayre, L. M., Lin, D., Yuan, Q., Zhu, X., and Tang, X. (2006). Protein adducts generated from products of lipid oxidation: focus on HNE and one. *Drug. Metab. Rev.* 38, 651–675. doi: 10.1080/03602530600959508

Scher, J. U., and Pillinger, M. H. (2005). 15d-PGJ2: the anti-inflammatory prostaglandin? *Clin. Immunol.* 114, 100–109. doi: 10.1016/j.clim.2004.09.008

Seet, R. C., Lee, C. Y., Lim, E. C., Tan, J. J., Quek, A. M., Chong, W. L., et al. (2010). Oxidative damage in Parkinson disease: Measurement using accurate biomarkers. *Free Radic. Biol. Med.* 48, 560–566. doi: 10.1016/j.freeradbiomed.2009.11.026

Seif, F., Khoshmirsafa, M., Aazami, H., Mohsenzadegan, M., Sedighi, G., and Bahar, M. (2017). The role of JAK-STAT signaling pathway and its regulators in the fate of T helper cells. *Cell Commun. Signal.* 15, 23.

Senftleben, U., Cao, Y., Xiao, G., Greten, F. R., Krahn, G., Bonizzi, G., et al. (2001). Activation by IKKalpha of a second, evolutionary conserved, NF-kappa B signaling pathway. *Science* 293, 1495–1499. doi: 10.1126/science.1062677

Seo, S. K., Seo, D. I., Park, W. S., Jung, W. K., Lee, D. S., Park, S. G., et al. (2014). Attenuation of IFN-gamma-induced B7-H1 expression by 15-deoxy-delta(12,14)-prostaglandin J2 via downregulation of the Jak/STAT/IRF-1 signaling pathway. *Life Sci.* 112, 82–89. doi: 10.1016/j.lfs.2014.07.021

Sharma, A. M., and Staels, B. (2007). Review: Peroxisome proliferator-activated receptor gamma and adipose tissue–understanding obesity-related changes in regulation of lipid and glucose metabolism. *J. Clin. Endocrinol. Metab.* 92, 386–395. doi: 10.1210/jc.2006-1268

Shi, J., Johansson, J., Woodling, N. S., Wang, Q., Montine, T. J., and Andreasson, K. (2010). The prostaglandin E2 E-prostanoid 4 receptor exerts anti-inflammatory effects in brain innate immunity. *J. Immunol.* 184, 7207–7218. doi: 10.4049/jimmunol.0903487

Shibata, T., Kondo, M., Osawa, T., Shibata, N., Kobayashi, M., and Uchida, K. (2002). 15-deoxy-delta 12,14-prostaglandin J2. A prostaglandin D2 metabolite generated during inflammatory processes. *J. Biol. Chem.* 277, 10459–10466.

Shibata, T., Yamada, T., Ishii, T., Kumazawa, S., Nakamura, H., Masutani, H., et al. (2003a). Thioredoxin as a molecular target of cyclopentenone prostaglandins. *J. Biol. Chem.* 278, 26046–26054. doi: 10.1074/jbc.m303690200

Shibata, T., Yamada, T., Kondo, M., Tanahashi, N., Tanaka, K., Nakamura, H., et al. (2003b). An endogenous electrophile that modulates the regulatory mechanism of protein turnover: inhibitory effects of 15-deoxy-Delta 12,14-prostaglandin J2 on proteasome. *Biochemistry* 42, 13960–13968. doi: 10.1021/bi035215a

Signorini, C., De Felice, C., Leoncini, S., Giardini, A., D'Esposito, M., Filosa, S., et al. (2011). F(4)-neuroprostanes mediate neurological severity in Rett syndrome. *Clin. Chim. Acta.* 412, 1399–1406. doi: 10.1016/j.cca.2011.04.016

Stamatakis, K., and Perez-Sala, D. (2006). Prostanoids with cyclopentenone structure as tools for the characterization of electrophilic lipid-protein interactomes. *Ann. N. Y. Acad. Sci.* 1091, 548–570. doi: 10.1196/annals.1378.096

Stocker, R., and Keaney, J. F. Jr. (2004). Role of oxidative modifications in atherosclerosis. *Physiol. Rev.* 84, 1381–1478. doi: 10.1152/physrev.00047.2003

Straus, D. S., and Glass, C. K. (2001). Cyclopentenone prostaglandins: new insights on biological activities and cellular targets. *Med. Res. Rev.* 21, 185–210. doi: 10.1002/med.1006

Straus, D. S., Pascual, G., Li, M., Welch, J. S., Ricote, M., Hsiang, C. H., et al. (2000). 15-deoxy-delta 12,14-prostaglandin J2 inhibits multiple steps in the NF-kappa B signaling pathway. *Proc. Natl. Acad. Sci. U S A.* 97, 4844–4849. doi: 10.1073/pnas.97.9.4844

Sun, S. C. (2017). The non-canonical NF-kappaB pathway in immunity and inflammation. *Nat. Rev. Immunol.* 17, 545–558. doi: 10.1038/nri.2017.52

Tae, I. H., Park, E. Y., Dey, P., Son, J. Y., Lee, S. Y., Jung, J. H., et al. (2018). Novel SIRT1 inhibitor 15-deoxy-Delta12,14-prostaglandin J2 and its derivatives exhibit anticancer activity through apoptotic or autophagic cell death pathways in SKOV3 cells. *Int. J. Oncol.* 53, 2518–2530.

Takashima, T., Fujiwara, Y., Higuchi, K., Arakawa, T., Yano, Y., Hasuma, T., et al. (2001). PPAR-gamma ligands inhibit growth of human esophageal adenocarcinoma cells through induction of apoptosis, cell cycle arrest and reduction of ornithine decarboxylase activity. *Int. J. Oncol.* 19, 465–471.

Tauber, D., and Parker, R. (2019). 15-Deoxy-Delta(12,14)-prostaglandin J2 promotes phosphorylation of eukaryotic initiation factor 2alpha and activates the integrated stress response. *J. Biol. Chem.* 294, 6344–6352. doi: 10.1074/jbc.ra118.007138

Vane, J. R., and Botting, R. M. (1990). The mode of action of anti-inflammatory drugs. *Postgrad. Med. J.* 66(Suppl. 4), S2–S17.

Varmus, H. E., Shank, P. R., Hughes, S. E., Kung, H. J., Heasley, S., Majors, J., et al. (1979). Synthesis, structure, and integration of the DNA of RNA tumor viruses. *Cold Spr. Harb. Symp. Quant. Biol.* 43(Pt 2), 851–864. doi: 10.1101/sqb.1979.043.01.091

Vijay, R., Fehr, A. R., Janowski, A. M., Athmer, J., Wheeler, D. L., Grunewald, M., et al. (2017). Virus-induced inflammasome activation is suppressed by prostaglandin D2/DP1 signaling. *Proc. Natl. Acad. Sci. U S A.* 114, E5444–E5453.

Wang, N., Liang, H., and Zen, K. (2014). Molecular mechanisms that influence the macrophage m1-m2 polarization balance. *Front. Immunol.* 5:614.

Wang, S. Z., Hallsworth, P. G., Dowling, K. D., Alpers, J. H., Bowden, J. J., and Forsyth, K. D. (2000). Adhesion molecule expression on epithelial cells infected with respiratory syncytial virus. *Eur. Respir. J.* 15, 358–366. doi: 10.1034/j.1399-3003.2000.15b23.x

Welch, J. S., Ricote, M., Akiyama, T. E., Gonzalez, F. J., and Glass, C. K. (2003). PPARgamma and PPARdelta negatively regulate specific subsets of lipopolysaccharide and IFN-gamma target genes in macrophages. *Proc. Natl. Acad. Sci. U S A.* 100, 6712–6717. doi: 10.1073/pnas.1031789100

Welliver, R. C., Garofalo, R. P., and Ogra, P. L. (2002). Beta-chemokines, but neither T helper type 1 nor T helper type 2 cytokines, correlate with severity of illness during respiratory syncytial virus infection. *Pediatr. Infect Dis. J.* 21, 457–461. doi: 10.1097/00006454-200205000-00033

Wen, Z., Song, H., and Ming, G. L. (2017). How does Zika virus cause microcephaly? *Genes Dev.* 31, 849–861. doi: 10.1101/gad.298216.117

Wesselingh, S. L., Glass, J., McArthur, J. C., Griffin, J. W., and Griffin, D. E. (1994). Cytokine dysregulation in HIV-associated neurological disease. *Adv. Neuroimmunol.* 4, 199–206. doi: 10.1016/s0960-5428(06)80258-5

Whelan, J., and Fritsche, K. (2013). Linoleic acid. *Adv. Nutr.* 4, 311–312.

Wu, H. C., Wang, Q., Yang, H. I., Ahsan, H., Tsai, W. Y., Wang, L. Y., et al. (2008). Urinary 15-F2t-isoprostane, aflatoxin B1 exposure and hepatitis B virus infection and hepatocellular carcinoma in Taiwan. *Carcinogenesis* 29, 971–976. doi: 10.1093/carcin/bgn057

Xin, X., Yang, S., Kowalski, J., and Gerritsen, M. E. (1999). Peroxisome proliferator-activated receptor gamma ligands are potent inhibitors of angiogenesis in vitro and in vivo. *J. Biol. Chem.* 274, 9116–9121. doi: 10.1074/jbc.274.13.9116

Yagami, T., Yamamoto, Y., and Koma, H. (2018). Physiological and Pathological Roles of 15-Deoxy-Delta(12,14)-Prostaglandin J2 in the Central Nervous System and Neurological Diseases. *Mol. Neurobiol.* 55, 2227–2248. doi: 10.1007/s12035-017-0435-4

Yamakawa, K., Hosoi, M., Koyama, H., Tanaka, S., Fukumoto, S., Morii, H., et al. (2000). Peroxisome proliferator-activated receptor-gamma agonists increase vascular endothelial growth factor expression in human vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.* 271, 571–574. doi: 10.1006/bbrc.2000.2665

Yamamoto, N., Fukushima, M., Tsurumi, T., Maeno, K., and Nishiyama, Y. (1987). Mechanism of inhibition of herpes simplex virus replication by delta 7-prostaglandin A1 and delta 12-prostaglandin J2. *Biochem. Biophys. Res. Commun.* 146, 1425–1431. doi: 10.1016/0006-291x(87)90809-6

Yamamoto, Y., Koma, H., and Yagami, T. (2020). 15-Deoxy-Delta(12,14)-prostaglandin J2 Inhibits Cell Migration on Renal Cell Carcinoma via Down-Regulation of Focal Adhesion Kinase Signaling. *Biol. Pharm. Bull.* 43, 153–157. doi: 10.1248/bpb.b19-00748

Yen, C. C., Hsiao, C. D., Chen, W. M., Wen, Y. S., Lin, Y. C., Chang, T. W., et al. (2014). Cytotoxic effects of 15d-PGJ2 against osteosarcoma through ROS-mediated AKT and cell cycle inhibition. *Oncotarget* 5, 716–725. doi: 10.18632/oncotarget.1704

Yoshida, M., and Seiki, M. (1987). Recent advances in the molecular biology of HTLV-1: trans-activation of viral and cellular genes. *Annu Rev. Immunol.* 5, 541–559. doi: 10.1146/annurev.iy.05.040187.002545

Yuan, J., Takahashi, A., Masumori, N., Uchida, K., Hisasue, S., Kitamura, H., et al. (2005). Ligands for peroxisome proliferator-activated receptor gamma have potent antitumor effect against human renal cell carcinoma. *Urology* 65, 594–599. doi: 10.1016/j.urology.2004.10.019

Zheng, J., Sariol, A., Meyerholz, D., Zhang, Q., Abrahante Llorens, J. E., Narumiya, S., et al. (2020). Prostaglandin D2 signaling in dendritic cells is critical for the development of EAE. *J. Autoimmun.* 114:102508. doi: 10.1016/j.jaut.2020.102508

Zimta, A. A., Cenariu, D., Irimie, A., Magdo, L., Nabavi, S. M., Atanasov, A. G., et al. (2019). The Role of Nrf2 Activity in Cancer Development and Progression. *Cancers* 11:11.

Zingarelli, B., and Cook, J. A. (2005). Peroxisome proliferator-activated receptor-gamma is a new therapeutic target in sepsis and inflammation. *Shock* 23, 393–399. doi: 10.1097/01.shk.0000160521.91363.88

Conflict of Interest: 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.

frontiers in Physiology

REVIEW
published: 22 July 2021
doi: 10.3389/fphys.2021.688946

The Role of Innate Immunity and Bioactive Lipid Mediators in COVID-19 and Influenza

Sabina Sahanic[1], Judith Löffler-Ragg[1], Piotr Tymoszuk[1], Richard Hilbe[1], Egon Demetz[1], Rebecca K Masanetz[1], Markus Theurl[2], Johannes Holfeld[3], Can Gollmann-Tepeköylü[3], Alexandar Tzankov[4], Guenter Weiss[1], Martin Giera[5] and Ivan Tancevski[1]**

[1]Department of Internal Medicine II, Medical University of Innsbruck, Innsbruck, Austria, [2]Department of Internal Medicine III, Medical University of Innsbruck, Innsbruck, Austria, [3]Department of Cardiac Surgery, Medical University of Innsbruck, Innsbruck, Austria, [4]Institute of Medical Genetics and Pathology, University Hospital Basel, Basel, Switzerland, [5]Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, Netherlands

OPEN ACCESS

Edited by:
Xu Zhang,
Tianjin Medical University, China

Reviewed by:
Antonio Recchiuti,
University of Studies G. d'Annunzio Chieti and Pescara, Italy
Shabbir Ahmed Ansari,
Harvard Medical School, United States

***Correspondence:**
Martin Giera
m.a.giera@lumc.nl
Ivan Tancevski
ivan.tancevski@i-med.ac.at

Specialty section:
This article was submitted to Lipid and Fatty Acid Research, a section of the journal Frontiers in Physiology

Received: *31 March 2021*
Accepted: *16 June 2021*
Published: *22 July 2021*

Citation:
Sahanic S, Löffler-Ragg J, Tymoszuk P, Hilbe R, Demetz E, Masanetz RK, Theurl M, Holfeld J, Gollmann-Tepeköylü C, Tzankov A, Weiss G, Giera M and Tancevski I (2021) The Role of Innate Immunity and Bioactive Lipid Mediators in COVID-19 and Influenza. Front. Physiol. 12:688946. doi: 10.3389/fphys.2021.688946

In this review, we discuss spatiotemporal kinetics and inflammatory signatures of innate immune cells specifically found in response to SARS-CoV-2 compared to influenza virus infection. Importantly, we cover the current understanding on the mechanisms by which SARS-CoV-2 may fail to engage a coordinated type I response and instead may lead to exaggerated inflammation and death. This knowledge is central for the understanding of available data on specialized pro-resolving lipid mediators in severe SARS-CoV-2 infection pointing toward inhibited E-series resolvin synthesis in severe cases. By investigating a publicly available RNA-seq database of bronchoalveolar lavage cells from patients affected by COVID-19, we moreover offer insights into the regulation of key enzymes involved in lipid mediator synthesis, critically complementing the current knowledge about the mediator lipidome in severely affected patients. This review finally discusses different potential approaches to sustain the synthesis of 3-PUFA-derived pro-resolving lipid mediators, including resolvins and lipoxins, which may critically aid in the prevention of acute lung injury and death from COVID-19.

Keywords: innate immunity, COVID-19, lipid mediator, metabololipidomics, influenza virus, macrophages and neutrophils

INTRODUCTION: GENERAL CONCEPTS OF SARS-CoV-2 INFECTION AND ASSOCIATED INFLAMMATION

COVID-19, the infectious disease caused by the novel coronavirus SARS-CoV-2, currently represents a worldwide medical, economic, social, and political challenge (Mahase, 2020). SARS-CoV-2 was discovered in Wuhan, China, in December 2019 and has rapidly spread all over the world. In January 2020, the World Health Organization has declared a "Public Health Event of International Concern" and since March 11, 2020, COVID-19 has been characterized as a worldwide pandemic. Overall mortality rates are highly variable and range from 0.5 to 7%, very much depending on the stringency of testing in a given region, age, comorbidities of the patient, and access to medical treatment. When focusing on hospitalized patients, 20–40% were admitted to the intensive care unit (ICU) due to severe lung pathology needing ventilatory assists.

Of these, >50% required invasive mechanical ventilation (Karagiannidis et al., 2021) and 15–22% of these patients were reported to die in hospital, highlighting the potential threat to public health (Huang et al., 2020).

As of March 2021, the number of global deaths due to COVID-19 exceeds already 2,700,000 people (Johns Hopkins Coronavirus Resource Center, 2021). In the 2002–2003 SARS-CoV-1 outbreak, the clinical course was characterized by fever, cough, myalgia, and other systemic symptoms that generally improved after a few days, followed by a second phase with recurrence of fever and severe pneumonia, associated with a high case fatality rate of 11% (Peiris et al., 2003; Hui et al., 2005). SARS-CoV-1 and SARS-CoV-2 are phylogenetically closely related and cause a similar biphasic clinical course, but they are phylogenetically closely related (Walls et al., 2020). From a structural point of view, both coronaviridae share high homology for their transmembrane spike (S) glycoprotein, a viral surface protein crucial for entry into host cells and for initiation of immune response (Jaimes et al., 2020; Monteil et al., 2020; Walls et al., 2020). Specifically, both SARS-CoV interact directly with angiotensin-converting enzyme 2 (ACE2) *via* the S protein to enter alveolar cells and are believed to induce acute respiratory distress syndrome (ARDS) through ACE2 downregulation and shedding (Imai et al., 2005, 2008; Kuba et al., 2005, 2006; Blanco-Melo et al., 2020; Fu et al., 2020). Intrapulmonary loss of ACE2 leads to accumulation of angiotensin II, which appears to play a central role in the release of inflammatory cytokines, resulting in the activation of the IL-6 amplifier, which describes stimulation of the NF-κB and the JAK-STAT3 pathways resulting in inflammatory cytokine formation (Imai et al., 2005, 2008; Kuba et al., 2005, 2006; Blanco-Melo et al., 2020; Fu et al., 2020; Moore and June, 2020).

SARS-CoV-2 patients suffering from a complicated course of infection either fail to exert a robust, interferon (IFN)-mediated anti-viral response in the early phase of infection and present with an overwhelming immune activation termed as "cytokine storm" (Blanco-Melo et al., 2020; Fu et al., 2020). The latter is defined by increased levels of circulating cytokines accompanied by systemic and pulmonary immune cell activation in a similar setting as described in subjects suffering from ARDS or sepsis (Wilson et al., 2020). Importantly, patients with severe COVID-19 show loss-of-function variants in Toll-like receptor (TLR)- and IFN-dependent genes, or neutralizing antibodies to type I IFN (α and ω; Bastard et al., 2020; Zhang et al., 2020a). In addition, there are marked variance and temporal changes in the IFN gene signature during the course of COVID-19, possibly driving immunopathology (Nienhold et al., 2020).

Since its appearance in late 2019, COVID-19 has been repeatedly compared to other viral infections and among others mainly to influenza. From an epidemiological perspective, it seems reasonable to compare seasonal flu with COVID-19, given that they are respiratory diseases with similar modes of transmission. However, patients affected by COVID-19 exert strikingly different predisposing comorbidities and a more severe clinical course with higher morbidity and mortality rates as compared to seasonal influenza (Piroth et al., 2021). Patients affected by COVID-19 are more frequently obese or overweight, and show higher incidences of diabetes, hypertension, and dyslipidemia compared to patients with severe influenza (Piroth et al., 2021). The most obvious reason for the marked differences observed in epidemiology and fatality rates relies on the fact that SARS-CoV-2 engages immunological and thrombo-inflammatory circuits (Bösmüller et al., 2021) that differ from the well-known IFN-based response to influenza virus. Therefore, this review will initially focus on innate immunity in influenza and in SARS-CoV-2 infection comparing the respective host immune signatures, and subsequently depict recent findings on the mediator lipidome in COVID-19.

INNATE IMMUNITY IN INFLUENZA VIRUS INFECTION

Despite both bearing the potential of causing a severe infection of the lung, influenza and SARS-CoV-2 elicit several pathways of innate immunity that differ in many aspects. The main immunological differences between the "classical" immune response to influenza virus and the aberrant, "SIRS-like" response to SARS-CoV-2 important for this review will be outlined as follows:

Immunological responses to influenza are mainly driven by coordinated type I and III IFN release following TLR3, TLR7, and TLR8 activation. Besides TLR3 and TLR7/8, RIG-I-like receptors as well as nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are known to initiate the immune response to influenza virus. Whereas endosomal TLR3 detects double-stranded RNA (dsRNA), TLR7 and TLR8 sense single-stranded RNA (ssRNA); RIG-I specifically recognizes 5'-triphosphate RNA; and NLRs may directly recognize viral products, leading to the formation of inflammasomes (Biondo et al., 2019). RIG-I and NLRs have been comprehensively reviewed elsewhere (Root-Bernstein, 2021) and will not be covered in more detail here.

TLR3 differs from other TLRs in using TRIF (TIR domain-containing adaptor protein-inducing IFN-β) as signal adaptor, leading to synthesis of type I IFN mainly through the activation of NF-κB and IRF3 (Kawai and Akira, 2010; Ullah et al., 2016). In addition, dsRNA released from damaged cells may also activate TLR3 in resident or recruited macrophages in the lungs, which actively phagocytose dying and apoptotic cells (Schulz et al., 2005; Gosu et al., 2019). TLR3 appears to play an important role in influenza A virus (or seasonal influenza, H1N1)-induced innate host defense: Mice deficient in TLR3 had an unexpected survival advantage in the H1N1 infection model, despite a higher viral load in the lungs (Le Goffic et al., 2006). Mechanistically, TLR3-induced secretion of type I IFNs promotes the expression of the so-called IFN-stimulated genes (ISGs) within infected cells, including the serine–threonine kinase protein kinase R, IFITM3, and the myxovirus resistance protein 1 (MX1; Iwasaki and Pillai, 2014; Schoggins, 2014). ISGs inhibit viral entry into the cytosol as well as virus replication in the different cellular compartments of the lung. Moreover, type I IFNs potently activate natural killer (NK) cells, which kill virus-infected cells. Besides NK cells, also

neutrophils play an important role in response to acute H1N1 infection.

The contribution of neutrophils to the pathology conferred by influenza is exemplified by the 1918 pandemic virus, which induces a massive neutrophil recruitment to the lungs (Kobasa et al., 2004). Neutrophils may be protective at low virus titers by ingesting apoptotic cells, whereas they may further destroy the lung parenchyma when recruited at high numbers. Specifically, the release of reactive oxygen species and neutrophil extracellular traps may aid in the development of ARDS (Tate et al., 2009; Narasaraju et al., 2011).

Finally, alveolar macrophages appear to play a pivotal role in the host defense against influenza. Alveolar macrophages, together with epithelial and dendritic cells, were found to produce anti-viral type I IFNs, but also several proinflammatory cytokines and chemokines capable of attracting neutrophils and monocytes (De Jong et al., 2006; Jayasekera et al., 2006; Narasaraju et al., 2011). Recruited monocytes differentiate into inflammatory macrophages, which greatly amplify cytokine production. Both macrophages and neutrophils can ingest H1N1-infected cells, particularly when they are damaged or apoptotic, thereby promoting viral clearance and elimination of cell debris (Tumpey et al., 2005; Watanabe et al., 2005).

INNATE IMMUNITY IN SARS-CoV-2 INFECTION

In contrast to influenza virus, SARS-CoV-2 was found to activate TLR4 (**Figure 1**) and TLR4-related pathways through binding of its spike protein in human and murine macrophages, resulting in IL-6-mediated hyperinflammation (Shirato and Kizaki, 2021). TLR4 activation by SARS-CoV-2 spike subunit S1 can be suppressed by selective inhibitors of NF-κB and JNK pathways (Shirato and Kizaki, 2021). Interestingly, *in silico* studies had predicted TLR4 to recognize molecular patterns of SARS-CoV-2 (Choudhury and Mukherjee, 2020). Direct activation of the TLR4 may switch the anti-viral response of a cell from a response otherwise dominated by type I IFNs to the release of mainly pro-inflammatory mediators, explaining at least in part the hyperinflammation associated with severe COVID-19. In addition, type I IFN response may further be blunted by changes in the Fc component of SARS-CoV-2-directed antibodies, as a recent study suggested (Combes et al., 2021). During the course of a disease, the characteristics of newly produced antibodies may fine-tune the immune response. One aspect of these changes is an alteration in the antibody Fc component that determines which Fc receptors will be engaged (Gentili and Hacohen, 2021). In this regard, engagement with the Fc receptors CD64, CD16, and CD32 can determine how the immune system combats viral infections. Using immune cells from healthy donors exposed to IFN-α and plasma from patients with severe COVID-19, Combes et al. individually blocked CD64, CD16, and CD32 Fc receptors and found that CD32 blockade enabled the expression of IFN-regulated genes (Combes et al., 2021; Gentili and Hacohen, 2021). Importantly, the CD32 Fc receptor exists in the two forms, CD32A and CD32B, respectively. CD32A engagement activates the immune system, whereas CD32B dampens immune responses (Gentili and Hacohen, 2021). Combes and colleagues showed that the inhibition of IFN-regulated genes, including IFITM3 and MX1, in severe COVID-19 cases was due to CD32B engagement. These data indicate that patients with severe COVID-19 may develop antibodies that interact with CD32B Fc receptors and thereby blunt IFN-mediated host defense (Combes et al., 2021). Accordingly, a subset of ISG-expressing monocytes and neutrophils was identified only in blood samples of patients with moderate disease and was almost absent in patients with severe COVID-19 (Combes et al., 2021).

Corroborating a failure in IFN response in severe COVID-19 cases, Casanova and coworkers recently identified patients with severe COVID-19 that bear mutations in genes involved in the regulation of type I and III IFN immunity. Specifically, loss-of-function mutations were found in genes that govern TLR3- and IFN regulatory factor 7 (IRF7)-dependent type I IFN immunity to influenza virus (Zhang et al., 2020a). In addition, another study by this laboratory identified individuals with high titers of neutralizing autoantibodies against type I IFN-α2 and IFN-ω in about 10% of patients with severe COVID-19 pneumonia (Bastard et al., 2020). These autoantibodies were found neither in infected people who were asymptomatic nor in those with mild infection or in healthy individuals (Bastard et al., 2020).

Further evidence for a dysregulated type I IFN response in severe SARS-CoV-2 infection comes from studies in patients previously infected with phylogenetically closely related SARS-CoV-1 and MERS-CoV, where blunted IFN response was associated with severe pathology and disease (Cameron et al., 2007; Channappanavar et al., 2016; Kindler et al., 2016). Overall, patients with severe COVID-19 infection may present with typical features of macrophage activation syndrome which is partly resulting from overwhelming IFNγ formation (Webb et al., 2020) as reflected by higher levels of the IFNγ-inducible macrophage-derived biomarker neopterin in subjects with severe COVID-19 (Bellmann-Weiler et al., 2021).

COMPARISON OF INNATE IMMUNITY IN INFLUENZA AND SARS-CoV-2 INFECTION

Altogether, mild-to-moderate SARS-CoV-2 infections appear to resolve at least in part due to an adequate anti-viral IFN-mediated response. In contrast, severe cases of COVID-19 not only fail to build up a robust type I IFN response in the initial phase but also show an uncontrolled hyperinflammatory response in the subsequent course of infection leading to multi-organ damage and death. Some reports moreover suggest that in severe COVID-19 patients, uncoordinated IFN response may further amplify TNF/IL-1β-centered hyperinflammatory signatures (Lee et al., 2020). This is *per se* not contradictory to the total absence of IFNs in patients born with loss-of-function mutations in type I IFN pathways. In the latter setting, patients will experience a delayed clearance of virus together

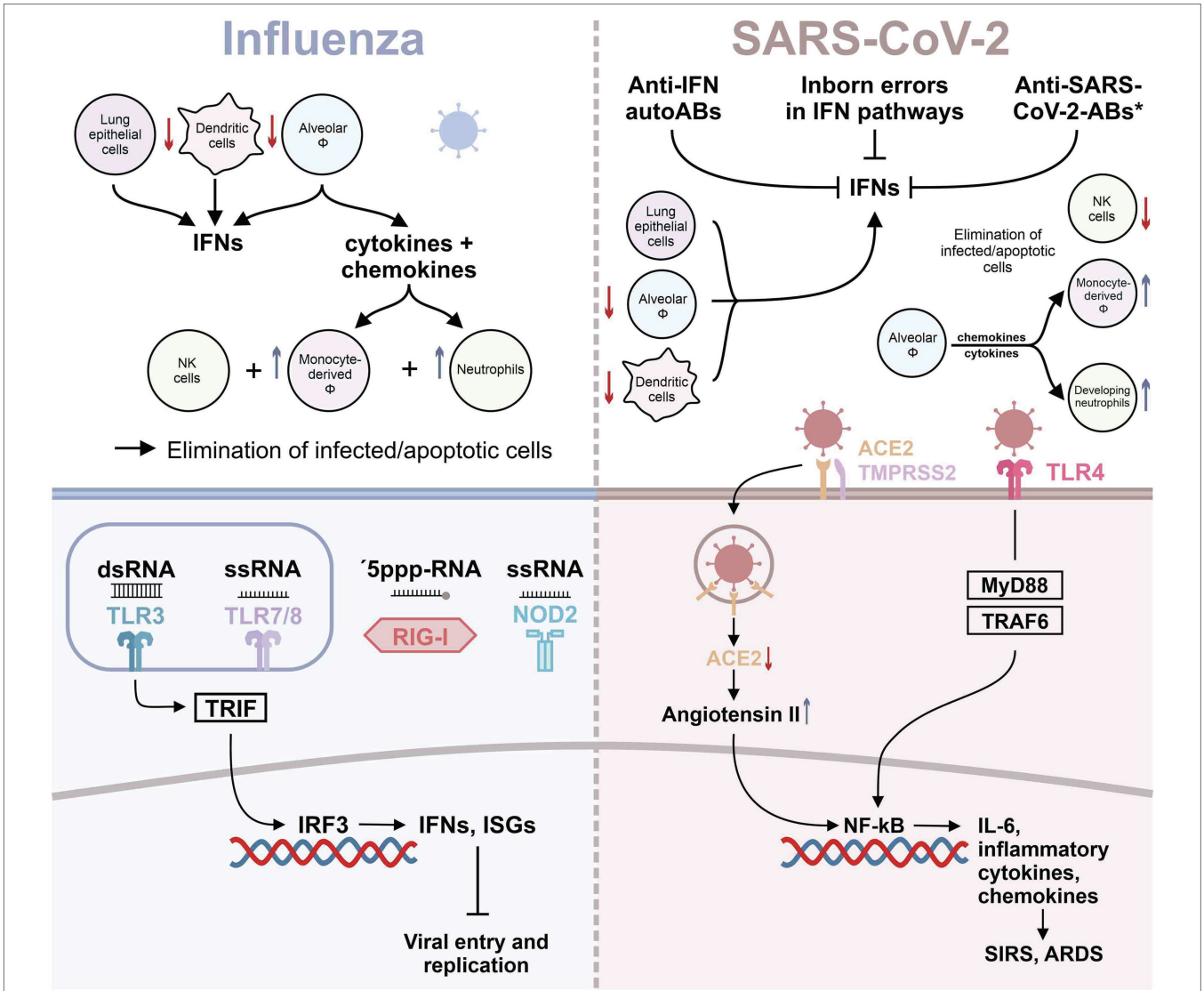


FIGURE 1 | Innate immunity in influenza and SARS-CoV-2 infection. The graph summarizes the current knowledge on innate immunity responses in influenza virus and in SARS-CoV-2 infection of the lung. *refers to anti-SARS-Cov-2 antibodies engaging with CD32B Fc receptors (Combes et al., 2021).

with an exaggerated immune response further amplified by IFNs. On the other hand, in patients with inborn errors in IFN-related genes unrestrained viral replication will end up in an overwhelming infection leading to an exaggerated myeloid cell activation. The initial failure to mount an efficient anti-viral immune response is also supported by the finding of low lymphocyte and specifically $CD4^+$ counts to be associated with higher cytokine levels and a more severe course of the infection (Zhang et al., 2020b).

Again emphasizing the difference between influenza and COVID-19, a recent report shows that in circulating leukocytes of COVID-19 patients, ISGs are expressed at a higher level than in healthy subjects but are more muted than seen with seasonal coronaviruses and much lower than seen in influenza infection (McClain et al., 2020). Shedding light on the impact of dysregulated immune responses on the recruitment of innate immune cells, Blanco-Melo et al. showed that COVID-19 patients exhibit elevated serum levels of pro-inflammatory cytokines: Increased levels of CXCL9 and CXCL16 may serve to attract NK cells, CCL8 and CCL2 to recruit monocytes and macrophages, and CXCL8 to recruit neutrophils (Blanco-Melo et al., 2020). Interestingly, using a comprehensive single-cell RNA sequencing approach, Blish and coworkers showed that during COVID-19 infection, several innate immune cell subsets are depleted, including γδ T cells, plasmacytoid dendritic cells (pDCs), and NK cells (Wilk et al., 2020). Loss of pDCs and NK cells may hamper viral elimination first by absent IFN I signal amplification *via* IRF7 (Zhang et al., 2020a) and second by reduced elimination of virus-infected cells. Moreover, the authors showed a significant loss of anti-inflammatory $CD16^+$ non-classical monocytes which are recognized as the first line of defense in recognition

and clearance of pathogens (Narasimhan et al., 2019; Winkler et al., 2021).

Finally, a novel cell population annotated as "developing neutrophils" was significantly increased only in COVID-19 patients with ARDS, which seems to represent emergency granulopoiesis (Yvan-Charvet and Ng, 2019; Wilk et al., 2020). Single-cell RNA sequencing of immune cells retrieved from bronchoalveolar lavage fluid (BALF) indicated loss of alveolar macrophages in severe cases accompanied by recruitment of inflammatory monocytes and neutrophils, resulting in a highly proinflammatory microenvironment in the lung (Liao et al., 2020). Corroborating the results by Blish and coworkers (Wilk et al., 2020), the latter study described reduced pDCs and NK cell numbers also in BALF of severe COVID-19 cases, inferring a systemic depletion of these leukocyte subsets rather than intrapulmonary exhaustion (Liao et al., 2020). Similar to what was described in humans with severe COVID-19, macrophages were found to drive inflammation within the lungs also in African green monkeys subjected to SARS-CoV-2 infection. Monocyte-derived newly recruited rather than resident alveolar macrophages were found to clear infected cells and debris, aiding in the resolution of infection (Speranza et al., 2021). Loss of alveolar macrophages during SARS-CoV-2 infection was present also in K18-hACE2-transgenic mice, with intrapulmonary neutrophil and Ly6C$^+$ monocyte [the murine equivalent to CD14$^+$CD16$^-$ classical monocytes in men (Ziegler-Heitbrock et al., 2010)] numbers increasing over 7 days post-infection (Winkler et al., 2020; The main mechanisms of inflammation in influenza as opposed to SARS-CoV-2 infection are summarized in **Figure 1**).

BIOACTIVE LIPID MEDIATORS IN VIRUS INFECTION, FROM INFLUENZA VIRUS TO SARS-CoV-2

With respect to alveolar inflammation, bioactive lipids are of highest significance. This is exemplified by the fact that eicosanoid metabolism has taken center stage as druggable axis in asthmatic disease. Activation of immune cells of myeloid and monocytic origin results in the release of arachidonic acid (AA) mediated by phospholipases (Demetz et al., 2014). Subsequent metabolism of AA with 5-lipoxygenase (5-LOX) as central enzyme results in the formation of leukotrienes, such as leukotriene B4 (LTB4) or the cysteinyl-leukotrienes as, for example, leukotriene D4 (Haeggström, 2018). Leukotrienes act *via* the BLT and cysteinyl leukotrienes (CysLT) receptors. LTB4 is a pivotal chemotactic agent for neutrophils in the initial phase of inflammation, which under physiological conditions is followed by a temporal switch in lipid mediators finally leading to resolution and tissue homeostasis (Spite et al., 2014). While maximal levels of LTB4 are reached as neutrophils infiltrate the infected lung, other eicosanoids, including the prostaglandins PGE2 and PGD2, lead to a lipid mediator class switch (Levy et al., 2001). This class switch initiates translational regulation of the enzymes required for the production of pro-resolving lipid mediators, including lipoxin A4 (LXA4; Spite et al., 2014) and inhibition of platelets (Braune et al., 2020). LXA4 serves as endogenous regulator of neutrophil trafficking, and its production is associated with cessation of neutrophil infiltration during the inflammatory response (Levy et al., 2001). The resolution phase of inflammation is characterized by the recruitment of monocytes to the injured tissue which then differentiate into macrophages and actively clear apoptotic cells (Spite et al., 2014). Besides LXA4, further so-called specialized pro-resolving lipid mediators (SPMs) are synthetized during this phase of inflammation, including resolvins, protectins, and maresins. SPMs blunt neutrophil infiltration, decrease pro-inflammatory mediator production, and stimulate macrophage-dependent uptake of apoptotic leukocytes as well of cell debris (Levy et al., 2001; Spite et al., 2014). Failure of such a tightly orchestrated resolution will end up in chronic inflammation and tissue damage.

Inflammatory stimuli of the lung may moreover lead to the production of CysLT, such as LTD4 mainly causing smooth muscle cell contraction in the respiratory tract (Gentile et al., 2003; Haeggström, 2018). This finding has led to the development of the CysLT 1 receptor antagonists (e.g., montelukast) as well as the 5-LOX inhibitor zileuton, highly useful drugs in the management of asthmatic disease (Wenzel and Kamada, 1996; Hon et al., 2014; Theron et al., 2014; Dahlin et al., 2016). A combination therapy has recently also been suggested for the use in patients infected with SARS-CoV-2 (Funk and Ardakani, 2020). Finally, eicosanoid metabolism is well known to play an important role in platelet activation which may be an additional link to the observed frequent thrombotic complications during SARS-CoV-2 infection (Gupta et al., 2020; Bösmüller et al., 2021). In turn, lipid and in particular eicosanoid metabolism deserve increased attention, as possible druggable pathways in SARS-CoV-2. For additional information, please refer to a recent overview of eicosanoid metabolism in SARS-CoV-2 (Hoxha, 2020).

Upon viral infection of the lung, SPMs appear to be involved in immunopathology, which include docosahexaenoic acid (DHA)-derived protectins and D-series resolvins (RvD1-RvD6), and the eicosapentaenoic acid (EPA)-derived E-series resolvins (Serhan et al., 2002, 2006, 2015; Duffield et al., 2006; Schwab et al., 2007; Serhan, 2007; Serhan and Petasis, 2011; Arita, 2012; Isobe et al., 2012; Imai, 2015; Libreros et al., 2021). In a systems biology approach, Imai and colleagues identified protectin D1 (PD1) to protect from lethal H5N1 influenza infection in mice by impairing virus replication *via* the RNA export machinery (Morita et al., 2013). Interestingly, by comparing PR8/H1N1 with the low-pathogenicity influenza strain X31/H3N2, Tam et al. showed that 5-LOX metabolites correlated with the pathogenic phase of infection, whereas 12/15-LOX metabolites were associated with the resolution phase (Tam et al., 2013).

In her review *Role of omega-3 PUFA-derived mediators, the protectins, in influenza virus infection*, Yumiko Imai concluded that despite their main limitation of a short half-life, omega-3-derived PUFA, including PD1 and stable analogs, may represent an attractive strategy to treat influenza infection (Imai, 2015).

Besides being implicated in the synthesis of lipoxins, 5-LOX main action lies in the production of omega-6 PUFA-derived leukotrienes and prostaglandins upon infection, driving leukocyte recruitment and activation, vasodilation, bronchoconstriction, and vasopermeability, as outlined above. Thus, increasing omega-3 PUFA and decreasing omega-6 PUFA levels may represent a possible mean to skew the immune response toward resolution of inflammation, which led to the conception of the *COVID-Omega-F* Trial with the aim of resolving the cytokine storm in COVID-19 patients by supplementation of hospitalized patients with high-dose omega-3 PUFA i.v. over 5 days (Arnardottir et al., 2021), ClinicalTrials.gov Identifier: NCT04647604, estimated study termination date April 30, 2021. However, the situation seems more complex than a simplified omega-3/omega-6 classification would reflect. For example, while prostaglandin E2 is frequently regarded as pro-inflammatory mediator, several recent studies have underlined its anti-inflammatory and tissue regenerative functions (FitzGerald, 2015; Duffin et al., 2016). Along these lines, a recent phase II trial by Haeberle et al. is testing the synthetic prostacyclin (PGI2) analog iloprost for the treatment of ARDS (Haeberle et al., 2020). Moreover, just recently, the omega-6 PUFA adrenic acid and its derivatives have been shown to exert anti-inflammatory properties (Brouwers et al., 2020). Additionally, linolenic acid has been reported as potential substrate for 15-LOX, producing trihydroxyoctadecenoic acids in eosinophils with potential anti-inflammatory/pro-resolving functions (Fuchs et al., 2020). Overall, cell-specific as well as spatiotemporal effects will ultimately sketch a detailed picture of SARS-CoV-2-related changes in lipid mediator biosynthesis; up to now, only a limited number of studies have addressed such changes, as outlined below.

Due to the striking differences in the response of the innate immune system, lipidomics data derived from lethal influenza infection may not directly be applicable to a severe infection with SARS-CoV-2. However, lipid mediators, including PD1, RvDs, and RvEs, exert strong anti-inflammatory activity. In this regard, supplementation with omega-3 PUFA showed controversial results in patients affected by ARDS. While IV emulsions with DHA and EPA were shown to be protective (Pontes-Arruda and Hirasawa, 2011), dietary supplementation with fishoil or with n-3 fatty acids, γ-linolenic acid, and antioxidants did not show a clear benefit in ARDS (Rice et al., 2011; Stapleton et al., 2011). On the other hand, independent meta-analyses indicated that supplementation with omega-3 PUFA in patients with ARDS would associate with improvements in the PaO2/FiO2 ratio, with a shorter ICU stay, a shorter duration of mechanical ventilation, and a trend toward reduced mortality (Dushianthan et al., 2019; Langlois et al., 2019; Arnardottir et al., 2021).

Up to date, little is known about changes in the mediator lipidome during acute SARS-CoV-2 infection. There is however one comprehensive analysis showing that serum from patients with a moderate and severe COVID-19 course displays specific differences in abundance of immune regulatory and pro-inflammatory lipid mediators (Schwarz et al., 2021). The authors show that moderate versus severe infections were characterized by unique lipidomic profiles. Of particular interest was the observation that specific pro-resolving mediators, including RvE3 and RvD4, were increased in serum from patients with moderate COVID-19 compared to subjects with severe disease. Moderate disease furthermore was associated with increased levels of PGs, particularly PGE2. In contrast, severe COVID-19 was associated with a trend to increased serum levels of D-series resolvins RvD1-3 and LXA4 (Schwarz et al., 2021). Bioactive lipid mediators are generated by sequential activity of different enzymes, namely, 5-LOX, 12-LOX, 15-LOX, COX, and cytochrome p450 (Cyp450). Grouping according to different enzymatic pathways showed that moderate disease was characterized by higher levels of lipid mediators that require COX and 12-LOX activity, whereas severe disease was characterized by lipid mediators that require activity of 5-LOX and Cyp450 (Schwarz et al., 2021). By mining a published single-cell RNA sequencing dataset in PBMCs from severe COVID-19 patients, the authors found increased *ALOX5* expression in $CD14^+$ and $CD16^+$ monocytes and in neutrophils reflecting emergency granulopoiesis (Schwarz et al., 2021).

In a similar approach, our group interrogated a published single-cell RNA sequencing dataset from BALF in patients affected by COVID-19 (Liao et al., 2020) and found *ALOX5* to be downregulated in BALF macrophages and DCs from patients affected by severe COVID-19, compared to healthy individuals and to patients with moderate COVID-19. Vice versa, *ALOX5* expression was increased in BALF neutrophils in severe disease, although at an overall low expression level (**Figure 2**). 5-LOX requires a set of stimulatory factors for full activity and is supported by accessory proteins, including 5-LOX-activating protein (FLAP; *ALOX5AP*; Haeggström, 2018). Importantly, we found a decrease in *ALOX5AP* expression levels in macrophages and DCs in BALF from severe COVID-19 patients, whereas RNA expression of this central activating protein tended to be increased in neutrophils of patients affected by severe disease (**Figure 3**). Finally, the expression of a LOX involved in the synthesis of pro-resolving lipid mediators, namely, *ALOX15*, was found highest in lung epithelia of patients affected by moderate COVID-19, potentially conferring an anti-inflammatory role to this cellular lung compartment upon SARS-CoV-2 infection (**Figure 4**). These data indicate differences in expression and potentially activity of 5-LOX between pulmonary macrophages and circulating monocytes in patients with severe COVID-19. Moreover, the differences in expression of *ALOX5* and *ALOX5AP* in cells of myeloid origin found in the lungs of patients with moderate and severe disease may contribute to the specific differences in abundance and immune-modulatory functions of resolvins and lipoxins (Schwarz et al., 2021; An overview on the main lipid metabolome changes in influenza and SARS-CoV-2 infection is given in **Figure 5**).

Interestingly, LXA4 and isomers of the D-series resolvin RvD6 were previously found to reduce the expression of ACE2 and to counteract the binding of the receptor-binding domain of SARS-CoV-2 spike protein to injured tissue

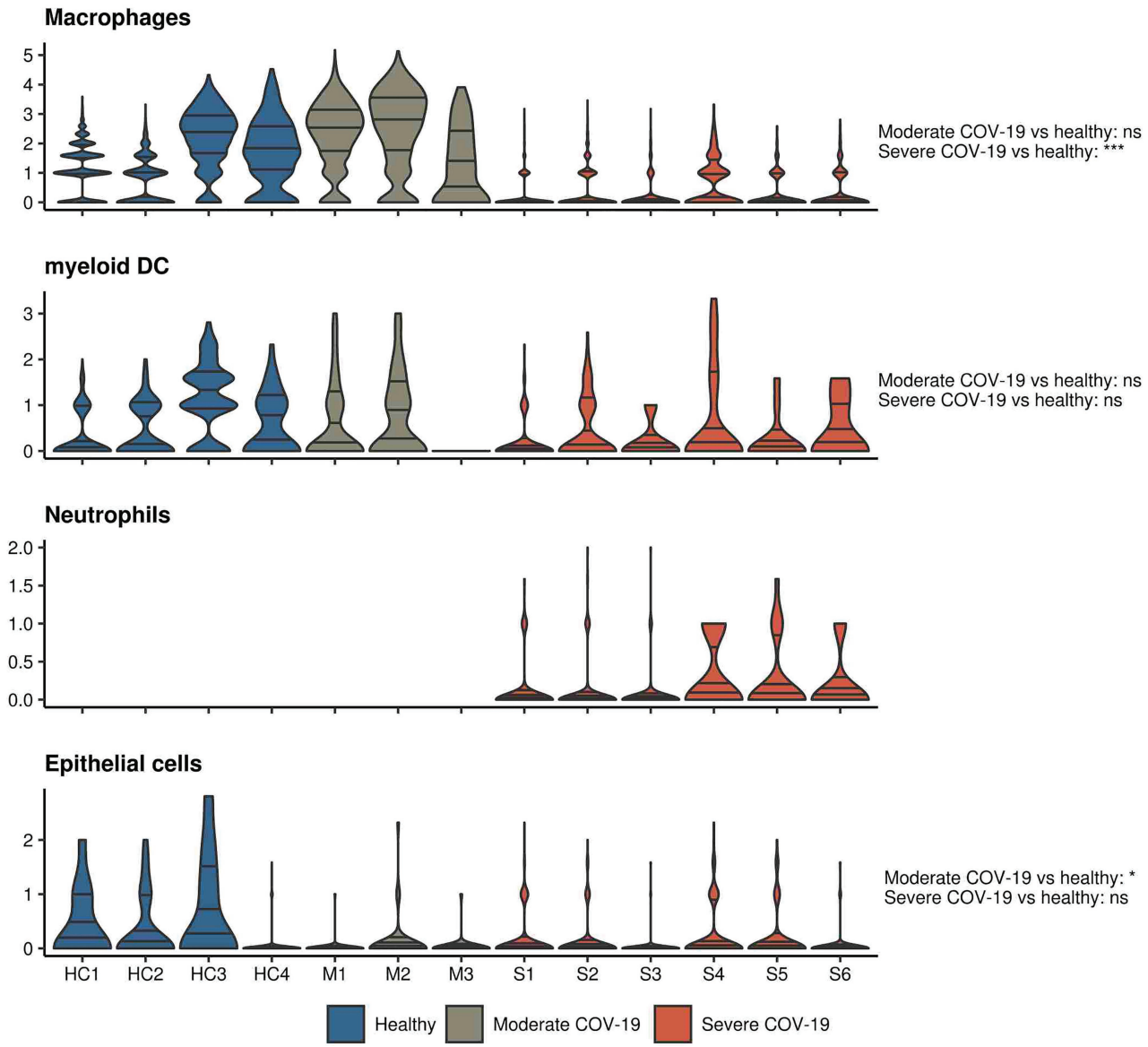


FIGURE 2 | *ALOX5* expression in bronchoalveolar lavage fluid (BALF) cells from patients affected by COVID-19. Violin plots depicting *ALOX5* expression levels within specific cellular populations in BALF cells from healthy (blue, $n = 4$), moderate (gray, $n = 3$), or severe (red, $n = 6$) COVID-19 patients. RNA sequencing data are publicly available (Liao et al., 2020). ns = non-significant, $^{***}p < 0.001$.

(Pham et al., 2020). The SARS-CoV-2 spike protein S1 subunit was moreover found to induce RvD1 in macrophages from patients affected by cystic fibrosis (CF). Importantly, RvD1 and RvD2 counteracted the inflammatory response to SARS-CoV-2 spike protein in both CF and non-CF macrophages, while potentiating their host defensive, phagocytic functions (Recchiuti et al., 2021).

From a clinical perspective, it will be important to develop biomarkers allowing to predict the course of a COVID-19 infection, to stratify patients for specific treatments and deduce novel potential targets to prevent a severe course of infection. Given the fact that LOX and FLAP proteins have been identified as key players in SARS-CoV-2 infections, several possibilities arise. With respect to disease and patient stratification biomarkers, LOX pathway markers, such as 5-HETE, 15-HETE, and other mono-hydroxylated PUFA derivatives, should be considered as possible diagnostic tools to predict the subsequent course of the infection and to

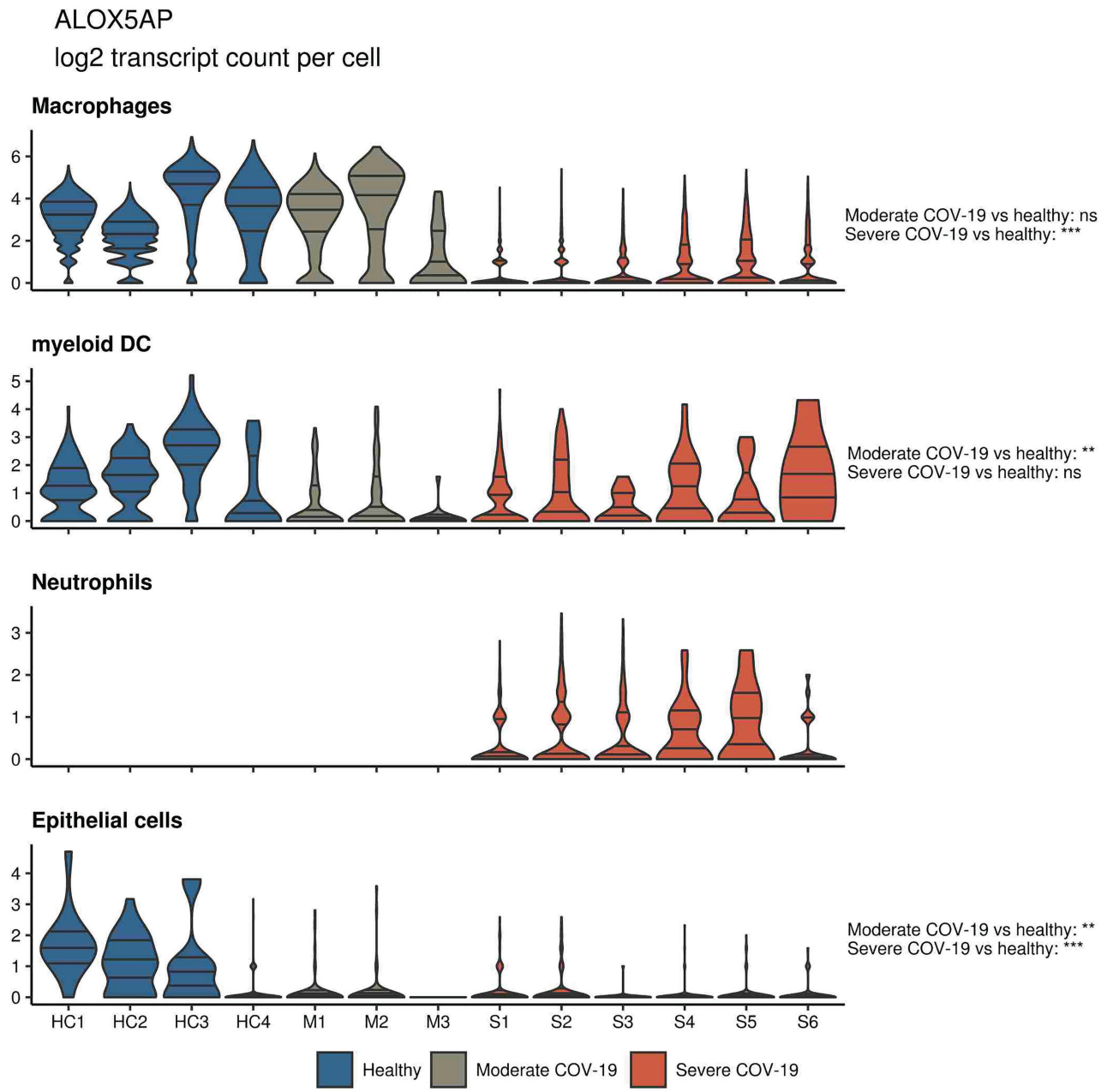


FIGURE 3 | *ALOX5AP* expression in BALF cells from patients affected by COVID-19. Violin plots depicting *ALOX5AP* expression levels within specific cellular populations in BALF cells from healthy (blue, $n = 4$), moderate (gray, $n = 3$), or severe (red, $n = 6$) COVID-19 patients. RNA sequencing data are publicly available (Liao et al., 2020). ns = non-significant, $^{**}p < 0.01$, $^{***}p < 0.001$.

induce specific targeted therapies. With regard to therapeutic interventions, omega-3 PUFA as investigated in the *COVID-Omega-F* Trial may represent an attractive approach to counteract pathologic inflammation thereby preventing lung dysfunction and need for mechanical ventilation. This view has just recently been backed up by Darwesh et al. proposing omega-3 PUFA as adjuvant therapy (Darwesh et al., 2021). Additionally, several drugs, such as for example montelukast or zileuton, have been designed to target leukotrienes in inflammatory lung diseases. As outlined by Funk and Ardakani (2020), a dual-treatment paradigm targeting leukotrienes as the final pro-inflammatory mediators of the 5-LOX pathway might pose a scientifically sound approach, which to our knowledge has however been neglected so far. Unfortunately, this counts for the entire eicosanoid pathway as recently outlined by Hammock et al. (2020), even though it bears a central role in pro- and anti-inflammatory responses triggered by infectious agents (Dennis and Norris, 2015).

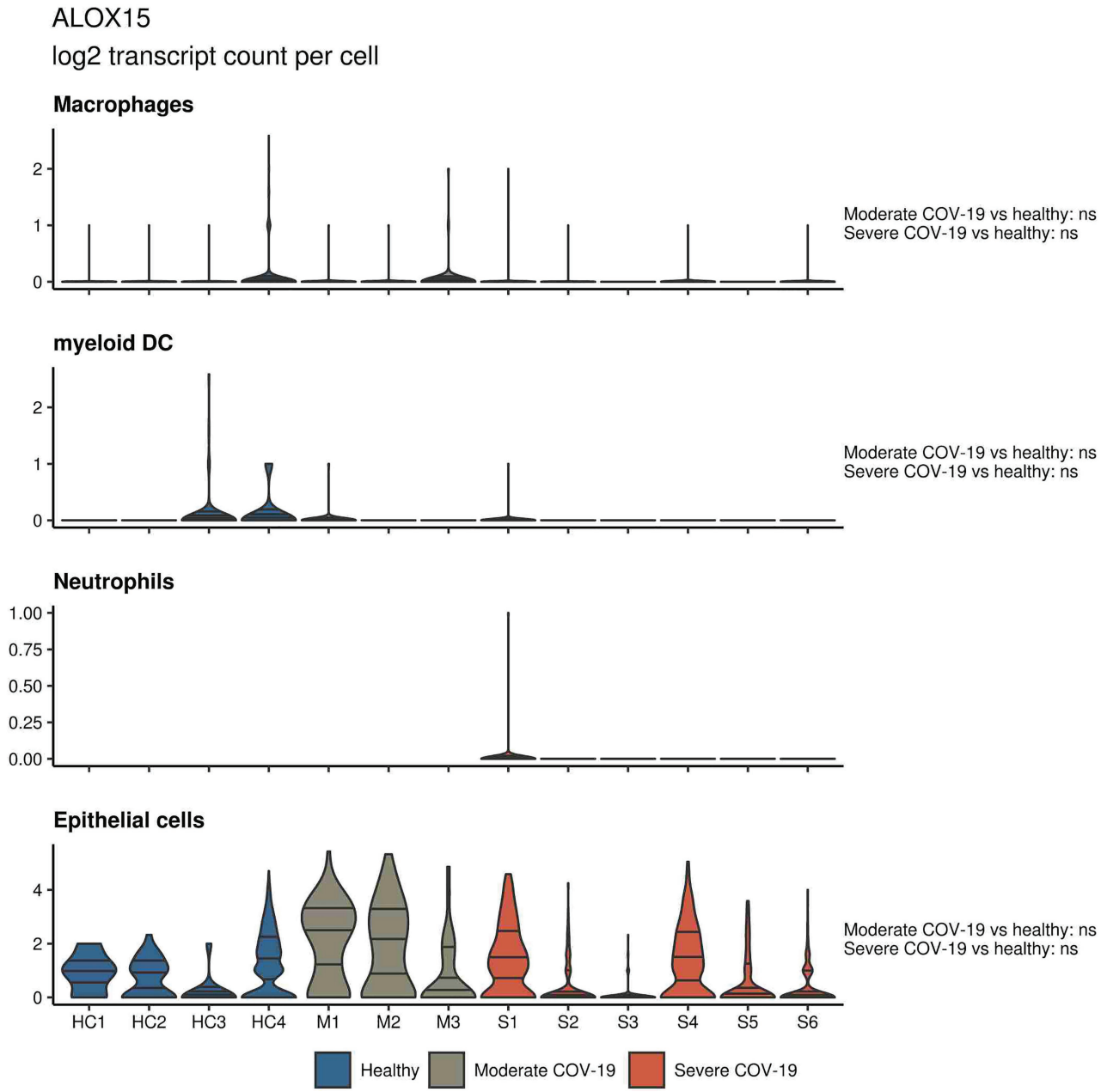


FIGURE 4 | *ALOX15* expression in BALF cells from patients affected by COVID-19. Violin plots depicting *ALOX15* expression levels within specific cellular populations in BALF cells from healthy (blue, *n* = 4), moderate (gray, *n* = 3), or severe (red, *n* = 6) COVID-19 patients. RNA sequencing data are publicly available (Liao et al., 2020). ns = non-significant.

In fact, the eicosanoid pathway presents several interesting targets for the treatment of COVID-19. In addition to the targets suggested by Funk and Ardakani, COX, microsomal prostaglandin E2 synthase-1 (mPGES-1; Bergqvist et al., 2020) as well as soluble epoxide hydrolase inhibitors (Hammock et al., 2020) present interesting novel avenues for the treatment of COVID-19. Particularly, the latter might ideally be combined with omega-3 PUFA substitution, boosting the production of epoxyeicosanoids exerting anti-inflammatory as well as tissue regenerative functions (Morisseau and Hammock, 2013).

Besides the extensive research on SARS-CoV-2 and the gained knowledge, there are still points that require further investigation to complete the picture of SARS-CoV-2 infection, COVID-19 disease progression and resolution. Increasing the knowledge about the complex interplay between lipid mediators, the immune system and SARS-CoV-2 infection will yield novel insights into underlying

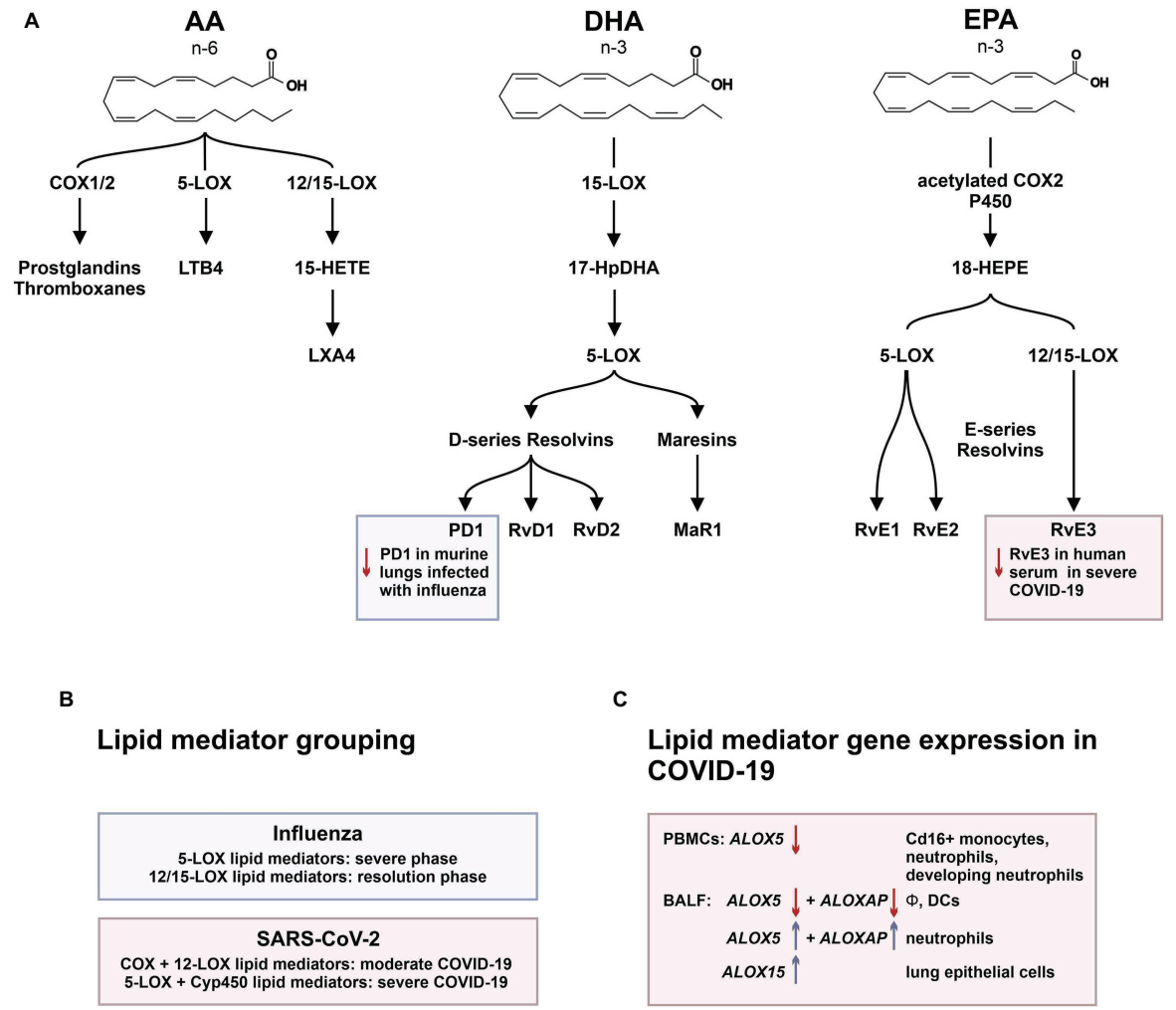


FIGURE 5 | The mediator lipidome in influenza and SARS-CoV-2 infection. **(A)** The graph summarizes common 6-PUFA- and 3-PUFA-derived bioactive and pro-resolving lipid mediators (AA, arachidonic acid; DHA, docosahexaenoic acid; and EPA, eicosapentaenoic acid). While severe influenza infection showed inhibition of protectin D1 (PD1) synthesis, severe SARS-CoV-2 infection was associated with a significant reduction in E-series resolvin 3 (RvE3). **(B)** Lipid mediator grouping according to synthetic pathways. **(C)** Transcriptional regulation of key enzymes involved in lipid mediator synthesis in PBMCs and BALF cells from patients affected by COVID-19.

pathomechanisms and will provide the basis for novel therapeutic strategies.

MATERIALS AND METHODS

Single-cell sequencing data provided by Liao et al. (2020) were analyzed with R programing suite version 4.0.3 and tidyverse package bundle. In brief, transcript counts per cell in BALF macrophages from healthy and COVID-19 individuals were extracted from the table with normalized expression and sample-cell-individual assignment table provided by the authors. For visualization as violin plots (package ggplot2), transcript counts were transformed with the log2(x + 1) function. Statistical significance for differences in transcript numbers per cell between the COVID-19 severity groups and healthy controls was assessed by mixed-effects generalized linear modeling (log link function, expected distribution of residuals: Poisson, package lme4) with the fixed effect of the study group and the random effect of the cell donor.

AUTHOR CONTRIBUTIONS

SS, IT, RM, GW, AT, and MG wrote the manuscript. PT performed bioinformatics analyses. All authors critically reviewed the final version of the manuscript.

FUNDING

IT's work on COVID-19 has been supported by Boehringer Ingelheim RCV GmbH & Co KG, and by AstraZeneca. AT was supported by the Botnar Research Centre for Child Health (BRCCH) Basel. CG-T has been supported by the Austrian Science Fund (FWF; grant P32821).

REFERENCES

Arita, M. (2012). Mediator lipidomics in acute inflammation and resolution. *J. Biochem.* 152, 313–319. doi: 10.1093/jb/mvs092

Arnardottir, H., Pawelzik, S. C., Öhlund Wistbacka, U., Artiach, G., Hofmann, R., et al. (2021). Stimulating the resolution of inflammation through Omega-3 polyunsaturated fatty acids in COVID-19: rationale for the COVID-omega-F trial. *Front. Physiol.* 11:624657. doi: 10.3389/fphys.2020.624657

Bastard, P., Rosen, L. B., Zhang, Q., Michailidis, E., Hoffmann, H. H., Zhang, Y., et al. (2020). Autoantibodies against type I IFNs in patients with life-threatening COVID-19. *Science* 370:eabd4585. doi: 10.1126/science.abd4585

Bellmann-Weiler, R., Lanser, L., Burkert, F., Seiwald, S., Fritsche, G., Wildner, S., et al. (2021). Neopterin predicts disease severity in hospitalized patients with COVID-19. *Open Forum Infect. Dis.* 8:aa521. doi: 10.1093/ofid/ofaa521

Bergqvist, F., Morgenstern, R., and Jakobsson, P. J. (2020). A review on mPGES-1 inhibitors: from preclinical studies to clinical applications. *Prostaglandins Other Lipid Mediat.* 147:106383. doi: 10.1016/j.prostaglandins.2019.106383

Biondo, C., Lentini, G., Beninati, C., and Teti, G. (2019). The dual role of innate immunity during influenza. *Biom. J.* 42, 8–18. doi: 10.1016/j.bj.2018.12.009

Blanco-Melo, D., Nilsson-Payant, B. E., Liu, W. C., Uhl, S., Hoagland, D., Møller, R., et al. (2020). Imbalanced host response to SARS-CoV-2 drives development of COVID-19. *Cell* 181, 1036.e9–1045.e9. doi: 10.1016/j.cell.2020.04.026

Bösmüller, H., Matter, M., Fend, F., and Tzankov, A. (2021). The pulmonary pathology of COVID-19. *Virchows Arch.* 478, 137–150. doi: 10.1007/s00428-021-03053-1

Braune, S., Küpper, J. H., and Jung, F. (2020). Effect of prostanoids on human platelet function: an overview. *Int. J. Mol. Sci.* 21:9020. doi: 10.3390/ijms21239020

Brouwers, H., Jónasdóttir, H. S., Kuipers, M. E., Kwekkeboom, J. C., Auger, J. L., Gonzalez-Torres, M., et al. (2020). Anti-inflammatory and proresolving effects of the Omega-6 polyunsaturated fatty acid adrenic acid. *J. Immunol.* 205, 2840–2849. doi: 10.4049/jimmunol.1801653

Cameron, M. J., Ran, L., Xu, L., Danesh, A., Bermejo-Martin, J. F., Cameron, C. M., et al. (2007). Interferon-mediated Immunopathological events are associated with atypical innate and adaptive immune responses in patients with severe acute respiratory syndrome. *J. Virol.* 81, 8692–8706. doi: 10.1128/JVI.00527-07

Channappanavar, R., Fehr, A. R., Vijay, R., Mack, M., Zhao, J., Meyerholz, D. K., et al. (2016). Dysregulated type i interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in sars-cov-infected Mice. *Cell Host Microbe* 19, 181–193. doi: 10.1016/j.chom.2016.01.007

Choudhury, A., and Mukherjee, S. (2020). In silico studies on the comparative characterization of the interactions of SARS-CoV-2 spike glycoprotein with ACE-2 receptor homologs and human TLRs. *J. Med. Virol.* 92, 2105–2113. doi: 10.1002/jmv.25987

Combes, A. J., Courau, T., Kuhn, N. F., Hu, K. H., Ray, A., Chen, W. S., et al. (2021). Global absence and targeting of protective immune states in severe COVID-19. *Nature* 591, 124–130. doi: 10.1038/s41586-021-03234-7

Dahlin, A., Litonjua, A., Irvin, C. G., Peters, S. P., Lima, J. J., Kubo, M., et al. (2016). Genome-wide association study of leukotriene modifier response in asthma. *Pharm. J.* 16, 151–157. doi: 10.1038/tpj.2015.34

Darwesh, A. M., Bassiouni, W., Sosnowski, D. K., and Seubert, J. M. (2021). Can N-3 polyunsaturated fatty acids be considered a potential adjuvant therapy for COVID-19-associated cardiovascular complications? *Pharmacol. Ther.* 219:107703. doi: 10.1016/j.pharmthera.2020.107703

De Jong, M. D., Simmons, C. P., Thanh, T. T., Hien, V. M., Smith, G. J. D., Chau, T. N. B., et al. (2006). Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nat. Med.* 12, 1203–1207. doi: 10.1038/nm1477

Demetz, E., Schroll, A., Auer, K., Heim, C., Patsch, J. R., Eller, P., et al. (2014). The arachidonic acid metabolome serves as a conserved regulator of cholesterol metabolism. *Cell Metab.* 20, 787–798. doi: 10.1016/j.cmet.2014.09.004

Dennis, E. A., and Norris, P. C. (2015). Eicosanoid storm in infection and inflammation. *Nat. Rev. Immunol.* 15, 511–523. doi: 10.1038/nri3859

Duffield, J. S., Hong, S., Vaidya, V. S., Lu, Y., Fredman, G., Serhan, C. N., et al. (2006). Resolvin D series and Protectin D1 mitigate acute kidney injury. *J. Immunol.* 177, 5902–5911. doi: 10.4049/jimmunol.177.9.5902

Duffin, R., O'Connor, R. A., Crittenden, S., Forster, T., Yu, C., Zheng, X., et al. (2016). Prostaglandin E2 constrains systemic inflammation through an innate lymphoid cell-IL-22 axis. *Science* 351, 1333–1338. doi: 10.1126/science.aad9903

Dushianthan, A., Cusack, R., Burgess, V. A., Grocott, M. P. W., and Calder, P. C. (2019). Immunonutrition for acute respiratory distress syndrome (ARDS) in adults. *Cochrane Database Syst. Rev.* 1:CD012041. doi: 10.1002/14651858.CD012041.pub2

FitzGerald, G. A. (2015). Bringing PGE2in from the cold. *Science* 348, 1208–1209. doi: 10.1126/science.aac5515

Fu, Y., Cheng, Y., and Wu, Y. (2020). Understanding SARS-CoV-2-mediated inflammatory responses: from mechanisms to potential therapeutic tools. *Virol. Sin.* 35, 266–271. doi: 10.1007/s12250-020-00207-4

Fuchs, D., Tang, X., Johnsson, A. K., Dahlén, S. E., Hamberg, M., and Wheelock, C. E. (2020). Eosinophils synthesize trihydroxyoctadecenoic acids (TriHOMEs) via a 15-lipoxygenase dependent process. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1865:158611. doi: 10.1016/j.bbalip.2020.158611

Funk, C. D., and Ardakani, A. (2020). A novel strategy to mitigate the Hyperinflammatory response to COVID-19 by targeting leukotrienes. *Front. Pharmacol.* 11:1214. doi: 10.3389/fphar.2020.01214

Gentile, D. A., Fireman, P., and Skoner, D. P. (2003). Elevations of local leukotriene C4 levels during viral upper respiratory tract infections. *Ann. Allergy Asthma Immunol.* 91, 362–367. doi: 10.1016/S1081-1206(10)61683-3

Gentili, M., and Hacohen, N. (2021). Some antibodies can dampen antiviral defences in people with severe COVID. *Nature* 591, 37–39. doi: 10.1038/d41586-021-00352-0

Gosu, V., Son, S., Shin, D., and Song, K. D. (2019). Insights into the dynamic nature of the dsRNA-bound TLR3 complex. *Sci. Rep.* 9:3652. doi: 10.1038/s41598-019-39984-8

Gupta, A., Madhavan, M. V., Sehgal, K., Nair, N., Mahajan, S., Sehrawat, T. S., et al. (2020). Extrapulmonary manifestations of COVID-19. *Nat. Med.* 26, 1017–1032. doi: 10.1038/s41591-020-0968-3

Haeberle, H., Prohaska, S., Martus, P., Straub, A., Zarbock, A., Marx, G., et al. (2020). Therapeutic iloprost for the treatment of acute respiratory distress syndrome (ARDS; the ThIlo trial): a prospective, randomized, multicenter phase II study. *Trials* 21:242. doi: 10.1186/s13063-020-4163-0

Haeggström, J. Z. (2018). Leukotriene biosynthetic enzymes as therapeutic targets. *J. Clin. Invest.* 128, 2680–2690. doi: 10.1172/JCI97945

Hammock, B. D., Wang, W., Gilligan, M. M., and Panigrahy, D. (2020). Eicosanoids: the overlooked storm in coronavirus disease 2019 (COVID-19)? *Am. J. Pathol.* 190, 1782–1788. doi: 10.1016/j.ajpath.2020.06.010

Hon, K. L. E., Leung, T. F., and Leung, A. K. C. (2014). Clinical effectiveness and safety of montelukast in asthma. What are the conclusions from clinical trials and meta-analyses? *Drug Des. Devel. Ther.* 8, 839–850. doi: 10.2147/DDDT.S39100

Hoxha, M. (2020). What about COVID-19 and arachidonic acid pathway? *Eur. J. Clin. Pharmacol.* 76, 1501–1504. doi: 10.1007/s00228-020-02941-w

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506. doi: 10.1016/S0140-6736(20)30183-5

Hui, D. S., Joynt, G. M., Wong, K. T., Gomersall, C. D., Li, T. S., Antonio, G., et al. (2005). Impact of severe acute respiratory syndrome (SARS) on pulmonary function, functional capacity and quality of life in a cohort of survivors. *Thorax* 60, 401–409. doi: 10.1136/thx.2004.030205

Imai, Y. (2015). Role of omega-3 PUFA-derived mediators, the protectins, in influenza virus infection. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1851, 496–502. doi: 10.1016/j.bbalip.2015.01.006

Imai, Y., Kuba, K., and Penninger, J. M. (2008). The discovery of angiotensin-converting enzyme 2 and its role in acute lung injury in mice. *Exp. Physiol.* 93, 543–548. doi: 10.1113/expphysiol.2007.040048

Imai, Y., Kuba, K., Rao, S., Huan, Y., Guo, F., Guan, B., et al. (2005). Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 436, 112–116. doi: 10.1038/nature03712

Isobe, Y., Kato, T., and Arita, M. (2012). Emerging roles of eosinophils and eosinophil-derived lipid mediators in the resolution of inflammation. *Front. Immunol.* 3:270. doi: 10.3389/fimmu.2012.00270

Iwasaki, A., and Pillai, P. S. (2014). Innate immunity to influenza virus infection. *Nat. Rev. Immunol.* 14, 315–328. doi: 10.1038/nri3665

Jaimes, J. A., André, N. M., Chappie, J. S., Millet, J. K., and Whittaker, G. R. (2020). Phylogenetic analysis and structural modeling of SARS-CoV-2 spike protein reveals an evolutionary distinct and proteolytically sensitive activation loop. *J. Mol. Biol.* 432, 3309–3325. doi: 10.1016/j.jmb.2020.04.009

Jayasekera, J. P., Vinuesa, C. G., Karupiah, G., and King, N. J. C. (2006). Enhanced antiviral antibody secretion and attenuated immunopathology during influenza virus infection in nitric oxide synthase-2-deficient mice. *J. Gen. Virol.* 87, 3361–3371. doi: 10.1099/vir.0.82131-0

Johns Hopkins Coronavirus Resource Center (2021). Available at: https://coronavirus.jhu.edu/map.html

Karagiannidis, C., Windisch, W., McAuley, D. F., Welte, T., and Busse, R. (2021). Major differences in ICU admissions during the first and second COVID-19 wave in Germany. *Lancet Respir. Med.* 9, e47–e48. doi: 10.1016/S2213-2600(21)00101-6

Kawai, T., and Akira, S. (2010). The role of pattern-recognition receptors in innate immunity: update on toll-like receptors. *Nat. Immunol.* 11, 373–384. doi: 10.1038/ni.1863

Kindler, E., Thiel, V., and Weber, F. (2016). Interaction of SARS and MERS coronaviruses with the antiviral interferon response. in *Adv. Virus Res.* 96, 219–243. doi: 10.1016/bs.aivir.2016.08.006

Kobasa, D., Takada, A., Shinya, K., Hatta, M., Halfmann, P., Theriault, S., et al. (2004). Enhanced virulence of influenza a viruses with the haemagglutinin of the 1918 pandemic virus. *Nature* 431, 703–707. doi: 10.1038/nature02951

Kuba, K., Imai, Y., and Penninger, J. M. (2006). Angiotensin-converting enzyme 2 in lung diseases. *Curr. Opin. Pharmacol.* 6, 271–276. doi: 10.1016/j.coph.2006.03.001

Kuba, K., Imai, Y., Rao, S., Gao, H., Guo, F., Guan, B., et al. (2005). A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat. Med.* 11, 875–879. doi: 10.1038/nm1267

Langlois, P. L., D'Aragon, F., Hardy, G., and Manzanares, W. (2019). Omega-3 polyunsaturated fatty acids in critically ill patients with acute respiratory distress syndrome: A systematic review and meta-analysis. *Nutrition* 61, 84–92. doi: 10.1016/j.nut.2018.10.026

Le Goffic, R., Balloy, V., Lagranderie, M., Alexopoulou, L., Escriou, N., Flavell, R., et al. (2006). Detrimental contribution of the toll-like receptor (TLR)3 to influenza A virus-induced acute pneumonia. *PLoS Pathog.* 2:e53. doi: 10.1371/journal.ppat.0020053

Lee, J. S., Park, S., Jeong, H. W., Ahn, J. Y., Choi, S. J., Lee, H., et al. (2020). Immunophenotyping of covid-19 and influenza highlights the role of type i interferons in development of severe covid-19. *Sci. Immunol.* 5:eabd1554. doi: 10.1126/sciimmunol.abd1554

Levy, B. D., Clish, C. B., Schmidt, B., Gronert, K., and Serhan, C. N. (2001). Lipid mediator class switching during acute inflammation: signals in resolution. *Nat. Immunol.* 2, 612–619. doi: 10.1038/89759

Liao, M., Liu, Y., Yuan, J., Wen, Y., Xu, G., Zhao, J., et al. (2020). Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. *Nat. Med.* 26, 842–844. doi: 10.1038/s41591-020-0901-9

Libreros, S., Shay, A. E., Nshimiyimana, R., Fichtner, D., Martin, M. J., Wourms, N., et al. (2021). A new E-series Resolvin: RvE4 stereochemistry and function in Efferocytosis of inflammation-resolution. *Front. Immunol.* 11:631319. doi: 10.3389/fimmu.2020.631319

Mahase, E. (2020). Covid-19: WHO declares pandemic because of "alarming levels" of spread, severity, and inaction. *BMJ* 368:m1036. doi: 10.1136/bmj.m1036

McClain, M. T., Constantine, F. J., Henao, R., Liu, Y., Tsalik, E. L., Burke, T. W., et al. (2020). Dysregulated transcriptional responses to SARS-CoV-2 in the periphery. *Nat. Commun.* 2:1079. doi: 10.1038/s41467-021-21289-y

Monteil, V., Kwon, H., Prado, P., Hagelkrüys, A., Wimmer, R. A., Stahl, M., et al. (2020). Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. *Cell* 181, 905.e7–913.e7. doi: 10.1016/j.cell.2020.04.004

Moore, J. B., and June, C. H. (2020). Cytokine release syndrome in severe COVID-19. *Science* 368, 473–474. doi: 10.1126/science.abb8925

Morisseau, C., and Hammock, B. D. (2013). Impact of soluble epoxide hydrolase and epoxyeicosanoids on human health. *Annu. Rev. Pharmacol. Toxicol.* 53, 37–58. doi: 10.1146/annurev-pharmtox-011112-140244

Morita, M., Kuba, K., Ichikawa, A., Nakayama, M., Katahira, J., Iwamoto, R., et al. (2013). The lipid mediator protectin D1 inhibits influenza virus replication and improves severe influenza. *Cell* 153, 112–125. doi: 10.1016/j.cell.2013.02.027

Narasaraju, T., Yang, E., Samy, R. P., Ng, H. H., Poh, W. P., Liew, A. A., et al. (2011). Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonitis. *Am. J. Pathol.* 179, 199–210. doi: 10.1016/j.ajpath.2011.03.013

Narasimhan, P. B., Marcovecchio, P., Hamers, A. A. J., and Hedrick, C. C. (2019). Nonclassical monocytes in health and disease. *Annu. Rev. Immunol.* 37, 439–456. doi: 10.1146/annurev-immunol-042617-053119

Nienhold, R., Ciani, Y., Koelzer, V. H., Tzankov, A., Haslbauer, J. D., Menter, T., et al. (2020). Two distinct immunopathological profiles in autopsy lungs of COVID-19. *Nat. Commun.* 11:5086. doi: 10.1038/s41467-020-18854-2

Peiris, J. S. M., Chu, C. M., Cheng, V. C. C., Chan, K. S., Hung, I. F. N., Poon, L. L. M., et al. (2003). Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet* 361, 1767–1772. doi: 10.1016/S0140-6736(03)13412-5

Pham, T. L., He, J., Kakazu, A. H., Calandria, J., Do, K. V., Nshimiyimana, R., et al. (2020). Elovanoid-N32 or RvD6-isomer decrease ACE2 and binding of S protein RBD after injury or INFγ in the eye. *Res. Sq.* [Preprint]. doi:10.21203/rs.3.rs-55764/v1

Piroth, L., Cottenet, J., Mariet, A. S., Bonniaud, P., Blot, M., Tubert-Bitter, P., et al. (2021). Comparison of the characteristics, morbidity, and mortality of COVID-19 and seasonal influenza: a nationwide, population-based retrospective cohort study. *Lancet Respir. Med.* 9, 251–259. doi: 10.1016/S2213-2600(20)30527-0

Pontes-Arruda, A., and Hirasawa, H. (2011). The effects of enteral feeding with eicosapentaenoic acid. GAMMA.-linolenic acid and antioxidant vitamins on mechanically ventilated patients with severe sepsis and septic shock: a retrospective analysis of Japanese-descendant patients. *Nihon Shuchu Chiryo Igakukai zasshi.* 18, 207–213. doi:10.3918/jsicm.18.207

Recchiuti, A., Patruno, S., Mattoscio, D., Isopi, E., Pomilio, A., Lamolinara, A., et al. (2021). Resolvin D1 and D2 reduce SARS-CoV-2-induced inflammatory responses in cystic fibrosis macrophages. *FASEB J.* 35:e21441. doi: 10.1096/fj.202001952R

Rice, T. W., Wheeler, A. P., Thompson, B. T., DeBoisblanc, B. P., Steingrub, J., and Rock, P. (2011). Enteral omega-3 fatty acid, γ-linolenic acid, and antioxidant supplementation in acute lung injury. *JAMA* 306, 1574–1581. doi: 10.1001/jama.2011.1435

Root-Bernstein, R. (2021). Innate receptor activation patterns involving tlr and nlr synergisms in covid-19, ali/ards and sepsis cytokine storms: a review and model making novel predictions and therapeutic suggestions. *Int. J. Mol. Sci.* 22:2108. doi: 10.3390/ijms22042108

Schoggins, J. W. (2014). Interferon-stimulated genes: roles in viral pathogenesis. *Curr. Opin. Virol.* 6, 40–46. doi: 10.1016/j.coviro.2014.03.006

Schulz, O., Diebold, S. S., Chen, M., Näslund, T. I., Nolte, M. A., Alexopoulou, L., et al. (2005). Toll-like receptor 3 promotes cross-priming to virus-infected cells. *Nature* 433, 887–892. doi: 10.1038/nature03326

Schwab, J. M., Chiang, N., Arita, M., and Serhan, C. N. (2007). Resolvin E1 and protectin D1 activate inflammation-resolution programmes. *Nature* 447, 869–874. doi: 10.1038/nature05877

Schwarz, B., Sharma, L., Roberts, L., Peng, X., Bermejo, S., Leighton, I., et al. (2021). Cutting edge: severe SARS-CoV-2 infection in humans is defined by a shift in the serum Lipidome, resulting in dysregulation of eicosanoid immune mediators. *J. Immunol.* 206, 329–334. doi: 10.4049/jimmunol.2001025

Serhan, C. N. (2007). Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Annu. Rev. Immunol.* 25, 101–137. doi: 10.1146/annurev.immunol.25.022106.141647

Serhan, C. N., Dalli, J., Colas, R. A., Winkler, J. W., and Chiang, N. (2015). Protectins and maresins: new pro-resolving families of mediators in acute inflammation and resolution bioactive metabolome. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1851, 397–413. doi: 10.1016/j.bbalip.2014.08.006

Serhan, C. N., Gotlinger, K., Hong, S., Lu, Y., Siegelman, J., Baer, T., et al. (2006). Anti-inflammatory actions of Neuroprotectin D1/Protectin D1 and its natural stereoisomers: assignments of Dihydroxy-containing Docosatrienes. *J. Immunol.* 176, 1848–1859. doi: 10.4049/jimmunol.176.3.1848

Serhan, C. N., Hong, S., Gronert, K., Colgan, S. P., Devchand, P. R., Mirick, G., et al. (2002). Resolvins: a family of bioactive products of omega-3 fatty

acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *J. Exp. Med.* 196, 1025–1037. doi: 10.1084/jem.20020760

Serhan, C. N., and Petasis, N. A. (2011). Resolvins and protectins in inflammation resolution. *Chem. Rev.* 111, 5922–5943. doi: 10.1021/cr100396c

Shirato, K., and Kizaki, T. (2021). SARS-CoV-2 spike protein S1 subunit induces pro-inflammatory responses via toll-like receptor 4 signaling in murine and human macrophages. *Heliyon* 7:e06187. doi: 10.1016/j.heliyon.2021.e06187

Speranza, E., Williamson, B. N., Feldmann, F., Sturdevant, G. L., Pérez-Pérez, L., Meade-White, K., et al. (2021). Single-cell RNA sequencing reveals SARS-CoV-2 infection dynamics in lungs of African green monkeys. *Sci. Transl. Med.* 13:eabe8146. doi: 10.1126/scitranslmed.abe8146

Spite, M., Clària, J., and Serhan, C. N. (2014). Resolvins, specialized proresolving lipid mediators, and their potential roles in metabolic diseases. *Cell Metab.* 19, 21–36. doi: 10.1016/j.cmet.2013.10.006

Stapleton, R. D., Martin, T. R., Weiss, N. S., Crowley, J. J., Gundel, S. J., Nathens, A. B., et al. (2011). A phase II randomized placebo-controlled trial of omega-3 fatty acids for the treatment of acute lung injury. *Crit. Care Med.* 39, 1655–1662. doi: 10.1097/CCM.0b013e318218669d

Tam, V. C., Quehenberger, O., Oshansky, C. M., Suen, R., Armando, A. M., Treuting, P. M., et al. (2013). XLipidomic profiling of influenza infection identifies mediators that induce and resolve inflammation. *Cell* 154, 213–227. doi: 10.1016/j.cell.2013.05.052

Tate, M. D., Deng, Y.-M., Jones, J. E., Anderson, G. P., Brooks, A. G., and Reading, P. C. (2009). Neutrophils ameliorate lung injury and the development of severe disease during influenza infection. *J. Immunol.* 183, 7441–7450. doi: 10.4049/jimmunol.0902497

Theron, A. J., Steel, H. C., Tintinger, G. R., Gravett, C. M., Anderson, R., and Feldman, C. (2014). Cysteinyl leukotriene receptor-1 antagonists as modulators of innate immune cell function. *J. Immunol. Res.* 2014:608930. doi: 10.1155/2014/608930

Tumpey, T. M., García-Sastre, A., Taubenberger, J. K., Palese, P., Swayne, D. E., Pantin-Jackwood, M. J., et al. (2005). Pathogenicity of influenza viruses with genes from the 1918 pandemic virus: functional roles of alveolar macrophages and neutrophils in limiting virus replication and mortality in mice. *J. Virol.* 79, 14933–14944. doi: 10.1128/JVI.79.23.14933-14944.2005

Ullah, M. O., Sweet, M. J., Mansell, A., Kellie, S., and Kobe, B. (2016). TRIF-dependent TLR signaling, its functions in host defense and inflammation, and its potential as a therapeutic target. *J. Leukoc. Biol.* 100, 27–45. doi: 10.1189/jlb.2RI1115-531R

Walls, A. C., Park, Y. J., Tortorici, M. A., Wall, A., McGuire, A. T., and Veesler, D. (2020). Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell* 181, 281.e6–292.e6. doi: 10.1016/j.cell.2020.02.058

Watanabe, Y., Hashimoto, Y., Shiratsuchi, A., Takizawa, T., and Nakanishi, Y. (2005). Augmentation of fatality of influenza in mice by inhibition of phagocytosis. *Biochem. Biophys. Res. Commun.* 337, 881–886. doi: 10.1016/j.bbrc.2005.09.133

Webb, B. J., Peltan, I. D., Jensen, P., Hoda, D., Hunter, B., Silver, A., et al. (2020). Clinical criteria for COVID-19-associated hyperinflammatory syndrome: a cohort study. *Lancet Rheumatol.* 2, e754–e763. doi: 10.1016/S2665-9913(20)30343-X

Wenzel, S. E., and Kamada, A. K. (1996). Zileuton: the first 5-lipoxygenase inhibitor for the treatment of asthma. *Ann. Pharmacother.* 30, 858–864. doi: 10.1177/106002809603000725

Wilk, A. J., Rustagi, A., Zhao, N. Q., Roque, J., Martínez-Colón, G. J., McKechnie, J. L., et al. (2020). A single-cell atlas of the peripheral immune response in patients with severe COVID-19. *Nat. Med.* 26, 1070–1076. doi: 10.1038/s41591-020-0944-y

Wilson, J. G., Simpson, L. J., Ferreira, A. M., Rustagi, A., Roque, J., Asuni, A., et al. (2020). Cytokine profile in plasma of severe COVID-19 does not differ from ARDS and sepsis. *JCI Insight.* 5:e140289. doi: 10.1172/jci.insight.140289

Winkler, E. S., Bailey, A. L., Kafai, N. M., Nair, S., McCune, B. T., Yu, J., et al. (2020). SARS-CoV-2 infection of human ACE2-transgenic mice causes severe lung inflammation and impaired function. *Nat. Immunol.* 21, 1327–1335. doi: 10.1038/s41590-020-0778-2

Winkler, E. S., Gilchuk, P., Yu, J., Bailey, A. L., Chen, R. E., Chong, Z., et al. (2021). Human neutralizing antibodies against SARS-CoV-2 require intact fc effector functions for optimal therapeutic protection. *Cell* 184, 1804.e16–1820.e16. doi: 10.1016/j.cell.2021.02.026

Yvan-Charvet, L., and Ng, L. G. (2019). Granulopoiesis and neutrophil homeostasis: a metabolic, daily balancing act. *Trends Immunol.* 40, 598–612. doi: 10.1016/j.it.2019.05.004

Zhang, Q., Liu, Z., Moncada-Velez, M., Chen, J., Ogishi, M., Bigio, B., et al. (2020a). Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. *Science* 370:eabd4570. doi: 10.1126/science.abd4570

Zhang, X., Tan, Y., Ling, Y., Lu, G., Liu, F., Yi, Z., et al. (2020b). Viral and host factors related to the clinical outcome of COVID-19. *Nature* 583, 437–440. doi: 10.1038/s41586-020-2355-0

Ziegler-Heitbrock, L., Ancuta, P., Crowe, S., Dalod, M., Grau, V., Hart, D. N., et al. (2010). Nomenclature of monocytes and dendritic cells in blood. *Blood* 116, e74–e80. doi: 10.1182/blood-2010-02-258558

Conflict of Interest: 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.

frontiers in Physiology

ORIGINAL RESEARCH
published: 26 July 2021
doi: 10.3389/fphys.2021.669652

Increased Epoxyeicosatrienoic Acids and Hydroxyeicosatetraenoic Acids After Treatment of Iodide Intake Adjustment and 1,25-Dihydroxy-Vitamin D_3 Supplementation in High Iodide Intake–Induced Hypothyroid Offspring Rats

Qing Liu[1†], *Yue Zhang*[2†], *Hailing Zhao*[1] *and Xiaomei Yao*[1*]

[1] *Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Tianjin Medical University, Tianjin, China,* [2] *Tianjin Key Laboratory of Ionic-Molecular of Cardiovascular Disease, Department of Cardiology, Tianjin Institute of Cardiology, the Second Hospital of Tianjin Medical University, Tianjin, China*

OPEN ACCESS

Edited by:
Jun Yang,
University of California, Davis, United States

Reviewed by:
John D. Imig,
Medical College of Wisconsin, United States
Hu Xu,
Dalian Medical University, China

***Correspondence:**
Xiaomei Yao
jupx@163.com

[†] *These authors have contributed equally to this work and share first authorship*

Specialty section:
This article was submitted to Lipid and Fatty Acid Research, a section of the journal Frontiers in Physiology

Received: *19 February 2021*
Accepted: *22 June 2021*
Published: *26 July 2021*

Citation:
Liu Q, Zhang Y, Zhao H and Yao X (2021) Increased Epoxyeicosatrienoic Acids and Hydroxyeicosatetraenoic Acids After Treatment of Iodide Intake Adjustment and 1,25-Dihydroxy-Vitamin D_3 Supplementation in High Iodide Intake–Induced Hypothyroid Offspring Rats. Front. Physiol. 12:669652. doi: 10.3389/fphys.2021.669652

Aim: This study aimed to investigate the potential role of fatty acids in high iodide intake–induced hypothyroidism and its complications and also in the intervention of iodide intake adjustment and 1,25-dihydroxy-vitamin D_3 [1,25$(OH)_2D_3$] supplementation.

Methods: Pregnant rats were allocated to two groups, namely, normal iodide (NI, 7.5 μg/day) intake and 100 times higher-than-normal iodide (100 HI, 750 μg/day) intake. The offspring were continuously administered potassium iodide from weaning [i.e., postnatal day 21 (PN21)] to PN90. After PN90, the offspring were either administered iodide intake adjustment (7.5 μg/day) or 1,25$(OH)_2D_3$ supplementation (5 $\mu g \cdot kg^{-1} \cdot day^{-1}$), or both, for 4 weeks. Thyroid function tests (free triiodothyronine, free thyroxine, thyrotropin, thyroid peroxidase antibody, and thyroglobulin antibody), blood lipids (triglyceride, total cholesterol, free fatty acid, and low-density lipoprotein cholesterol), and vitamin D3 (VD3) levels were detected by ELISA. Cardiac function was measured by echocardiography. Blood pressure was measured using a non-invasive tail-cuff system. The serum fatty acids profile was analyzed by liquid chromatography–mass spectrometry.

Results: In the offspring rats with continued 100 HI administration, the levels of 8,9-dihydroxyeicosatrienoic acid (8,9-DHET) and thromboxane B2 (TXB2) were decreased, while those of prostaglandin J2 (PGJ2), prostaglandin B2 (PGB2), 4-hydroxydocosahexaenoic acid (4-HDoHE), 7-HDoHE, 8-HDoHE, and 20-HDoHE were increased. Significant correlations were found between PGB2, 8,9-DHET, 7-HDoHE levels and thyroid dysfunction, between PGJ2, 20-HDoHE, PGB2, 8,9-DHET levels and cardiac dysfunction, between PGJ2, 20-HDoHE levels and hypertension, between 4-HDoHE, 8-HDoHE, TXB2 levels and dyslipidemia, and between PGB2 and decreased VD3 level. After the treatment of iodide intake adjustment and 1,25$(OH)_2D_3$ supplementation, the levels of 16-hydroxyeicosatetraenoic acids (16-HETE), 18-HETE, 5,6-epoxyeicosatrienoic acid (5,6-EET), 8,9-EET, 11,12-EET,

14,15-EET, PGE2, 5-oxo-ETE, and 15-oxo-ETE were increased. The significant associations between PGE2, 16-HETE, 18-HETE and improved thyroid function and also between 5,6-EET, 11,12-EET, 14,15-EET, 16-HETE, 15-oxo-ETE and attenuated dyslipidemia were detected.

Conclusion: Increased levels of prostaglandins (PGs) and HDoHEs and decreased levels of 8,9-DHET and TXB2 might occur in the progression of cardiac dysfunction, hypertension, and dyslipidemia in high iodide intake–induced hypothyroidism. The increased levels of EETs and HETEs might help to ameliorate these complications after iodide intake adjustment and $1,25(OH)_2D_3$ supplementation.

Keywords: hypothyroidism, $1,25(OH)_2D_3$, cardiac dysfunction, hypertension, dyslipidemia, iodide intake adjustment, fatty acids

INTRODUCTION

Excess iodine consumption may lead to hypothyroidism (Bürgi, 2010), hyperthyroidism (Roti and Uberti, 2001), and autoimmune thyroid diseases (Laurberg et al., 2010). Serrano-Nascimento et al. investigated the effects of administering five times higher-than-normal iodide (5 HI) [i.e., sodium iodide (NaI)] during the pregnancy and lactation period of rats. The results showed hypothyroidism with the decreased circulating levels of free triiodothyronine (FT3) and free thyroxine (FT4) in offspring at postnatal day 90 (PN90) (Serrano-Nascimento et al., 2017). Our study has shown that 100 HI [i.e., potassium iodide (KI)] during the pregnancy and lactation period of rats can induce the decrease of FT3, FT4, and vitamin D3 (VD3) and also the increase of thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TgAb) levels in offspring at PN120. In addition, we demonstrated the protective effect of iodide intake adjustment, 1,25-dihydroxy-vitamin D_3 [$1,25(OH)_2D_3$] supplementation, or both in offspring rats following excess iodide intake (Wang et al., 2020). Hypothyroidism is a commonly encountered clinical condition, and it can impact cardiac function (Klein and Danzi, 2007; Udovcic et al., 2017), blood pressure (Klein and Danzi, 2007), lipid parameters (Jabbar et al., 2017), and vitamin D level (Salma et al., 2020).

Fatty acids occur in the form of mixtures of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) (Chen and Liu, 2020). PUFA can be classified into n-3 fatty acids and n-6 fatty acids. Arachidonic acid (AA) is synthesized from the n-6 fatty acid. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are synthesized from the n-3 fatty acid (Coras et al., 2021). AA and EPA were 20 carbons in the chain, and DHA was 22 carbons in the chain. Cyclooxygenase (COX) is the oxidase in the pathway for producing prostaglandin (PG) and thromboxane (TX) from AA. Lipoxygenase (LOX) is responsible for producing hydroxyeicosatetraenoic acids (HETEs). Cytochrome P450 (CYP) metabolizes AA to epoxyeicosatrienoic acids (EETs). DHA was metabolized by non-enzymatic (NE) (**Figure 1**).

Epoxyeicosatrienoic acids (EETs), the CYP epoxygenase metabolites of AA (Neckár et al., 2019), can prevent various cardiovascular diseases (CVDs) and can modulate cardiac and vascular physiology to maintain cardiovascular homeostasis, due to their vasodilator, antihypertensive, and other beneficial biological actions (Imig, 2012). PGs and TXs from AA have been associated with CVD (Dasilva and Medina, 2019), hypertension (Cavalca et al., 2010), and hypercholesterolemia (Davi et al., 1997).

However, the studies to determine the profile of fatty acids in hypothyroidism are scarce. The formation of fatty acids, their role in high iodide intake–induced hypothyroidism and its related complications, and the impact of iodide intake adjustment and $1,25(OH)_2D_3$ supplementation on the modulation of fatty acid synthesis remain unclear. The application of fatty acids as a therapeutic option for high iodide intake–induced hypothyroidism and its complications may be promising.

MATERIALS AND METHODS

Animals and Administration

Healthy Wistar rats (Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China) were housed in the specific pathogen-free (SPF) level of the Experimental Animal Center of Tianjin Medical University. In this study, 7-week-old female Wistar rats were mated with fertile males (1:1). The presence of a vaginal plug or sperm in the vaginal smear of the female rats was indicative of pregnancy (Day 0 of gestation, GD 0). The pregnant rats were randomly assigned to two groups, namely, normal iodide (NI) intake ($n = 6$) and 100 HI intake ($n = 12$). The offspring were continuously administered potassium iodide (KI) from weaning [i.e., postnatal day 21 (PN21)] to PN90. After PN90, the rats with NI were held as the control group (group 1, $n = 6$), and the rats with 100 HI were randomly divided into four treatment groups, namely, continued 100 HI administration (group 2, $n = 6$), continued 100 HI administration + $1,25(OH)_2D_3$ supplementation (group 3, $n = 6$), adjustment from 100 HI to NI administration (group 4, $n = 6$), and adjustment from 100 HI to NI administration + $1,25(OH)_2D_3$ supplementation (group 5, $n = 6$) (**Figure 2**). The rats in the NI group received dietary feed containing iodide (7.5 μg/day), in addition to the oral administration of deionized water. The rats in the 100 HI group received deionized water containing KI (24,750 μg/L) and dietary

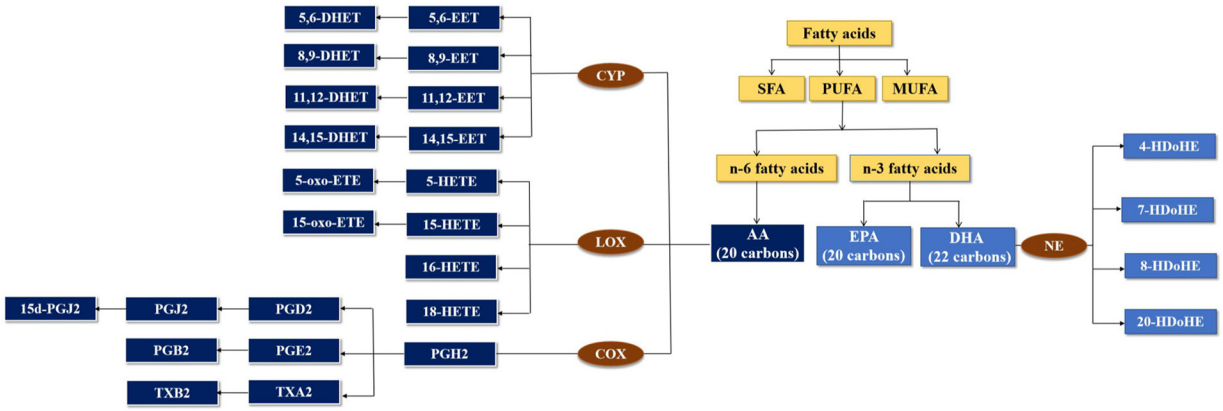


FIGURE 1 | Metabolism of fatty acids by cytochrome P450 (CYP), lipoxygenase (LOX), and cyclooxygenase (COX) enzymes.

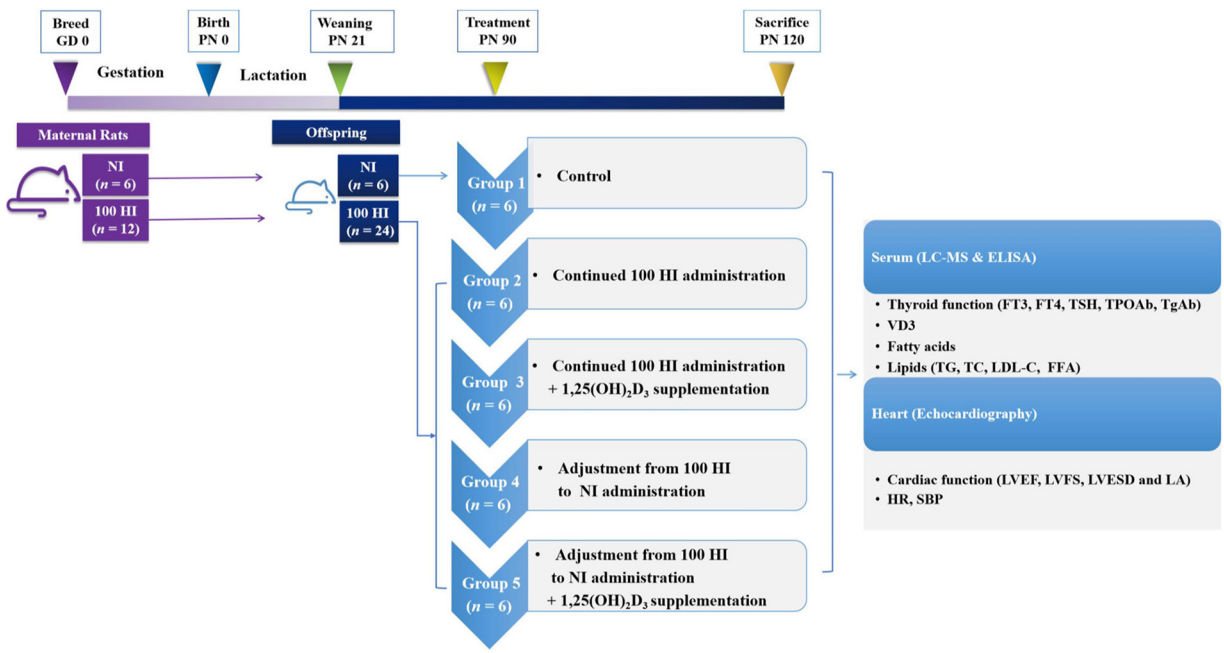


FIGURE 2 | Study plan and timing.

iodide. Therefore, the intake of iodide was 750 μg/day (Wang et al., 2018). The rats in groups 3 and 5 were supplemented with $1,25(OH)_2D_3$ (MedChemExpress, Monmouth Junction, NJ, USA) by gavage (5 $\mu g \cdot kg^{-1} \cdot day^{-1}$) (Wang et al., 2020). The animal study was reviewed and approved by the Institutional Animal Care and Use Committee of Tianjin Medical University (No. TMUaMEC 2016054).

Measurements of Thyroid Function, Lipid, and VD3 Levels in Serum by ELISA

Blood samples were drawn from the orbital sinus, centrifuged, and stored at −80°C. The levels of FT3, FT4 (Meilian Biological Technology, Shanghai, China), thyrotropin (TSH) (ImmunoWay Biotechnology Company, TX, USA), TPOAb, and TgAb (MyBioSource, San Diego, CA, USA), the lipid levels of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and free fatty acid (FFA) (Nanjing Jiancheng Bioengineering Institute, China), and the VD3 level (Meilian Biological Technology, Shanghai, China) in the serum were determined by the rat-specific ELISA kits.

Analysis of Fatty Acids in Serum by Liquid Chromatography–Mass Spectrometry (LC–MS)

Serum was extracted by solid-phase extraction (SPE) as described by Zhang X. et al. (2015). A UPLC BEH C18 column (1.7 μm, 100 × 2.1 mm i.d.; Waters, Milford, MA, USA) consisting of ethylene-bridged hybrid particles (Waters, Milford, MA,

USA) was used for chromatographic separations. Solvent A was water, and solvent B was acetonitrile. The mobile-phase flow rate was 0.6 mL/min (column temperature, 25°C). The injection volume was set to 10 μl. A total of 27 AA and 24 n-3 PUFA metabolites were profiled by multiple reaction monitoring (MRM) scans in negative mode, which involved the use of a 5,500 QTRAP hybrid triple quadrupole linear ion-trap mass spectrometer (AB Sciex, Foster City, CA, USA) equipped with a turbo ion-spray electrospray ionization source. The ion source parameters were as follows: CUR = 40 psi, GS1 = 30 psi, GS2 = 30 psi, IS = −4,500 V, CAD = medium, and temperature = 500°C.

Cardiac Function Measured by Echocardiography

The rats were anesthetized with inhaled isoflurane and fixed onto the operation table, and the probe was placed on the left chest. The M-mode images were obtained from the short axis of the left ventricle at the level of the papillary muscle. A VisualSonics echocardiographic system equipped with a 30-MHz transducer (RMV-707B, Toronto, Canada) and the Vevo2100 version 3.0.0 software (VisualSonics Inc., Canada) was used.

Measurement of Systolic Blood Pressure

The rats were trained to become familiar with the restrainer of the rat and to remain calm during the monitoring of blood pressure. The systolic blood pressure (SBP) for each rat was monitored by the "non-invasive tail-cuff system" (Visitech, BP-2000 Series II, Ohio, USA). The SBP measurement was always carried out in the afternoon, and the data were obtained from the average of measuring three times.

Statistical Analysis

Fatty acids were quantified with the use of MultiQuant 2.1 software (AB Sciex, Foster City, CA, USA). Fold change (FC) >2 and p-value <0.05 represent a significant result (Shaker et al., 2020). Metaboanalyst 3.0 (http://www.metaboanalyst.ca) was used for the metabolomic data analysis, interpretation, and visualization, which is presented as a heat map and a volcano plot (Xia et al., 2015). The data were compared and analyzed using IBM SPSS Statistics for Windows Version 22.0 (IBM Corp. Armonk, NY, USA). The control group and the continued 100 HI administration group were compared by using the independent samples t-test. The normal distribution of continuous variables was verified using the Kolmogorov–Smirnov test ($p < 0.05$). The two-way ANOVA was used to analyze the effects of treatment with iodide intake adjustment and/or $1,25(OH)_2D_3$ supplementation. Pearson's correlations were used to detect the relationship between fatty acids, thyroid function, VD3, lipids, and parameters of cardiac function. The data were expressed as the mean ± SD and $n = 6$ for each group. The $p < 0.05$ was considered significant.

RESULTS

No Significant Change in Body Weight Following Iodide Adjustment and/or $1,25(OH)_2D_3$ Supplementation for 4 Weeks

The body weight of offspring rats at PN120 per group were as follows: 329.8 ± 63.27 g (group 1, $n = 6$), 301.11 ± 75.15 g (group 2, $n = 6$), 309.68 ± 87.29 g (group 3, $n = 6$), 303.93 ± 60.76 g (group 4, $n = 6$), and 301.22 ± 64.27 g (group 5, $n = 6$). There was no significant difference in body weight among the five groups ($p > 0.05$).

Fatty Acids Profile in the Continued 100 HI Administration Group

The top 25 fatty acids were visualized in a heat map, which enabled effective differentiation between the control group (group 1) and the continued 100 HI administration group (group 2) (**Figure 3I**). The upregulation or downregulation with significance was identified in a volcano plot, which was compared with the log2-FC of the levels of fatty acids of significance (log t-test). In the continued 100 HI administration group (group 2), six fatty acids were significantly upregulated, including two fatty acids derived from AA: PGB2, PGJ2; four fatty acids derived from DHA: 4-hydroxydocosahexaenoic acids (HDoHE), 7-HDoHE, 8-HDoHE, and 20-HDoHE, while another two fatty acids derived from AA were significantly downregulated, namely, 8,9-dihydroxyeicosatrienoic acid (DHET) and TXB2 (**Figure 3J**). The median levels of 8,9-DHET and TXB2 were significantly decreased, while those of PGJ2, PGB2, 4-HDoHE, 7-HDoHE, 8-HDoHE, and 20-HDoHE were significantly increased in the continued 100 HI administration group (group 2) when compared with the control group (group 1). No significant alteration was detected in other fatty acids (**Figures 3A–H**).

Effects of Fatty Acids With Significant Change on Thyroid Function, Cardiac Function, Blood Lipids, and VD3 Levels in the Continued 100 HI Administration Group

Compared with the control group (group 1), FT3, FT4, and VD3 levels were significantly decreased, TSH, TPOAb, and TgAb levels were significantly increased, and blood lipid levels (i.e., TG, TC, FFA, and LDL-C) were also significantly increased in the continued 100 HI administration group (group 2) ($p < 0.05$) (**Table 1**). Although there was no significant change in the left ventricular end-systolic diameter (LVESD), the left ventricular ejection fraction (LVEF) and the left ventricular fractional shortening (LVFS) were significantly reduced, and the left atrial (LA) dimension and the heart rate (HR) were significantly increased in cardiac function measured by echocardiography. For blood pressure, SBP was significantly increased (**Figure 4**).

The correlation analysis was used to investigate the effect of metabolites with significant change. We showed that thyroid function was correlated with PGB2, 8,9-DHET, and 7-HDoHE, cardiac function was correlated with PGB2, PGJ2, and 8,9-DHET, blood pressure was correlated with PGJ2 and 20-HDoHE, blood

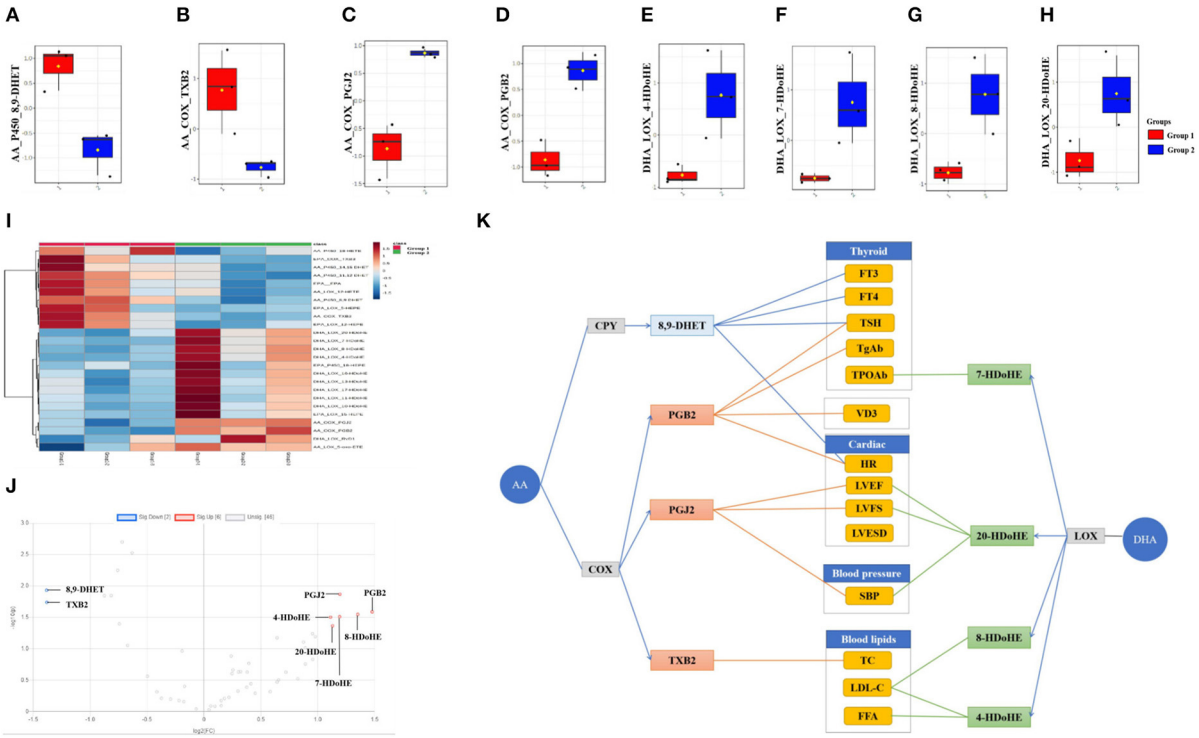


FIGURE 3 | Fatty acids profile and correlation analysis in the continued 100 HI administration group (group 2). **(A–H)** Box plots of the fatty acids levels. The bar in the quartile-indicating box is the median value. **(I)** Heat map shows the fatty acids profile from group 1 and group 2. **(J)** Volcano plot shows the significance and fold change in expression of fatty acids. **(K)** Correlation analysis between significantly changed fatty acids and thyroid function, cardiac function, blood lipids, and vitamin D3 (VD3) levels in the continued 100 HI administration group (group 2). $n = 6$ for each group.

lipid was correlated with 4-HDoHE, 8-HDoHE, and TXB2, and VD3 was correlated with PGB2 (**Figure 3K**).

Alteration of Fatty Acids in Different Treatment Groups

Compared with the continued 100 HI administration group (group 2), although no significant alteration was detected in both the continued 100 HI administration + $1,25(OH)_2D_3$ supplementation group (group 3) and the adjustment from 100 HI to NI administration group (group 4), nine significantly upregulated fatty acids were determined in the adjustment from 100 HI to NI administration + $1,25(OH)_2D_3$ supplementation group (group 5) in a volcano plot (**Figures 5J,K**). The median levels of 16-HETE, 18-HETE, 5,6-EET, 8,9-EET, 11,12-EET, 14,15-EET, PGE2, 5-oxo-ETE, and 15-oxo-ETE were significantly increased in the adjustment from 100 HI to NI administration + $1,25(OH)_2D_3$ supplementation group (group 5) in box plots (**Figures 5A–I**).

Effects of Significantly Changed Fatty Acids in the Adjustment From 100 HI to NI Administration + $1,25(OH)_2D_3$ Supplementation Group

Compared with the continued 100 HI administration group, the levels of FT3 and VD3 were significantly increased, and the levels of blood lipids (i.e., TG, TC, FFA, and LDL-C) were significantly decreased in the continued 100 HI administration + $1,25(OH)_2D_3$ supplementation (group 3), the adjustment from 100 HI to NI administration (group 4), and the adjustment from 100 HI to NI administration + $1,25(OH)_2D_3$ supplementation (group 5) ($p < 0.05$) (**Table 1**).

In the adjustment from 100 HI to NI administration + $1,25(OH)_2D_3$ supplementation group (group 5), thyroid function correlated with PGE2, 16-HETE, and 18-HETE, and blood lipid correlated with 5,6-EET, 11,12-EET, 14,15-EET, 16-HETE, and 15-oxo-ETE (**Figure 5L**).

DISCUSSION

Our findings reported in this study showed that 100 HI iodide intake during the pregnancy and lactation period of rats can induce hypothyroidism with decreased FT3 and FT4 levels and increased TSH levels, resulting in the complication of cardiac dysfunction in offspring rats at PN120. Significant correlations were found between PGJ2, 20-HDoHE, PGB2, 8,9-DHET, and cardiac dysfunction. Triiodothyronine (T3) is the main regulator of gene expression in myocardial muscle, and the decreased T3 in hypothyroidism can affect myocardial contractility and remodeling (Kahaly and Dillmann, 2005; Udovcic et al., 2017). Hypothyroidism is related to cardiac

TABLE 1 | Thyroid hormone, autoantibody, blood lipids, and vitamin D3 (VD3) levels in different treatment groups.

Groups	Thyroid hormone and autoantibody					Blood lipids and VD3				
	FT3 (pmol/L)	FT4 (pmol/L)	TSH (ng/mL)	TPOAb (IU/mL)	TgAb (ng/L)	TG (mmol/L)	TC (mmol/L)	FFA (mmol/L)	LDL-C (mmol/L)	VD3 (ng/mL)
Group 1	8.75 ± 0.46	12.75 ± 1.40	9.67 ± 0.95	47.30 ± 1.60	10.95 ± 1.11	0.70 ± 0.07	2.59 ± 0.20	2.59 ± 0.20	1.20 ± 0.12	26.62 ± 1.74
Group 2	7.54 ± 0.30*	9.85 ± 1.17*	12.53 ± 0.44*	59.58 ± 5.73*	18.52 ± 2.26*	1.26 ± 0.05*	4.68 ± 0.15*	4.68 ± 0.15*	2.09 ± 0.37*	14.27 ± 0.74*
Group 3	9.48 ± 0.59#	11.80 ± 0.85#	14.22 ± 2.30	50.46 ± 5.94	18.19 ± 1.50	0.84 ± 0.05#	3.28 ± 0.20#	3.28 ± 0.20#	1.47 ± 0.18#	17.62 ± 0.71#
Group 4	9.45 ± 0.80#	11.58 ± 0.18#	12.73 ± 1.23	49.48 ± 3.89	17.12 ± 2.87	0.96 ± 0.05#	3.61 ± 0.14#	3.61 ± 0.14#	1.39 ± 0.09#	17.85 ± 1.89#
Group 5	9.10 ± 0.69#	12.90 ± 0.37	14.47 ± 0.81	57.35 ± 6.27	15.73 ± 1.18	0.88 ± 0.05#	2.98 ± 0.21#	2.98 ± 0.21#	1.36 ± 0.17#	18.30 ± 1.53#

*Group 1, control; Group 2, continued 100 HI administration; Group 3, continued 100 HI administration + 1,25(OH)$_2$D$_3$ supplementation; Group 4, adjustment from 100 HI to NI administration; Group 5, adjustment from 100 HI to NI administration + 1,25(OH)$_2$D$_3$ supplementation. *$p < 0.05$ vs. group 1. #$p < 0.05$ vs. group 2. $n = 6$ for each group.*

dysfunction. Bassel et al. (2007) reported that, in propylthiouracil (PTU)-induced hypothyroidism rats, LVEF and LVFS were decreased significantly. Fu et al. (2017) reported that, in children with chronic viral myocarditis complicated with arrhythmia, the significantly decreased levels of FT3 and FT4 and the significantly increased levels of TSH, TPOAb, and TgAb were the independent risk factors of malignant arrhythmia. Ren et al. (2019) demonstrated that, in 2,663 euthyroid individuals, SBP was positively correlated with TSH and was an independent predictor of the serum TSH levels. The increased PGB2 and the decreased 8,9-DHET were correlated with HR, the increased PGJ2 was correlated with LVEF and LVFS, and the increased LA and HR, the increased HDoHEs (4-HDoHE, 7-HDoHE, 8-HDoHE, and 20-HDoHE), and the decreased TXB2 were also observed in high iodide intake–induced hypothyroidism. DHETs are the metabolites of EETs hydrolyzed by soluble epoxide hydrolase (sEH) (Yang et al., 2013). PGB2 is the metabolite of PGE2 (Coras et al., 2021), and PGE2 can promote cell growth and elevate the expression of hypertrophic marker genes by inhibiting the COX2/PGE2 pathway (Zhang et al., 2019). PGJ2 is formed by dehydration within the cyclopentenone ring of PGD2 (Abdelrahman et al., 2004). Stelling et al. (2020) reported that the PGD2 level was significantly increased in male mice cardiomyocytes with a cardiomyocyte-specific transcription factor-3 (STAT3) deficiency conditional knockout (CKO), which is an impairment of the endogenous cardiac regeneration potential as it shifts the differentiation potential of the cardiac progenitor cell (CPC) pool from endothelial cells toward white adipocytes, thereby promoting heart failure, a condition that impairs androgen receptor (AR) signaling in the absence of STAT3, which reduces the expression of the PG-degrading enzyme 15-hydroxyprostaglandin-dehydrogenase (HPGD). HDoHEs are biosynthesized from DHA, and the function is still not clearly elucidated. Reynaud et al. (1993) reported that 20-HDoHE was increased during the early period of oxidative stress *in vitro*, indicating that it probably played a pro-inflammatory role. Yao et al. (2015) reported that the serum TXB2 level was significantly elevated in patients with hyperthyroidism. Following the treatment with iodide intake adjustment + 1,25(OH)$_2$D$_3$ supplementation, 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET levels were significantly increased. Although there was no significant change detected for PGB2, a downward trend was apparent. The heart can metabolize EETs, which in turn may play an important role in modulating the electrophysiological properties of the heart. Lee et al. (1999) reported that 8,9-EET and the other EET regioisomers are the potent voltage-dependent inhibitors of the cardiac Na^+ channels. Na^+ channel-blocking drugs are the most commonly used pharmacological agents for the treatment of arrhythmias. 11,12-EET has been shown to enhance the recovery of cardiac function following global ischemia (Wu et al., 1997). Neckár et al. (2019) demonstrated that 2-week oral treatment with the EET analog EET-B improved cardiac function in spontaneously hypertensive rats with congestive heart failure induced by myocardial infarction. This study reveals that the increased EETs may serve to alleviate cardiac dysfunction associated with high iodide intake–induced hypothyroidism.

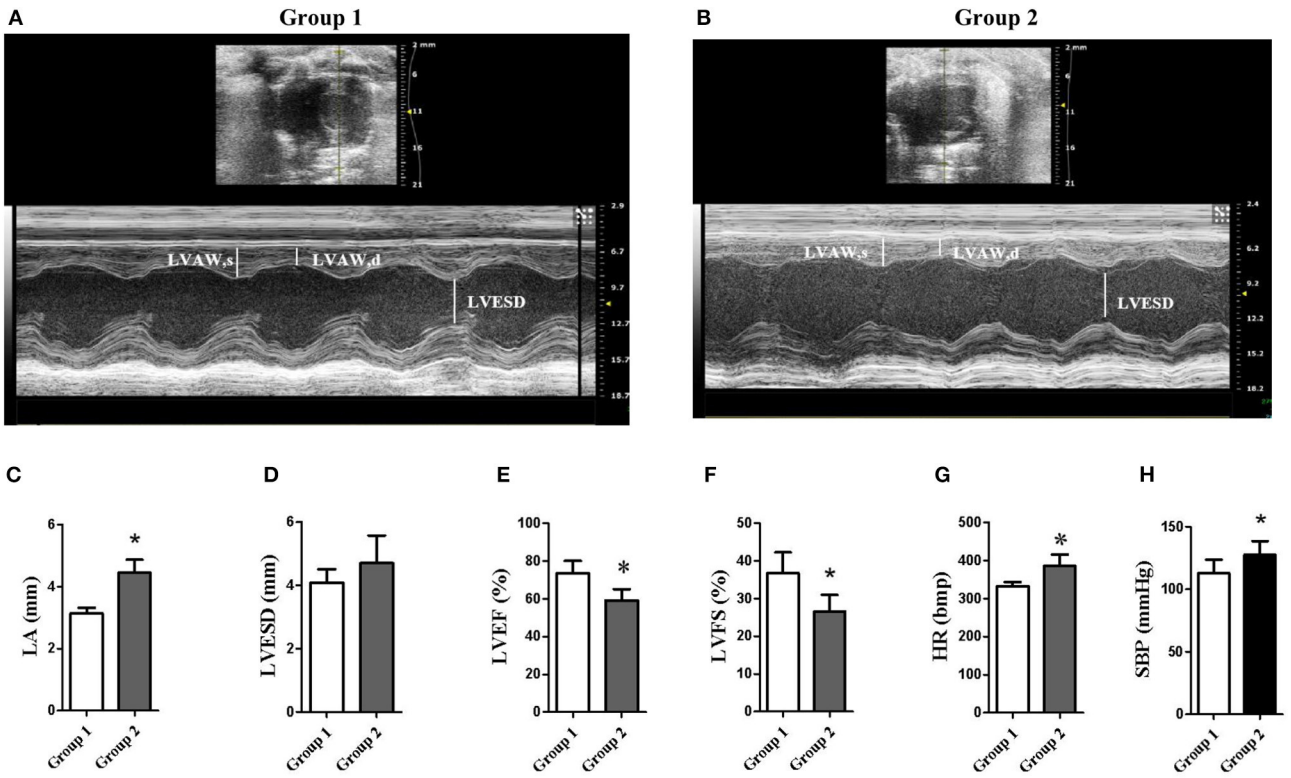


FIGURE 4 | Echocardiography data. **(A,B)** Representative examples of M-mode and B-mode echocardiographic tracings from group 1 (control) and group 2 (continued 100 HI administration). **(C–G)** Measurements were assessed by the echocardiographic data. *$p < 0.05$. **(H)** Systolic blood pressure (SBP). LVAW, left ventricular anterior wall. $n = 6$ for each group.

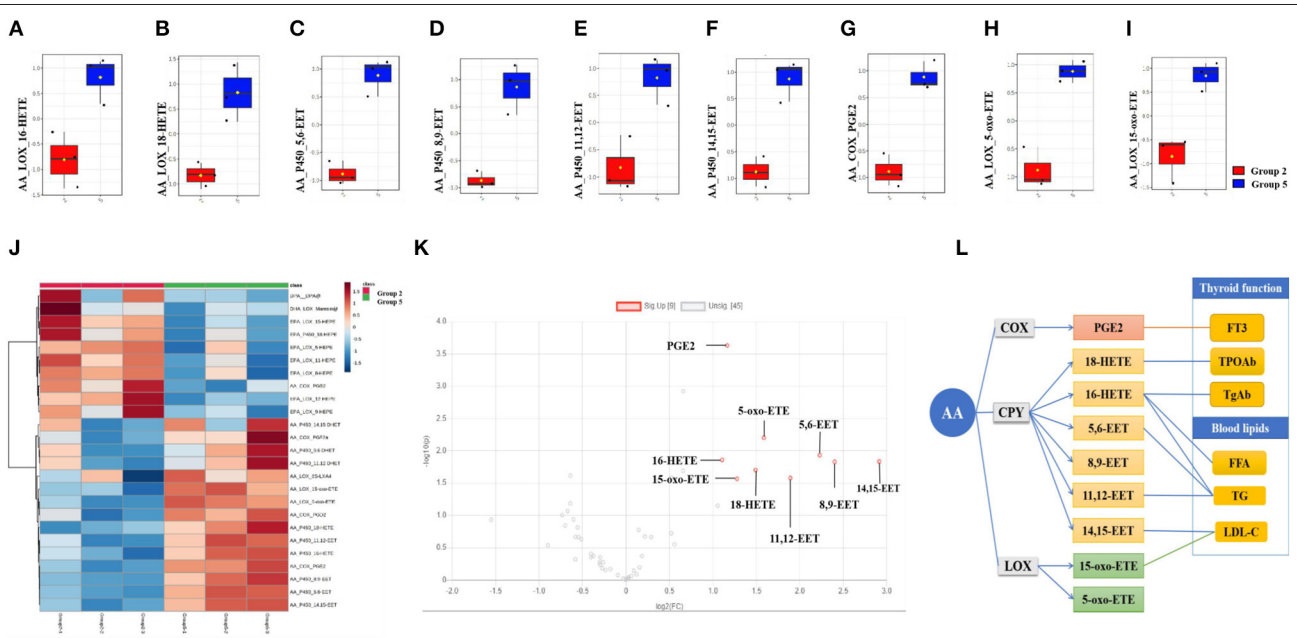


FIGURE 5 | Fatty acids profile and correlation analysis in adjustment from 100 HI to NI administration +1,25$(OH)_2D_3$ supplementation group (group 5). **(A–I)** Box plots of the fatty acids levels in serum. The bar in the quartile-indicating box is the median value. **(J)** Heat map from group 5 and group 2. **(K)** Volcano plot. **(L)** Correlation analysis. $n = 6$ for each group.

It was reported that there is some relationship between metabolites of AA in both serum and the heart. Hu et al. (2017) reported that 46 and 50 eicosanoids were detected and quantified in the plasma and heart tissue, respectively, and 43 overlapped eicosanoids were detected in plasma and the heart. The vast majority of plasma and heart tissue eicosanoids presented positive correlations with each other. In present study, 8,9-DHET, PGB2, 8-HDoHE, 20-HDoHE, 5,6-EET, 8,9-EET, 11,12-EET, 16-HETE, 18-HETE, and 15-oxo-ETE are among the overlapped eicosanoids. Al-Lawati et al. (2020) reported that, in adjuvant arthritis rats, the changes of total EET concentration in both the plasma and the heart were parallel to each other. The concentrations of the cardioprotective 8,9-EET, 11,12-EET, and 14,15-EET were reported to be parallel to each other in the plasma as well as in the heart (Theken et al., 2011; Aghazadeh-Habashi et al., 2018). In this study, although we did not measure the contents in the heart, according to the previous studies, the changes of cardioprotective 8,9-EET, 11,12-EET, and 14,15-EET in serum may be parallel with those in the heart.

Besides, in our experiment, blood pressure was significantly increased in high iodide intake–induced hypothyroidism. Significant correlations were found between PGJ2, 20-HDoHE, and hypertension. It is well known that thyroid hormones can impact the renin-angiotensin-aldosterone system, and the renin substrates are synthesized in the liver under the stimulus of T3, which results in hypertension in a hypothyroid state (Klein and Danzi, 2007). Iqbal et al. (2006) reported that both the systolic and the diastolic blood pressure were elevated in hypothyroidism. We have shown that the increased PGJ2 was correlated with blood pressure in high iodide intake–induced hypothyroidism. PGJ2 is formed by dehydration within the cyclopentenone ring of PGD2 (Abdelrahman et al., 2004). PGD2 is a vasoconstrictor (Ogletree, 1982). Asirvatham-Jeyaraj et al. (2019) reported that the PGD2 level was increased during the developmental stage of angiotensin II-salt hypertension in Sprague–Dawley rats. We found that EETs (i.e., 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET) and HETEs (i.e., 16-HETE and 18-HETE) levels were significantly increased after the treatment of iodide intake adjustment + $1,25(OH)_2D_3$ supplementation. These findings suggest that the increased EETs and HETEs may help to improve hypertension. The derivative of EETs was found to be antihypertensive, to protect vascular endothelial function, and to inhibit renal tubular sodium channel [i.e., epithelial sodium channel (ENaC)] in angiotensin II-dependent hypertension (Hye Khan et al., 2014). Besides, EETs are the potent endothelium-derived vasodilators that modulate vascular tone through the enhancement of Ca^{2+}-activated K^+ channels in vascular smooth muscle (Baron et al., 1997). In addition, 16-HETE and 18-HETE were shown to produce renal vasodilation, and they exhibited the inhibition of proximal tubule ATPase activity. Subterminal HETEs may participate in renal mechanisms affecting vasomotion (Carroll et al., 1996). Zhang et al. (2005) reported that the levels of 18-HETE were significantly decreased in renal interlobar arteries of spontaneously hypertensive rats.

Moreover, we demonstrated hyperlipidemia with significantly increased PGJ2 level in high iodide intake–induced hypothyroidism and found significant correlations between 4-HDoHE, 8-HDoHE, TXB2, 5,6-EET, 11,12-EET, 14,15-EET, 16-HETE, 15-oxo-ETE, and dyslipidemia. It was reported that the causes of hyperlipidemia in hypothyroidism are the decreased expression of hepatic LDL receptors, which reduces cholesterol clearance, and the reduced activity of cholesterol-α-monooxygenase, an enzyme that breaks down cholesterol (Canaris et al., 2000; Jabbar et al., 2017). PGJ2 metabolized further to yield $^{\Delta 12}$-PGJ2 and 15-deoxy-$^{\Delta 12,14}$-PGJ2 (15d-PGJ2) (Abdelrahman et al., 2004). PGJ2 and PGD2 exhibited an effect similar to 15d-PGJ2 (Kasai et al., 2000). 15d-PGJ2 is a natural ligand for peroxisome proliferator-activated receptor γ (PPARγ), which functions as a transcriptional regulator of genes linked to lipid metabolism (Ricote et al., 1999). There are findings which indicate that 15d-PGJ2 may stimulate the production of TG (Kasai et al., 2000). In this study, high iodide intake–induced hypothyroidism associated with hyperlipidemia was significantly improved after the treatment of iodide intake adjustment + $1,25(OH)_2D_3$ supplementation, with significantly increased EETs (i.e., 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET), 5-oxo-ETE, and 15-oxo-ETE. It was reported that 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET can be metabolized by cytochrome P450 2J2 (CYP2J2). Zhang S. S. et al. (2015) reported that endothelial-specific CYP2J2 overexpression can decrease TG, TC, and FFA levels in the liver of hyperlipidemic mice by enhanced FFA β-oxidation, which was mediated by the AMPK and PPARα pathway. 5-oxo-ETE and 15-oxo-ETE are the metabolites of 5-HETE and 15-HETE, respectively. Grzesiak et al. reported that TG was correlated with 5-HETE and 15-HETE, TC was correlated with 15-HETE in patients with both benign prostatic hyperplasia (BPH) and metabolic syndrome (MetS), and lipid mediators of inflammation, which influence the levels of biochemical parameters, may contribute to the mechanism (Grzesiak et al., 2019).

Furthermore, our results indicated that PGB2, PGE2, 16-HETE, 18-HETE, 8,9-DHET, and 7-HDoHE were correlated with the function of the thyroid. In addition, the significantly increased TSH level with increased PGB2 and the significantly decreased VD3 level with increased PGB2 and PGJ2 were detected in high iodide intake–induced hypothyroidism. PGB2 is the metabolite of PGE2 (Coras et al., 2021), and PGJ2 is formed by dehydration within the cyclopentenone ring of PGD2 (Abdelrahman et al., 2004). Tahara et al. (1991) reported that TSH stimulates the production of PGD2 and PGE2 in the Fischer rat thyroid follicular cell line (FRTL-5). Menon et al. (2019) reported that PGI2 analog was the mainstay of treatment for severe pulmonary arterial hypertension (PAH). PGs, in particular, prostacyclin, and their analogs cause a variety of side effects, such as hyperthyroidism, autoimmune goiter, Graves' disease, Hashimoto's disease, and thyrotoxicosis, in patients with PAH, and therapy with medications targeting the prostacyclin pathway is a potential risk factor for the development of symptomatic thyroid disease. The activation of PG receptors in the thyroid gland leads to the production of cyclic AMP (cAMP), which, in turn, stimulates the production of thyroid hormone and may contribute to the mechanism (Menon et al., 2019). The elevated levels of VD3 and PGE2

were detected. Although there was no significant change detected for PGB2, a downward trend was apparent after the treatment of iodide intake adjustment + 1,25$(OH)_2D_3$ supplementation. Liu et al. (2014) reported that all three forms of vitamin D reduced the production of PGE2 by stimulating HPGD, an enzyme that degrades PGE2. The onset of autoimmune thyroid disease with vitamin D deficiency is very common (Clinckspoor et al., 2012). Some studies indicated that vitamin D deficiency is a predisposing condition for autoimmune diseases (Peterlik et al., 2009). PGE2 can serve both pro-inflammatory and anti-inflammatory functions (Frolov et al., 2013). Qian et al. (2011) reported that PGE2 negatively regulates inflammation by inhibiting C-C chemokine ligand 5 (CCL5) expression in activated macrophages. Loynes et al. (2018) illustrated that the production of PGE2 at sites of tissue injury promotes an anti-inflammatory neutrophil phenotype and determines the outcome of inflammation resolution *in vivo*.

However, the studies to determine the mechanism of fatty acids in hypothyroidism and its complications are largely unknown. It is reported that the Ca/phosphoinositide/AA signal system is important to both the function and the growth of FRTL-5 rat thyroid cells and to the action of both TSH and alpha-1 adrenergic agents. This action was accompanied by the increases in cytosolic Ca^{++}, the release of AA from the cells, and the action of AA metabolites in processes important to the formation and growth of thyroid hormone (Tahara et al., 1989). Coria et al. reported that thyroid hormones are the important regulators of lipid metabolism, and hypothyroidism may reduce the relative contents of AA (Coria et al., 2012). In PTU-induced hypothyroidism, all the enzyme activities involved in the biosynthesis of fatty acids (i.e., acetyl-CoA carboxylase, fatty acid synthetase, and microsomal chain elongation and desaturation reactions) are strongly reduced after 3 days of drug administration.

Most studies on thyroid cancer were focused on AA. The AA is the precursor of PGs, which is a class of oncogenic lipid signaling molecules. Sun et al. reported that AA is a biomarker of papillary thyroid cancer (PTC). AA was significantly increased in PTC tissues from an iodine excess area compared with tissues from an iodine adequate area. The high levels of iodine may inhibit the activity of metabolic enzymes, such as COX, LOX, and LYP450, which in turn leads to a significant decrease in the synthesis of PGs (Sun et al., 2021). While AA was significantly decreased in PTC tissue compared with para-PTC tissue in both tissues from iodine adequate area and iodine excess area, a decrease in AA could be explained by the increased generation of PGs in PTC (Sun et al., 2021). Chen et al. reported that the relative levels of AA decreased in PTC. PG-endoperoxide synthase 2 (PTGS2; also known as COX-2) (Kunzmann et al., 2013) catalyzes the conversion of AA to PG, the mRNA level of PTGS2 was increased in PTC, and an increased consumption of AA was observed, which forms the oncogenic lipid in PTC (Chen et al., 2015). Krawczyk-Rusiecka et al. (2014) reported that there was a significantly higher expression level of the COX-2 gene in the PTC group, in comparison with Hashimoto's thyroiditis (HT) and non-toxic nodular goiter (NNG) groups. Reyes et al. (2019) also reported that an elevated arachidonate 5-lipoxygenase (ALOX5) was detected in patients with PTC. Kummer et al. (2012) reported that ALOX5 protein and mRNA were upregulated in PTC and that ALOX5 expression positively correlated with invasive tumor histopathology. Kim et al. (2003) reported that the levels of AA and DHA were significantly decreased in the urine profiles of the patients with thyroid cancer compared with normal female subjects, and the decreased level of glucocorticoids induced from the decreasing urinary concentration of DHA may play an important role in thyroid cancer. Berg et al. (1994) found that the high serum levels of AA and DHA provide a protective effect, and the low serum levels provide the risk of developing thyroid cancer. AA and DHA possibly may prevent thyroid cancer by reducing the estrogen receptor contents in thyroid tissues. Ji et al. (2012) reported that COX-2 expressions were stronger in thyroid carcinoma than in thyroid adenomas and normal tissues and that the COX-2 expressions in thyroid carcinoma were correlated with the tumor type and tumor-node-metastasis (TNM) stage. They also suggested that the expression of COX-2 may promote angiogenesis, infiltration, and metastasis of of thyroid carcinoma. Puxeddu et al. (2003) reported that COX-2 is overexpressed in thyroid malignancies compared with benign nodules and normal thyroid tissues. Alexanian et al. (2012) reported that the expression of CYP4A/4F genes was markedly elevated in the samples of thyroid cancer in comparison with matched normal tissues.

This study has some limitations. Further investigations on the measurements of echocardiography, blood pressure, and serum fatty acids in other time points and also on the expression of COXs, CYP450, and LOX need to be validated in our future research.

CONCLUSION

The increased PGs (PGB2 and PGJ2) and decreased 8,9-DHET levels might take part in the progression of cardiac dysfunction, hypertension, and dyslipidemia in high iodide intake–induced hypothyroidism. Significantly increased EETs (i.e., 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET) and HETEs (5-oxo-ETE, 15-oxo-ETE, 16-HETE, and 18-HETE) might represent the key regulators of these complications after iodide intake adjustment + 1,25$(OH)_2D_3$ supplementation. This novel aspect of fatty acids may provide new insights into high iodide intake–induced hypothyroidism and its complications.

DATA AVAILABILITY STATEMENT

The original contributions generated for the study are included in the article, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by the Institutional Animal Care and Use Committee of Tianjin Medical University (no. TMUaMEC 2016054).

AUTHOR CONTRIBUTIONS

QL, YZ, and HZ performed the experiments and analyzed the data. QL, HZ, and XY wrote the manuscript. XY designed the theme and experimental methods of the study. All authors carried out this research.

FUNDING

This work was supported by the National Natural Science Foundation of China (Nos. 81874257, 81273009, and 81800297) and by the Principal Investigators, XY and YZ.

ACKNOWLEDGMENTS

The authors thank the Large Research Equipment Sharing Platform in Tianjin Medical University for their help.

REFERENCES

Abdelrahman, M., Collin, M., and Thiemermann, C. (2004). The peroxisome proliferator-activated receptor-gamma ligand 15-deoxyDelta12,14 prostaglandin J2 reduces the organ injury in hemorrhagic shock. *Shock* 22, 555–561. doi: 10.1097/01.shk.0000144132.13900.24

Aghazadeh-Habashi, A., Asghar, W., and Jamali, F. (2018). Drug-disease interaction: effect of inflammation and nonsteroidal anti-inflammatory drugs on cytochrome P450 metabolites of arachidonic acid. *J. Pharm. Sci.* 107, 756–763. doi: 10.1016/j.xphs.2017.09.020

Alexanian, A., Miller, B., Roman, R. J., and Sorokin, A. (2012). 20-HETE-producing enzymes are up-regulated in human cancers. *Cancer Genomics Proteomics* 9, 163–169. doi: 10.1096/fasebj.25.1_supplement.829.1

Al-Lawati, H., Vakili, M. R., Lavasanifar, A., Ahmed, S., and Jamali, F. (2020). Reduced heart exposure of diclofenac by its polymeric micellar formulation normalizes CYP-mediated metabolism of arachidonic acid imbalance in an adjuvant arthritis rat model: implications in reduced cardiovascular side effects of diclofenac by nanodrug delivery. *Mol. Pharm*. 17, 1377–1386. doi: 10.1021/acs.molpharmaceut.0c00069

Asirvatham-Jeyaraj, N., Jones, A. D., Burnett, R., and Fink, G. D. (2019). Brain prostaglandin D2 increases neurogenic pressor activity and mean arterial pressure in angiotensin ii-salt hypertensive rats. *Hypertension* 74, 1499–1506. doi: 10.1161/HYPERTENSIONAHA.119.13175

Baron, A., Frieden, M., and Bény, J. L. (1997). Epoxyeicosatrienoic acids activate a high-conductance, Ca(2+)-dependent K + channel on pig coronary artery endothelial cells. *J. Physiol.* 504, 537–543. doi: 10.1111/j.1469-7793.1997.537bd.x

Bassel, K., Ankit, P., Rebecca, R., and Martin, G. (2007). Effect of low thyroid function on cardiac structure and function in spontaneously hypertensive heart failure rats. *J. Card. Fail*. 14, 167–171. doi: 10.1016/j.cardfail.2007.10.018

Berg, J. P., Glattre, E., Haldorsen, T., Høstmark, A. T., Bay, I. G., Johansen, A. F., et al. (1994). Longchain serum fatty acids and risk of thyroid cancer: a population-based case-control study in Norway. *Cancer Causes Control* 5, 433–438. doi: 10.1007/BF01694757

Bürgi, H. (2010). Iodine excess best pract. *Res. Clin. Endocrinol. Metab*. 24, 107–115. doi: 10.1016/j.beem.2009.08.010

Canaris, G. J., Manowitz, N. R., Mayor, G., and Ridgway, E. C. (2000). The Colorado thyroid disease prevalence study. *Arch. Intern. Med*. 160, 526–534. doi: 10.1001/archinte.160.4.526

Carroll, M. A., Balazy, M., Margiotta, P., Huang, D. D., Falck, J. R., and McGiff, J. C. (1996). Cytochrome P-450-dependent HETEs: profile of biological activity and stimulation by vasoactive peptides. *Am. J. Physiol.* 271, R863–869. doi: 10.1152/ajpregu.1996.271.4.R863

Cavalca, V., Minardi, F., Scurati, S., Guidugli, F., Squellerio, I., Veglia, F., et al. (2010). Simultaneous quantification of 8-iso-prostaglandin-F(2alpha) and 11-dehydro thromboxane B(2) in human urine by liquid chromatography-tandem mass spectrometry. *Anal. Biochem.* 397, 168–174. doi: 10.1016/j.ab.2009.10.014

Chen, J., and Liu, H. (2020). Nutritional Indices for assessing fatty acids: a mini-review. *Int. J. Mol. Sci*. 21:5695. doi: 10.3390/ijms21165695

Chen, M., Shen, M., Li, Y., Liu, C., Zhou, K., Hu, W., et al. (2015). GC-MS-based metabolomic analysis of human papillary thyroid carcinoma tissue. *Int. J. Mol. Med*. 36, 1607–1614. doi: 10.3892/ijmm.2015.2368

Clinckspoor, I., Gérard, A. C., Van Sande, J., Many, M. C., Verlinden, L., Bouillon, R., et al. (2012). The vitamin d receptor in thyroid development and function. *Eur. Thyroid J.* 1, 168–175. doi: 10.1159/000342363

Coras, R., Pedersen, B., Narasimhan, R., Brandy, A., Mateo, L., Prior-Español, A., et al. (2021). Imbalance between Omega-6- and Omega-3-derived bioactive lipids in arthritis in older adults. *J. Gerontol. A Biol. Sci. Med. Sci*. 76, 415–425. doi: 10.1093/gerona/glaa113

Coria, M. J., Carmona Viglianco, Y. V., Marra, C. A., Gomez-Mejiba, S. E., Ramirez, D. C., Anzulovich, A. C., et al. (2012). Hypothyroidism modifies lipid composition of polymorphonuclear leukocytes. *Cell. Physiol. Biochem*. 29, 713–724. doi: 10.1159/000170987

Dasilva, G., and Medina, I. (2019). Lipidomic methodologies for biomarkers of chronic inflammation in nutritional research: ω-3 and ω-6 lipid mediators. *Free Radic. Biol. Med*. 144, 90–109. doi: 10.1016/j.freeradbiomed.2019.03.017

Davi, G., Alessandrini, P., Mezzetti, A., Minotti, G., Bucciarelli, T., Costantini, F., et al. (1997). In vivo formation of 8-Epi-prostaglandin F2 alpha is increased in hypercholesterolemia. *Arterioscler. Thromb. Vasc. Biol.* 17, 3230–3235. doi: 10.1161/01.ATV.17.11.3230

Frolov, A., Yang, L., Dong, H., Hammock, B. D., and Crofford, L. J. (2013). Anti-inflammatory properties of prostaglandin E2: deletion of microsomal prostaglandin E synthase-1 exacerbates non-immune inflammatory arthritis in mice. *Prostaglandins Leukot. Essent. Fatty Acids* 89, 351–358. doi: 10.1016/j.plefa.2013.08.003

Fu, M. Y., Wang, Q. W., Xue, Y., Xu, F., Li, C. L., and An, X. J. (2017). Relevant researches on chronic viral myocarditis (CVMC) in children, complicated with arrhythmia and thyroid hormone level. *Eur. Rev. Med. Pharmacol. Sci.* 21, 3083–3087.

Grzesiak, K., Ry,ł, A., Stachowska, E., Słojewski, M., Rotter, I., Ratajczak, W., et al. (2019). The relationship between eicosanoid levels and serum levels of metabolic and hormonal parameters depending on the presence of metabolic syndrome in patients with benign prostatic hyperplasia. *Int. J. Environ. Res. Public Health* 16:1006. doi: 10.3390/ijerph16061006

Hu, T., Tie, C., Wang, Z., and Zhang, J. L. (2017). Highly sensitive and specific derivatization strategy to profile and quantitate eicosanoids by UPLC-MS/MS. *Anal. Chim. Acta* 950, 108–118. doi: 10.1016/j.aca.2016.10.046

Hye Khan, M. A., Pavlov, T. S., Christain, S. V., Neckár, J., Staruschenko, A., Gauthier, K. M., et al. (2014). Epoxyeicosatrienoic acid analogue lowers blood pressure through vasodilation and sodium channel inhibition. *Clin. Sci.* 127, 463–474. doi: 10.1042/CS20130479

Imig, J. D. (2012). Epoxides and soluble epoxide hydrolase in cardiovascular physiology. *Physiol. Rev*. 92, 101–130. doi: 10.1152/physrev.00021.2011

Iqbal, A., Figenschau, Y., and Jorde, R. (2006). Blood pressure in relation to serum thyrotropin: the tromso study. *J. Hum. Hypertens.* 20, 932–936. doi: 10.1038/sj.jhh.1002091

Jabbar, A., Pingitore, A., Pearce, S. H., Zaman, A., Iervasi, G., and Razvi, S. (2017). Thyroid hormones and cardiovascular disease. *Nat. Rev. Cardiol.* 14, 39–55. doi: 10.1038/nrcardio.2016.174

Ji, B., Liu, Y., Zhang, P., Wang, Y., and Wang, G. (2012). COX-2 Expression and tumor angiogenesis in thyroid carcinoma patients among Northeast Chinese population-result of a single-center study. *Int. J. Med.* 9, 237–242. doi: 10.7150/ijms.4173

Kahaly, G. J., and Dillmann, W. H. (2005). Thyroid hormone action in the heart. *Endocr. Rev.* 26, 704–728. doi: 10.1210/er.2003-0033

Kasai, K., Banba, N., Hishinuma, A., Matsumura, M., Kakishita, H., Matsumura, M., et al. (2000). 15-Deoxy-Delta(12,14)-prostaglandin J(2) facilitates thyroglobulin production by cultured human thyrocytes. *Am. J. Physiol. Cell Physiol.* 279, C1859–1869. doi: 10.1152/ajpcell.2000.279.6.C1859

Kim, K. M., Jung, B. H., Lho, D. S., Chung, W. Y., Paeng, K. J., and Chung, B. C. (2003). Alteration of urinary profiles of endogenous steroids and polyunsaturated fatty acids in thyroid cancer. *Cancer Lett.* 202, 173–179. doi: 10.1016/j.canlet.2003.08.002

Klein, I., and Danzi, S. (2007). Thyroid disease and the heart. *Circulation. Curr. Probl. Cardiol.* 116, 1725–1735. doi: 10.1161/CIRCULATIONAHA.106.678326

Krawczyk-Rusiecka, K., Wojciechowska-Durczynska, K., Cyniak-Magierska, A., Zygmunt, A., and Lewinski, A. (2014). Assessment of cyclooxygenase-1 and 2 gene expression levels in chronic autoimmune thyroiditis, papillary thyroid carcinoma and nontoxic nodular goiter. *Thyroid Res.* 7:10. doi: 10.1186/s13044-014-0010-2

Kummer, N. T., Nowicki, T. S., Azzi, J. P., Reyes, I., Iacob, C., Xie, S., et al. (2012). Arachidonate 5 lipoxygenase expression in papillary thyroid carcinoma promotes invasion via MMP-9 induction. *J. Cell. Biochem.* 113, 1998–2008. doi: 10.1002/jcb.24069

Kunzmann, A. T., Murray, L. J., Cardwell, C. R., McShane, C. M., McMenamin, U. C., and Cantwell, M. M. (2013). PTGS2 (Cyclooxygenase-2) Expression and survival among colorectal cancer patients: a systematic review. *Cancer Epidemiol. Biomarkers Prev.* 22, 1490–1497. doi: 10.1158/1055-9965.EPI-13-0263

Laurberg, P., Cerqueira, C., Ovesen, L., et al. (2010). Iodine intake as a determinant of thyroid disorders in populations. *Best Pract. Res. Clin. Endocrinol. Metab.* 24, 13–27. doi: 10.1016/j.beem.2009.08.013

Lee, H. C., Lu, T., Weintraub, N. L., VanRollins, M., Spector, A. A., and Shibata, E. F. (1999). Effects of epoxyeicosatrienoic acids on the cardiac sodium channels in isolated rat ventricular myocytes. *J. Physiol.* 519, 153–168. doi: 10.1111/j.1469-7793.1999.0153o.x

Liu, X., Nelson, A., Wang, X., Farid, M., Gunji, Y., Ikari, J., et al. (2014). Vitamin D modulates prostaglandin E2 synthesis and degradation in human lung fibroblasts. *Am. J. Respir. Cell Mol. Biol.* 50, 40–50. doi: 10.1165/rcmb.2013-0211OC

Loynes, C. A., Lee, J. A., Robertson, A. L., Steel, M. J., Ellett, F., Feng, Y., et al. (2018). PGE(2) production at sites of tissue injury promotes an anti-inflammatory neutrophil phenotype and determines the outcome of inflammation resolution *in vivo*. *Sci. Adv.* 4:eaar8320. doi: 10.1126/sciadv.aar8320

Menon, A. A., Sahay, S., Braverman, L. E., and Farber, H. W. (2019). Thyroid Dysfunction in patients with pulmonary artery hypertension (PAH): the effect of therapies affecting the prostanoid pathway. *Lung* 197, 761–768. doi: 10.1007/s00408-019-00283-8

Neckár, J., Hye Khan, M. A., Gross, G. J., Cyprová, M., Hrdlička, J., Kvasilová, A., et al. (2019). Epoxyeicosatrienoic acid analog EET-B attenuates post-myocardial infarction remodeling in spontaneously hypertensive rats. *Clin. Sci.* 133, 939–951. doi: 10.1042/CS20180728

Ogletree, M. L. (1982). Pharmacology of prostaglandins in the pulmonary microcirculation. *Ann. N. Y. Acad. Sci.* 384, 191–206. doi: 10.1111/j.1749-6632.1982.tb21372.x

Peterlik, M., Boonen, S., Cross, H. S., and Lamberg-Allardt, C. (2009). Vitamin D and calcium insufficiency-related chronic diseases: an emerging world-wide public health problem. *Int. J. Environ. Res. Public Health* 6, 2585–2607. doi: 10.3390/ijerph6102585

Puxeddu, E., Mitsutake, N., Knauf, J. A., Moretti, S., Kim, H. W., Seta, K. A., et al. (2003). Microsomal prostaglandin E 2 synthase-1 is induced by conditional expression of RET/PTC in Thyroid PCCL3 cells through the activation of the MEK-ERK pathway. *J. Biol. Chem.* 278, 52131–52138. doi: 10.1074/jbc.M306003200

Qian, X., Zhang, J., and Liu, J. (2011). Tumor-secreted PGE2 inhibits CCL5 production in activated macrophages through cAMP/PKA signaling pathway. *J. Biol. Chem.* 286, 2111–2120. doi: 10.1074/jbc.M110.154971

Ren, R., Ma, Y., Deng, F., Li, T., Wang, H. Y., Wei, J., et al. (2019). Association between serum TSH levels and metabolic components in euthyroid subjects: a nationwide population-based study. *Diabetes. Metab. Syndr. Obes.* 22, 1563–1569. doi: 10.2147/DMSO.S202769

Reyes, I., Reyes, N., Suriano, R., Iacob, C., Suslina, N., Policastro, A., et al. (2019). Gene expression profiling identifies potential molecular markers of papillary thyroid carcinoma. *Cancer Biomark* 24, 71–83. doi: 10.3233/CBM-181758

Reynaud, D., Thickitt, C. P., and Pace-Asciak, C. R. (1993). Facile preparation and structural determination of monohydroxy derivatives of docosahexaenoic acid (HDoHE) by alpha-tocopherol-directed autoxidation. *Anal. Biochem.* 214, 165–170. doi: 10.1006/abio.1993.1472

Ricote, M., Huang, J. T., Welch, J. S., and Glass, C. K. (1999). The peroxisome proliferator-activated receptor(PPARgamma) as a regulator of monocyte/macrophage function. *J. Leukoc. Biol.* 66, 733–739. doi: 10.1002/jlb.66.5.733

Roti, E., and Uberti, E. D. (2001). Iodine excess and hyperthyroidism. *Thyroid* 11, 493–500. doi: 10.1089/105072501300176453

Salma, A., Mohammad, R. D., and Naser, H. (2020). Vitamin D deficiency in non-autoimmune hypothyroidism: a case-control study. *BMC Endocr. Disord.* 20:41. doi: 10.1186/s12902-020-0522-9

Serrano-Nascimento, C., Salgueiro, R. B., Pantaleão, T., Corrêa da Costa, V. M., and Nunes, M. T. (2017). Maternal exposure to iodine excess throughout pregnancy and lactation induces hypothyroidism in adult male rat offspring. *Sci. Rep.* 7:15591. doi: 10.1038/s41598-017-15529-9

Shaker, O. G., Golam, R. M., Ayoub, S., Daker, L. I., Elguaad, M. K. A., Said, E. S., et al. (2020). Correlation between LincR-Gng2-5′ and LincR-Epas1-3′ as with the severity of multiple sclerosis in Egyptian patients. *Int. J. Neurosci.* 130, 515–521. doi: 10.1080/00207454.2019.1695610

Stelling, E., Ricke-Hoch, M., Erschow, S., Hoffmann, S., Bergmann, A. K., Heimerl, M., et al. (2020). Increased prostaglandin-D2 in male STAT3-deficient hearts shifts cardiac progenitor cells from endothelial to white adipocyte differentiation. *PLoS Biol.* 18:e3000739. doi: 10.1371/journal.pbio.3000739

Sun, Q., Zhao, H., Liu, Z., Wang, F., He, Q., and Xiu, C. (2021). Identifying potential metabolic tissue biomarkers for papillary thyroid cancer in different iodine nutrient regions. *Endocrine* doi: 10.1007/s12020-021-02773-3. [Epub ahead of print].

Tahara, K., Grollman, E. F., Saji, M., and Kohn, L. D. (1991). Regulation of prostaglandin synthesis by thyrotropin, insulin or insulin-like growth factor-I, and serum in FRTL-5 rat thyroid cells. *J. Biol. Chem.* 266, 440–448. doi: 10.1016/S0021-9258(18)52454-6

Tahara, K., Saji, M., Aloj, S. M., and Kohn, L. D. (1989). The arachidonic acid signal system in the thyroid: regulation by thyrotropin and insulin/IGF-I. *Adv. Exp. Med. Biol.* 261, 295–326. doi: 10.1007/978-1-4757-2058-7_12

Theken, K. N., Deng, Y., Kannon, M. A., Miller, T. M., Poloyac, S. M., and Lee, C. R. (2011). Activation of the acute inflammatory response alters cytochrome P450 expression and eicosanoid metabolism. *Drug Metab. Dispos.* 39, 22–29. 110.035287 doi: 10.1124/dmd.110.035287

Udovcic, M., Pena, R. H., Patham, B., Tabatabai, L., and Kansara, A. (2017). Hypothyroidism and the Heart. *Methodist Debakey Cardiovasc. J.* 13, 55–59. doi: 10.14797/mdcj-13-2-55

Wang, T. T., Liang, X., Abeysekera, I. R., Iqbal, U., Duan, Q., Naha, G., et al. (2018). Activation of the Nrf2-Keap 1 pathway in short-term iodide excess in thyroid in rats. *Oxid. Med. Cell. Longev.* 2017:4383652. doi: 10.1155/2017/4383652

Wang, Y., Liu, Q., Dong, H., Feng, Y. N., Raguthuet, C., and Liang, X. (2020). The protective effect of iodide intake adjustment and 1,25$(OH)_2D_3$ supplementation in rat offspring following excess iodide intake. *Ther. Adv. Endocrinol. Metab.* 11, 1–12. doi: 10.1177/2042018820958295

Wu, S., Chen, W., Murphy, E., Gabel, S., Tomer, K. B., Foley, J., et al. (1997). Molecular cloning, expression, and functional significance of a cytochrome P450 highly expressed in rat heart myocytes. *J. Biol. Chem.* 272, 12551–12559. doi: 10.1074/jbc.272.19.12551

Xia, J. G., Sinelnikov, I. V., Han, B., and Wishart, D. S. (2015). MetaboAnalyst 3.0–making metabolomics more meaningful. *Nucleic Acids Res.* 43, W251-W257. doi: 10.1093/nar/gkv380

Yang, T., Peng, R., Guo, Y., Shen, L., Zhao, S., and Xu, D. (2013). The role of 14,15-dihydroxyeicosatrienoic acid levels in inflammation and its relationship to lipoproteins. *Lipids Health Dis*. 12:151. doi: 10.1186/1476-511X-12-151

Yao, X., Sa, R., Ye, C., Zhang, D., Zhang, S., Xia, H., et al. (2015). Effects of thyroid hormone status on metabolic pathways of arachidonic acid in mice and humans: a targeted metabolomic approach. *Prostaglandins Other Lipid Mediat*. 118, 11–18. doi: 10.1016/j.prostaglandins.2015.03.005

Zhang, F., Deng, H., Kemp, R., Singh, H., Gopal, V. R., Falck, J. R., et al. (2005). Decreased levels of cytochrome P450 2E1-derived eicosanoids sensitize renal arteries to constrictor agonists in spontaneously hypertensive rats. *Hypertension* 45, 103–108. doi: 10.1161/01.HYP.0000150782.28485.91

Zhang, L. L., Deng, M., Lu, A. H., Chen, Y. T., Chen, Y., Wu, C. Y., et al. (2019). Sodium butyrate attenuates angiotensin II-induced cardiac hypertrophy by inhibiting COX2/PGE2 pathway via a HDAC5/HDAC6-dependent mechanism. *J. Cell. Mol. Med.* 23, 8139–8150. doi: 10.1111/jcmm.14684

Zhang, S. S., Chen, G. Z., Li, N., Dai, M. Y., Chen, C., Wang, P. H., et al. (2015). CYP2J2 overexpression ameliorates hyperlipidemia via increased fatty acid oxidation mediated by the AMPK pathway. *Obesity* 23, 1401–1413. doi: 10.1002/oby.21115

Zhang, X., Yang, N., Ai, D., and Zhu, Y. (2015). Systematic metabolomic analysis of eicosanoids after omega-3 polyunsaturated fatty acid supplementation by a highly specific liquid chromatography-tandem mass spectrometry-based method. *J. Proteome Res.* 14, 1843-1853. doi: 10.1021/pr501200u

Conflict of Interest: 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.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership