UNDERSTANDING THE HETEROGENEITY IN EXERCISE-INDUCED CHANGES IN GLUCOSE METABOLISM TO HELP OPTIMIZE TREATMENT OUTCOMES

EDITED BY: Thomas P. J. Solomon, Kristian Karstoft, Jacob Haus and John P. Thyfault

PUBLISHED IN: Frontiers in Endocrinology and Frontiers in Physiology

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88971-062-1
DOI 10.3389/978-2-88971-062-1

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

UNDERSTANDING THE HETEROGENEITY IN EXERCISE-INDUCED CHANGES IN GLUCOSE METABOLISM TO HELP OPTIMIZE TREATMENT OUTCOMES

Topic Editors:
Thomas P. J. Solomon, Blazon Scientific, United Kingdom
Kristian Karstoft, Centre for Physical Activity Research, Denmark
Jacob Haus, University of Michigan, United States
John P. Thyfault, University of Kansas Medical Center, United States

Topic editor Dr. Thomas Solomon is the owner of Blazon Scientific company. All other topic editors declare no competing interests with regards to the Research Topic subject.

Citation: Solomon, T. P. J., Karstoft, K., Haus, J., Thyfault, J. P., eds. (2021). Understanding the Heterogeneity in Exercise-Induced Changes in Glucose Metabolism to Help Optimize Treatment Outcomes. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88971-062-1

Table of Contents

frontiers in Endocrinology

EDITORIAL
published: 27 May 2021
doi: 10.3389/fendo.2021.699354

Editorial: Understanding the Heterogeneity in Exercise-Induced Changes in Glucose Metabolism to Help Optimize Treatment Outcomes

Thomas P. J. Solomon[1]*, *John P. Thyfault*[2], *Jacob M. Haus*[3] and *Kristian Karstoft*[4]

[1] Blazon Scientific, London, United Kingdom, [2] Departments of Molecular & Integrative Physiology and Internal Medicine, University of Kansas Medical Center, Kansas City, KS, United States, [3] School of Kinesiology, University of Michigan, Ann Arbor, MI, United States, [4] Centre for Physical Activity Research, Rigshospitalet, Copenhagen, Denmark

Keywords: exercise training, heterogeneity, variability, inter-individual, glucose control, metformin, hyperglycemia, diabetes

Editorial on the Research Topic

Understanding the Heterogeneity in Exercise-Induced Changes in Glucose Metabolism to Help Optimize Treatment Outcomes

OPEN ACCESS

Edited and reviewed by:
Hans Ulrich Häring, Tübingen University Hospital, Germany

***Correspondence:**
Thomas P. J. Solomon
info@blazon-scientific.com

Specialty section:
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Endocrinology

Received: *23 April 2021*
Accepted: *28 April 2021*
Published: *27 May 2021*

Citation:
Solomon TPJ, Thyfault JP, Haus JM and Karstoft K (2021) Editorial: Understanding the Heterogeneity in Exercise-Induced Changes in Glucose Metabolism to Help Optimize Treatment Outcomes. Front. Endocrinol. 12:699354. doi: 10.3389/fendo.2021.699354

Regular exercise improves several aspects of glucose metabolism. The evidence to date has culminated in clear public health physical activity guidelines (1). However, for patients with diabetes, physical activity guidelines are no different from those issued for the general population. While many randomized controlled trials (RCTs) have demonstrated the benefits of exercise for the prevention and the treatment of diabetes, some trials have not and several studies now highlight the large variability that exists in the inter-individual changes in blood glucose control following exercise (2–5). A 2018 narrative review (6) speculated that this variability is explained by exercise dose, meal-exercise timing, drug-exercise interactions, and more. But these speculations were largely based on observational and correlative evidence because prospective trials were lacking. Identifying and understanding the causes of this *response heterogeneity* is critical for maximizing therapeutic outcomes for patients with, or at risk of, diabetes. Optimizing the therapeutic effect of exercise may also reduce diabetes incidence, complications, and health care burdens. Therefore, this Research Topic aimed to publish papers that: (i) advance our understanding of the inter-individual heterogeneity of exercise-induced changes in blood glucose control, (ii) identify factors influencing such heterogeneity, and (iii) test the causality of such factors.

WHAT DID THIS TOPIC FIND?

We accepted ten papers from experts in the field. Three focussed on exercise intensity, exercise duration, and exercise type. For example, the meta-analysis by Liu et al. found that high-intensity interval training better improves blood glucose in children and adolescents with obesity when compared to moderate-intensity continuous training. The narrative review by Paquin et al. argued for the use of resistance exercise protocols aimed at invoking both a high oxygen demand and improves muscle function for inducing the greatest muscle adaptations favouring glucose control.

The narrative review by Warner et al. examined the impact of exercise on the liver, highlighting the urgent need for clinical studies to unravel the complexity of hepatic glucose metabolism. They argued that the heterogeneity in exercise effects on hepatic insulin sensitivity and splanchnic glucose metabolism in patients with type 2 diabetes may be attributable to between-study variations in exercise mode, duration, intensity, and weight loss. Further to the review by Warner et al.; Brennan et al. completed an RCT to objectively characterise the inter-individual heterogeneity of several health-related variables in response to energy restriction-induced weight loss with or without exercise, in older-aged adults with obesity. They found that the addition of exercise to energy restriction-induced weight loss increased the proportion of patients showing improvements in blood glucose control and cardiometabolic risk compared to weight loss alone.

To help deepen our understanding of *response heterogeneity*, Munan et al. completed a meta-analysis of single-bout and training studies that used continuous glucose monitoring (CGM) to assess glucose control. The meta-analysis showed that acute exercise and short-term training is sufficient to improve 24-hour glucose profiles in adults with type 2 diabetes but that there is high inter-individual heterogeneity, which was explained in part by the sex of participants, the timing of exercise, and the extent to which glycaemia is impaired on non-exercise days. Two RCTs included in this topic helped probe the causality of these sources of *response heterogeneity*. The RCT by Carter and Solomon showed that experimentally-induced pre-exercise hyperglycaemia blunted the glucoregulatory benefits of a single exercise bout, while the RCT by Porter et al. examined exercise-meal timing, finding that moderate-intensity exercise after an evening meal caused transient asymptomatic hypoglycaemia to a greater extent in women with diabetes than in men.

Finally, three papers in our topic focussed on drug-exercise interactions. The narrative review by Pitt et al. discussed the pharmacokinetics of subcutaneously-administered insulin in the context of type 1 diabetes, contending that exercise may increase circulating insulin concentrations and therefore contribute to exercise-related hyperinsulinemia and consequent hypoglycaemia in insulin-dependent patients. They argued that the location and depth of insulin injection cause variability in insulin absorption rates, which are influenced during exercise by several factors that must be studied in prospective trials. The narrative review by Malin and Stewart postulated that while metformin attenuates the insulin-sensitising effect of exercise, it has variable outcomes on exercise-induced changes in blood glucose control (i.e., HbA1c). Given that metformin is not always used in isolation and given that other medications used to treat diabetes (inc. GLP-1 receptor agonists and SGLT-2 inhibitors) may also interact with exercise, Malin and Stewart emphasised the urgent need for prospective trials in this area. Further to these narrative reviews, Pilmark et al. conducted an RCT to objectively examine the interaction between metformin and exercise. They found that 17 days of metformin treatment increased participants' ratings of perceived exertion (RPE) during exercise at a fixed intensity but had no effect on self-selected exercise intensity. Therefore, metformin may have implications for exercise adherence but this phenomenon must be prospectively studied in a longer-term trial.

WHAT NEXT?

Exercise can be a useful tool for improving glucose control but, for some patients, exercise does not provide the intended therapeutic outcome. It is indeed frustrating for a patient who invests great effort in implementing and maintaining a lifestyle change only to see no obvious benefit to their glucose control. We are a long way from fully understanding *response heterogeneity* to exercise and this topic only scratches the surface in the arduous task of testing the causality of factors responsible. This collection of papers indicates that to help maximise the therapeutic benefit of exercise for all people, we must advance the scientific understanding in this field with basic science mechanistic studies coupled with high-quality long-term RCTs specifically designed to tackle key questions. Namely, the interaction between exercise and glucose-lowering drugs, the interaction between ambient hyperglycaemia and exercise adaptations, and the causal roles of sex, exercise-meal timing, and diurnal timing of exercise on the *response heterogeneity* of blood glucose control deserve increased attention.

AUTHOR CONTRIBUTIONS

TS, JT, JH, and KK made substantial contributions to the conception of this topic and the drafting of this editorial. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Bull FC, Al-Ansari SS, Biddle S, Borodulin K, Buman MP, Cardon G, et al. World Health Organization 2020 Guidelines on Physical Activity and Sedentary Behaviour. *Br J Sports Med* (2020) 54:1451–62. doi: 10.1136/bjsports-2020-102955
2. Boulé NG, Weisnagel SJ, Lakka TA, Tremblay A, Bergman RN, Rankinen T, et al. Effects of Exercise Training on Glucose Homeostasis: The HERITAGE Family Study. *Diabetes Care* (2005) 28:108–14. doi: 10.2337/diacare.28.1.108
3. Solomon TPJ, Malin SK, Karstoft K, Kashyap SR, Haus JM, Kirwan JP. Pancreatic Beta-Cell Function is a Stronger Predictor of Changes in Glycemic Control After an Aerobic Exercise Intervention Than Insulin Sensitivity. *J Clin Endocrinol Metab* (2013) 98(10):4176–86. doi: 10.1210/jc.2013-2232
4. Álvarez C, Ramírez-Campillo R, Ramírez-Vélez R, Izquierdo M. Prevalence of Non-Responders for Glucose Control Markers After 10 Weeks of High-Intensity Interval Training in Adult Women With Higher and Lower Insulin Resistance. *Front Physiol* (2017) 8:479. doi: 10.3389/fphys.2017.00479

5. Phillips BE, Kelly BM, Lilja M, Ponce-González JG, Brogan RJ, Morris DL, et al. A Practical and Time-Efficient High-Intensity Interval Training Program Modifies Cardio-Metabolic Risk Factors in Adults With Risk Factors for Type II Diabetes. *Front Endocrinol (Lausanne)* (2017) 8:229. doi: 10.3389/fendo.2017.00229
6. Solomon TPJ. Sources of Inter-Individual Variability in the Therapeutic Response of Blood Glucose Control to Exercise in Type 2 Diabetes: Going Beyond Exercise Dose. *Front Physiol* (2018) 9:896. doi: 10.3389/fphys.2018.00896

Conflict of Interest: TS is the owner of Blazon Scientific company (https://www.blazon-scientific.com).

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.

frontiers in Physiology

SYSTEMATIC REVIEW
published: 03 April 2020
doi: 10.3389/fphys.2020.00214

Comparative Effectiveness of High-Intensity Interval Training and Moderate-Intensity Continuous Training for Cardiometabolic Risk Factors and Cardiorespiratory Fitness in Childhood Obesity: A Meta-Analysis of Randomized Controlled Trials

Jingxin Liu[1], *Lin Zhu*[1]* *and Yu Su*[2]

[1] Research Center for Physical Fitness and Health Promotion of Adolescent, Guangzhou Sport University, Guangzhou, China, [2] College of Physical Education, Shaoguan University, Shaoguan, China

OPEN ACCESS

Edited by:
Kristian Karstoft,
Rigshospitalet, Denmark

Reviewed by:
Roya Kelishadi,
Isfahan University of Medical Sciences, Iran
Moritz Schumann,
German Sport University Cologne, Germany

***Correspondence:**
Lin Zhu
40848567@qq.com

Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

Received: 26 October 2019
Accepted: 24 February 2020
Published: 03 April 2020

Citation:
Liu J, Zhu L and Su Y (2020) Comparative Effectiveness of High-Intensity Interval Training and Moderate-Intensity Continuous Training for Cardiometabolic Risk Factors and Cardiorespiratory Fitness in Childhood Obesity: A Meta-Analysis of Randomized Controlled Trials. Front. Physiol. 11:214. doi: 10.3389/fphys.2020.00214

Purpose: The main objective of this meta-analysis was to compare the effectiveness of high-intensity interval training (HIIT) and of moderate-intensity continuous training (MICT) on cardiometabolic health in childhood obesity and determine whether HIIT is a superior form of training in managing obese children's metabolic health.

Methods: Relevant studies published in PubMed, Web of Science, Embase, the Cochrane Library, EBSCO, and CNKI were searched, restricted to those published from inception to 1 October 2019. Only randomized controlled trials (RCTs) depicting the effect of HIIT on childhood obesity were included.

Results: Nine RCTs involving 309 participants were included in the meta-analysis. Among the 309 participants, 158 subjects were randomized for HIIT, while the others were randomized for MICT. Significant differences were observed in the body weight (mean difference [MD] = −5.45 kg, $p = 0.001$), body mass index (BMI; MD = −1.661 kg/m^2, $p = 0.0001$), systolic blood pressure (SBP; MD = −3.994 mmHg, $p = 0.003$), and diastolic blood pressure (DBP; MD = −3.087 mmHg, $p = 0.0001$) in the HIIT group relative to the baseline values. Similar effects were found in the MICT group, as depicted by the significantly decreased values for body weight (MD = −4.604 kg, $p = 0.0001$), BMI (MD = −2.366 kg/m^2, p = 0.0001), SBP (MD = −3.089 mmHg, $p = 0.019$), and DBP (MD = −3.087 mmHg, $p = 0.0001$). However, no significant differences were observed in the changes in body weight, BMI, SBP, or DBP between the HIIT and MICT groups. Furthermore, our studies showed that both HIIT and MICT could significantly improve VO_{2peak} (HIIT, MD = 4.17 ml/kg/min, 95% CI: 3.191 to 5.163, $p = 0.0001$; MICT, MD = 1.704 ml/kg/min, 95% CI: 0.279 to 3.130, $p = 0.019$). HIIT also showed more positive effects on VO_{2peak} (SMD = 0.468, 95% CI: 0.040 to 0.897, $p = 0.006$) than MICT.

Conclusion: HIIT positively affects the cardiometabolic risk factors in childhood obesity. Similar positive effects on body composition and blood pressure were established. Moreover, HIIT can improve cardiorespiratory fitness more significantly than MICT. These findings indicate that HIIT may be an alternative and effective training method for managing childhood obesity.

PROSPERO Registration Number: CRD42018111308.

Keywords: high-intensity interval training, pediatric obesity, weight loss, cardiorespiratory fitness, lipid metabolism

INTRODUCTION

Childhood obesity, defined by the World Health Organization (WHO) as abnormal or excessive fat accumulation that can eventually pose health risks, is one of the most serious global public health challenges of the twenty first century. Childhood obesity is highly prevalent; the latest epidemiological studies demonstrated that 107.7 million children worldwide were obese in 2015 and that the growth rate of childhood obesity was greater than that of adult obesity (Afshin et al., 2017). Strong evidence indicates that excess weight during childhood is a predictor of future obesity and can increase cardiometabolic risks, such as insulin resistance, dyslipidemia, hypertension, and poor cardiorespiratory fitness, in obese children (Gepstein and Weiss, 2019; Wibaek et al., 2019). Emerging evidence also shows that cardiorespiratory fitness, as an important predictor of cardiovascular disease, not only helps prevent cardiovascular disease (Castro-Pinero et al., 2017; Kachur et al., 2017; Lavie et al., 2019) but also plays a regulatory role in reducing the risk of obesity in children (Lahoz-Garcia et al., 2018; Yu et al., 2018; Prieto-Benavides et al., 2019). Therefore, adverse changes in the aforementioned contributing factors will inevitably increase the risk of cardiometabolic diseases, such as type 2 diabetes and cardiovascular disease, in adulthood (Juonala et al., 2011; Chung et al., 2018).

Exercise is a critical component of childhood obesity management because it can improve body composition and maintain cardiometabolic health. Both the American College of Sports Medicine and the WHO have strongly recommended that children allocate at least 60 min per day to moderate to vigorous physical activities and engage in high-intensity exercises at least three times per week. Moderate-intensity continuous training (MICT) is the traditional method of increasing physical activity. It is an effective way of reducing body fat and cardiometabolic risk in obese children. However, the effectiveness of MICT relies on long-duration sessions (Alberga et al., 2013; Sigal et al., 2014), and only a few children can achieve the required effective duration (Fan and Cao, 2017). Therefore, other time-efficient exercise modalities for obese children and adolescents should be explored.

High-intensity interval training (HIIT), defined as alternating short bursts of high-intensity exercise and light exercise or passive recovery periods, has been considered a good alternative, more time-efficient strategy to MICT. Existing systematic reviews and meta-analyses have revealed that HIIT has more significant effects on abdominal and visceral fat reduction and cardiorespiratory fitness improvement in overweight and obese adults than MICT (Maillard et al., 2018; Roy et al., 2018). Moreover, HIIT can reduce metabolic risk factors in type 2 diabetes more effectively than MICT (Costigan et al., 2015; Hannan et al., 2018). Wewege et al. (2017) showed that HIIT can save 40% of the time committed to MICT, with similar magnitude changes in body fat and waist circumference (WC).

HIIT studies have focused more on adults and patients with chronic disease than on obese children and adolescents. Moreover, there is no consensus or indication as to whether HIIT is superior or a good alternative training modality to MICT for reducing the cardiometabolic risk factors in childhood obesity. The purpose of our meta-analysis was to compare the effectiveness of HIIT and MICT in reducing the abovementioned cardiometabolic risk factors and determine which HIIT modality is effective and time-efficient in managing the abovementioned risks.

METHODS

The meta-analysis protocol was registered with the International Prospective Register of Systematic Reviews (CRD42018111308), and the study was conducted according to the recommendations of the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) protocols (Shamseer et al., 2015). The details of the meta-analysis protocol have been published previously by Liu et al. (2019a).

Search Strategy

Relevant studies published in PubMed, Web of Science, Embase, the Cochrane Library, EBSCO, and CNKI were searched, restricted to those published from inception to 1 December 2019. A systematic literature search strategy was employed using the patient/problem, intervention, control/comparison, outcome, study design principle. The search strategy is detailed in **Supplementary Appendix e-1**. Moreover, we screened the list of included articles cited in the relevant journals and references to identify other potentially eligible studies.

Inclusion Criteria

The studies were regarded eligible for inclusion if they met the following criteria. (1) Participants: The participants were 8- to

16-year-old children and adolescents diagnosed with childhood obesity. Childhood obesity in the present research was defined on the basis of a body mass index (BMI) ≥95th percentile for the age and gender subgroups (see Centers for Disease Control and Prevention in Kuczmarski et al., 2002) and age- and gender-specific cutoff points (males, >21.6 kg/m^2; females, >21.57 kg/m^2; see International Obesity Task Force in Cole et al., 2000) and a BMI standard deviation score (BMI-SDS) of >2 (see WHO in de Onis et al., 2007). (2) Intervention: The participants only received HIIT interventions, and HIIT was compared with MICT. We excluded the combination of HIIT or MICT with other types of exercises. HIIT intensity was defined

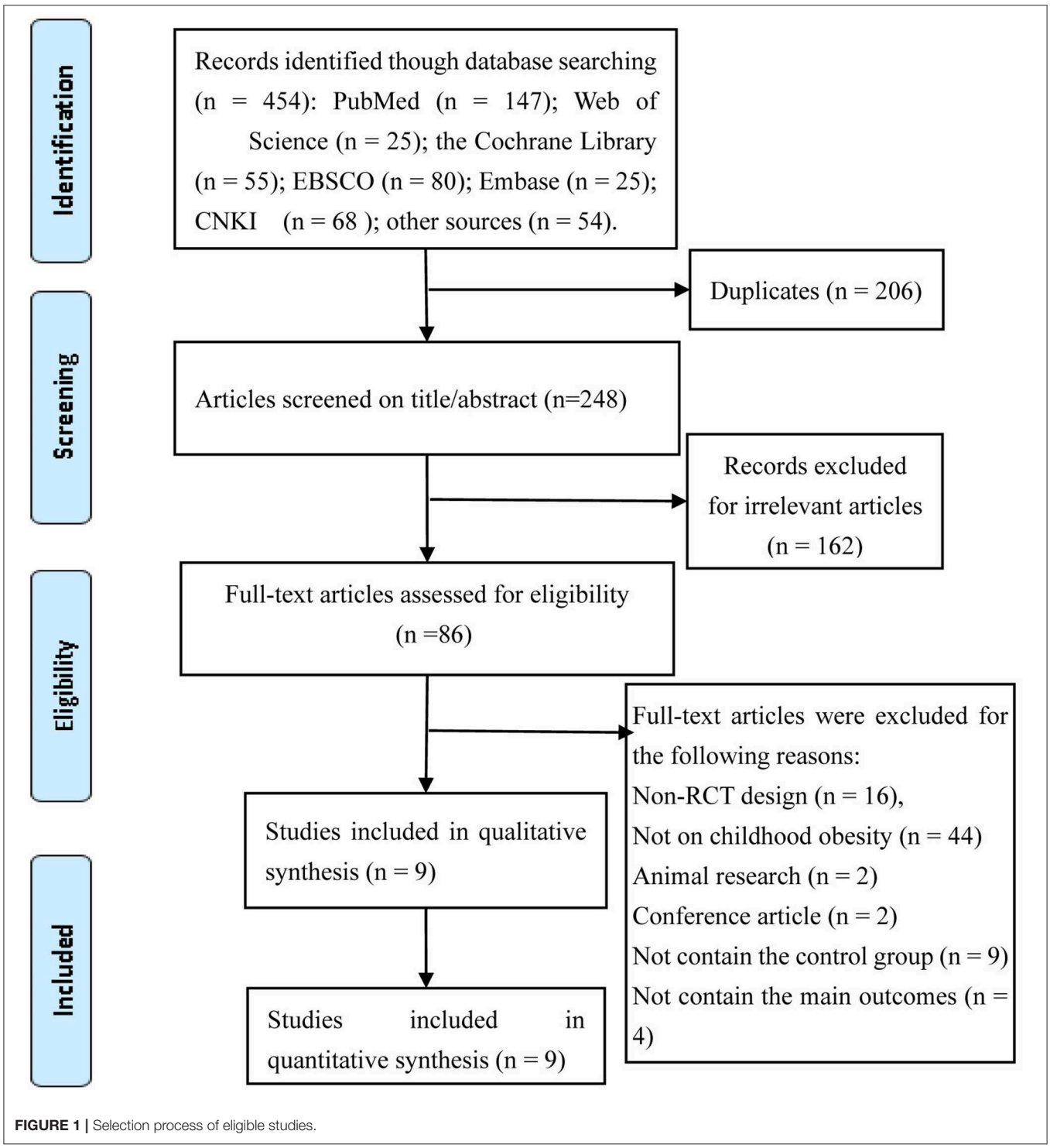


FIGURE 1 | Selection process of eligible studies.

TABLE 1 | Characteristics of the included studies.

References	Country	Study design	Main characteristics of the subjects	Interventions		Outcomes	Drop-out subjects
				HIIT	MICT		
Morrissey et al. (2018)	France	RCT	HIIT: mean age was 15.0 ± 1.4 years, mean BMI was 35.0 ± 3.0 kg/m^2, four males and 12 females, $n = 16$ MICT: mean age was 15.0 ± 1.6 years, mean BMI was 34.0 ± 1.0 kg/m^2, four males and nine females, $n = 13$	Training at 90–95% HR_{max} for 120–150 s, with a recovery interval at 55% HR_{max} for 90 s. 4–6 bouts per session, three times a week for 12 week	Training at 60–70% HR_{max}, 40–60 min per session, 3 times a week for 12 weeks	BW; BMI; BF (%); SBP; DBP; FG; FI; HOMA-IR; TG; TC	0
Dias et al. (2018)	Norway and Australia	RCT	HIIT: mean age was 12.4 ± 1.9 years, 16 males and 17 females, $n = 33$ MICT: mean age was 11.9 ± 2.4 years, 15 males and 17 females, $n = 32$.	4*4 min bouts at 85–95% HR_{max}, with an active recovery interval at 50–70% HR_{max} for 3 min, three times a week for 12 weeks	Training at 60–70% HR_{max} for 44 min, three times a week for 12 weeks	BW; BMI; BF (%); HDL-c; IR; FG; TG; LDL-c; TC; VO_{2peak}	24
Lazzer et al. (2017)	Italy	RCT	HIIT: mean age was 16.8 ± 0.7 years, 10 males, $n = 10$ MICT: mean age was 16.1 ± 1.1 years, nine males, $n = 9$	Training at 100% VO_{2peak} for 40 s, interspersed with 5 min of walking at 40% VO_{2peak}, 37 min/session, two sessions per day for 3 weeks	Training at 70% VO_{2peak} for 30 min, 31 min per session, two sessions per day for 3 weeks	BW; BMI; VO_{2peak}	0
Mahgoub and Aly (2015)	Egypt	RCT	HIIT: mean age was 13.66 ± 1.11 years, mean BMI was 30.42 ± 1.58 kg/m^2, six males and nine females, $n = 15$ MICT: mean age was 13.73 ± 1.03 years, five males and 10 females, mean BMI was 30.18 ± 1.67 kg/m^2, $n = 15$	Training at 80% VO_{2peak} for 2 min, with 1 min rest intervals, 30 min per session, 8 weeks Note: 75% VO_{2peak} in the first 4 weeks	Training at 50–60% VO_{2peak} for 30 min for 8 weeks	TC; TG; LDL-c; HDL-c	0
Starkoff et al. (2014)	USA	RCT	HIIT: mean age was 14.9 ± 1.6 years, mean BMI was 36.5 ± 5.4 kg/m^2, eight males and 10 females, $n = 18$ MICT: mean age was 14.5 ± 1.4 years, mean BMI was 38.7 ± 6.7 kg/m^2, six males and 10 females, $n = 16$	Training at 90–95% HR_{max} for 2 min, with an active recovery interval at 55% HR_{max} for 1 min, 10 bouts per session, 3 times a week for 6 weeks	Training at 65–70% HR_{max} for 30 min, three times a week for 6 weeks	VO_{2peak}	7
Xiuming (2014)	China	RCT	HIIT: mean age was 10.20 ± 0.45 years, mean BMI was 28.0 ± 1.19 kg/m^2, 20 males and 10 females, n = 30 MICT: mean age was 10.40 ± 1.34 years, mean BMI was 28.50 ± 1.11 kg/m^2, 22 males and eight females, $n = 30$	Training 90–95% HR_{max} for 1 min, and then gradually to 50% HR_{max} within 1, 30 min per session, twice a week for 12 weeks	Training at 80% HR_{max} for 30–60 min, twice a week for 12 weeks	BW; BMI; SBP; FG; DBP; FI; TC; HDL-c; LDL-c; TG; HOMA-IR	0
Murphy et al. (2015)	USA	RCT	HIIT: mean age was 13.7 ± 2.0 years, two males and five female, $n = 7$ MICT: mean age was 14.3 ± 1.2 years, one male and five females, $n = 6$	Training at 80–90% HR_{max} for 1 min, with an active interval at 60% HR_{max} for 2, 30 min per session, three times a week for 4 weeks	Training at 65% HR_{max} for 30 min, three times a week for 4 weeks.	BW; BMI; BF (%); SBP; SDP; VO_{2peak}	0
Koubaa et al. (2013)	Tunisia	RCT	HIIT: mean age was 13 ± 0.8 years, mean BMI was 30.2 ± 3.6 kg/m^2, 14 males, $n = 14$ MICT: mean age was 12.9 ± 0.5 years, mean BMI was 30.8 ± 2.9 kg/m^2, 15 males, $n = 15$	Training at 80% VO_{2max} for 2 min, interspersed with 1 min recovery, three times per week for 12 weeks	Training at 60–70% VO_{2max} for 30–40 min, three times per week for 12 weeks	BW; BMI; TC; HDL-c; LDL-c; SBP; WC; DBP; TG; VO_{2peak}	1

(Continued)

TABLE 1 | Continued

References	Country	Study design	Main characteristics of the subjects	Interventions		Outcomes	Drop-out subjects
				HIIT	MICT		
Corte de Araujo et al. (2012)	Brazil	RCT	HIIT: mean age was 10.7 ± 0.7 years, mean BMI was 30.8 ± 3.7 kg/m^2, five males and 10 females, $n = 15$ MICT: mean age was 10.4 ± 0.9 years, mean BMI was 29.6 ± 4.0 kg/m^2, four males and 11 females, $n = 15$	Training at 100% peak velocity for 1 min, with a 3 min interval at 50% peak velocity, twice a week for 12 weeks	Training at 80% HR_{max} for 30–60 min, twice a week for 12 weeks	BW; BMI; WC; SBP; DBP; FG; FI; HDL-c; LDL-c; HOMA- IR; TG; TC	0

BW, body weight; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FG, fasting glucose; FI, fasting insulin; TG, triglyceride; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; RCT, randomized controlled trial; HIIT, high-intensity interval training; MICT, moderate-intensity interval training.

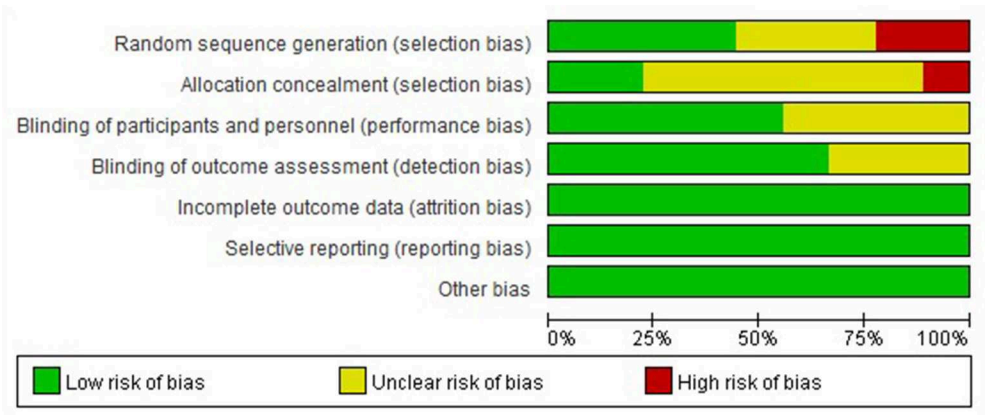


FIGURE 2 | Review judgment of risk bias for each item: percentages across all included studies.

as maintaining 80–100% of the peak heart rate (HR_{peak}) or VO_{2peak} (Keating et al., 2017) for 30 s to 4 min, interspersed to a maximum of 4 min of passive recovery or low-intensity aerobic exercise. Exercise intensity, prescribed as a percentage of heart rate (HR) reserve, maximal aerobic speed, and rate of perceived exertion equivalent to 80–100% of HR_{peak} and VO_{2peak}, was included in HIIT (Garber et al., 2011). MICT intensity was defined as maintaining 40–79% of HR_{peak} or VO_{2peak} for 20–60 min. The HIIT and MICT interventions entailed the same training frequencies and durations. (3) Outcomes: The studies reported at least one of the following data types related to the cardiometabolic risk factors: body composition (e.g., body weight, BMI, WC, and body fat percentage), glucose metabolism (e.g., blood fasting glucose, blood fasting insulin, and homeostatic model assessment of insulin resistance [HOMA-IR]), blood pressure (e.g., systolic blood pressure [SBP] and diastolic blood pressure (DBP), lipid metabolism (e.g., high-density lipoprotein cholesterol [HDL-c], low-density lipoprotein cholesterol [LDL-c], triglyceride [TG], and total cholesterol [TC]), and cardiorespiratory fitness (e.g., VO_{2peak}). (4) Design: Only randomized controlled trials (RCTs) were included.

Selection of Studies and Data Extraction

Two independent reviewers performed a study screening process following the PRISMA guidelines. A bibliographic reference manager (EndNote X7, Thomson Reuters) was used to remove duplicate entries. After screening the titles and abstracts, the studies that did not meet our eligibility criteria were excluded. The remaining studies were evaluated by reading their full texts and making a final decision. All differences between the reviewers' viewpoints were resolved through discussions or consultation with a third reviewer.

The data were extracted from each study following the predesigned guideline on unified standardization by two independent reviewers. The following data were extracted: first author's name, publication year, country, participant characteristics (gender and age), number of participants, intervention protocols (training intensity and time, interval intensity and time, and frequency and duration), main outcomes, and dropout rates. If duplicate data were observed in the different studies during data collection, then additional comprehensive studies were extracted, and the authors were consulted if data were missing.

Risk of Bias Assessment

The methodological quality of the included studies was evaluated by two independent authors (JL and LZ) who used Cochrane Collaboration's tools to check for random sequence generation, allocation concealment, blinding, incomplete outcome data, selective reporting, and other biases; the evaluation results were categorized into high-risk, low-risk, and unclear grades (Higgins et al., 2011).

Data Analysis and Synthesis

Data analysis was performed using Review Manager 5.3.5 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark) and Stata 12.0. The mean difference (MD) with a 95% confidence interval (95% CI) was calculated for the effect size either between HIIT and MICT or between pre-intervention and post-intervention in each group. The standard mean difference (SMD) with 95% CI was selected due to the varying units or the large differences among the studies. The change in values from the baseline in each group was calculated with the formula $M = \mid M_1 - M_2 \mid$, where M is the effect mean, M_1 is the effect mean of the baseline, and M_2 is the end value mean, followed by the formula $S^2 = S_1^2 + S_2^2 - 2 \times R \times S_1 \times S_2$, where S is the standard deviation of the effect, S_1 is the standard deviation of the baseline value, S_2 is the final standard deviation, and R is a constant (0.4 or 0.5). I^2 statistic and Q statistic were used to estimate the heterogeneity between two studies.

Values of $0\% \leq I^2 < 25\%$ indicate trivial heterogeneity, $25\% \leq I^2 < 50\%$ indicate small heterogeneity, $50\% \leq I^2 < 75\%$ indicate moderate heterogeneity, and $75\% \leq I^2 < 100\%$ indicate high heterogeneity. If moderate or high heterogeneity exists between the studies, then the random effect model is used; otherwise, the fixed-effect model is adopted. If moderate or high heterogeneity exists between the studies, then sensitivity analysis and subgroup analysis are conducted. Here, sensitivity analysis was performed by changing the pooled model or by adopting a 1 × 1 exclusion approach.

Subgroup analysis was performed to examine whether the training parameters in the included studies positively affect the cardiometabolic risk factors. The following intervention features were examined: training session time, total training duration, and type of interval. The subgroup analysis of each outcome was carried out by referring to at least two studies, and a chi-square test was conducted to assess heterogeneity between subgroups. Egger's test was carried out to assess publication bias. Univariate meta-regression analyses were not conducted due to the limited number of studies.

RESULTS

Study Selection

A total of 454 studies were identified in accordance with our search strategy. Among the 454 studies, nine RCTs were considered eligible after the duplicates were removed, the titles/abstracts were screened, and the full texts were reviewed. The selection process of identifying eligible studies is shown in **Figure 1**.

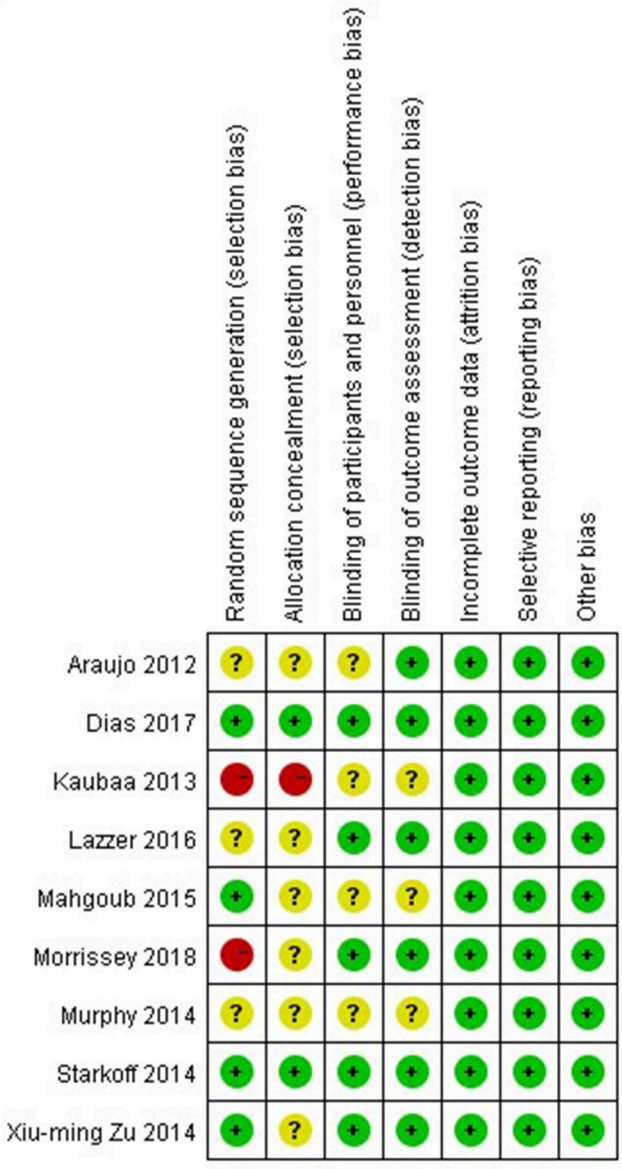


FIGURE 3 | Summary of risk bias: review authors' judgment of risk bias for each item and adolescent.

Study Characteristics and Participants

The primary characteristics of the included studies are shown in **Table 1**. A total of 309 participants were included in the meta-analysis, of whom 166 participants were males and the remaining were females. The gender composition of the participants in the HIIT group was 85 males and 73 females, while that of the MICT group was 81 males and 73 females. The mean age of the participants was 13.31 ± 1.94 years old, and the mean BMI was 31.89 ± 3.09 kg/m^2.

Most of the studies monitored training intensity with the use of HR monitors or monitored oxygen consumption to ensure adequate training intensity. The nine studies applied

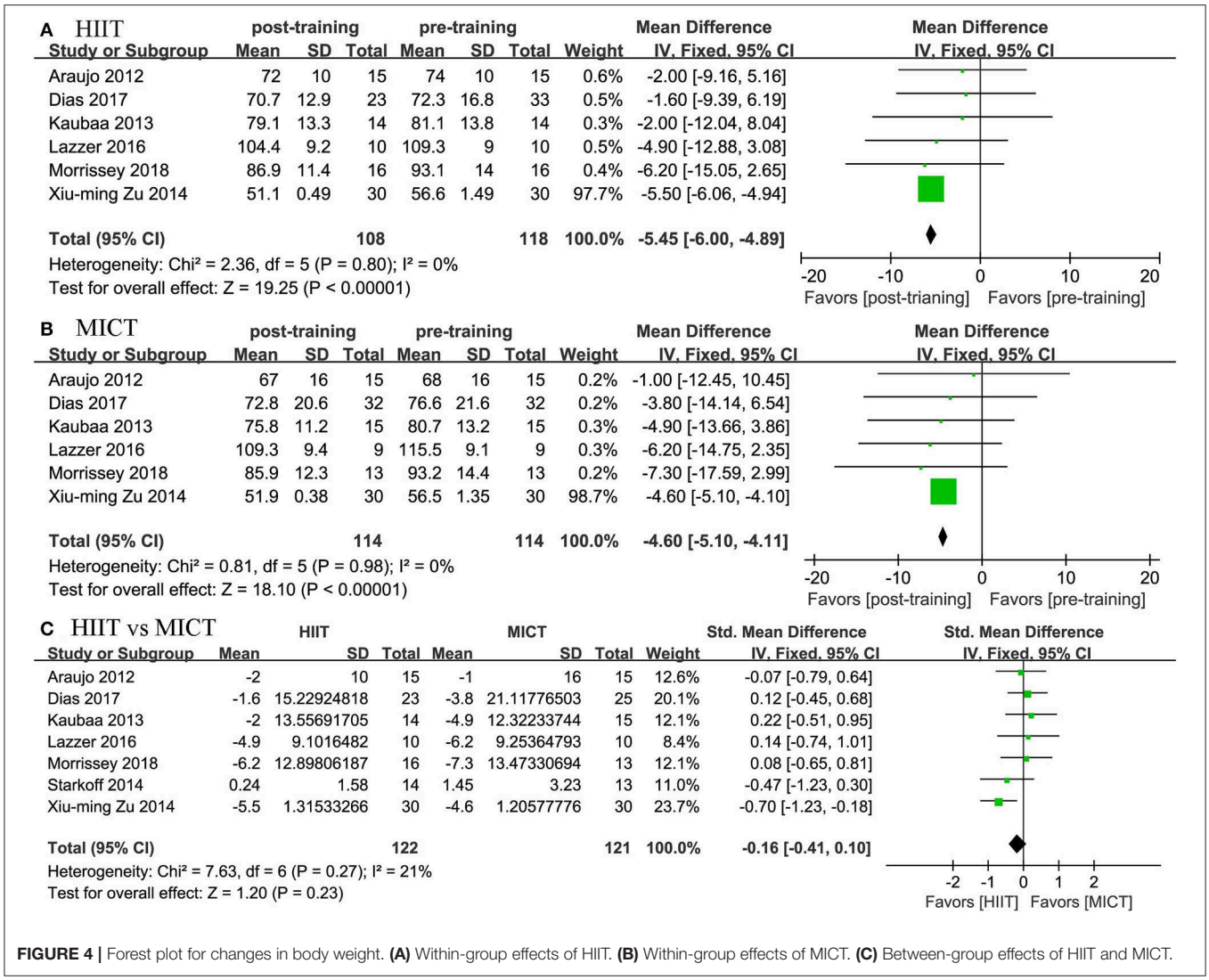


FIGURE 4 | Forest plot for changes in body weight. **(A)** Within-group effects of HIIT. **(B)** Within-group effects of MICT. **(C)** Between-group effects of HIIT and MICT.

HIIT strategies involving a variety of intensities and interval durations; HIIT strategies could be divided into short-interval training programs and long-interval training programs. The short-interval training programs consisted of training performed at intensities >90% VO_{2peak} or HR_{max} for 30 s to 2 min intervals with 30 s to 1 min recovery (Corte de Araujo et al., 2012; Xiuming, 2014; Murphy et al., 2015; Lazzer et al., 2017). The long-interval training programs consisted of training performed at intensities of 80% VO_{2peak} or 85–100% HR_{max} fort 2–4 min intervals with 1–3 min recovery (Koubaa et al., 2013; Starkoff et al., 2014; Mahgoub and Aly, 2015; Dias et al., 2018; Morrissey et al., 2018). The majority of the MICT programs consisted of training performed at 60–80% HR_{max} or 60–70% VO_{2peak} for 30–40 min. The frequency range of HIIT was two to three times per week; most of the reviewed studies mentioned three times per week. The duration of HIIT usually lasted for 3–12 weeks; most of the reviewed studies mentioned 12 weeks.

Among the included studies, only Corte de Araujo et al. (2012), Dias et al. (2018), and Lazzer et al. (2017) reported methods to assess dietary intake or energy intake. No adverse events were mentioned in any of the studies.

Methodological Quality of Included Studies

The nine studies were assessed for risk bias (**Figures 2**, **3**). Among the included studies, four studies cited random sequence generation, two studies mentioned concealment, five studies involved the blind participation of respondents and personnel, and six studies described the blind participation of outcome evaluators. None of the included studies reported incomplete outcome data, selective reports bias, or other biases.

Meta-Analysis

Body Composition

Eight studies assessed the effects of HIIT and MICT on body composition, as measured by body weight ($n = 7$), BMI ($n = 8$), WC ($n = 3$), and body fat percentage ($n = 5$). Significant differences were observed for body weight (MD = −5.45 kg, 95% CI: −6.003 to −4.894, $p = 0.001$) and BMI (MD =

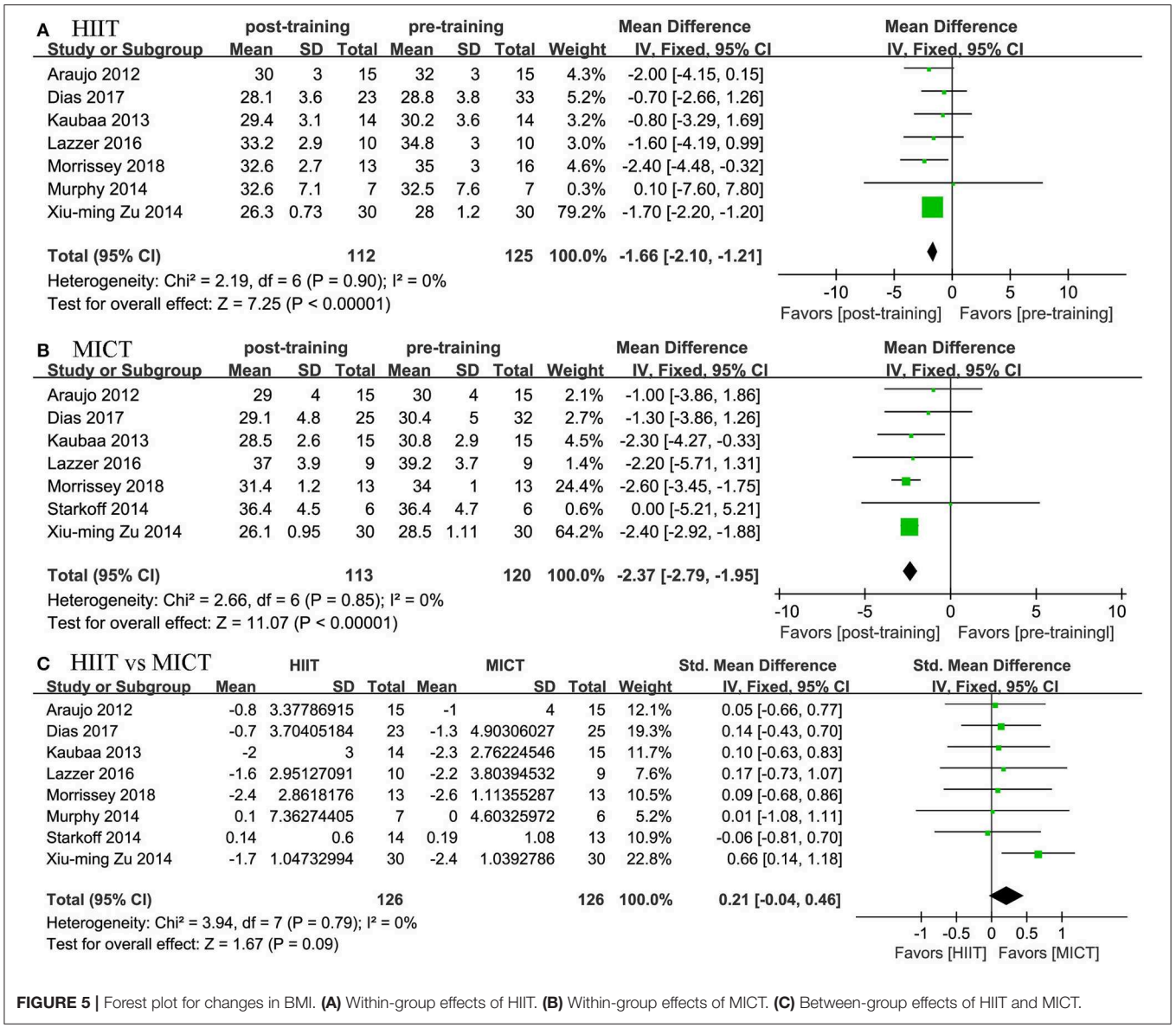


FIGURE 5 | Forest plot for changes in BMI. **(A)** Within-group effects of HIIT. **(B)** Within-group effects of MICT. **(C)** Between-group effects of HIIT and MICT.

−1.661 kg/m^2, 95% CI: −2.109 to −1.213, p = 0.0001) in the HIIT group (**Figures 4**, **5**) relative to the baseline values. Similar effects were found in the MICT group (**Figures 4**, **5**), as indicated by the significantly decreased body weight (MD = −4.604 kg, 95% CI: −5.103 to −4.106, p = 0.0001), BMI (MD = −2.366 kg/m^2, 95% CI: −2.785 to −1.947, p = 0.0001), and WC (MD = −6.468 cm, 95% CI: −11.546 to −1.389, p = 0.013). However, no significant differences were observed in the changes in body weight (SMD = −0.16, 95% CI: −0.41 to 0.10, p = 0.23), BMI (SMD = 0.21, 95% CI: −0.04 to 0.46, p = 0.09), WC (MD = −0.342 cm, 95% CI: −3.204 to 2.520, p = 0.815), or body fat percentage (MD = −0.253%, 95% CI: −1.392 to 0.885, p = 0.663) between the HIIT and MICT interventions (**Table 2**).

Glucose Metabolism

Four studies assessed the effects of HIIT and MICT on glycemic control, as measured by fasting glucose (n = 4), fasting insulin (n = 3), and HOMA-IR (n = 3). The meta-analysis showed significantly reduced values for fasting glucose (MD = −0.445 mmol/L, 95% CI: −0.834 to −0.0.056, p = 0.025) in the HIIT group relative to the baseline values. The meta-analysis also showed that MICT had no significant effects based on the fasting glucose, fasting insulin, or HOMA-IR values. In the between-group comparison, the pooled results from the meta-analysis showed that HIIT elicited a higher change trend for fasting glucose (MD = −0.479 mmol/L), fasting insulin (SMD = −0.694), and HOMA-IR (SMD = −0.554); however, none of the changes in the effect values were significant (**Table 2**).

TABLE 2 | Summary of the meta-analysis.

Outcomes			Within-group effects		HIIT vs. MICT
			HIIT	MICT	
Body weight	N		6	6	7
	ES (95% CI)		MD: −5.45 (−6.003, −4.894)	MD: −4.604 (−5.103, −4.106)	SMD: −0.16 (−0.41, 0.10)
	Heterogeneity	I^2	0	0	21%
		P	0.797	0.977	0.27
	P		0.0001	0.0001	0.23
BMI	N		7	7	8
	ES (95% CI)		MD: −1.661 (−2.109, −1.213)	MD: −2.366 (−2.785, −1.947)	SMD: 0.21 (−0.04, 0.46)
	Heterogeneity	I^2	0	0	0
		P	0.851	0.851	0.79
	P		0.0001	0.0001	0.09
WC	N		2	2	3
	ES (95% CI)		MD: −4.575 (−9.506, 0.356)	MD: −6.468 (−11.546, −1.389)	MD: −0.342 (−3.204, 2.520)
	Heterogeneity	I^2	2.4%	0	28.6%
		P	0.311	0.818	0.247
	P		0.069	0.013	0.815
Body fat (%)	N		4	4	5
	ES (95% CI)		MD: −0.792 (−2.551, 0.967)	MD: −0.156 (−1.985, 1.674)	MD: −0.253 (−1.392, 0.885)
	Heterogeneity	I^2	12.4%	29.8%	0
		P	0.331	1.042	0.607
	P		0.378	0.867	0.663
VO_{2peak}	N		4	4	4
	ES (95% CI)		MD: 4.17 (3.191, 5.163)	MD: 1.704 (0.279, 3.130)	MD:2.497 (1.151, 3.843)
	Heterogeneity	I^2	0	0	0
		P	0.822	0.819	0.636
	P		0.0001	0.019	0.0001
SBP	N		4	4	4
	ES (95% CI)		MD: −3.994 (−6.942, −1.045)	MD: −3.089 (−5.679, −0.498)	MD: −1.208 (−2.603, 0.186)
	Heterogeneity	I^2	78.4%	60%	47.2%
		P	0.003	0.058	0.128
	P		0.008	0.019	0.089
DBP	N		4	4	4
	ES (95% CI)		MD: −3.087 (−4.083, −2.092)	MD: −2.481 (−3.551, −1.410)	MD: 1.213 (−2.597, 5.023)
	Heterogeneity	I^2	24.0%	23.1%	77.3%
		P	0.267	0.272	0.004
	P		0.0001	0.0001	0.533
TC	N		6	6	6
	ES (95% CI)		MD: −0.221 (−0.594, 0.108)	MD: −0.265 (−0.635, 0.124)	MD: −0.141 (−0.619, 0.337)
	Heterogeneity	I^2	95.8%	97.2%	95.4
		P	0.0001	0.1963	0.0001
	P		0.1442	0.182	0.563
HDL–c	N		5	5	5
	ES (95% CI)		MD: 0.198 (−0.162, 0.557)	MD: 0.120 (−0.035, 0.275)	MD: 0.086 (−0.164, 0.337)
	Heterogeneity	I^2	98.6%	92.9%	95.7
		P	0.0001	0.0277	0.0001
	P		0.281	0.130	0.499
LDL–c	N		5	5	5
	ES (95% CI)		MD: −0.495 (−1.059, 0.068)	SMD: −1.142 (−2.277, −0.007)	MD: −0.142 (−0.348, 0.063)
	Heterogeneity	I^2	98.3%	91.9%	69.0%
		P	0.000	0.000	0.012
	P		0.3913	0.049	0.174

(Continued)

TABLE 2 | Continued

Outcomes			Within-group effects		HIIT vs. MICT
			HIIT	MICT	
TG	N		6	6	6
	ES (95% CI)		MD: −0.085 (−0.271, 0.1)	MD: −0.048 (−0.110, 0.013)	MD: −0.052 (−0.113, 0.009)
	Heterogeneity	I^2	89.6%	48.1%	20.7
		P	0.0001	0.086	0.278
	P		0.365	0.123	0.096
HOMA–IR	N		4	4	4
	ES (95% CI)		MD: −1.296 (−3.186, 0.595)	−0.814 (−2.187, 0.559)	SMD: −0.554 (−1.202, 0.093)
	Heterogeneity	I^2	98.0%	95.9%	77.0%
		P	0.179	0.0001	0.005
	P		0.215	0.245	0.093
Fasting glucose	N		4	4	4
	ES (95% CI)		MD: −0.445 (−0.834, −0.056)	MD: 0.035 (−0.316, 0.387)	MD: −0.479 (−0.975, 0.017)
	Heterogeneity	I^2	98.9%	91.2%	95.6%
		P	0.1508	0.001	0.0001
	P		0.025	0.843	0.059
Fasting insulin	N		3	3	3
	ES (95% CI)		SMD: −1.548 (−3.551, 0.454)	SMD: −0.343 (−711, 0.026)	SMD: −0.694 (−1.816, 0.428)
	Heterogeneity	I^2	95.2%	0%	87.7%
		P	0.001	0.414	0.001
	P		0.130	0.068	0.225

Lipid Metabolism

Six studies reported the effects of HIIT and MICT on blood lipids, as measured by TC ($n = 6$), HDL-c ($n = 5$), LDL-c ($n = 5$), and TG ($n = 6$). As the heterogeneity was large in all the comparisons ($I^2 = 95.8\%$ for TC, $I^2 = 98.6\%$ for HDL-c, $I^2 = 98.3\%$ for LDL-c, and $I^2 = 89.6\%$ for TG in HIIT vs. the baseline; $I^2 = 97.2\%$ for TC, $I^2 = 92.9\%$ for HDL-c, and $I^2 = 91.9\%$ for LDL-c in MICT vs. the baseline), no evidence was derived from the pooled results for the effects of TC, HDL-c, LDL-c, or TG. MICT was determined to have no effects on TC, HDL-c, and TG relative to the baseline values. The change in HIIT was not significant; that is, no significant effects on the change in HIIT were found in any of the measures, unlike the change in MICT (**Table 2**).

Blood Pressure

Four studies reported the effects of HIIT on SBP and DBP and compared them with the effects of MICT. We found positive effects of HIIT and MICT on blood pressure. In terms of SBP, we found a positive effect of HIIT (MD = −3.994 mmHg, 95% CI: −6.942 to −1.045, $p = 0.003$) and MICT (MD = −3.089 mmHg, 95% CI: −5.679 to −0.498, $p = 0.019$). In terms of DBP, we also found a positive effect of HIIT (MD = −3.087 mmHg, 95% CI: −4.083 to −2.092, $p = 0.0001$) and MICT (MD = −2.481 mmHg, 95% CI: −3.551 to −1.410, $p = 0.0001$). The two groups did not differ significantly; no significant difference was established by the meta-analysis for the change in blood pressure between the HIIT and MICT interventions (HIIT, MD = −1.208 mmHg, 95% CI: −2.603 to 0.186, $p = 0.089$; MICT, MD = 1.213 mmHg, 95% CI: −2.597 to 5.023, $p = 0.533$; **Figures 6, 7**).

Cardiorespiratory Fitness

Four studies reported the effects of HIIT on VO_{2peak} and compared them with the effects of MICT. The meta-analysis showed that both HIIT and MICT could significantly improve VO_{2peak} (HIIT, MD = 4.17 mL/kg/min, 95% CI: 3.191 to 5.163, $p = 0.0001$; MICT, MD = 1.704 mL/kg/min, 95% CI: 0.279 to 3.130, $p = 0.019$). The pooled results of the meta-analysis also revealed that HIIT had a more positive effect on VO_{2peak} than MICT (MD = 2.497 mL/kg/min, 95% CI: 1.151 to 3.843, $p = 0.0001$; **Figure 8**).

Subgroup Analysis

Subgroup analysis was conducted based on the HIIT training parameters of the training session time, duration, and interval protocol. The subgroup analysis results revealed that duration was a key parameter associated with cardiorespiratory fitness improvement. HIIT programs of ≥8 weeks showed positive effects on VO_{2peak} (SMD = 0.805, 95% CI: 0.334 to 1.276, $p = 0.001$) compared with MICT. However, HIIT programs of <8 weeks did not show any positive effects on VO_{2peak} (SMD = 0.276, 95% CI: −0.346 to 0.904, $p = 0.381$). Furthermore, long-interval HIIT programs seemed to more effectively improve VO_{2peak} (SMD = 0.691, 95% CI: 0.290 to 1.092, $p = 0.006$) than short-interval HIIT programs. HIIT also demonstrated positive effects on VO_{2peak} (SMD = 0.468, 95% CI: 0.040 to 0.897, $p = 0.006$) and LDL-c (SMD = −0.777, 95% CI: −1.456 to −0.098, $p = 0.028$), which were greater than the effects of MICT (**Table 3**).

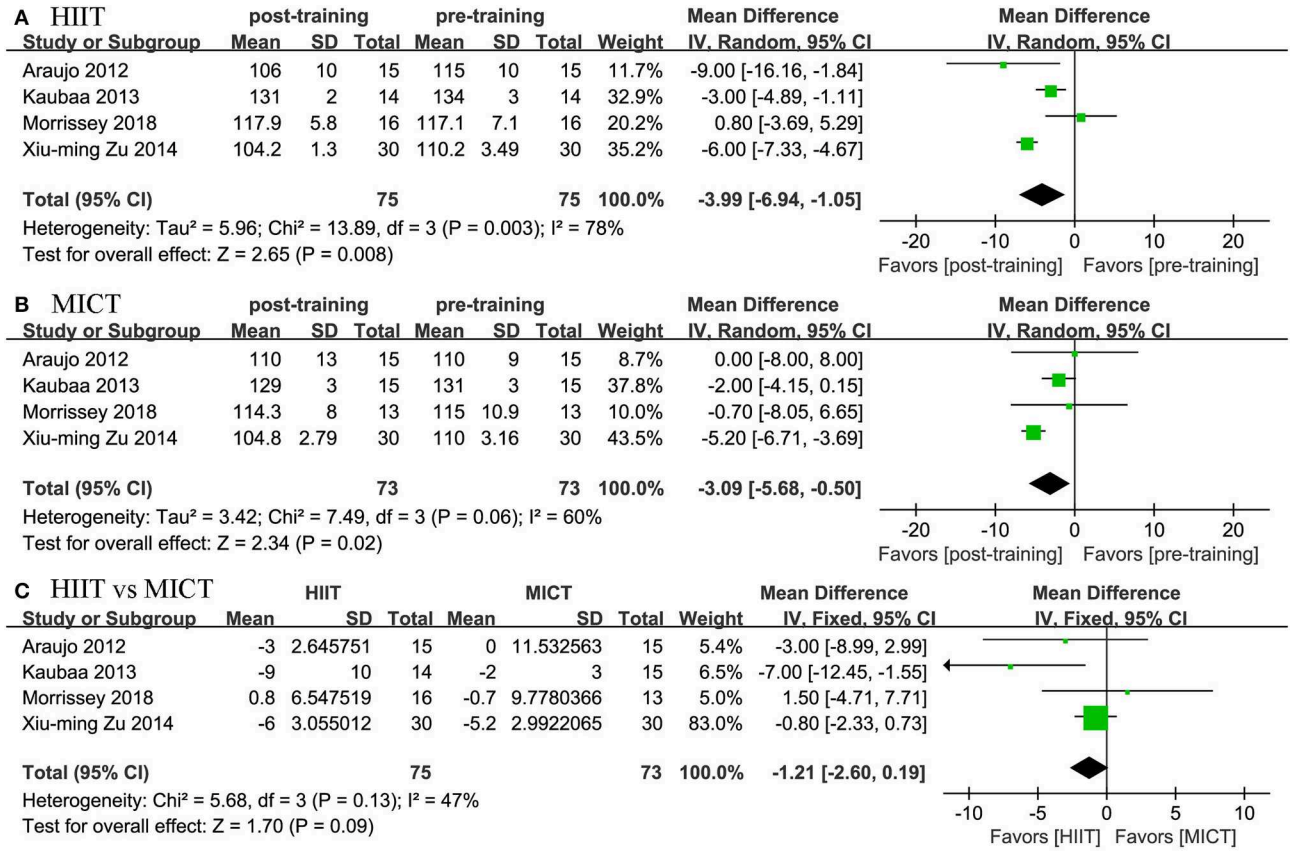


FIGURE 6 | Forest plot for changes in SBP. **(A)** Within-group effects of HIIT. **(B)** Within-group effects of MICT. **(C)** Between-group effects of HIIT and MICT.

DISCUSSION

This meta-analysis is the first study to systematically compare the effectiveness of HIIT and MICT on cardiometabolic risk factors and cardiorespiratory fitness in childhood obesity. The results showed that HIIIT and MICT were effective interventions in reducing cardiometabolic risk through body weight, BMI, SBP, and DBP reduction. However, no significant differences were noted in these effects in the comparison between the HIIT and MICT interventions. Furthermore, the meta-analysis revealed that the post-intervention change in VO_{2peak} was significantly greater following HIIT than following MICT at the same training session. With no significant adverse events reported, these findings indicated that HIIT is an appropriate and alternative training modality to MICT for reducing cardiometabolic risk in childhood obesity.

We found meaningful reductions from pre- to post-intervention of −5.45 and −4.604 kg in body weight and −1.661 and −2.366 kg/m^2 in BMI in the HIIT and MICT groups, respectively, with no difference in the change in body weight or BMI in the pooled results and subgroup analysis. Therefore, our results confirmed that both HIIT and MICT improved body composition to a similar extent in childhood obesity. These findings were consistent with those reported by Keating et al. (2017), whose meta-analysis combined 31 studies involving 873 participants and demonstrated that both HIIT and MICT are equally beneficial for eliciting a small reduction in body fat when a similar time commitment or energy expenditure is used in young adults and adults who are overweight or obese. A recent meta-analysis conducted by Wewege et al. (2017) also found that HIIT and MICT induce a similar magnitude of change in body fat and WC in overweight and obese adults. Given the magnitude and statistics of the effect sizes associated with the training session time, duration, and interval protocol, subgroup analysis was conducted to explore whether different HIIT training parameters cause changes in body composition compared with MICT. The subgroup analysis demonstrated that none of the body composition measures in any subgroup elicited greater changes in HIIT than in MICT. These findings suggested that HIIT may be an effective alternative to MICT, achieving equivalent levels of body composition improvement. Moreover, although there was no difference in body composition improvement between the HIIT and MICT interventions, the physiological nature of HIIT and MICT differed. First, moderate-intensity exercise may involve elevated rates of burning of fat as a substrate, with a sustained high release of free fatty acids (FFAs) and subsequent oxidation of FFAs, whereas high-intensity exercise may be associated with the increased secretion of catecholamine

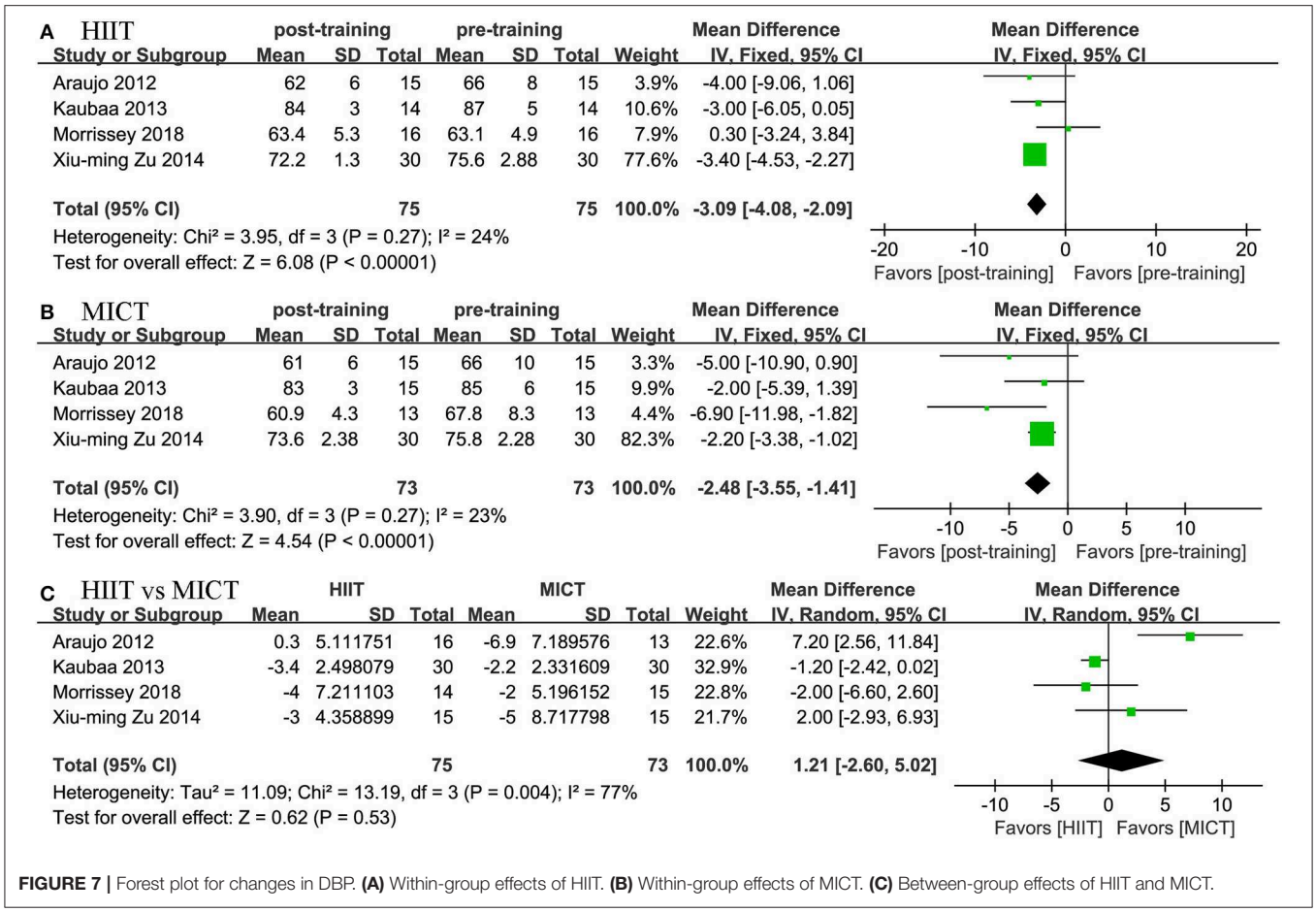


FIGURE 7 | Forest plot for changes in DBP. **(A)** Within-group effects of HIIT. **(B)** Within-group effects of MICT. **(C)** Between-group effects of HIIT and MICT.

and growth hormone, which could improve the rates of adipose lipolysis (Jensen, 2003; Trapp et al., 2007; van Hall, 2015). Second, high-intensity exercise elicits high excess post-exercise oxygen consumption, which promotes a substrate shift that favors fat utilization during the recovery period (Saris and Schrauwen, 2004; Islam et al., 2018). The findings of Treuth et al. (1996), Saris and Schrauwen (2004) supported this inference, demonstrating that high-intensity exercise and moderate-intensity exercise are similar for fat consumption within 24 h after exercise. These factors might explain why HIIT could achieve similar effects on body composition as MICT in obese children and adolescents.

Our studies indicated that both HIIT and MICT led to a small but significant reduction in SBP and DBP, which may have a positive effect on preventing hypertension in childhood obesity. The potential for blood pressure reduction is low, which may be related to the fact that our study did not include work based on hypertension. The absence of a significant difference in blood pressure reduction between HIIT and MICT was similar to the findings of previous systematic reviews and meta-analyses, indicating that HIIT and MICT could provide a similar decrease in SBP and DBP in adults with pre- to established hypertension (Costa et al., 2018). Campbell et al. (2019) also demonstrated that a HIIT-induced blood pressure reduction was comparable with that of MICT in adults; people who were overweight or obese were more responsive in terms of blood pressure reduction than people with a normal weight. All of these findings suggested that the effects of HIIT and MICT on blood pressure reduction were comparable; our study proved that those effects also exist in childhood obesity. The mechanism of HIIT in blood pressure reduction may be related to nitric oxide (NO). Previous research demonstrated that high-intensity exercise can increase the blood flow velocity, resulting in increased NO production by vascular endothelial cells, further vasodilation of blood vessels, and lowered blood pressure (Ghardashi Afousi et al., 2018; Izadi et al., 2018). Furthermore, HIIT could increase the shear stress of the vascular endothelium, reduce sympathetic nerve activity and peripheral blood vessel resistance, and lower blood pressure (Nishida et al., 1992; Halliwill, 2001; Green et al., 2004; Pal et al., 2013; Sawyer et al., 2016).

Unfortunately, our study failed to provide sufficient evidence to confirm the effects of HIIT and MICT on glucose metabolism and lipid metabolism, even if an improvement was observed in both interventions. These findings were inconsistent with the results of previous studies, which found that MICT and HIIT, when implemented in early life, are effective in lowering the relative weight of adipose tissue and improving glucose metabolism, thereby reverting or preventing metabolic alterations (Marcinko et al., 2015; de Lade et al., 2018). Recent

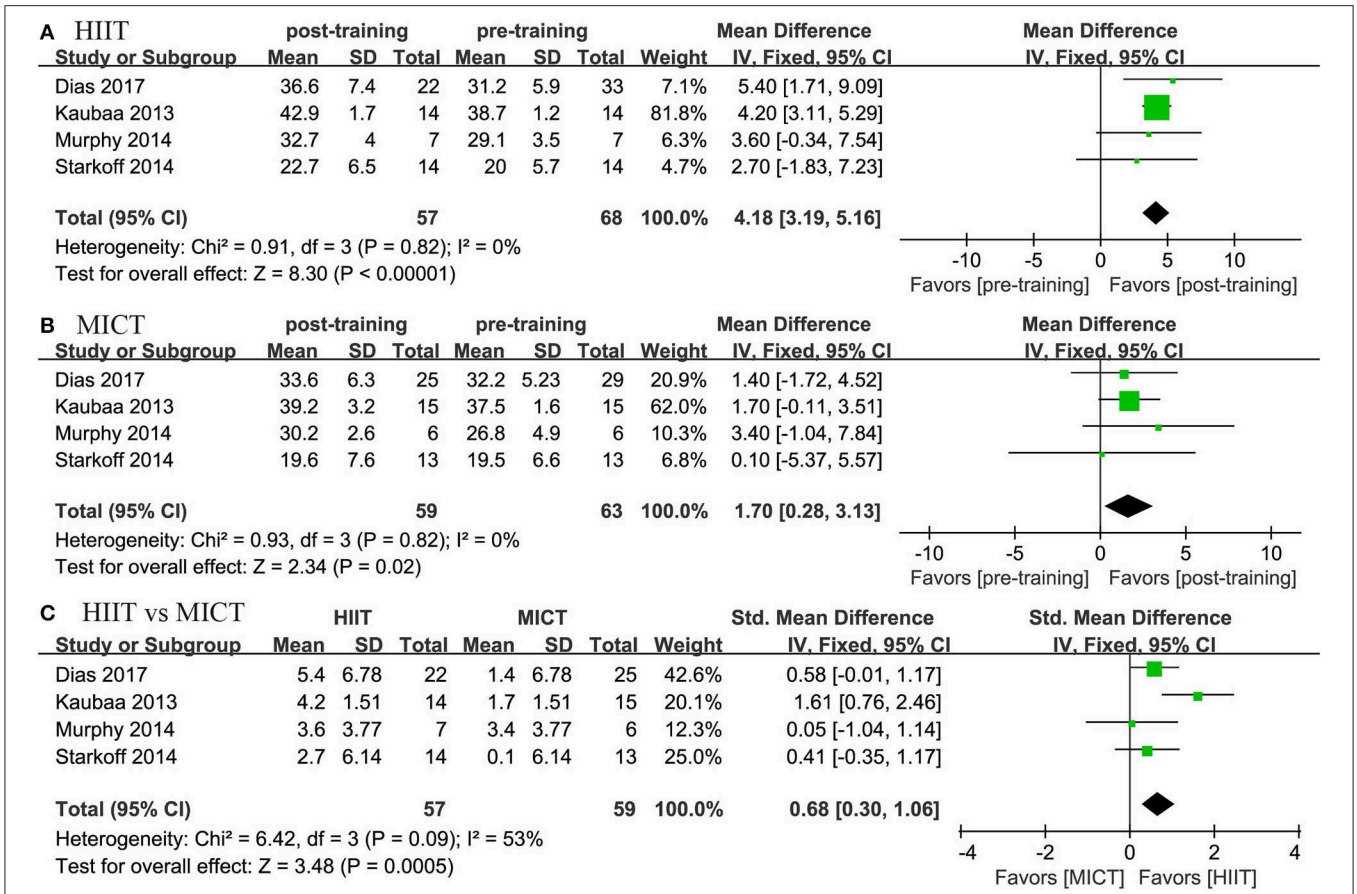


FIGURE 8 | Forest plot for changes in VO_{2peak}. **(A)** Within-group effects of HIIT. **(B)** Within-group effects of MICT on body weight. **(C)** Between-group effects of HIIT and MICT.

studies have found that a large proportion of individuals do not respond (non-responders) or respond adversely to exercise in terms of glycemic control (Atkinson and Batterham, 2015; Bohm et al., 2016). Some variables, such as exercise dose and other phenomena like genetics and gut microbiota, have been identified as the causes of response heterogeneity. We attempted to use subgroups to analyze the effects of different HIIT training parameters on glucose metabolism and lipid metabolism, but no significant changes were found. Recent studies indicated that the dysbiosis of gut microbiota plays a critical role in response to exercise; moreover, HIIT-induced alterations in the gut microbiota are correlated closely with improvements in glucose homeostasis and insulin sensitivity, and participants who do not respond to HIIT are characterized by increased production of metabolically detrimental compounds (Bouter et al., 2017; Liu Y. et al., 2019). However, research on gut microbiota in obese adolescents remains limited. Therefore, the mechanism of the gut microbiota in improving glycemic and lipid metabolism in non-response children with obesity should be further explored by high-quality studies.

Notably, a significant improvement from pre- to post-intervention of 4.17 and 1.704 mL/kg/min for VO_{2peak} was found in both of the HIIT and MICT interventions, with greater improvement observed in the HIIT intervention than in MICT. Therefore, both HIIT and MICT could effectively improve cardiorespiratory fitness in obese children and adolescents. From a clinical perspective, the improvement of cardiorespiratory fitness in childhood is associated with enhanced cardiometabolic health in later life. Schmidt et al. (2016) showed that low cardiorespiratory fitness in childhood is a significant independent predictor of metabolic syndrome (MS) in early adulthood; obese children with low cardiorespiratory fitness who increase their relative fitness by adulthood present substantially reduced risk of MS compared with those who maintain low fitness. Moreover, Lahoz-Garcia et al. (2018) demonstrated that cardiorespiratory fitness is a partial mediator of the relationship of energy and macronutrient intake with obesity. Previous studies have revealed that cardiorespiratory fitness as a mediator could reduce the rate of cardiovascular disease-induced mortality by 15% for every one metabolic equivalent in adults. All of these findings suggest the importance of improving cardiorespiratory fitness in obese children and adolescents for cardiometabolic risk reduction. Our meta-analysis demonstrated that HIIT improved cardiorespiratory fitness compared with MICT for the first time in obese children and adolescents; these results were in accordance with previous

TABLE 3 | Subgroup analysis for the change in cardiometabolic risk factors in HIIT and MICT.

Outcomes		N	ES (95% CI)	Heterogeneity		p
				I^2 (%)	P	
Body weight	>8 weeks	5	SMD: −0.146 (−0.429, 0.138)	40.9	0.149	0.314
	≤8 weeks	2	SMD: −0.212 (−0.789, 0.365)	9.1	0.294	0.472
	Long interval	4	SMD: −0.373 (−0.753, 0.008)	44.7	0.164	0.055
	Short interval	3	SMD: 0.015 (−0.327, 0.357)	0	0.554	0.993
	Same time	3	SMD: −0.044 (−0.448, 0.361)	47.0	0.152	0.833
	Less time	3	SMD: −0.348 (−0.714, 0.018)	0	0.419	0.062
BMI	>8 weeks	5	SMD:0.273 (−0.012, 0.557)	0	0.518	0.061
	≤8 weeks	3	SMD:0.034 (−0.477, 0.545)	0	0.925	0.896
	Long interval	4	SMD:0.080 (−0.264, 0.425)	0	0.982	0.648
	Short interval	4	SMD:0.365 (−0.005, 0.725)	0	0.456	0.047
	Same time	3	SMD:0.349 (−0.075, 0.772)	21.1	0.282	0.106
	Less time	4	SMD:0.08 (−0.299, 0.46)	0	0.973	0.678
WC	>8 weeks	2	SMD: 0.2 (−0.435, 0.835)	34.1	0.218	0.538
	≤8weeks	1	SMD: −0.321 (−1.081, 0.439)	32.5	NA	0.408
	Long interval	2	SMD: 0.109 (−0.726, 0.945)	59.5	0.116	0.797
	Short interval	1	SMD: −0.117 (−0.833, 0.599)	NA	NA	0.749
	Same time	1	SMD: −0.321 (−1.081, 0.439)	NA	NA	0.408
	Less time	1	SMD: −0.117 (−0.833, 0.559)	NA	NA	0.749
Body fat	>8 weeks	3	SMD: −0.095 (−0.302, 0.492)	0	0.478	0.639
	≤8 weeks	2	SMD: −0.317 (−0.942, 0.309)	0	0.754	0.321
	Long interval	3	SMD: −0.115 (−0.517, 0.287)	0	0.897	0.575
	Short interval	2	SMD: 0.099 (−0.789, 0.988)	46.9	0.170	0.826
	Same time	3	SMD: −0.165 (−0.607, 0.276)	21.4	0.762	0.463
	Less time	2	SMD: 0.170 (−0.412, 0.752)	21.4	0.259	0.566
VO_{2peak}	>8 weeks	2	SMD: 0.805 (0.334, 1.276)	0	0.345	0.001
	≤8 weeks	2	SMD:0.276 (−0.346, 0.904)	0	0.615	0.381
	Long interval	3	SMD: 0.691 (0.290, 1.092)	0	0.425	0.001
	Short interval	1	SMD: 0.50 (−1.041, 1.41)	NA	NA	0.928
	Same time	3	SMD: 0.468 (0.040, 0.897)	0	0.633	0.032
	less time	1	SMD: 1.109 (0.323, 1.895)	NA	NA	0.006
SBP	>8 weeks	4	SMD: −0.330 (−0.742, 0.082)	33.9	0.209	0.117
	≤8 weeks	0	NA	NA	NA	NA
	Long interval	1	SMD: 0.184 (−0.549, 0.918)	NA	NA	0.063
	Short interval	3	SMD: −0.330 (−0.742, 0.082)	11.8	0.322	0.024
	Same time	NA	NA	0	NA	NA
	Less time	3	SMD: −0.179 (−0.541, 0.183)	0	0.525	0.332
DBP	>8 weeks	4	MD: 0.128 (−0.589, 0.846)	77.5	0.004	0.726
	≤8 weeks	NA	NA	NA	NA	NA
	Long interval	3	SMD: 0.370 (−0.460, 1.20)	72.8	0.025	0.383
	Short interval	1	SMD: −0.497 (−1.011, 0.017)	NA	NA	0.058
	Same time	NA	NA	NA	NA	NA
	Less time	3	SMD: 0.286 (−0.679, 1.251)	83.9	0.002	0.561
TC	>8 weeks	5	SMD: 0.027 (−0.442, 0.496)	60.1	0.040	0.911
	≤8 weeks	1	SMD: −4.292 (−5.624, −2.959)	NA	NA	0.001
	Long interval	4	SMD: −0.802 (−2.387, 0.368)	31.2	0.228	0.545
	Short interval	2	SMD: −0.160 (−1.456, 0.337)	98.0	0.0001	0.430
	Same time	2	SMD: −2.314 (−6.097, 1.468)	96.2	0.0001	0.230
	Less time	3	SMD: 0.161 (−0.589, 0.912)	73.8	0.022	0.674
HDL–c	>8 weeks	4	SMD: −0.072 (−0.383, 0.240)	0	0.468	0.653
	≤8 weeks	1	SMD: 4.204 (2.891, 5.517)	NA	NA	0.001
	Long interval	3	SMD: 1.217(−0.888, 3.322)	94.8	0.0001	0.257
	Short interval	2	SMD: −0.067(−0.481, 0.346)	0	0.651	0.750
	Same time	2	SMD: 2.173 (−1.721, 6.066)	96.5	0.0001	0.274
	Less time	2	SMD: −0.067(−0.481, 0.346)	0	0.651	0.750
LDL–c	>8 weeks	4	SMD: −0.158 (−0.548, 0.232)	33.3	0.213	0.427

(Continued)

TABLE 3 | Continued

Outcomes		N	ES (95% CI)	Heterogeneity		p
				I^2 (%)	P	
	≤8 weeks	1	SMD: −1.166 (−1.944, −0.388)	NA	NA	0.003
	Long interval	2	SMD: −0.042 (−0.921, 0.836)	0	0.627	0.925
	Short interval	3	SMD: −0.526 (−1.170, 0.117)	64.4	0.060	0.109
	Same time	2	SMD: −0.777 (−1.456, −0.098)	46.9	0.170	0.025
	Less time	2	SMD: −0.209 (−0.639, 0.222)	5.4	0.304	0.342
TG	>8 weeks	5	SMD: −0.092(−0.379, 0.194)	0	0.799	0.529
	≤8 weeks	1	SMD: −0.966 (−1.725, −0.207)	NA	NA	0.013
	Long interval	3	SMD: −0.258 (−0.746, 0.230)	47.6	0.126	0.301
	Short interval	4	SMD: −0.126 (−0.540, 0.288)	0	0.681	0.550
	Same time	2	SMD: −0.588 (−1.251, 0.074)	46.1	0.173	0.082
	Less time	3	SMD: −0.024 (−0.385, 0.336)	0	0.566	0.894
HOMA–IR	>8 weeks	4	SMD: −1.102(−2.715, 0.511)	94.9	0.0001	0.180
	≤8 weeks	0	NA	NA	NA	NA
	Long interval	2	SMD: −0.264 (−0.740, 0.212)	0	0.603	0.277
	Short interval	2	SMD: −1.948 (−5.782, 1.886)	97.8	0.0001	0.319
	Same time	1	SMD: −0.158 (−0.780, 0.463)	NA	NA	0.618
	Less time	3	SMD: −1.430 (−3.709, 0.849)	96.2	0.001	0.219
Fasting glucose	>8 weeks	4	SMD: −2.507 (−5.108, 0.094)	97.2	0.0001	0.059
	≤8 weeks	0	NA	NA	NA	NA
	Long interval	2	SMD: −1.467 (−4.265, 1.321)	95.1	0.0001	0.302
	Short interval	2	SMD: −3.633 (−11.013, 3.747)	98.8	0.0001	0.335
	Same time	1	MD: −0.079 (−0.693, 0.535)	NA	NA	0.800
	Less time	3	MD: −3.374 (−7.401, 0.653)	97.8	0.0001	0.101
Fasting insulin	>8 weeks	3	SMD: −0.694 (−1.816, 0.428)	87.7	0.0001	0.225
	≤8 weeks	0	NA	NA	NA	NA
	Long interval	2	SMD: −0.140 (−0.653, 0.373)	0	0.581	0.592
	Short interval	1	SMD: −1.746 (−2.344, −1.149)	NA	NA	0.001
	Same time	0	NA	NA	NA	NA
	Less time	3	SMD: −0.694 (−1.816, 0.428)	87.7	0.0001	0.225

systematic reviews that investigated the effect of HIIT vs. MICT on VO_{2peak} in patients with heart failure (Gomes Neto et al., 2018) and type 2 diabetes (Liu et al., 2019b). Although the effects of HIIT on cardiorespiratory fitness have been confirmed in a variety of individuals, findings on cardiorespiratory fitness improvement compared with MICT are inconsistent. A recent meta-analysis conducted by Gomes-Neto et al. (2017) showed that HIIT is superior to MICT on VO_{2peak} gain in patients with heart failure, whereas this advantage disappears in comparison with an isocaloric MICT protocol. Moreover, the meta-analysis of Milanovic et al. (2015) demonstrated that both HIIT and endurance training can elicit a large improvement in VO_{2max} in healthy adults aged 18–45 years and that HIIT has a small beneficial effect on VO_{2max} compared with endurance training. Given the magnitude and effect sizes associated with the training session time, duration, and interval protocol, we conducted a subgroup analysis to explore whether different HIIT training parameters cause changes in cardiometabolic health compared with those of MICT. Subgroup analysis indicated that the duration was a moderator for VO_{2peak}, with larger effects evident in studies of ≥8 weeks compared with those with a <8-week duration. These findings were partially consistent with Hannan et al.'s meta-analysis, which found that patients with coronary artery disease who engaged in HIIT intervention for ≤6 weeks did not experience significant changes compared with MICT intervention, while 7–12 weeks may be a reasonable duration for the largest improvement in cardiorespiratory fitness (Hannan et al., 2018). The underlying physiological mechanisms that may explain why HIIT elicited a greater improvement than MICT are not fully understood. Several physiological adaptations may partially explain the potential mechanism, which involves central adaption and peripheral adaptation. HIIT-induced central adaptation primarily increased the ejection volume due to the increased pre-load, decreased afterload, and cardiac enlargement (Nottin et al., 2002). Meanwhile, peripheral adaptation may be related to skeletal muscle remodeling, which primarily improves capillary and mitochondrial density and increases skeletal muscle oxidative capacity, as reflected by the maximal activity and protein content of mitochondrial enzymes (Gibala et al., 2012; Montero et al., 2015; Lundby and Jacobs, 2016).

The current study is the first meta-analysis of RCTs to evaluate the difference between the effects of HIIT and of MICT on cardiometabolic risk factors among children and adolescents with obesity. To ensure the robustness of our results, we investigated whether several HIIT training parameters (including the training session time, total training duration, and length of interval) affect the final results/pooled results. However, this study had several limitations that possibly affected the interpretation of our results. First, although we searched the relevant studies as thoroughly as possible, the small number of available RCTs limited the number of studies in the subgroups and prevented further meta-regression analysis to investigate the dose-response relationship between HIIT and cardiometabolic risk improvement. Second, the difference in the measurements of glucose metabolism and lipid metabolism of the included

studies possibly led to the high heterogeneity, which may partly explain the non-significant improvement in both the HIIT and MICT groups. Third, the puberty of subjects may be an important factor affecting the results. Given that only three of the included studies evaluated the pubertal growth of subjects, we were unable to further explore the impact of the puberty stage on the cardiometabolic risk improvement between HIIT and MICT. Fourth, most of the included studies lacked information on whether HIIT and MICT have the same workload and attendance rates, which may adversely affect our pooled results. Despite these limitations, this meta-analysis provided a comprehensive analysis of all of the included studies to compare the effects of HIIT vs. MICT on cardiometabolic risk and cardiorespiratory fitness in children and adolescents with obesity. Further studies with large samples and a high-quality methodology are needed to compare the effects of HIIT and MICT on glucose metabolism and lipid metabolism, determine the optimal HIIT protocol, and optimize the combination of training and intervals for maximum health benefits in children and adolescents with obesity.

In conclusion, the present meta-analysis supports the positive effects of HIIT on cardiorespiratory fitness and suggests that HIIT and MICT have similar effects on body composition and blood pressure in childhood obesity. These findings indicate that HIIT can be implemented in the management of childhood obesity as an alternative training modality to MICT to maintain cardiometabolic health.

AUTHOR CONTRIBUTIONS

JL contributed to the study conception and design, drafted the submitted article, and critically revised the draft for important intellectual content. LZ revised the draft for important intellectual content and gave the final approval of the version for publication. YS contributed to the acquisition, analysis, and interpretation of the data.

FUNDING

This work was supported by the National Office of Philosophy and Social Science of China (No. 18BTY075), the Project Supported by Guangdong Province Universities and Colleges Pearl River Scholar Funded Scheme (2019), and the Department of Education of Guangdong Province (2018WCXTD010).

ACKNOWLEDGMENTS

We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.00214/full#supplementary-material

REFERENCES

Afshin, A., Forouzanfar, M. H., Reitsma, M. B., Sur, P., Estep, K., Lee, A., et al. (2017). Health effects of overweight and obesity in 195 countries over 25 years. *N. Engl. J. Med.* 377, 13–27. doi: 10.1056/NEJMoa1614362

Alberga, A. S., Frappier, A., Sigal, R. J., Prud'homme, D., and Kenny, G. P. (2013). A review of randomized controlled trials of aerobic exercise training on fitness and cardiometabolic risk factors in obese adolescents. *Phys. Sportsmed.* 41, 44–57. doi: 10.3810/psm.2013.05.2014

Atkinson, G., and Batterham, A. M. (2015). True and false interindividual differences in the physiological response to an intervention. *Exp. Physiol.* 100, 577–588. doi: 10.1113/EP085070

Bohm, A., Weigert, C., Staiger, H., and Haring, H. U. (2016). Exercise and diabetes: relevance and causes for response variability. *Endocrine* 51, 390–401. doi: 10.1007/s12020-015-0792-6

Bouter, K. E., van Raalte, D. H., Groen, A. K., and Nieuwdorp, M. (2017). Role of the gut microbiome in the pathogenesis of obesity and obesity-related metabolic dysfunction. *Gastroenterology* 152, 1671–1678. doi: 10.1053/j.gastro.2016.12.048

Campbell, W. W., Kraus, W. E., Powell, K. E., Haskell, W. L., Janz, K. F., Jakicic, J. M., et al. (2019). High-intensity interval training for cardiometabolic disease prevention. *Med. Sci. Sports Exerc.* 51, 1220–1226. doi: 10.1249/MSS.0000000000001934

Castro-Pinero, J., Perez-Bey, A., Segura-Jimenez, V., Aparicio, V. A., Gomez-Martinez, S., Izquierdo-Gomez, R., et al. (2017). Cardiorespiratory fitness cutoff points for early detection of present and future cardiovascular risk in children: a 2-year follow-up study. *Mayo Clin. Proc.* 92, 1753–1762. doi: 10.1016/j.mayocp.2017.09.003

Chung, S. T., Onuzuruike, A. U., and Magge, S. N. (2018). Cardiometabolic risk in obese children. *Ann. N. Y. Acad. Sci.* 1411, 166–183. doi: 10.1111/nyas.13602

Cole, T. J., Bellizzi, M. C., Flegal, K. M., and Dietz, W. H. (2000). Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* 320, 1240–1243. doi: 10.1136/bmj.320.7244.1240

Corte de Araujo, A. C., Roschel, H., Picanco, A. R., do Prado, D. M., Villares, S. M., de Sa Pinto, A. L., et al. (2012). Similar health benefits of endurance and high-intensity interval training in obese children. *PLoS ONE* 7:e42747. doi: 10.1371/journal.pone.0042747

Costa, E. C., Hay, J. L., Kehler, D. S., Boreskie, K. F., Arora, R. C., Umpierre, D., et al. (2018). Effects of high-intensity interval training versus moderate-intensity continuous training on blood pressure in adults with pre- to established hypertension: a systematic review and meta-analysis of randomized trials. *Sports Med.* 48, 2127–2142. doi: 10.1007/s40279-018-0944-y

Costigan, S. A., Eather, N., Plotnikoff, R. C., Taaffe, D. R., and Lubans, D. R. (2015). High-intensity interval training for improving health-related fitness in adolescents: a systematic review and meta-analysis. *Br. J. Sports Med.* 49, 1253–1261. doi: 10.1136/bjsports-2014-094490

de Lade, C. G., Andreazzi, A. E., Bolotari, M., Costa, V. M. G., Peters, V. M., and Guerra, M. O. (2018). Effects of moderate intensity endurance training vs. *high intensity interval training on weight gain, cardiorespiratory capacity, and metabolic profile in postnatal overfed rats. Diabetol. Metab. Syndr.* 10:70. doi: 10.1186/s13098-018-0374-x

de Onis, M., Onyango, A. W., Borghi, E., Siyam, A., Nishida, C., and Siekmann, J. (2007). Development of a WHO growth reference for school-aged children and adolescents. *Bull. World Health Organ.* 85, 660–667. doi: 10.2471/BLT.07.043497

Dias, K. A., Ingul, C. B., Tjonna, A. E., Keating, S. E., Gomersall, S. R., Follestad, T., et al. (2018). Effect of high-intensity interval training on fitness, fat mass and cardiometabolic biomarkers in children with obesity: a randomised controlled trial. *Sports Med.* 48, 733–746. doi: 10.1007/s40279-017-0777-0

Fan, X., and Cao, Z. B. (2017). Physical activity among Chinese school-aged children: National prevalence estimates from the 2016 physical activity and fitness in China-the youth study. *J. Sport Health Sci.* 6, 388–394. doi: 10.1016/j.jshs.2017.09.006

Garber, C. E., Blissmer, B., Deschenes, M. R., Franklin, B. A., Lamonte, M. J., Lee, I. M., et al. (2011). American college of sports medicine

position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Med. Sci. Sports Exerc.* 43, 1334–1359. doi: 10.1249/MSS.0b013e318213fefb

Gepstein, V., and Weiss, R. (2019). Obesity as the main risk factor for metabolic syndrome in children. *Front. Endocrinol.* 10:568. doi: 10.3389/fendo.2019.00568

Ghardashi Afousi, A., Izadi, M. R., Rakhshan, K., Mafi, F., Biglari, S., and Gandomkar Bagheri, H. (2018). Improved brachial artery shear patterns and increased flow-mediated dilatation after low-volume high-intensity interval training in type 2 diabetes. *Exp. Physiol.* 103, 1264–1276. doi: 10.1113/EP087005

Gibala, M. J., Little, J. P., Macdonald, M. J., and Hawley, J. A. (2012). Physiological adaptations to low-volume, high-intensity interval training in health and disease. *J. Physiol.* 590, 1077–1084. doi: 10.1113/jphysiol.2011.224725

Gomes Neto, M., Duraes, A. R., Conceicao, L. S. R., Saquetto, M. B., Ellingsen, O., and Carvalho, V. O. (2018). High intensity interval training versus moderate intensity continuous training on exercise capacity and quality of life in patients with heart failure with reduced ejection fraction: a systematic review and meta-analysis. *Int. J. Cardiol.* 15, 134–141. doi: 10.1016/j.ijcard.2018.02.076

Gomes-Neto, M., Duraes, A. R., Reis, H., Neves, V. R., Martinez, B. P., and Carvalho, V. O. (2017). High-intensity interval training versus moderate-intensity continuous training on exercise capacity and quality of life in patients with coronary artery disease: a systematic review and meta-analysis. *Eur. J. Prev. Cardiol.* 24, 1696–1707. doi: 10.1177/2047487317728370

Green, D. J., Maiorana, A., O'Driscoll, G., and Taylor, R. (2004). Effect of exercise training on endothelium-derived nitric oxide function in humans. *J. Physiol.* 561, 1–25. doi: 10.1113/jphysiol.2004.068197

Halliwill, J. R. (2001). Mechanisms and clinical implications of post-exercise hypotension in humans. *Exerc. Sport Sci. Rev.* 29, 65–70. doi: 10.1097/00003677-200104000-00005

Hannan, A. L., Hing, W., Simas, V., Climstein, M., Coombes, J. S., Jayasinghe, R., et al. (2018). High-intensity interval training versus moderate-intensity continuous training within cardiac rehabilitation: a systematic review and meta-analysis. *Open Access J. Sports Med.* 9, 1–17. doi: 10.2147/OAJSM.S150596

Higgins, J. P., Altman, D. G., Gotzsche, P. C., Juni, P., Moher, D., Oxman, A. D., et al. (2011). The cochrane collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 343, 889–893. doi: 10.1136/bmj.d5928

Islam, H., Townsend, L. K., and Hazell, T. J. (2018). Excess postexercise oxygen consumption and fat utilization following submaximal continuous and supramaximal interval running. *Res. Q. Exerc. Sport* 89, 450–456. doi: 10.1080/02701367.2018.1513633

Izadi, M. R., Ghardashi Afousi, A., Asvadi Fard, M., and Babaee Bigi, M. A. (2018). High-intensity interval training lowers blood pressure and improves apelin and NOx plasma levels in older treated hypertensive individuals. *J. Physiol. Biochem.* 74, 47–55. doi: 10.1007/s13105-017-0602-0

Jensen, M. D. (2003). Fate of fatty acids at rest and during exercise: regulatory mechanisms. *Acta Physiol. Scand.* 178, 385–390. doi: 10.1046/j.1365-201X.2003.01167.x

Juonala, M., Magnussen, C. G., Berenson, G. S., Venn, A., Burns, T. L., Sabin, M. A., et al. (2011). Childhood adiposity, adult adiposity, and cardiovascular risk factors. *N. Engl. J. Med.* 365, 1876–1885. doi: 10.1056/NEJMoa1010112

Kachur, S., Lavie, C. J., de Schutter, A., Milani, R. V., and Ventura, H. O. (2017). Obesity and cardiovascular diseases. *Minerva Med.* 108, 212–228. doi: 10.23736/S0026-4806.17.05022-4

Keating, S. E., Johnson, N. A., Mielke, G. I., and Coombes, J. S. (2017). A systematic review and meta-analysis of interval training versus moderate-intensity continuous training on body adiposity. *Obes. Rev.* 18, 943–964. doi: 10.1111/obr.12536

Koubaa, A., Trabelsi, H., Masmoudi, L., Elloumi, M., Sahnoun, Z., Zeghal, K. M., et al. (2013). Effect of intermittent and continuous training on body composition cardiorespiratory fitness and lipid profile in obese adolescents. *IOSR J. Pharm.* 3, 31–37. doi: 10.9790/3013-32103137

Kuczmarski, R. J., Ogden, C. L., Guo, S. S., Grummer-Strawn, L. M., Flegal, K. M., Mei, Z., et al. (2002). 2000 CDC growth charts for the United States: methods and development. *Vital Health Stat.* 11, 1–190.

Lahoz-Garcia, N., Garcia-Hermoso, A., Milla-Tobarra, M., Diez-Fernandez, A., Soriano-Cano, A., and Martinez-Vizcaino, V. (2018). Cardiorespiratory fitness as a mediator of the influence of diet on obesity in children. *Nutrients* 10:E358. doi: 10.3390/nu10030358

Lavie, C. J., Ozemek, C., Carbone, S., Katzmarzyk, P. T., and Blair, S. N. (2019). Sedentary behavior, exercise, and cardiovascular health. *Circ. Res.* 124, 799–815. doi: 10.1161/CIRCRESAHA.118.312669

Lazzer, S., Tringali, G., Caccavale, M., De Micheli, R., Abbruzzese, L., and Sartorio, A. (2017). Effects of high-intensity interval training on physical capacities and substrate oxidation rate in obese adolescents. *J. Endocrinol. Invest.* 40, 217–226. doi: 10.1007/s40618-016-0551-4

Liu, J. X., Zhu, L., and Deng, J. M. (2019a). The effects of high-intensity interval training versus moderate-intensity continuous training on fat loss and cardiometabolic health in pediatric obesity: a protocol of systematic review and meta-analysis. *Medicine* 98:e14751. doi: 10.1097/MD.0000000000014751

Liu, J. X., Zhu, L., Li, P. J., Li, N., and Xu, Y. B. (2019b). Effectiveness of high-intensity interval training on glycemic control and cardiorespiratory fitness in patients with type 2 diabetes: a systematic review and meta-analysis. *Aging Clin. Exp. Res.* 31, 575–593. doi: 10.1007/s40520-018-1012-z

Liu, Y., Wang, Y., Ni, Y., Cheung, C. K. Y., Lam, K. S. L., Wang, Y., et al. (2019). Gut microbiome fermentation determines the efficacy of exercise for diabetes prevention. *Cell Metab.* 31, 77–91. doi: 10.1016/j.cmet.2019.11.001

Lundby, C., and Jacobs, R. A. (2016). Adaptations of skeletal muscle mitochondria to exercise training. *Exp. Physiol.* 101, 17–22. doi: 10.1113/EP085319

Mahgoub, M. S. E., and Aly, S. (2015). The effects of continuous vs intermittent exercise on lipid profile in obese children. *Int. J. Ther. Rehabil.* 22, 272–275. doi: 10.12968/ijtr.2015.22.6.272

Maillard, F., Pereira, B., and Boisseau, N. (2018). Effect of high-intensity interval training on total, abdominal and visceral fat mass: a meta-analysis. *Sports Med.* 48, 269–288. doi: 10.1007/s40279-017-0807-y

Marcinko, K., Sikkema, S. R., Samaan, M. C., Kemp, B. E., Fullerton, M. D., and Steinberg, G. R. (2015). High intensity interval training improves liver and adipose tissue insulin sensitivity. *Mol. Metab.* 4, 903–915. doi: 10.1016/j.molmet.2015.09.006

Milanovic, Z., Sporis, G., and Weston, M. (2015). Effectiveness of high-intensity interval training (HIT) and continuous endurance training for VO_2max improvements: a systematic review and meta-analysis of controlled trials. *Sports Med.* 45, 1469–1481. doi: 10.1007/s40279-015-0365-0

Montero, D., Cathomen, A., Jacobs, R. A., Fluck, D., de Leur, J., Keiser, S., et al. (2015). Haematological rather than skeletal muscle adaptations contribute to the increase in peak oxygen uptake induced by moderate endurance training. *J. Physiol.* 593, 4677–4688. doi: 10.1113/JP270250

Morrissey, C., Montero, D., Raverdy, C., Masson, D., Amiot, M. J., and Vinet, A. (2018). Effects of exercise intensity on microvascular function in obese adolescents. *Int. J. Sports Med.* 39, 450–455. doi: 10.1055/a-0577-4280

Murphy, A., Kist, C., Gier, A. J., Edwards, N. M., Gao, Z., and Siegel, R. M. (2015). The feasibility of high intensity interval exercise in obese adolescents. *Clin. Pediatr.* 54, 87–90. doi: 10.1177/0009922814528038

Nishida, K., Harrison, D. G., Navas, J. P., Fisher, A. A., Dockery, S. P., Uematsu, M., et al. (1992). Molecular cloning and characterization of the constitutive bovine aortic endothelial cell nitric oxide synthase. *J. Clin. Invest.* 90, 2092–2096. doi: 10.1172/JCI116092

Nottin, S., Vinet, A., Stecken, F., N'Guyen, L. D., Ounissi, F., Lecoq, A. M., et al. (2002). Central and peripheral cardiovascular adaptations to exercise in endurance-trained children. *Acta Physiol. Scand.* 175, 85–92. doi: 10.1046/j.1365-201X.2002.00975.x

Pal, S., Radavelli-Bagatini, S., and Ho, S. (2013). Potential benefits of exercise on blood pressure and vascular function. *J. Am. Soc. Hypertens.* 7, 494–506. doi: 10.1016/j.jash.2013.07.004

Prieto-Benavides, D. H., Garcia-Hermoso, A., Izquierdo, M., Alonso-Martinez, A. M., Agostinis-Sobrinho, C., Correa-Bautista, J. E., et al. (2019). Cardiorespiratory fitness cut-points are related to body adiposity parameters in latin american adolescents. *Medicina* 55:508. doi: 10.3390/medicina55090508

Roy, M., Williams, S. M., Brown, R. C., Meredith-Jones, K. A., Osborne, H., Jospe, M., et al. (2018). High-intensity interval training in the real world: outcomes from a 12-month intervention in overweight adults. *Med. Sci. Sports Exerc.* 50, 1818–1826. doi: 10.1249/MSS.0000000000001642

Saris, W. H., and Schrauwen, P. (2004). Substrate oxidation differences between high- and low-intensity exercise are compensated over 24 hours in obese men. *Int. J. Obes. Relat. Metab. Disord.* 28, 759–765. doi: 10.1038/sj.ijo.0802631

Sawyer, B. J., Tucker, W. J., Bhammar, D. M., Ryder, J. R., Sweazea, K. L., and Gaesser, G. A. (2016). Effects of high-intensity interval training and moderate-intensity continuous training on endothelial function and cardiometabolic risk markers in obese adults. *J. Appl. Physiol.* 121, 279–288. doi: 10.1152/japplphysiol.00024.2016

Schmidt, M. D., Magnussen, C. G., Rees, E., Dwyer, T., and Venn, A. J. (2016). Childhood fitness reduces the long-term cardiometabolic risks associated with childhood obesity. *Int. J. Obes.* 40, 1134–1140. doi: 10.1038/ijo.2016.61

Shamseer, L., Moher, D., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., et al. (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ* 350:g7647. doi: 10.1136/bmj.g7647

Sigal, R. J., Alberga, A. S., Goldfield, G. S., Prud'homme, D., Hadjiyannakis, S., Gougeon, R., et al. (2014). Effects of aerobic training, resistance training, or both on percentage body fat and cardiometabolic risk markers in obese adolescents: the healthy eating aerobic and resistance training in youth randomized clinical trial. *JAMA Pediatr.* 168, 1006–1014. doi: 10.1001/jamapediatrics.2014.1392

Starkoff, B., Eneli, I. U., Bonny A. E., Hoffman, R., Devor S. T. (2014). Estimated aerobic capacity changes in adolescents with obesity following high intensity interval exercise. *Int. J. Kinesiol. Sports Sci.* 2, 2202–2946. doi: 10.7575/aiac.ijkss.v.2n.3p.1

Trapp, E. G., Chisholm, D. J., and Boutcher, S. H. (2007). Metabolic response of trained and untrained women during high-intensity intermittent cycle exercise. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 293, R2370–R2375. doi: 10.1152/ajpregu.00780.2006

Treuth, M. S., Hunter, G. R., and Williams, M. (1996). Effects of exercise intensity on 24-h energy expenditure and substrate oxidation. *Med. Sci. Sports Exerc.* 28, 1138–1143. doi: 10.1097/00005768-199609000-00009

van Hall, G. (2015). The physiological regulation of skeletal muscle fatty acid supply and oxidation during moderate-intensity exercise. *Sports Med* 45(Suppl. 1), S23–S32. doi: 10.1007/s40279-015-0394-8

Wewege, M., van den Berg, R., Ward, R. E., and Keech, A. (2017). The effects of high-intensity interval training vs. moderate-intensity continuous training on body composition in overweight and obese adults: a systematic review and meta-analysis. *Obes. Rev.* 18, 635–646. doi: 10.1111/obr.12532

Wibaek, R., Vistisen, D., Girma, T., Admassu, B., Abera, M., Abdissa, A., et al. (2019). Body mass index trajectories in early childhood in relation to cardiometabolic risk profile and body composition at 5 years of age. *Am. J. Clin. Nutr.* 110, 1175–1185. doi: 10.1093/ajcn/nqz170

Xiuming, Z. (2014). Effects of endurance training and high-intensity interval training on health-related indexs of obese children. *Med. J. Natl. Defend. Forces Southwest China* 24, 408–411. doi: 10.3969/j.issn.1004-0188.2014.04.023

Yu, H. J., Cai, L. B., Yang, X. H., Yuan, S., Li, Q. X., and He, Q. Q. (2018). Cardiorespiratory fitness attenuates the obesity risk in chinese children who have parents with overweight/obesity. *J Pediatr* 200, 150–4.e151. doi: 10.1016/j.jpeds.2018.05.015

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 Endocrinology

SYSTEMATIC REVIEW
published: 04 August 2020
doi: 10.3389/fendo.2020.00495

Acute and Chronic Effects of Exercise on Continuous Glucose Monitoring Outcomes in Type 2 Diabetes: A Meta-Analysis

Matthew Munan[1,2], *Camila L. P. Oliveira*[2,3], *Alexis Marcotte-Chénard*[4,5], *Jordan L. Rees*[1,2], *Carla M. Prado*[2,3], *Eléonor Riesco*[4,5] *and Normand G. Boulé*[1,2]*

[1] Faculty of Kinesiology, Sport, and Recreation, University of Alberta, Edmonton, AB, Canada, [2] Alberta Diabetes Institute, University of Alberta, Edmonton, AB, Canada, [3] Faculty of Agricultural, Life & Environmental Sciences, University of Alberta, Edmonton, AB, Canada, [4] Faculty of Physical Activity Sciences, University of Sherbrooke, Sherbrooke, QC, Canada, [5] Research Center on Aging, CIUSSS de l'Estrie - CHUS, Sherbrooke, QC, Canada

OPEN ACCESS

Edited by:
John P. Thyfault,
University of Kansas Medical Center,
United States

Reviewed by:
Richard M. Bracken,
Swansea University, United Kingdom
Steven K. Malin,
University of Virginia, United States

***Correspondence:**
Normand G. Boulé
nboule@ualberta.ca

Specialty section:
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Endocrinology

Received: 30 April 2020
Accepted: 22 June 2020
Published: 04 August 2020

Citation:
Munan M, Oliveira CLP, Marcotte-Chénard A, Rees JL, Prado CM, Riesco E and Boulé NG (2020) Acute and Chronic Effects of Exercise on Continuous Glucose Monitoring Outcomes in Type 2 Diabetes: A Meta-Analysis. Front. Endocrinol. 11:495. doi: 10.3389/fendo.2020.00495

Objective: To examine the acute and chronic effects of structured exercise on glucose outcomes assessed by continuous glucose monitors in adults with type 2 diabetes.

Methods: PubMed, Medline, EMBASE were searched up to January 2020 to identify studies prescribing structured exercise interventions with continuous glucose monitoring outcomes in adults with type 2 diabetes. Randomized controlled trials, crossover trials, and studies with pre- and post-designs were eligible. Short-term studies were defined as having exercise interventions lasting ≤2 weeks. Longer-term studies were defined as >2 weeks.

Results: A total of 28 studies were included. Of these, 23 studies were short-term exercise interventions. For all short-term studies, the same participants completed a control condition as well as at least one exercise condition. Compared to the control condition, exercise decreased the primary outcome of mean 24-h glucose concentrations in short-term studies (−0.5 mmol/L, [−0.7, −0.3]; $p < 0.001$). In longer-term studies, mean 24-h glucose was not significantly reduced compared to control (−0.9 mmol/L [−2.2, 0.3], $p = 0.14$) but was reduced compared to pre-exercise values (−0.5 mmol/L, [−0.7 to −0.2] $p < 0.001$). The amount of time spent in hyperglycemia and indices of glycemic variability, but not fasting glucose, also improved following short-term exercise. Among the shorter-term studies, subgroup, and regression analyses suggested that the timing of exercise and sex of participants explained some of the heterogeneity among trials.

Conclusion: Both acute and chronic exercise can improve 24-h glucose profiles in adults with type 2 diabetes. The timing of exercise and sex of participants are among the factors that may explain part of the heterogeneity in acute glycemic improvements following exercise.

Keywords: exercise, type 2 diabetes, systematic review, meta-analysis, continuous glucose monitoring

INTRODUCTION

Meta-analyses have repeatedly confirmed that, on average, regular exercise training causes meaningful improvements in glycemic control in people with type 2 diabetes (T2D) (1, 2). These meta-analyses typically included glycated hemoglobin (A1C) as a primary outcome and showed a high degree of heterogeneity among trials (2). A1C reflects the average glucose concentrations over the last 2–3 months. However, A1C does not provide information on what aspect of glycemic control has been improved (i.e., two people with very different daily glucose profiles can have the same A1C) and does not allow direct comparisons between short-term and longer-term responses to exercise. A better understanding of how exercise affects shorter-term indicators of glycemic control could help better understand how exercise affects longer-term indicators of glycemic control, as well as the heterogenous responses to exercise.

Continuous glucose monitors (CGM) can measure interstitial glucose concentrations at frequent intervals over several days. In addition to mean daily glucose concentration, CGM permit measures such as glucose concentrations over specific periods (e.g., post-prandial periods), the amount of time within specific glucose ranges (e.g., below 3.9 mmol/L), or other outcomes such as glucose variability, which can be associated with oxidative stress (3) and potentially other diabetes-related complications (4).

In 2013, members of our team published the first meta-analysis on the effects of exercise on CGM outcomes based on eight short-term studies and three longer-term studies (5). Synthesis of results from short-term studies revealed that exercise reduced outcomes such as mean 24-h glucose and time spent in hyperglycemia but did not affect other outcomes such as fasting glucose. Due to the low number of studies, we had limited our subgroup comparisons to aerobic vs. resistance exercise. Since then, the number of exercise and CGM studies in T2D has increased rapidly.

Therefore, the purpose of this meta-analysis was to provide an updated systematic review of the effects of exercise on CGM outcomes in T2D. Given the heterogeneity identified in previous meta-analyses, we explored differences among the short-term trials with pre-specified and novel subgroup comparisons, as well as meta-regression analyses, to examine the impact of factors such as exercise timing, dietary standardization, medications, type of CGM, sex, and baseline glycemic control.

METHODS

Search Strategy

On January 9 of 2020, a literature search of EMBASE, PubMed, and Medline were performed using terms relating to exercise, T2D and CGM. Search results were combined into a bibliographic software (Endnotes X6, Thomson Reuters, Toronto, Canada) and duplicates were eliminated using an automated feature. Details of the literature search strategy are available in **Supplementary Table 1**.

Two reviewers independently read titles and abstracts. Any record that was deemed to meet the inclusion criteria was selected for a full-text review (i.e., agreement between reviewers was not required at this stage). Two reviewers then reviewed all selected full-text articles for eligibility and any disagreement was resolved through discussion with a third reviewer.

Study Selection

Eligibility was determined according to the following inclusion criteria:

- **Population:** Only studies with data from adults with T2D were eligible. Studies were not eligible if data were combined for people with and without diabetes, or with people above and below 18 years of age.
- **Intervention:** Both short-term (i.e., $\leq$2 weeks) and longer-term studies (>2 weeks) were included if they examined the effects of structured exercise interventions defined in terms of frequency, intensity, type, and duration. Interventions that encouraged participants to become more active without providing structured prescriptions or monitoring (e.g., direct supervision or logs) were not eligible. Since developing this criterion for our previous meta-analysis (5) several studies examined the effects of breaking up sedentary time with exercise. These studies were not included to facilitate comparisons with our previous meta-analysis and because they often involved restricting activities during the control condition (e.g., prolonged sitting). In such studies, it was unclear if differences between conditions were due to the activity itself or the impact of prolonged sitting in the control condition.
- **Comparison:** A non-exercise control condition was required for comparison to the exercise condition. Both randomized and non-randomized (e.g., pre vs. post) comparisons were eligible, as were trials that employed parallel or crossover designs. Studies comparing combined exercise and dietary interventions to a control condition not receiving the dietary intervention were not eligible.
- **Outcome:** Studies were required to provide data from CGM or "Flash" glucose monitoring over a day (i.e., approximately 24 h) from both the exercise and control conditions. Mean 24-h glucose was considered the primary outcome of interest.

Data Extraction

Two reviewers extracted the following CGM outcomes in duplicate: mean 24-h glucose, time in hyperglycemia, time in hypoglycemia, time in range, post-prandial glucose, fasting glucose, nocturnal glucose, and glucose variability. Recent international consensus statements (6) suggest values of 3.9–10.0 mmol/L for time in range, but we also extracted data from articles who had similar definitions but slightly different cutoffs (e.g., 4.0 instead of 3.9 mmol/L, or 9.0 instead of 10.0 mmol/L). Indicators of glucose variability included mean amplitude of glucose excursions (MAGE), continuous overall net glycemic action (CONGA), or standard deviation (SD). Participant characteristics and details of the interventions were extracted by a single reviewer and verified by a second reviewer. Participant characteristics included age, sex, body mass index (BMI), duration of diabetes, menopausal status, the type of CGM,

and type of glucose lowering medication they were treated with, and A1C. Characteristics of the exercise intervention included the type of exercise, the frequency and duration of exercise sessions, as well as the intensity. We noted if meals were provided as a means of standardizing diet between the exercise and control conditions and categorized groups into: all meals provided, meals partially provided, or no meals provided. The timing of exercise in relation to meals was categorized as fasting, after breakfast, afternoon (i.e., before dinner), or evening (i.e., after dinner).

Several data transformations were made before combining data from trials. Glucose concentrations in mg/dL were converted and presented as mmol/L by dividing by 18. Since CGM measures are provided in constant time intervals (e.g., 5 min), the area under the curve data was converted to mean glucose by dividing the total area by the amount of time. The percent time in hyper- or hypoglycemia was transformed into minutes by multiplying the percentage by the total amount of time.

Based on our previous meta-analysis (5), we expected participants in the short-term studies to complete both the exercise and control conditions (e.g., crossover trials) even if some would not be in randomized order. The primary analyses for these studies were based on the within-person difference in glucose concentrations. In instances where the SD or standard error (SE) of the change was not reported, it was estimated from *p*-values as described in section 7.7.3.3 of the Cochrane Handbook (7). In cases where information was displayed in a figure, mean difference and SD was estimated using plot digitizer software (Plot Digitizer Version 2.1 ©Joseph Huwaldt). In infrequent cases, we were unable to estimate the SE of the change from any of the above methods. In such cases, we used the correlation coefficient between exercise and control values that we calculated from other studies to estimate the SE of the change as described in section 16.1.3.2 of the Cochrane Handbook (7).

Risk of Bias

Two authors independently performed risk of bias assessment. Risk of bias was assessed using a domain-based evaluation, in which seven specific domains were addressed: (1) sequence generation, (2) allocation concealment, (3) blinding of participants and personnel, (4) blinding of outcome assessment, (5) incomplete outcome data, (6) selective outcome reporting, and (7) other bias. A judgement of "low risk," "high risk," and "unclear risk" of bias was assigned for each study, according to the criteria in the Cochrane Collaboration's Risk of Bias Tool; section 8.5 in the Cochrane Handbook (7). These criteria had been updated since our previous review (5). For example, describing a trial as randomized was no longer sufficient to be categorized as "low risk" for "sequence generation"; the authors were required to describe an appropriate method for randomization.

Statistical Analysis

Statistical analyses were performed using Review Manager Software (Revman 5.3, Cochrane Collaboration, Copenhagen Denmark). For all shorter-term studies, participants completed both conditions (crossover trials or pre- and post-designs). For these trials, the mean difference (MD) and the within participant SE of this difference were pooled using the generic inverse variance method to calculate a weighted mean difference (WMD).

For the longer-term trials that randomly assigned participants to either exercise vs. control conditions, the primary analyses considered mean differences between conditions which was pooled using a random effects model. When a control condition was compared to multiple exercise conditions, the sample size of the control condition was divided by the number of comparisons. Three of the five longer-term trials did not include a control condition. Therefore, secondary analysis compared pre- vs. post-exercise data from all longer-term trials using the generic inverse variance method.

Heterogeneity was examined through the chi-square test and also presented using the I^2 statistic, which describes the percentage of the variability that is due to heterogeneity rather than chance (7). When the I^2 was above 40%, heterogeneity was explored with subgroup and meta-regression analyses. As in previous meta-analyses (5, 8), subgroups were pre-defined according to type of exercise (i.e., aerobic, vs. high-intensity interval training, vs. resistance). As suggested in the study by Rees et al. (9), other factors such as exercise timing, and dietary intervention may have influenced the results and were therefore included in subgroup analyses. Lastly factors such as the type of CGM (real time vs. blinded vs. intermittently scanned) and the type of glucose lowering medications taken by participants were added during the review process. Meta-regression analyses included the proportion of participants who were female, A1C, and glucose concentrations from the control condition as predictors. For all the short-term studies, the same participants completed the control and exercise conditions.

RESULTS

Description of Studies

The literature search retrieved 657 records (see PRISMA Trial Flow diagram in **Figure 1**). After duplicates were removed, 435 records were reviewed. Fifty-four full text articles were screened and 26 were excluded for the following reasons:

- **Population.** Studies that were not exclusively conducted in adults with T2D (10–14) were excluded. Of these, the study by Newton and White (12) was included in our first meta-analyses, but excluded this time because the age range was from 14 to 20 years old.
- **Intervention.** Studies were excluded when they had co-interventions, such as changes in medication or insulin (15, 16), which influenced the changes caused by exercise. There was also one study in three records (17–19) examining the effect of Yoga, but it was excluded since we were unable to extract sufficient detail on the structure of exercise component, or control for any effect of the breathing exercise or meditation components of the intervention. Two studies examined the effect of breaking up sedentary time (20, 21) with several short bouts of activity. The control condition in these studies involved restricting movement by sitting from 8 to 14 h

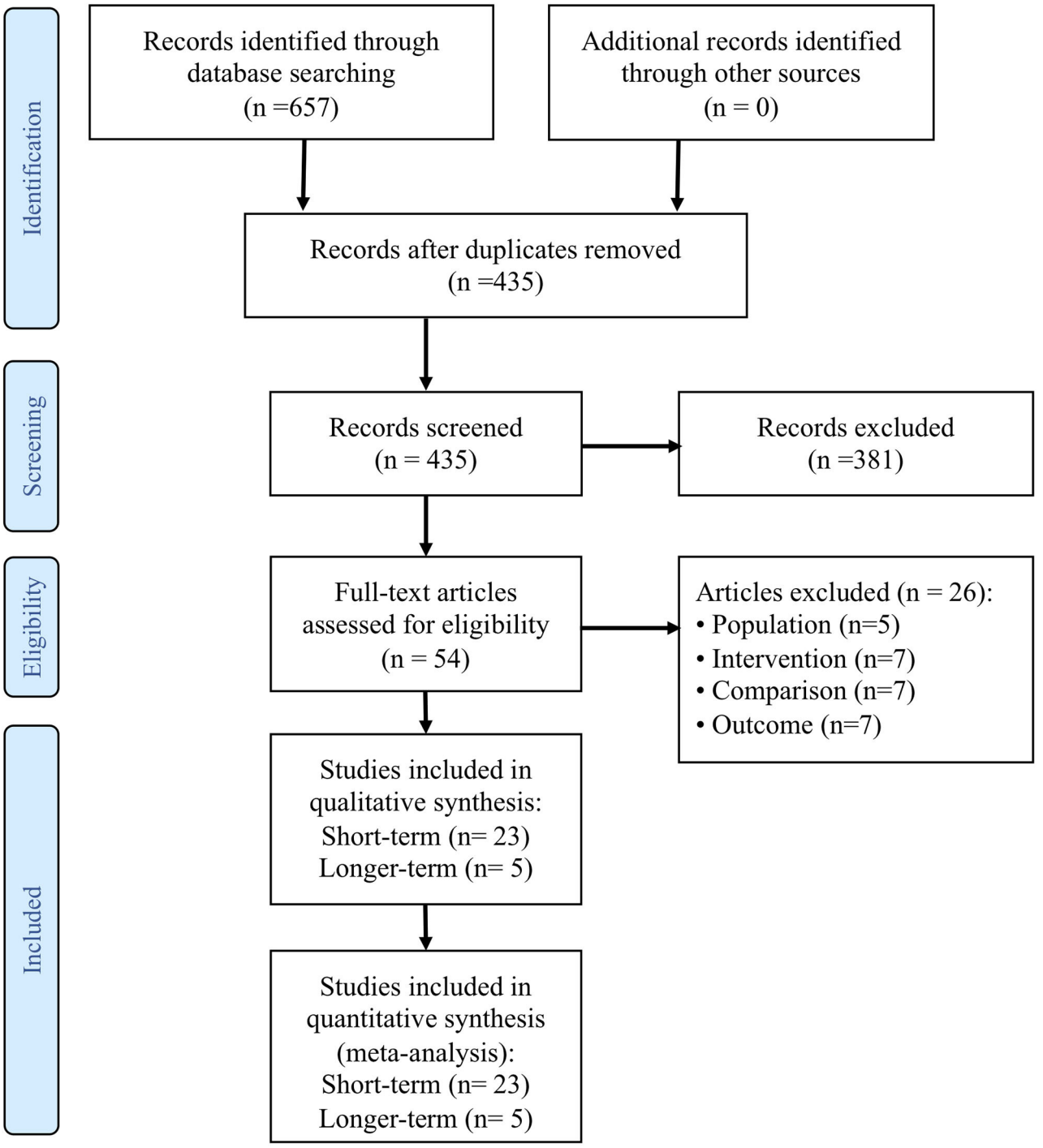


FIGURE 1 | PRISMA study flow diagram.

(20, 21). It therefore became difficult to know how much of the difference between the activity and control conditions was due to the physical activity itself or the prolonged sedentary behavior, which was likely greater than in free-living conditions. The study by Blankenship et al. (22) included a continuous walking condition and another activity condition with 12 breaks in sedentary time. However, the control condition asked participants to maintain their habitual physical activity behavior and we therefore chose to include the control vs. walking comparison.

- **Comparison.** Studies that did not include a non-exercise control condition were excluded (23–29). Of these trials, the

one by Bacchi et al. (25) had been included in the qualitative synthesis of our 2013 systematic review. We excluded it in the present analysis because the control condition started 24 h after the exercise condition and we could not rule out that the effect of exercise did not persist beyond 24 h. In the study by Godkin et al. (30), the effect of a single bout of exercise was compared to control after the first session of exercise and after a session of exercise performed after 6 weeks of exercise training. We only included the effects of the first session of exercise since this was more comparable to the other included studies.

- **Outcomes.** Some studies did not have usable CGM data (31, 32) or presented data which was available from the same population as another included study (33–37). For example, Little et al. (34) included the same participants as the study by Gillen et al. (38). These articles were different in that Gillen et al. examined participants after one bout of exercise while Little et al. examined participants after six bouts of exercise. Another difference was that Little et al. assessed glycemic control starting ∼48 h after the last training bout; a period that was inconsistent with the rest of the short-term studies. To favor homogeneity among studies only the results from Gillen et al. was included. The study from Savikj et al. (39) provided data from week 1 and week 2 of training but we only included the data from week 1.

Table 1 includes characteristics of the 23 eligible short-term studies. A total of 373 participants were included. The majority of these participants were males (264 males vs. 109 females). Many of the studies included multiple exercise groups for a total of 40 exercise groups. There were a variety of exercise prescriptions, with studies prescribing low, moderate, and high-intensity aerobic exercise, including different forms of high-intensity interval training (HIIT). The timing in relation to meals varied among studies but was reported in all but 2 studies. Eleven studies provided all of the meals to the participants throughout the 24-h period, 6 studies provided some meals but not all, and 6 studies did not provide any meals. In the studies that did not provide meals, or partially provided meals, participants were often asked to maintain similar dietary intakes across conditions. Of the 23 short-term studies, one study used an intermittently scanned CGM (Freestyle Libre, Abbott). Three studies used the Guardian or MiniMed (Medtronic) CGM which provided real-time data to participants. Five studies used GlucoDay S (A. Menarini Diagnostics) CGM, which has the capability of showing real time glucose concentrations but was likely blinded. An additional 12 studies used iPro (Medtronic) CGM technology, which are blinded to participants and researchers until the data is download after removal of the sensor. An additional three studies did not specify the type of Medtronic CGM but provided enough detail to suggest that the data were also examined retrospective and not available in real-time.

Twenty of the 23 short-term studies provided some information on the type of medication. Of the 373 participants from the 23 short-term trials, we were able to determine that the most common medications were: metformin (taken by at least 70% of participants), sulfonylureas (taken by at least 17% of participants), insulin (taken by at least 11% of participants), and DPP4 inhibitors (taken by at least 10% of participants). Other classes of medications were each taken by ≤5% of the participants. Menopausal status was reported in 6 of the 15 short-term studies that included women. In these 6 studies, almost all participants were postmenopausal (a total of only 3 women were not).

Table 2 describes the five eligible longer-term studies. Interventions ranged from 8 to 16 weeks in duration. A total of 99 participants (57 males and 42 females) were included in 9 different exercise interventions, but only 15 participants in two separate control groups (60, 62). Francois et al. (59) had three separate groups performing the same HIIT training protocol, but we only included one of these groups in our analyses because the others received a skimmed-milk supplement or a macronutrient matched control beverage, making it unclear what effects were due to the exercise or supplements. Consequently, we only included the HIIT group that received the flavored water placebo from Francois et al. (59). Of the five longer-term studies, two used the blinded iPro CGM, two used the Guardian CGM and one used a MiniMed system that also included a portable monitor (all from Medtronic).

Effect of Short-Term Exercise (≤2 Weeks) on Glucose Concentrations

Among the 23 short-term studies, 22 reported 24-h glucose concentrations. Several studies had multiple exercise conditions, which led to a total of 39 exercise groups included in the overall analyses. Compared to control, exercise reduced 24-h glucose concentrations by 0.5 mmol/L, 95% CI [−0.7 to −0.3] ($p < 0.001$, see complete details in **Figure 2**). However, there was a high degree of heterogeneity among trials ($Chi^2 = 140.8$, $p < 0.001$); $I^2 = 73\%$). This heterogeneity was only partially reduced ($Chi^2 = 76.1$, $p < 0.001$, $I^2 = 51\%$) after removing a visual outlier [i.e., the group performing resistance training at 40% of their 1-repetition maximum from Cruz et al. (40)].

Due to the significant heterogeneity among studies, analysis was performed by dividing studies into subgroups according to the timing of exercise, type of exercise, dietary control, and type of CGM (see **Table 3**). Of these subgroups, only the exercise timing analyses identified heterogeneity among subgroups ($p < 0.001$). There were significant reductions in mean 24-h glucose when exercise was performed in the fasted state (−0.7 mmol/L [−1.1, −0.2], $p = 0.004$) and in the morning (−0.6 mmol/L [−0.9, −0.4], $p < 0.001$) but not in the afternoon (−0.1 mmol/L [−0.2, 0.1], $p = 0.54$). Heterogeneity remained elevated in the morning subgroup but was reduced from $I^2 = 75\%$ to $I^2 = 38\%$ when the outlier from Cruz et al. (40) was removed.

Meta-regression was performed to predict changes in 24-h glucose concentrations following exercise with other variables such as 24-h glucose concentrations in the control condition, baseline A1C, age, BMI, or the percentage of female participants. Greater mean 24-h glucose concentration in the control condition predicted a greater decrease in 24-h glucose concentrations following exercise ($r = -0.61$, $p < 0.001$), as shown in **Figure 3**. Note that the same participants completed

TABLE 1 | Characteristics of included short-term (≤2 weeks) studies.

Source	(*N*) M/F	Age (yr)	BMI (kg/m^2)	Duration T2D (yr)	A1C (%)	Type of exercise	Exercise intensity	Exercise duration	Timing of exercise	Meals during CGM
1. Blankenship et al. (22)*	(30) 14/16	64 ± 8.2	31.7 ± 5.4	10.0 ± 7.8	7.4 ± 1.1	- Walking	- "Faster than usual walking speed"	- 1 bout (20, 40, or 60 min)	- Morning (30–60 min post-breakfast)	Partially provided
2. Cruz et al. (40)	(12) 0/12	55.2 ± 4.0	29.0 ± 5.4	5.7 ± 3.7	NR	- Resistance - Resistance	- 40% 1RM - 80% 1RM	- 1 bout (40 min) - 1 bout (40 min)	- Morning - Morning	Partially provided
3. Erickson et al. (41)	(8) 5/3	60 ± 10.7	33.8 ± 10.3	NR	7.9 ± 2.3	- Walking	- 50% $V0_2$ Peak	1 bout (3 × 10 min)	- Morning	All provided
4. Figueira et al. (42)	(14) 5/9	56 ± 7	30 ± 4	4.5 [3.1–5.9]	7.9 ± 2.6	- Cycling - Cycling and Resistance	- 70% Peak HR - 70% Peak HR and 4 exercises 65% 1RM	- 1 bout (40 min) - 1 bout (20 min) and 3 sets of 12 reps	- Morning - Morning	None provided
5. Gillen et al. (38)	(7) 4/3	62 ± 3	30.5 ± 1.9	>3 month	6.9 ± 0.7	- Cycling	- 85% max HR	- 1 bout, 10 × 60 s intervals (10 min)	- Morning	All Provided
6. Godkin et al. (30)	(7) 5/2	21 to 70	31 ± 5	6 ± 9	6.5 ± 0.7	- Stair climbing	- HIIT: Mean HR = 74 ± 5% of max HR	- 1 bout: 3 × 1:1 min stairs: walking	- Morning	All provided
7. Haxhi et al. (43)	(9) 9/0	52.8 ± 6.6	30.2 ± 3.1	5.2 ± 4.3	7.0 ± 0.6	- Walking - Walking	- 50% HRR - 50% HRR	- 2 bouts (20 min) - 1 bout (40 min)	- Split before and after lunch - Afternoon	Partially provided
8. Karstoft et al. (44)	(10) 7/3	60.3 ± 2.3	28.3 ± 1.1	6 ± 0.9	6.3 ± 0.6	- Walking - Walking	- Interval @ 54–89% VO_{2peak} (3:3 min) - 73% VO_{2peak}	- 1 bout (60 min) - 1 bout (60 min)	- Fasting - Fasting	None provided
9. Karstoft et al. (45)	(14) 11/3	65 ± 2	18 to 39.9	9 ± 1	6.6 ± 1.1	- Walking - Walking	- Interval @ 54–89% VO_{2peak} (3:3 min) - 73%VO_{2peak}	- 10 bouts (60 min) - 10 bouts (60 min)	- Not specified - Not specified	Partially provided
10. Li et al. (46)	(29) 22/7	51.0 ± 11.2	24.8 ± 3.4	5.7 ± 3.4	7.3 ± 1.3	- Walking	- 40% HRR	- 1 bout (20 min)	- Evening (post-dinner)	All provided
11. Macdonald et al. (47)	(6) 5/1	59 ± 3	32 ± 1.4	2.0 ± 0.5	8.4 ± 1.7	- Cycling	- 90% LT	- 1 bout (60 min)	- Fasted (morning)	None provided
12. Manders et al. (48)	(9) 9/0	57 ± 6	29 ± 3.0	9 ± 12	7.1 ± 1.2	- Cycling - Cycling	- 35% Wmax - 70% Wmax	- 1 bout (60 min) - 1 bout (30 min)	- Morning (1 h post-breakfast)	All provided
13. Metcalfe et al. (49)	(11) 11/0	52 ± 6	29.7 ± 3.1	4 ± 3	7.0 ± 0.8	- Cycling - Cycling - Cycling	- REHIT-all out - HIIT-85% Wmax - MICT-50% Wmax	- 1 bout (10 min) - HIIT (10 min) - MICT (30 min)	- Morning (30 min post-breakfast)	All provided
14. Mikus et al. (50)	(13) 8/5	53.0 ± 7.2	34.1 ± 4.7	NR	6.6 ± 0.6	- Alternating walk/cycle	- 60–75% HRR	- 7 days (60 min/day)	- Not specified	None provided
15. Myette-Côté et al. (51)	(10) 5/5	59 ± 9.6	29.5 ± 4.7	7.5 ± 5.2	6.6 ± 0.6	- Walking	- 85% VT	- 1 bout (50 min)	- Morning	Partially provided
16. Oberlin et al. (52)	(9) 5/4	60.3 ± 3	36.0 ± 1.1	NR	6.3 ± 0.6	- Alternating walk/cycle	- 60% HRR	- 1 bout (60 min) - (20:20:20 min of walk:cycle:walk)	- Fasting	All provided

(Continued)

TABLE 1 | Continued

Source	(*N*) M/F	Age (yr)	BMI (kg/m^2)	Duration T2D (yr)	A1C (%)	Type of exercise	Exercise intensity	Exercise duration	Timing of exercise	Meals during CGM
17. Praet et al. (53)	(11) 11/0	59.1 ± 7.6	32.2 ± 4.0	12.1 ± 7.0	7.6 ± 1.0	- HIIT and Resistance	- HIIT: 30:60 s @ 50%Wmax:15 W; - Resistance: 2 sets × 10 reps @ 50% 1RM	- 1 bout (45 min)	- Morning	None provided
18. Rees et al. (9)	(63) 29/34	64.4 ± 8.0	30.5 ± 6.5	9.7 ± 6.1	6.8 ± 0.7	- Walking	- 5.0 km/h, 0.5% incline	- 1 bout (50 min)	- Afternoon	All provided
19. Savikj et al. (39)	(11) 11/0	60 ± 7	27.5 ± 2.0	11 ± 10	6.6 ± 1.3	- HIIT cycling - HIIT cycling	- 180–350 W - 180–350 W	- 6 × 60:60 s intervals - 6 × 60:60 s intervals	- Morning, with snack available - Afternoon	None provided
20. Terada et al. (54)	(10) 8/2	60 ± 6	30.8 ± 5.4	6.8 ± 4.6	7.1 ± 1.0	- Walking - HIIT Walk - Walking - HIIT Walk	- 55%VO_{2peak} - 3:1 min @ 40:100% - 55%VO_{peak} - 3:1 min 40–100%	- 1 bout (60 min) - 1 bout (60 min) - 1 bout (60 min) - 1 bout (60 min)	- Fasting - Fasting - Morning - Morning	Partially provided
21. Van Dijk et al. (55)	(15) 15/0 (15) 15/0	Insulin 61 ± 4 No-Ins 60 ± 4	Insulin 29.7 ± 4.3 No-Ins 29.7 ± 3.5	Insulin 13.5 ± 8.5 No-Ins 6.5 ± 3.9	Insulin 7.6 ± 1.2 No-Ins 7.5 ± 0.8	- Cycling - Resistance - Cycling - Resistance	- 50% Wmax - 5 sets of 10 reps (55–75% 1RM) - 50% Wmax - 5 sets of 10 reps (55–75% 1RM)	- 1 bout (45 min) - 1 bout (45 min) - 1 bout (45 min) - 1 bout (45 min)	- Morning - Morning - Morning - Morning	All provided
22. Van Dijk et al. (56)	(30) 30/0	60 ± 6	31.1 ± 3.8	8.1	7.2 ± 1.1	- Cycling - Cycling	- 50% Wmax - 50% Wmax	- 2 bouts (30 min on 2 days) - 1 bout (60 min)	- Morning - Morning	All provided
23. Van Dijk et al. (57)	(20) 20/0	61 ± 4	29.5 ± 4.0	8 ± 4	6.9 ± 0.4	- Cycling - Walking ("strolling")	- ~6.0 METS - ~3.0 METS	- 1 day (1 × 45 min) - 1 day (3 × 15 min)	- Morning - After Each Meal	All provided

Data presented as mean ± standard deviation, n, sample size; M, males; F, females; yr, years; A1C, glycated hemoglobin; BMI, body mass index; T2D, type 2 diabetes; METS, metabolic equivalent; $V0_2$, oxygen consumption; NR, not reported; HR, heart rate; HRR, heart rate Reserve; VT, ventilatory threshold; LT, lactate threshold; Wmax, peak workload; REHIT, reduced exertion high intensity interval training; HIIT, high intensity interval training; MICT, moderate-intensity continuous training.

**The Blankenship et al. (22) study also included a condition with breaking sedentary time, which was not included in the meta-analysis.*

TABLE 2 | Characteristics of longer-term (>2 weeks) exercise studies.

Source	Group	(*N*) M/F	Age (yr)	BMI (kg/m^2)	Duration T2D (yr)	A1C (%)	Duration of intervention	Frequency exercise	Length of exercise	Intensity of exercise	Meals during CGM
1. Cauza et al. (58)	Resistance	(8) 3/5	55.1 ± 4.8	29.9 ± 2.3	9 ± 11	7.5 ± 1.4	16 weeks	3/week	10 exercise	1–2 sets @ 10–15 reps	None provided
	Endurance	(7) 1/6	60.3 ± 8.2	36.3 ± 12.4	9 ± 11	8.0 ± 1.1	16 weeks	3/week	15–30 min	60% VO_{2max}	
2. Francois et al. (59)*	HIIT (with aerobic and resistance)	(19) 8/11	55 ± 9	33 ± 6	5 ± 6	6.9 ± 0.8	12 weeks	3/week: 2 aerobic, 1 resistance	20 min (1:1 min intervals)	Aerobic: 90%HRmax Resistance: RPE 5/10	None provided
3. Karstoft et al. (60)	Control	(8) 5/3	57.1 ± 8.5	29.7 ± 5.4	4.5 ± 4.2	6.4 ± 0.6	16 weeks	NA	NA	NA	None provided
	Walking	(12) 8/4	60.8 ± 7.6	29.9 ± 5.5	6.2 ± 5.2	6.6 ± 0.7	16 weeks	5/week	60 min	55% of peek EE	
	Interval Walking	(12) 7/5	57.5 ± 8.3	29.0 ± 4.5	3.5 ± 2.4	6.9 ± 0.7	16 weeks	5/week	60 min (3:3 min intervals)	70:40% peek EE	
4. Ruffino et al. (61)	Walking	(16) 16/0	55 ± 5	30.6 ± 2.8	4 ± 4	NR	8 weeks	3/week	30 min	40–55% of HRR	All provided
	REHIT						8 weeks	5/week	10 min	Cycling @ 25 Watt +2 sprints of 10–20 s @ 0.65 Nm/kg lean mass	
5. Winding et al. (62)	Control	(7) 5/2	57 ± 7	28.0 ± 3.5	7 ± 5	7.0 ± 1.2	11 weeks	NA	NA	NA	None provided
	Endurance	(12) 7/5	58 ± 8	27.4 ± 3.1	6 ± 4	6.6 ± 0.9	11 weeks	3/week	40 min	50% Wpeak	
	HIIT	(13) 7/6	54 ± 6	28.1 ± 3.5	8 ± 4	6.8 ± 0.8	11 weeks	3/week	20 min (1:1 min intervals)	95:20% Wpeak	

Data presented as mean ± standard deviation, n, sample size; M, males; F, females; yr, years; A1C, glycated hemoglobin; BMI, body mass index; T2D, type 2 diabetes; min, minutes; EE, energy expenditure; HIIT, high-intensity interval training; REHIT, reduced exertion high intensity interval training; HRmax, maximum heart rate; HRR, heart rate reserve; CGM, continuous glucose monitoring; NR, not reported; RPE, rating of perceived exertion; VO_2max, maximal oxygen consumption; Wpeak, peak workload. Some participants in the control group we randomized to one of the exercise groups in the Karstoft et al. (60) and Winding et al. (62) studies.

**Francois et al. (59) also had two other HIIT groups with dietary interventions that are not included in the meta-analysis.*

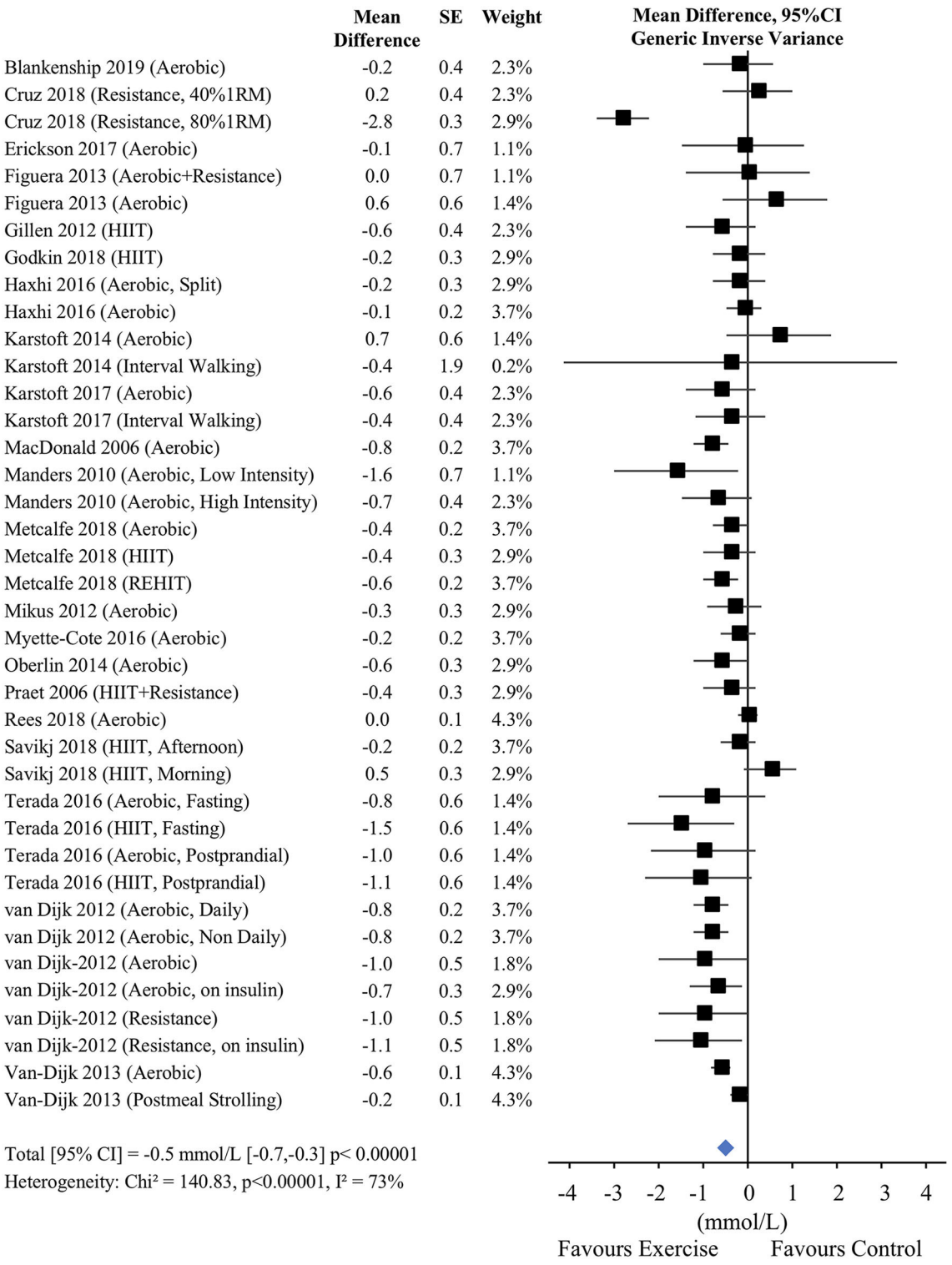


FIGURE 2 | Mean 24-h glucose concentrations in short-term (≤2 weeks) studies. CI, confidence interval; SE, standard error; 1RM, one repetition maximum; HIIT, high-intensity interval training; REHIT, reduced exertion high intensity interval training.

both the control and exercise conditions (i.e., repeated measures). When mean 24-h glucose from the control condition was replaced by A1C as an indicator of glycemic control, the relationship was in the same direction ($r = -0.33$, $p = 0.04$). The proportion of females within a study was not associated with improvements in 24-h glucose ($r = -0.10$, $p = 0.55$), but

TABLE 3 | Subgroup analyses for changes in mean 24-h glucose in short-term (≤2 weeks) studies.

Subgroup	Number of subgroups	Effect estimate	Heterogeneity
Overall	39	−0.5 [−0.7, −0.3], $p < 0.001$	$Chi^2 = 140.8$, $p < 0.001$, $I^2 = 73\%$
Exercise timing			
Fasting	6	−0.7 [−1.1, −0.2], $p = 0.004$	$Chi^2 = 7.6$, $p = 0.18$, $I^2 = 35\%$
Morning	24	−0.6 [−0.9, −0.4], $p < 0.001$	$Chi^2 = 91.0$, $p < 0.00001$ $I^2 = 75\%$
Afternoon	3	−0.1 [−0.2, 0.1], $p = 0.54$	$Chi^2 = 0.9$, $p = 0.65$, $I^2 = 0\%$
None of the above*	6	−0.2 [−0.4, −0.1], $p = 0.005$	$Chi^2 = 1.2$, $p = 0.94$, $I^2 = 0\%$
		Subgroup differences: $p < 0.001$	
Exercise type			
Continuous aerobic	24	−0.4 [−0.6, −0.3], $p < 0.001$	$Chi^2 = 54.2$, $p < 0.001$, $I^2 = 58\%$
HIIT/REHIT	9	−0.4 [−0.7, −0.1], $p < 0.02$	$Chi^2 = 15.8$, $p = 0.05$, $I^2 = 49\%$
Resistance	4	−1.2 [−2.6, 0.3], $p = 0.11$	$Chi^2 = 38.5$, $p < 0.001$, $I^2 = 92\%$
Aerobic and resistance	2	−0.3, [−0.9, 0.2], $p = 0.22$	$Chi^2 = 0.28$, $p = 0.60$, $I^2 = 0\%$
		Subgroup differences: $p = 0.76$	
Dietary control			
No meals provided	10	−0.2 [−0.5, 0.2], $p = 0.29$	$Chi^2 = 19.5$, $p < 0.02$, $I^2 = 54\%$
Meals partially provided	11	−0.7 [−1.3, −0.1], $p = 0.01$	$Chi^2 = 74.6$, $p < 0.001$, $I^2 = 87\%$
All meals provided	18	−0.5 [−0.7, −0.3], $p < 0.001$	$Chi^2 = 43.7$, $p < 0.001$, $I^2 = 61\%$
		Subgroup differences: $p = 0.16$	
Type of CGM			
Real-time	6	−0.3 [−1.9, 1.3], $p = 0.70$	$Chi^2 = 62.2$, $p < 0.001$, $I^2 = 92\%$
Blinded	31	−0.5 [−0.6, −0.4], $p < 0.001$	$Chi^2 = 58.1$, $p = 0.002$, $I^2 = 48\%$
Intermittently scanned	2	[−0.6, 0.8], $p = 0.74$	$Chi^2 = 3.8$, $p = 0.05$, $I^2 = 73\%$
		Subgroup differences: $p = 0.23$	
Randomization			
Low or unclear risk	33	−0.5 [−0.6, −0.4], $p < 0.001$	$Chi^2 = 56.4$, $p < 0.001$, $I^2 = 54\%$
High risk	6	−0.4 [−0.9, 0.1], $p < 0.001$	$Chi^2 = 83.3$, $p < 0.001$, $I^2 = 87\%$
		Subgroup differences: $p = 0.71$	

Analyses performed according to the generic inverse variance method. From the 22 included studies, several had multiple exercise interventions for a total of 39 subgroups. HIIT, high-intensity interval training; REHIT, reduced exertion high intensity interval training; CGM, continuous glucose monitor.

**The difference among exercise timing subgroup remained after removing the "none of the above" subgroup, which included exercise interventions for which the timing was not specified or split over different times of the day.*

when the aforementioned outlier was removed the correlation became positive and statistically significant ($r = 0.39$, $p = 0.016$) suggesting that studies with a greater proportion of females observed smaller improvements in mean 24-h glucose.

A greater proportion of participants treated with sulfonylureas within a study was associated with greater reductions in mean 24-h glucose following exercise ($r = -0.34$, $p = 0.04$). Use of other medications, including metformin ($r = 0.20$, $p = 0.25$), were not significantly associated with changes in 24-h glucose concentrations. Other variables such as exercise duration, age and BMI were not associated with changes in 24-h glucose concentration when examined among all studies or only among studies prescribing continuous aerobic exercise (all $p > 0.30$).

Change in secondary glycemic outcomes are summarized in **Table 4**. Time spent in hyperglycemia was analyzed from 16 studies, which included 30 exercise vs. control comparisons. There was a significant reduction in the daily time spent in hyperglycemia (−94 min [−115, −72], $I^2 = 53\%$). The subgroup differences reflected the findings from the 24-h glucose concentrations but are not presented as some of the subgroups were much smaller (e.g., only a single study). Indices of glycemic variability were reported in 11 studies with a total of 18 subgroups. Many different measures (e.g., MAGE, SD, and CONGA) were reported in the individual studies. MAGE was the most frequently reported index of glycemia variability and was available in all but two subgroups. MAGE was reduced by −0.41 [−0.63, −0.20] ($Chi^2 = 19.65$, $p = 0.19$; $I^2 = 24\%$). On the other hand, fasting glucose and time in hypoglycemia were not significantly affected by exercise.

Effect of Longer-Term (>2 Weeks) Exercise Training on Glucose Concentrations

Four of the studies started post-training CGM measures 48–72 h after the last bout of exercise and described the post-intervention measurements within 1 week of the last bout of exercise. There was no baseline difference in mean 24-h glucose concentrations between exercise and non-exercise control groups (−0.1 mmol/L [−1.5, 1.3], $p = 0.87$, $I^2 = 0\%$; **Figure 4A**). When the post-intervention results were pooled, mean 24-h glucose concentration was not significantly lower in the exercise groups compared with the control groups (−0.9 mmol/L [−2.2, 0.3] $p = 0.14$, $I^2 = 0\%$; **Figure 4B**). However, only 4 exercise conditions were included in this exercise vs. control comparison with a total of 49 participants in the exercise groups and 15 in the control groups.

Secondary analysis of the pre- and post-exercise comparisons resulted in the inclusion of 9 longer-term exercise conditions with a total of 115 participants. Compared to pre-exercise values, post-exercise 24-h mean glucose concentrations significantly decreased (−0.5 mmol/L [−0.7, −0.2], $p < 0.0002$,

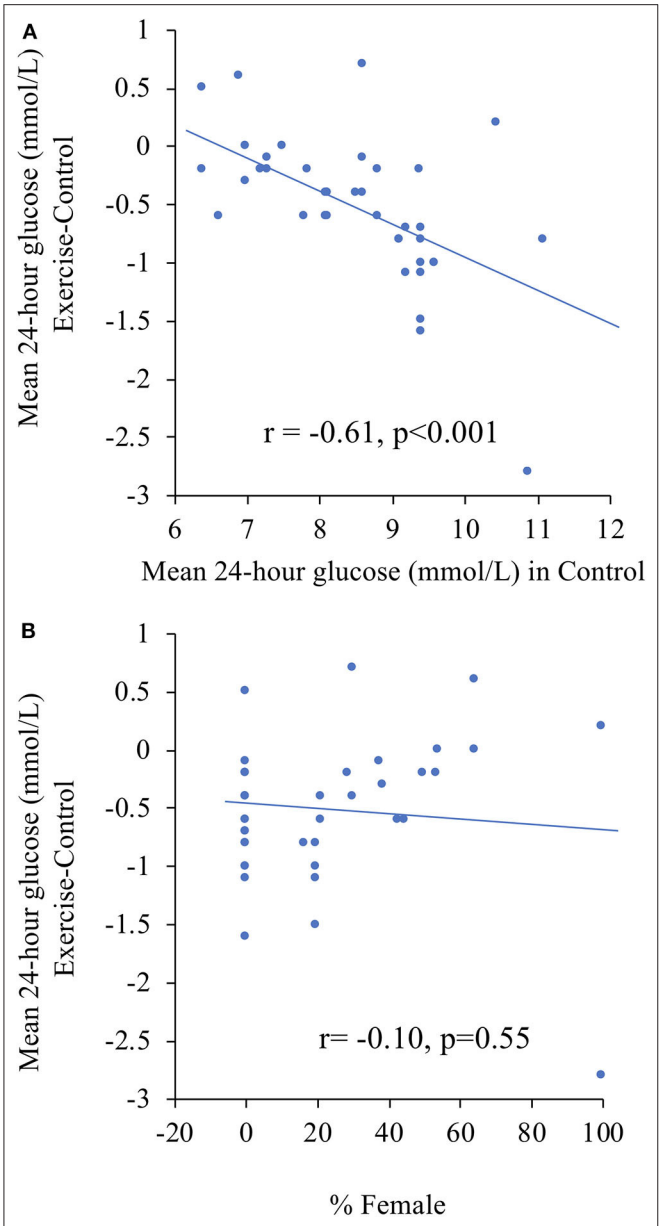


FIGURE 3 | Meta-regression to predict changes in mean 24-h glucose concentrations following exercise according to: **(A)** mean 24-h glucose concentrations in the control condition, and **(B)** percentage of females. The correlation coefficients were changed to $r = -0.53$ ($p < 0.001$) and $r = 0.39$ ($p = 0.016$), respectively, after removing the potential outlier with the largest decrease in mean 24-h glucose.

$I^2 = 1\%$; **Figure 5**). Subgroup analyses, regression analyses, and examination of other outcomes were not performed due to the low number of available comparisons.

Risk of Bias

Summaries according to the Cochrane Collaboration Risk of Bias tool are provided in **Supplementary Figures 1**, **2** for short and longer-term studies, respectively. Most of the included studies described their intervention as randomized but did not describe the methods of randomization, resulting in the categorization of "unknown" risk of bias on this criterion. Some of the trials that were described as randomized trials were actually categorized as "high" risk of bias because the randomization only affected the multiple exercise conditions and control condition always took place before exercise. When we performed subgroup analyses among the short-term studies to compare the "low" or "unknown" to "high" risk of biases on the random sequence generation criteria there was no difference between these types of studies on 24-h glucose concentrations (−0.5 mmol/L [−0.6, −0.3] vs. −0.4 mmol/L [−0.9, 0.1], respectively; see **Table 3**). As expected in exercise trials, blinding of participants to the exercise intervention is not feasible.

Funnel plots were also generated to examine the potential for publication bias. For the primary outcome of mean 24-h glucose concentrations, funnel plots are provided in **Supplementary Figures 3**, **4** for short and longer-term studies, respectively. Visual inspection of the funnel plots did not reveal any asymmetries, with the exception of the outlier from Cruz et al. (40) which found a comparatively large 2.8 mmol/L decrease in one of their short-term exercise groups. However, this group also had average size SE, which would not be expected in a typical publication bias scenario where studies with the largest SE tend to show more beneficial effects.

DISCUSSION

The present systematic review and meta-analyses confirms our previous findings that exercise reduces mean 24-h glucose and time spent in hyperglycemia (5), but also builds on this 2013 work in several ways:

1. The number of eligible short-term studies reporting the effects of exercise on CGM outcomes in T2D has approximately tripled (from 8 to 23 studies; or from 116 to 373 participants).
2. The greater number of short-term studies allowed for hypothesis generating subgroup and meta-regression analyses, which helped explain the heterogeneous responses among trials (e.g., the effects of exercise timing, sex, and glycemic control).
3. There were a sufficient number of trials to include outcomes that were not previously considered; including glycemic variability in short-term studies and mean 24-h glucose in longer-term studies.

The improvement in mean 24-h glucose concentrations following short-term exercise was 0.8 mmol/L in our 2013 meta-analyses and 0.5 mmol/L in the current one. These means were outside of each other's 95% confidence intervals. The differences may be due to the higher variability among trials in our current review as reflected in the higher I^2-value (i.e., 3 vs. 73%) and the addition of recent studies in which glucose concentrations were unchanged following exercise [e.g., Rees et al. (9)].

As in our previous meta-analysis (5), exercise did not affect fasting glucose (−0.2 mmol/L [−0.4, 0.1], $p = 0.14$). It is possible that this would have reached statistical significance had fasting

TABLE 4 | Analyses of secondary outcomes.

Outcome	Number of subgroups	Effect estimate	Heterogeneity
Time in hyperglycemia (min)	30	−94 [−115, −72], $p < 0.001$	$Chi^2 = 61.73$, $p = 0.0004$, $I^2 = 53\%$
Time in hypoglycemia (min)	12	−2 [−11, 7], $p = 0.67$	$Chi^2 = 14.81$, $p = 0.19$, $I^2 = 26\%$
Glycemic variability (MAGE)	16	−0.41 [−0.63, −0.20], $p < 0.001$	$Chi^2 = 19.65$, $p = 0.19$, $I^2 = 24\%$
Fasting glucose (mmol/L)	16	−0.2 [−0.4, 0.1], $p = 0.14$	$Chi^2 = 14.12$, $p = 0.52$, $I^2 = 0\%$

Min, minutes; MAGE, mean amplitude of glycemic excursions; hyperglycemia, typically defined as >10 mmol/L; hypoglycemia, typically defined as <3.9 mmol/L.

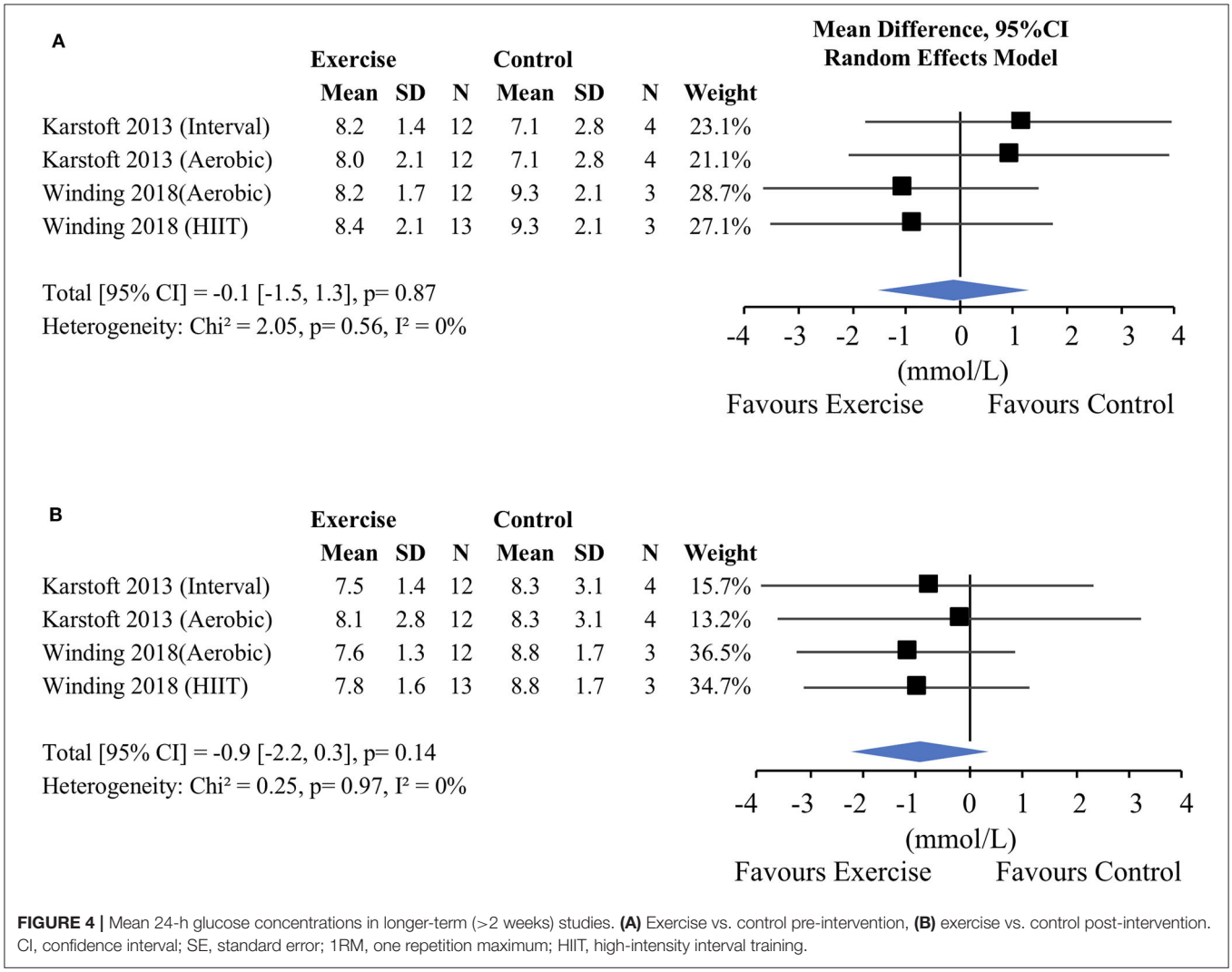


FIGURE 4 | Mean 24-h glucose concentrations in longer-term (>2 weeks) studies. **(A)** Exercise vs. control pre-intervention, **(B)** exercise vs. control post-intervention. CI, confidence interval; SE, standard error; 1RM, one repetition maximum; HIIT, high-intensity interval training.

glucose been reported in more short-term studies. Nonetheless, it may be that exercise has a greater impact on postprandial glucose, which is more strongly linked to muscle insulin resistance, whereas fasting glucose is believed to be more strongly associated with hepatic insulin resistance (63, 64). Longer-term studies have shown reductions in fasting glucose with exercise (65), but it is difficult to know to what extent this was due to weight loss.

To better understand the heterogeneity among short-term trials, we conducted a series of subgroup meta-regression analyses. It is important to note that since participants were not randomly assigned to the subgroups, we cannot determine if

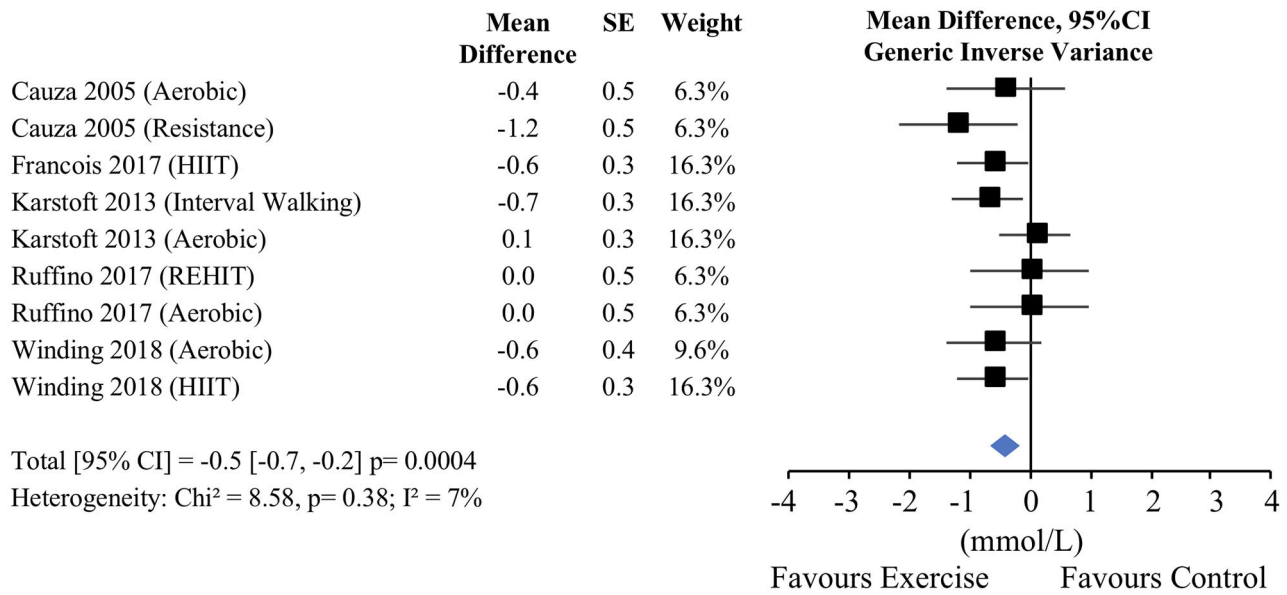


FIGURE 5 | Mean 24-h glucose concentrations in longer-term (>2 weeks) studies pre- vs. post-exercise. CI, confidence interval; SE, standard error; 1RM, one repetition maximum; HIIT, high-intensity interval training; REHIT, reduced exertion high intensity interval training.

it was a causal relationship. In addition, some variables in our subgroup and meta-regression analyses were not pre-specified. Consequently, results from our subgroup analyses should be interpreted with caution and confirmed by randomized trials. In our meta-regression analyses, the strongest predictor of greater improvements in glycemic control was the mean 24-h glucose concentrations from the control condition, suggesting that participants with elevated glucose concentrations had greater reductions following exercise. Although this may seem intuitive, it is potentially affected by a regression to the mean artifact [as previously reviewed by Sheppard (66)]. However, the association between baseline A1C and changes in mean 24-h glucose following exercise was in the same direction ($r = -0.33$, $p = 0.03$). Sex, but not age or BMI, was associated with changes in mean 24-h glucose. Studies that had a higher proportion of males were associated with greater reductions in mean 24-h glucose. Our meta-analysis does not permit us to identify the reasons why males may have responded more favorably compared to females. However, a greater effect of exercise on insulin sensitivity (67) and post-exercise glucose metabolism (68) has been previously observed in males compared to females. The reasons for these differences are not well-known, but may be related to differences in substrate oxidation during exercise and recovery (69). Of note, only 3 women were not postmenopausal among the 6 studies that reported menopausal status. Consequently, it is possible that the results are not generalizable to women before menopause.

However, we cannot rule out that the association with sex was caused by other confounders and we noted very high heterogeneity among the studies that only included males (see left side of **Figure 3B**). The association observed between the proportion of females and changes in mean 24-h glucose following exercise was only observed after removing of a potential outlier. Indeed, the study by Cruz et al. (40) was the only study that included only female participants ($n = 12$). They compared a single bout of exercise performed at 80 vs. 40% of the participants individualized one-repetition maximum (i.e., the heaviest weight that could be lifted once for each of 7 exercises). Resistance training was performed with a circuit in which each exercise was performed 3 times. While exercise at 80% increased mean 24-h glucose by 0.2 mmol/L, exercise at 40% reduced it by 2.8 mmol/L. To put this in perspective, this reduction is more than 5 times as much as the mean reduction in our meta-analyses and nearly twice as much as the next largest reduction among the 39 exercise conditions. The authors suggest that the greater counterregulatory hormone responses with the greater resistance exercise intensity may have contributed to the differences between conditions. It is also noteworthy that the participants in the Cruz et al. study were also the ones with the highest mean 24-h glucose during the control condition and therefore had the potential for greater reductions without experiencing hypoglycemia.

The timing of exercise was associated with some of the variance among short-term studies. Again, in our subgroup analyses, most participants were not randomly assigned to different exercise timing and therefore causality cannot be inferred. However, five studies directly compared two similar amounts of exercise performed at different times of the day (39, 43, 54, 56, 70). The results from Savikj et al. (39) contradict the findings from our meta-analyses and suggest exercise performed in the morning was less effective than afternoon exercise. However, this study involved HIIT training whereas most of the studies in our meta-analyses did not. They also offered a snack after morning exercise only. If changes in the timing of exercise can be found to consistently affect glycemic responses, this

could be encouraging for people with T2D who could use such strategies to get more benefits from the same amount of exercise. The decision to perform subgroup analyses based on exercise timing in relation to meals was *a priori* as a consequence of our findings in the study by Rees et al. (9), which used afternoon exercise. However, we were unsure of the exact subgroups that would be available (e.g., we expected to have evening/post-dinner exercise subgroups?) and divided our subgroups in a way to have multiple studies in each subgroup.

The reasons why fasting (i.e., before breakfast) exercise would lead to significant and consistent reductions in mean 24-h glucose, while afternoon exercise did not, are not well-understood. One potential explanation could be that, in the absence of exogenous fuels, fasting exercise must rely to a greater extent on endogenous fuels (e.g., intramuscular lipids and glycogen) and that these changes may favor an increase in insulin sensitivity. The first two longer-term training studies comparing fasting exercise to postprandial exercise in T2D have been recently published (71, 72). These longer-terms studies did not support a more favorable effect of fasting exercise compared to postprandial exercise. However, the postprandial exercise was performed shortly after breakfast (not in the afternoon) in both of these studies (71, 72). It is currently difficult to understand to what extent the effects of fasting exercise are due to fasting itself or to the time of day (i.e., diurnal variations). To further complicate matters, in people with T2D, many glucose lowering medications are taken with meals and we found an association with the use of sulfonylurea within a study in changes in 24-hr glucose following exercise vs. control, but not for other categories of medication.

Interpretation of differences among subgroups is based on comparing results from different exercise conditions that did not benefit from randomization, therefore subgroup comparisons may be affected by several confounding variables and should be confirmed by randomized trials. Several studies included in our meta-analysis did directly compare the effect of different exercise intensities. Some compared continuous exercise to different forms of higher intensity interval training (45, 49, 54, 73), one compared low vs. moderate intensity continuous exercise (48), and one compared different intensities of resistance exercise (40). As in the subgroup analyses from our meta-analysis, no clear pattern emerged when examining these studies individually. However, a previous meta-analysis of longer-term studies with head-to-head comparison of exercise of different intensities suggested that higher intensity exercise led to greater declines A1C (8). Another difference was that the trials in the earlier meta-analysis had similar or greater energy expenditures in the high intensity groups compared to the lower intensity groups from the same trial. Likewise, the aerobic vs. resistance training comparison in the short-term trials may not reflect longer term adaptations. The mechanisms leading to improvements in glycemic control following continuous aerobic, HIIT and resistance training may be different, and are beyond the scope of our meta-analysis.

Methodological aspects unrelated to exercise, such as the type of CGM (real-time vs. blinded vs. intermittently scanned) as well as the level of dietary control (i.e., the provision of meals), did not significantly explain the heterogeneity among trials in regards to changes in mean 24-h glucose. However, the absence of significant subgroup differences may be due to the presence of other confounders as there was high heterogeneity within many different subgroups. The type of CGM or the degree of dietary control may influence compensatory behaviors from participants (e.g., eating more if glucose is known to be low).

Glycemic variability may be independently associated with cardiovascular disease (74). When examining the change across all short-term studies, we observed a consistent and statistically significant reduction in MAGE. However, within each individual study the 95% confidence interval would often overlap with zero, suggesting that individual studies were often underpowered to detect differences. There were several indices of glycemic variability. Although these indices differ in their calculations, they were highly related to each other. For example, correlation coefficients were all above 0.85 among MAGE, CONGA, and SD (75).

There were fewer longer-term studies identified and only two with randomization to a non-exercise control condition. The pre- vs. post-analyses led to different conclusions than the randomized exercise vs. control comparison. The pre- vs. post-comparison had a smaller mean difference but reached statistical significance, in part due to the greater number of participants but also because of the increased statistical power within participant analyses. Interestingly, the weighted mean difference in the pre- and post-analyses was similar to the weighted mean difference found in the acute studies (i.e., 0.5 mmol/L). Based on conversions between A1C and estimated average glucose (76), such a reductions could correspond to a 0.3 percentage point reduction in A1C, which is lower than previous meta-analyses of exercise trials with A1C as a primary outcome (1, 2). This is not surprising given that the post-training CGM measures typically started at least 48-h after the last bout of exercise to minimize the acute effect from this last bout. Therefore, we would expect the weekly average glucose to be lower in these participants who prescribed exercise three times per week or more. Weight loss in longer-term exercise trials may mediate some of the improvements in glycemic control. For eight of the nine longer-term exercise conditions, changes in body weight were $\leq$1 kg. Consequently, we believe that most of the changes were observed in the absence of meaningful weight loss.

The main limitation of this meta-analysis is the high heterogeneity among the shorter-term studies and that we were only partially successful at explaining the heterogeneity. Consequently, interpreting the overall effects should be done with caution. Based on our findings, it is unlikely that exercise increases blood glucose; it is more likely that the heterogeneity is in the degree of the positive to no effects. The apparent heterogeneity may in fact be in part a result of the analytical approach that we chose. Indeed, the within participant mean change and SE used in the generic inverse method approach, leads to much narrower confidence internals than if we compared the mean glucose from the exercise vs. control using the between participant standard deviation in each condition. When the latter approach is used, the weighted mean difference remained similar

(0.4 mmol/L [−0.70, −0.20]) but the heterogeneity is greatly reduced ($Chi^2 = 24.5$, $p = 0.96$), $I^2 = 0\%$) since the mean difference found in each study has wider confidence intervals. The heterogeneity may also be caused my methodological issues. Several CGM devices require multiple calibrations per day and errors in calibration values can have a meaningful impact on 24-h outcomes. In addition, investigators often have to make difficult decisions on how to treat missing CGM values. Lastly, another limitation is the low number of longer-term studies and we would caution against inferring that chronic exercise training no more effective than shorter-term exercise due to the timing of the CGM measures in the longer-term studies.

In conclusion, both short-term and long-term exercise can reduce mean 24-h glucose concentrations. Short-term exercise also reduces other CGM-derived outcomes such as glycemic variability, while additional longer-term studies are needed to examine such outcomes. The glycemic response to short-term exercise can be variable, and exploratory analyses suggests that the heterogeneity among studies might in part be explained by the extent to which glycaemia is impaired on non-exercise days, or factors such as the timing of exercise and the sex of participants.

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.

AUTHOR CONTRIBUTIONS

MM, CO, AM-C, and JR contributed to data extraction. MM, CO, and NB performed the statistical analysis and wrote sections of the manuscript. All authors contributed to the conception and design of the study, manuscript revision, read, and approved the submitted version.

FUNDING

This research was performed without financial support. MM was supported by graduate student stipends from the Faculty of Kinesiology, Sport, and Recreation at the University of Alberta. AM-C was supported by the Fonds de recherche du Québec – Santé.

ACKNOWLEDGMENTS

We would like to thank Linda Slater who helped us develop previous search strategies from which the current one was produced. We also thank Dominic Tremblay who assisted AM-C with data extraction.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2020.00495/full#supplementary-material

REFERENCES

1. Boulé NG, Haddad E, Kenny GP, Wells GA, Sigal RJ. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. *JAMA*. (2001) 286:1218–27. doi: 10.1001/jama.286.10.1218
2. Umpierre D, Ribeiro PA, Kramer CK, Leitao CB, Zucatti AT, Azevedo MJ, et al. Physical activity advice only or structured exercise training and association with HbA1c levels in type 2 diabetes: a systematic review and meta-analysis. *JAMA*. (2011) 305:1790–9. doi: 10.1001/jama.2011.576
3. Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA*. (2006) 295:1681–7. doi: 10.1001/jama.295.14.1681
4. Suh S, Kim JH. Glycemic variability: how do we measure it and why is it important? *Diabet Metabol J*. (2015) 39:273–82. doi: 10.4093/dmj.2015.39.4.273
5. MacLeod SF, Terada T, Chahal BS, Boulé NG. Exercise lowers postprandial glucose but not fasting glucose in type 2 diabetes: a meta-analysis of studies using continuous glucose monitoring. *Diabet Metabol Res Rev*. (2013) 29:593–603. doi: 10.1002/dmrr.2461
6. Battelino T, Danne T, Bergenstal RM, Amiel SA, Beck R, Biester T, et al. Clinical targets for continuous glucose monitoring data interpretation: recommendations from the international consensus on time in range. *Diabetes care*. (2019) 42:1593–603. doi: 10.2337/dci19-0028
7. Higgins J, Green Se. *Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [Updated March 2011]*. The Cochrane Collaboration, 2011 (2009). Available online at: www.handbook.cochrane.org (accessed April 1, 2020).
8. Liubaoerjijin Y, Terada T, Fletcher K, Boule NG. Effect of aerobic exercise intensity on glycemic control in type 2 diabetes: a meta-analysis of head-to-head randomized trials. *Acta diabetologica*. (2016) 53:769–81. doi: 10.1007/s00592-016-0870-0
9. Rees JL, Chang CR, Francois ME, Marcotte-Chenard A, Fontvieille A, Klaprat ND, et al. Minimal effect of walking before dinner on glycemic responses in type 2 diabetes: outcomes from the multi-site E-PAraDiGM study. *Acta diabetologica*. (2019) 56:755–65. doi: 10.1007/s00592-019-01358-x
10. Francois ME, Baldi JC, Manning PJ, Lucas SJE, Hawley JA, Williams MJA, et al. 'Exercise snacks' before meals: a novel strategy to improve glycaemic control in individuals with insulin resistance. *Diabetologia*. (2014) 57:1437–45. doi: 10.1007/s00125-014-3244-6
11. Iscoe KE, Campbell JE, Jamnik V, Perkins BA, Riddell MC. Efficacy of continuous real-time blood glucose monitoring during and after prolonged high-intensity cycling exercise: spinning with a continuous glucose monitoring system. *Diabet Technol Ther*. (2006) 8:627–35. doi: 10.1089/dia.2006.8.627
12. Newton PG, White NH. Impact of acute moderate intensity exercise on blood glucose (BG) in youth with type 2 diabetes mellitus (T2D). *Diabetes*. (2012) 1:A334−5.
13. Farabi SS, Carley DW, Smith D, Quinn L. Impact of exercise on diurnal and nocturnal markers of glycaemic variability and oxidative stress in obese individuals with type 2 diabetes or impaired glucose tolerance. *Diabet Vasc Dis Res*. (2015) 12:381–5. doi: 10.1177/1479164115579003
14. Rafiei H, Robinson E, Barry J, Jung ME, Little JP. Short-term exercise training reduces glycaemic variability and lowers circulating endothelial microparticles in overweight and obese women at elevated risk of type 2 diabetes. *Eur J Sport Sci*. (2019) 19:1140–9. doi: 10.1080/17461391.2019.1576772
15. Gordon BA, Bird SR, Macisaac RJ, Benson AC. Glycemic response varies between resistance and aerobic exercise in inactive males with long-term type 2 diabetes. *Appl Physiol Nutr Metabol*. (2013) 38:900–4. doi: 10.1139/apnm-2012-0494

16. Gordon BA, Bird SR, MacIsaac RJ, Benson AC. Does a single bout of resistance or aerobic exercise after insulin dose reduction modulate glycaemic control in type 2 diabetes? A randomised cross-over trial. *J Sci Med Sport.* (2016) 19:795–9. doi: 10.1016/j.jsams.2016.01.004
17. Vijayakumar V, Mavathur R, Kannan S. Yoga helps in improving glycemic variability in type 2 diabetes mellitus-results of pilot study. *Int J Diabet Dev Countr.* (2018) 38(Suppl. 2):S171.
18. Vijayakumar V, Mavathur R, Sharma MNK, Kannan S. Reduced glycemic variability with yoga in patients with type 2 diabetes mellitus: results of a pilot study. *J Diabet Sci Technol.* (2019) 13:803–4. doi: 10.1177/1932296819852064
19. Vijayakumar V, Raju A, Mavathur R, Kannan S. Effect of short term yoga practice on ambulatory glucose profile in type 2 diabetes mellitus: report of pilot outcomes. *Indian J Endocrinol Metabol.* (2017) 21 (8 Suppl. 1):S26–7.
20. Duvivier BMFM, Schaper NC, Hesselink MKC, van Kan L, Stienen N, Winkens B, et al. Breaking sitting with light activities vs structured exercise: a randomised crossover study demonstrating benefits for glycaemic control and insulin sensitivity in type 2 diabetes. *Diabetologia.* (2017) 60:490–8. doi: 10.1007/s00125-016-4161-7
21. Dempsey PC, Blankenship JM, Larsen RN, Sacre JW, Sethi P, Straznicky NE, et al. Interrupting prolonged sitting in type 2 diabetes: nocturnal persistence of improved glycaemic control. *Diabetologia.* (2017) 60:499–507. doi: 10.1007/s00125-016-4169-z
22. Blankenship JM, Chipkin SR, Freedson PS, Staudenmayer J, Lyden K, Braun B. Managing free-living hyperglycemia with exercise or interrupted sitting in type 2 diabetes. *J Appl Physiol.* (2019) 126:616–25. doi: 10.1152/japplphysiol.00389.2018
23. Reynolds AN, Mann JI, Williams S, Venn BJ. Advice to walk after meals is more effective for lowering postprandial glycaemia in type 2 diabetes mellitus than advice that does not specify timing: a randomised crossover study. *Diabetologia.* (2016) 59:2572–8. doi: 10.1007/s00125-016-4085-2
24. Tay J, Luscombe-Marsh ND, Thompson CH, Noakes M, Buckley JD, Wittert GA, et al. A very low-carbohydrate, low-saturated fat diet for type 2 diabetes management: a randomized trial. *Diabet Care.* (2014) 37:2909–18. doi: 10.2337/dc14-0845
25. Bacchi E, Negri C, Trombetta M, Zanolin ME, Lanza M, Bonora E, et al. Differences in the acute effects of aerobic and resistance exercise in subjects with type 2 diabetes: results from the RAED2 randomized trial. *PLoS ONE.* (2012) 7:e49937. doi: 10.1371/journal.pone.0049937
26. Paing AC, McMillan KA, Kirk AF, Collier A, Hewitt A, Chastin SFM. Impact of free-living pattern of sedentary behaviour on intra-day glucose regulation in type 2 diabetes. *Eur J Appl Physiol.* (2020) 120:171–9. doi: 10.1007/s00421-019-04261-z
27. Paing AC, McMillan KA, Kirk AF, Collier A, Hewitt A, Chastin SFM. Dose-response between frequency of breaks in sedentary time and glucose control in type 2 diabetes: a proof of concept study. *J Sci Med Sport.* (2019) 22:808–13. doi: 10.1016/j.jsams.2019.01.017
28. Li Z, Hu Y, Ma J. [Effect of moderate exercise for 30 min at 30 min versus 60 min after dinner on glycemic control in patients with type 2 diabetes: a randomized, crossover, self-controlled study]. *Nan Fang Yi Ke Da Xue Xue Bao.* (2018) 38:1165–70. doi: 10.3969/j.issn.1673-4254.2018.10.03
29. Hu Y, Zhang DF, Dai L, Li Z, Li HQ, Li FF, et al. Pre-exercise blood glucose affects glycemic variation of aerobic exercise in patients with type 2 diabetes treated with continuous subcutaneous insulin infusion. *Diabet Res Clin Prac.* (2018) 141:98–105. doi: 10.1016/j.diabres.2018.04.043
30. Godkin FE, Jenkins EM, Little JP, Nazarali Z, Percival ME, Gibala MJ. The effect of brief intermittent stair climbing on glycemic control in people with type 2 diabetes: a pilot study. *Appl Physiol Nutr metabol.* (2018) 43:969–72. doi: 10.1139/apnm-2018-0135
31. Rodriguez KJ, Coombes J, Keating S. Personalised exercise as medicine for type 2 diabetes: finding the 'sweet spot' for glycaemic control. *J Sci Med Sport.* (2019) 22 (Supplement 2):S88–S9. doi: 10.1016/j.jsams.2019.08.103
32. Viana AA, Fernandes B, Alvarez C, Guimaraes GV, Ciolac EG. Prescribing high-intensity interval exercise by RPE in individuals with type 2 diabetes: metabolic and hemodynamic responses. *Appl Physiol Nutr metabol.* (2019) 44:348–56. doi: 10.1139/apnm-2018-0371
33. Karstoft K, Clark MA, Jakobsen I, Knudsen SH, van Hall G, Pedersen BK, et al. Glucose effectiveness, but not insulin sensitivity, is improved after short-term interval training in individuals with type 2 diabetes mellitus: a controlled, randomised, crossover trial. *Diabetologia.* (2017) 60:2432–42. doi: 10.1007/s00125-017-4406-0
34. Little JP, Gillen JB, Percival ME, Safdar A, Tarnopolsky MA, Punthakee Z, et al. Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. *J Appl Physiol.* (2011) 111:1554–60. doi: 10.1152/japplphysiol.00921.2011
35. Van Dijk JW, Manders RJF, Canfora EE, Van Mechelen W, Hartgens F, Stehouwer CDA, et al. Exercise and 24-h glycemic control: equal effects for all type 2 diabetes patients? *Med Sci Sports nd Exerc.* (2013) 45:628–35. doi: 10.1249/MSS.0b013e31827ad8b4
36. Cruz LC, Teixeira-Araujo AA, Andrade KTP, Rocha TCOG, Moreira SR. Low intensity resistance exercise attenuates the relationship between glucose and autonomic nervous system indicators during 24 hours in women with type 2 diabetes L'exercice de resistance a faible intensite attenue la relation entre le glucose et les indicateurs du systeme nerveux autonome pendant 24 heures chez les femmes atteintes de diabete de type 2. *Sci Sports.* (2018) 33:e75–83. doi: 10.1016/j.scispo.2017.10.004
37. Karstoft K, Winding K, Knudsen SH, Pedersen BK, Nielsen JS, Solomon TP. The effects and physiological mechanisms of free-living interval-walking training on glycaemic control in type 2 diabetes patients: a randomised, controlled trial. *Diabetologia.* (2012) 1):S248.
38. Gillen JB, Little JP, Punthakee Z, Tarnopolsky MA, Riddell MC, Gibala MJ. Acute high-intensity interval exercise reduces the postprandial glucose response and prevalence of hyperglycaemia in patients with type 2 diabetes. *Diabet Obe Metabol.* (2012) 14:575–7. doi: 10.1111/j.1463-1326.2012.01564.x
39. Savikj M, Gabriel BM, Alm PS, Smith J, Caidahl K, Bjornholm M, et al. Afternoon exercise is more efficacious than morning exercise at improving blood glucose levels in individuals with type 2 diabetes: a randomised crossover trial. *Diabetologia.* (2019) 62:233–7. doi: 10.1007/s00125-018-4767-z
40. Cruz LCD, Teixeira-Araujo AA, Passos Andrade KT, Rocha T, Puga GM, Moreira SR. Low-Intensity resistance exercise reduces hyperglycemia and enhances glucose control over a 24-hour period in women with type 2 diabetes. *J Strength Condition Res.* (2019) 33:2826–35. doi: 10.1519/JSC.0000000000002410
41. Erickson ML, Little JP, Gay JL, McCully KK, Jenkins NT. Effects of postmeal exercise on postprandial glucose excursions in people with type 2 diabetes treated with add-on hypoglycemic agents. *Diabet Res Clin Prac.* (2017) 126:240–7. doi: 10.1016/j.diabres.2017.02.015
42. Figueira FR, Umpierre D, Casali KR, Tetelbom PS, Henn NT, Ribeiro JP, et al. Aerobic and combined exercise sessions reduce glucose variability in type 2 diabetes: crossover randomized trial. *PLoS ONE.* (2013) 8:e57733. doi: 10.1371/journal.pone.0057733
43. Haxhi J, Leto G, di Palumbo AS, Sbriccoli P, Guidetti L, Fantini C, et al. Exercise at lunchtime: effect on glycemic control and oxidative stress in middle-aged men with type 2 diabetes. *Eur J Appl Physiology.* (2016) 116:573–82. doi: 10.1007/s00421-015-3317-3
44. Karstoft K, Christensen CS, Pedersen BK, Solomon TPJ. The acute effects of interval-vs continuous-walking exercise on glycemic control in subjects with type 2 diabetes: a crossover, controlled study. *J Clin Endocrinol Metab.* (2014) 99:3334–42. doi: 10.1210/jc.2014-1837
45. Karstoft K, Clark MA, Jakobsen I, Muller IA, Pedersen BK, Solomon TPJ, et al. The effects of 2 weeks of interval vs continuous walking training on glycaemic control and whole-body oxidative stress in individuals with type 2 diabetes: a controlled, randomised, crossover trial. *Diabetologia.* (2017) 60:508–17. doi: 10.1007/s00125-016-4170-6
46. Li Z, Hu Y, Yan R, Li H, Zhang D, Li F, et al. Twenty minute moderate-intensity post-dinner exercise reduces the postprandial glucose response in chinese patients with type 2 diabetes. *Med Sci Monit.* (2018) 24:7170–7. doi: 10.12659/MSM.910827
47. Macdonald AL, Philp A, Harrison M, Bone AJ, Watt PW. Monitoring exercise-induced changes in glycemic control in type 2 diabetes. *Med Sci Sports Exerc.* (2006) 38:201–7. doi: 10.1249/01.mss.0000183852.31164.5a
48. Manders RJF, Van Dijk JWM, Van Loon LJC. Low-intensity exercise reduces the prevalence of hyperglycemia in type 2 diabetes. *Med Sci Sports Exerc.* (2010) 42:219–25. doi: 10.1249/MSS.0b013e3181b3b16d
49. Metcalfe RS, Fitzpatrick B, Fitzpatrick S, McDermott G, Brick N, McClean C, et al. Extremely short duration interval exercise improves 24-h glycaemia

in men with type 2 diabetes. *Eur J Appl Physiol.* (2018) 118:2551–62. doi: 10.1007/s00421-018-3980-2
50. Mikus CR, Oberlin DJ, Libla J, Boyle LJ, Thyfault JP. Glycaemic control is improved by 7 days of aerobic exercise training in patients with type 2 diabetes. *Diabetologia.* (2012) 55:1417–23. doi: 10.1007/s00125-012-2490-8
51. Myette-Cote E, Terada T, Boule NG. The effect of exercise with or without metformin on glucose profiles in type 2 diabetes: a pilot study. *Can J Diabet.* (2016) 40:173–7. doi: 10.1016/j.jcjd.2015.08.015
52. Oberlin DJ, Mikus CR, Kearney ML, Hinton PS, Manrique C, Leidy HJ, et al. One bout of exercise alters free-living postprandial glycemia in type 2 diabetes. *Med Sci Sports Exerc.* (2014) 46:232–8. doi: 10.1249/MSS.0b013e3182a54d85
53. Praet SF, Manders RJ, Lieverse AG, Kuipers H, Stehouwer CD, Keizer HA, et al. Influence of acute exercise on hyperglycemia in insulin-treated type 2 diabetes. *Med Sci Sports Exerc.* (2006) 38:2037–44. doi: 10.1249/01.mss.0000235352.09061.1d
54. Terada T, Wilson BJ, Myette-Cote E, Kuzik N, Bell GJ, McCargar LJ, et al. Targeting specific interstitial glycemic parameters with high-intensity interval exercise and fasted-state exercise in type 2 diabetes. *Metabol Clin Exp.* (2016) 65:599–608. doi: 10.1016/j.metabol.2016.01.003
55. Van Dijk JW, Manders RJF, Tummers K, Bonomi AG, Stehouwer CDA, Hartgens F, et al. Both resistance- and endurance-type exercise reduce the prevalence of hyperglycaemia in individuals with impaired glucose tolerance and in insulin-treated and non-insulin-treated type 2 diabetic patients. *Diabetologia.* (2012) 55:1273–82. doi: 10.1007/s00125-011-2380-5
56. Van Dijk JW, Tummers K, Stehouwer CDA, Hartgens F, Van Loon LJC. Exercise therapy in type 2 diabetes: Is daily exercise required to optimize glycemic control? *Diabetes care.* (2012) 35:948–54. doi: 10.2337/dc11-2112
57. Van Dijk JW, Venema M, Van Mechelen W, Stehouwer CDA, Hartgens F, Van Loon LJC. Effect of moderate-intensity exercise versus activities of daily living on 24-hour blood glucose homeostasis in male patients with type 2 diabetes. *Diabet care.* (2013) 36:3448–53. doi: 10.2337/dc12-2620
58. Cauza E, Hanusch-Enserer U, Strasser B, Kostner K, Dunky A, Haber P. Strength and endurance training lead to different post exercise glucose profiles in diabetic participants using a continuous subcutaneous glucose monitoring system. *Eur J Clin Invest.* (2005) 35:745–51. doi: 10.1111/j.1365-2362.2005.01573.x
59. Francois ME, Durrer C, Pistawka KJ, Halperin FA, Chang C, Little JP. Combined interval training and post-exercise nutrition in type 2 diabetes: a randomized control trial. *Front Physiol.* (2017) 8:528. doi: 10.3389/fphys.2017.00528
60. Karstoft K, Winding K, Knudsen SH, Nielsen JS, Thomsen C, Pedersen BK, et al. The effects of free-living interval-walking training on glycemic control, body composition, and physical fitness in type 2 diabetic patients: a randomized, controlled trial. *Diabetes Care.* (2013) 36:228–36. doi: 10.2337/dc12-0658
61. Ruffino JS, Songsorn P, Haggett M, Edmonds D, Robinson AM, Thompson D, et al. A comparison of the health benefits of reduced-exertion high-intensity interval training (REHIT) and moderate-intensity walking in type 2 diabetes patients. *Appl Physiol Nutr Metabol.* (2017) 42:202–8. doi: 10.1139/apnm-2016-0497
62. Winding KM, Munch GW, Iepsen UW, Van Hall G, Pedersen BK, Mortensen SP. The effect on glycaemic control of low-volume high-intensity interval training versus endurance training in individuals with type 2 diabetes. *Diabet Obes Metabol.* (2018) 20:1131–9. doi: 10.1111/dom.13198
63. Abdul-Ghani MA, Jenkinson CP, Richardson DK, Tripathy D, DeFronzo RA. Insulin secretion and action in subjects with impaired fasting glucose and impaired glucose tolerance: results from the veterans administration genetic epidemiology study. *Diabetes.* (2006) 55:1430–5. doi: 10.2337/db05-1200
64. Nathan DM, Davidson MB, DeFronzo RA, Heine RJ, Henry RR, Pratley R, et al. Impaired fasting glucose and impaired glucose tolerance: implications for care. *Diabet Care.* (2007) 30:753–9. doi: 10.2337/dc07-9920
65. Snowling NJ, Hopkins WG. Effects of different modes of exercise training on glucose control and risk factors for complications in type 2 diabetic patients: a meta-analysis. *Diabet Care.* (2006) 29:2518–27. doi: 10.2337/dc06-1317
66. Shephard RJ. Regression to the mean. A threat to exercise science? *Sports Med.* (2003) 33:575–84. doi: 10.2165/00007256-200333080-00003
67. Boulé NG, Weisnagel SJ, Lakka TA, Tremblay A, Bergman RN, Rankinen T, et al. Effects of exercise training on glucose homeostasis: the heritage family study. *Diabet Care.* (2005) 28:108–14. doi: 10.2337/diacare.28.1.108
68. Henderson GC, Fattor JA, Horning MA, Faghihnia N, Johnson ML, Luke-Zeitoun M, et al. Glucoregulation is more precise in women than in men during postexercise recovery. *Am J clin Nutr.* (2008) 87:1686–94. doi: 10.1093/ajcn/87.6.1686
69. Henderson GC, Fattor JA, Horning MA, Faghihnia N, Johnson ML, Mau TL, et al. Lipolysis and fatty acid metabolism in men and women during the postexercise recovery period. *J Physiol.* (2007) 584:963–81. doi: 10.1113/jphysiol.2007.137331
70. Van Dijk JW, Venema M, Van Mechelen W, Stehouwer CD, Hartgens F, Van Loon LJ. Exercise or habitual physical activity to improve blood glucose homeostasis in patients with type 2 diabetes? *Diabetes.* (2013) 1:A184.
71. Verboven K, Wens I, Vandenabeele F, Stevens AN, Celie B, Lapauw B, et al. Impact of exercise-nutritional state interactions in patients with type 2 diabetes. *Med Sci Sports Exerc.* (2020) 52:720–8. doi: 10.1249/MSS.0000000000002165
72. Brinkmann C, Weh-Gray O, Brixius K, Bloch W, Predel HG, Kreutz T. Effects of exercising before breakfast on the health of T2DM patients-A randomized controlled trial. *Scand J Med Sci Sports.* (2019) 29:1930–6. doi: 10.1111/sms.13543
73. Karstoft K, Christensen CS, Pedersen BK, Solomon TP. The acute effects of interval-vs. continuouswalking exercise on glycemic control in subjects with type 2 diabetes: a cross-over, controlled study. *Diabet Technol Ther.* (2015) 17(Suppl. 1):S18–9.
74. Su G, Mi S, Tao H, Li Z, Yang H, Zheng H, et al. Association of glycemic variability and the presence and severity of coronary artery disease in patients with type 2 diabetes. *Cardiovasc Diabetol.* (2011) 10:19. doi: 10.1186/1475-2840-10-19
75. Saisho Y, Tanaka C, Tanaka K, Roberts R, Abe T, Tanaka M, et al. Relationships among different glycemic variability indices obtained by continuous glucose monitoring. *Primary Care* Diabet. (2015) 9:290–6. doi: 10.1016/j.pcd.2014.10.001
76. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ, et al. Translating the A1C assay into estimated average glucose values. *Diabetes Care.* (2008) 31:1473–8. doi: 10.2337/dc08-0545

Conflict of Interest: NB has received continuous glucose monitors from Medtronic Canada for previous studies.

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.

frontiers in Endocrinology

REVIEW
published: 11 August 2020
doi: 10.3389/fendo.2020.00519

Metformin May Contribute to Inter-individual Variability for Glycemic Responses to Exercise

Steven K. Malin[1,2,3]* *and Nathan R. Stewart*[1]

[1] Department of Kinesiology, University of Virginia, Charlottesville, VA, United States, [2] Division of Endocrinology and Metabolism, University of Virginia, Charlottesville, VA, United States, [3] Robert M. Berne Cardiovascular Research Center, University of Virginia, Charlottesville, VA, United States

Metformin and exercise independently improve glycemic control. Metformin traditionally is considered to reduce hepatic glucose production, while exercise training is thought to stimulate skeletal muscle glucose disposal. Collectively, combining treatments would lead to the anticipation for additive glucose regulatory effects. Herein, we discuss recent literature suggesting that metformin may inhibit, enhance or have no effect on exercise mediated benefits toward glucose regulation, with particular emphasis on insulin sensitivity. Importantly, we address issues surrounding the impact of metformin on exercise induced glycemic benefit across multiple insulin sensitive tissues (e.g., skeletal muscle, liver, adipose, vasculature, and the brain) in effort to illuminate potential sources of inter-individual glycemic variation. Therefore, the review identifies gaps in knowledge that require attention in order to optimize medical approaches that improve care of people with elevated blood glucose levels and are at risk of cardiovascular disease.

Keywords: pre-diabetes, type 2 diabetes, metabolic syndrome, insulin resistance, exercise, weight loss

OPEN ACCESS

Edited by:
John P. Thyfault, University of Kansas Medical Center, United States

Reviewed by:
Camila Manrique-Acevedo, University of Missouri, United States
Nathan C. Winn, Vanderbilt University, United States
Louise Lantier, Vanderbilt University, United States

***Correspondence:**
Steven K. Malin
skm6n@virginia.edu

Specialty section:
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Endocrinology

Received: *30 April 2020*
Accepted: *26 June 2020*
Published: *11 August 2020*

Citation:
Malin SK and Stewart NR (2020) Metformin May Contribute to Inter-individual Variability for Glycemic Responses to Exercise. Front. Endocrinol. 11:519. doi: 10.3389/fendo.2020.00519

INTRODUCTION

Nearly 34.2 million individuals in the U.S. have type 2 diabetes, and ∼88 million men and women have prediabetes (1). Perhaps more concerningly is the observation that new cases of type 2 diabetes have increased significantly among U.S. youth, particularly non-Hispanic black people (1). This is clinically concerning because people with hyperglycemia are at greatly elevated risk for not only retinopathy, nephropathy, renal disease, but also cardiovascular disease (CVD). Blood glucose regulation is considered to be a complex balance between endogenous glucose production and peripheral glucose uptake. Insulin resistance of organs regulating these processes is considered to a be a primary defect. In particular, insulin resistance contributes to compensatory hyperinsulinemia via taxation on the pancreatic beta-cells to secrete insulin. Over time, however, the beta-cells begin to "fail" and cannot compensate for the ambient levels of systemic insulin resistance resulting in severe hyperglycemia. Therefore, targeting insulin resistance is a reasonable approach to the prevention, treatment, and management of type 2 diabetes.

Although randomized clinical trials show the efficacy of exercise to treat type 2 diabetes (2) as well as prevent the progression from prediabetes to type 2 diabetes (3, 4), there is large inter-individual heterogeneity in response to conventional exercise aerobic (up to 5 d/wk at 60–85% HR_{max}) and strength (up to 2 d/wk at 60–80% 1-repetition max). Moreover, the optimal dose of exercise to improve glycemic control remains to be elucidated (5–7), and exercise adherence remains low. Patients with prediabetes and/or type 2 diabetes often exhibit multiple

pathophysiological abnormalities that contribute to the approximate 20% lower aerobic capacity compared to those without dysglycemia (8). These include: mitochondrial dysfunction, poor muscle perfusion, and low cardiac function in addition to declines in pancreatic insulin secretion and sensitivity. Together, these are mechanisms contributing to decreased oxidative capacity and may help explain barriers to starting exercise interventions (9). Subsequently, many individuals may require pharmacological therapy to manage blood glucose concentrations. The American Diabetes Association suggests that in addition to lifestyle modification, metformin be considered the "first-line" pharmacological treatment to manage blood glucose in those with type 2 diabetes as well as those with prediabetes and at least 1 CVD risk factor (e.g., hypertension, elevated triacylglycerol, low HDL, etc.) (10). Not surprisingly, metformin is the most widely used prescription drug to treat hyperglycemia in adults with type 2 diabetes (11). In addition, metformin has gained interest in cancer prevention/treatment (12) as well as lifespan within aging (13). This highlights that metformin is a multi-faceted drug with health effects. Despite the widespread popularity of metformin, the interaction with exercise has received little attention. If anything, the overarching thought is that recommending exercise plus metformin will enhance glycemic control, and be better than either intervention alone. Herein, we highlight recent data describing whether co-prescribing metformin with exercise blunts, enhances, or has negligible effects on glucose regulation for ultimate CVD risk reduction. In this review, we focus on the multiple tissues (i.e., skeletal muscle, liver, adipose, vasculature, and brain) that metformin may affect during exercise training to influence cardiometabolic health (**Figure 1**). Lastly, we hypothesize that combining metformin with exercise may induce cellular processes that regulate metabolic adaptation in relation to glycemia.

IMPACT OF METFORMIN ON EXERCISE MEDIATED GLYCEMIC CONTROL

Regulation of blood glucose is a tightly controlled process through "cross-talk" of pancreatic insulin secretion and insulin action on several tissues, including but not limited to: skeletal muscle, liver, adipose tissue, vasculature, and the brain. Fasting plasma glucose is maintained by endogenous (principally hepatic) glucose production, as glucose disposal by skeletal muscle and adipose tissue is minimal (14). Following mixed-meal absorption, insulin levels rise in response to carbohydrate (and to smaller extents protein) to reduce liver glucose production and lipolysis as well as stimulate blood flow to skeletal muscle for glucose uptake (15). Moreover, insulin acts on the brain to provide additional regulation of endogenous glucose production as well as inhibit additional food intake (16). Thus, considering treatments that impact liver and/or skeletal muscle glucose metabolism should, in theory, lead to enhanced glycemic control.

Current exercise prescription advised by the American College of Sports Medicine and the American Diabetes Association for men and women with prediabetes or T2D is to perform either 150 min/week or more of moderate intensity or 75 min/week of vigorous intensity aerobic exercise. The Look AHEAD study is a landmark clinical trial that reported a $\geq$ 7% reduction in weight loss through a nutrition intervention in combination with $\geq$175 min exercise/wk, reduced CVD risk by ~0.7%/yr (17), although more work is needed to understand the utility of lifestyle treatment for vasculature related events/mortality. Exercise can consist of either aerobic or resistance form, although the combination may result in the best HbA1c reductions (2). While there is still much debate as to whether exercise intensity is critical for glycemic control (18), we and others have shown either no effect (19) or that moderate intensity may have slightly better effects (20). Regardless, more recent work has suggested "exercise snacks" may be a novel approach to combat post-prandial hyperglycemia, and further work examining time of day to exercise is warranted (18).

Metformin predominantly reduces circulating glucose by lowering hepatic glucose production (11, 21), although it has also been reported to increase peripheral insulin sensitivity in some but not all work (22, 23). In the landmark U.S. diabetes prevention program, metformin was shown to decrease the incidence of T2D by 31% (1,700 mg/d) in adults with impaired glucose compared with 58% following lifestyle modification (7% weight loss and 150 min/wk of physical activity) (4). Although these findings suggest lifestyle was better than metformin alone, the Indian Diabetes Prevention Program (IDDP) observed that both regular physical activity (recommended >30 min/d) and metformin (500 mg/d) reduced the progression of impaired glucose tolerance to T2D in native Asian Indians (24).

Exercise and metformin both increase 5-adenosine monophosphate kinase (AMPK). This is important because AMPK is one of the several mechanisms by which each therapy act to suppress hepatic glucose output and increase insulin-stimulated glucose disposal (11, 23). As a result, it would be fair to expect a greater benefit to glycemic control since two of the major organs regulating blood glucose would be impacted compared with either treatment alone. However, the literature on co-prescribing lifestyle modification with metformin on blood glucose is equivocal (**Table 1**). Indeed, some (25) have shown that lifestyle modification plus metformin resulted in more weight loss than lifestyle modification alone, and the weight loss was associated with lower 2-h circulating glucose levels. This is somewhat consistent with recent work by Erickson et al. (26) showing that post-meal exercise and metformin resulted in the lowest peak post-prandial glucose excursion compared with either treatment alone in people with hyperglycemia. Furthermore, Ortega et al. sought to test the effects of combining metformin with exercise on free-living glycemic control in individuals with prediabetes or T2D (27). The results of this later work demonstrated that high intensity interval exercise in combination with metformin therapy lowered interstitial fluid glucose to a greater extent than exercise alone. Interestingly, others have suggested that in people with T2D treated with metformin that timing exercise 30 to 60 min following drug ingestion may impact plasma glucose and insulin

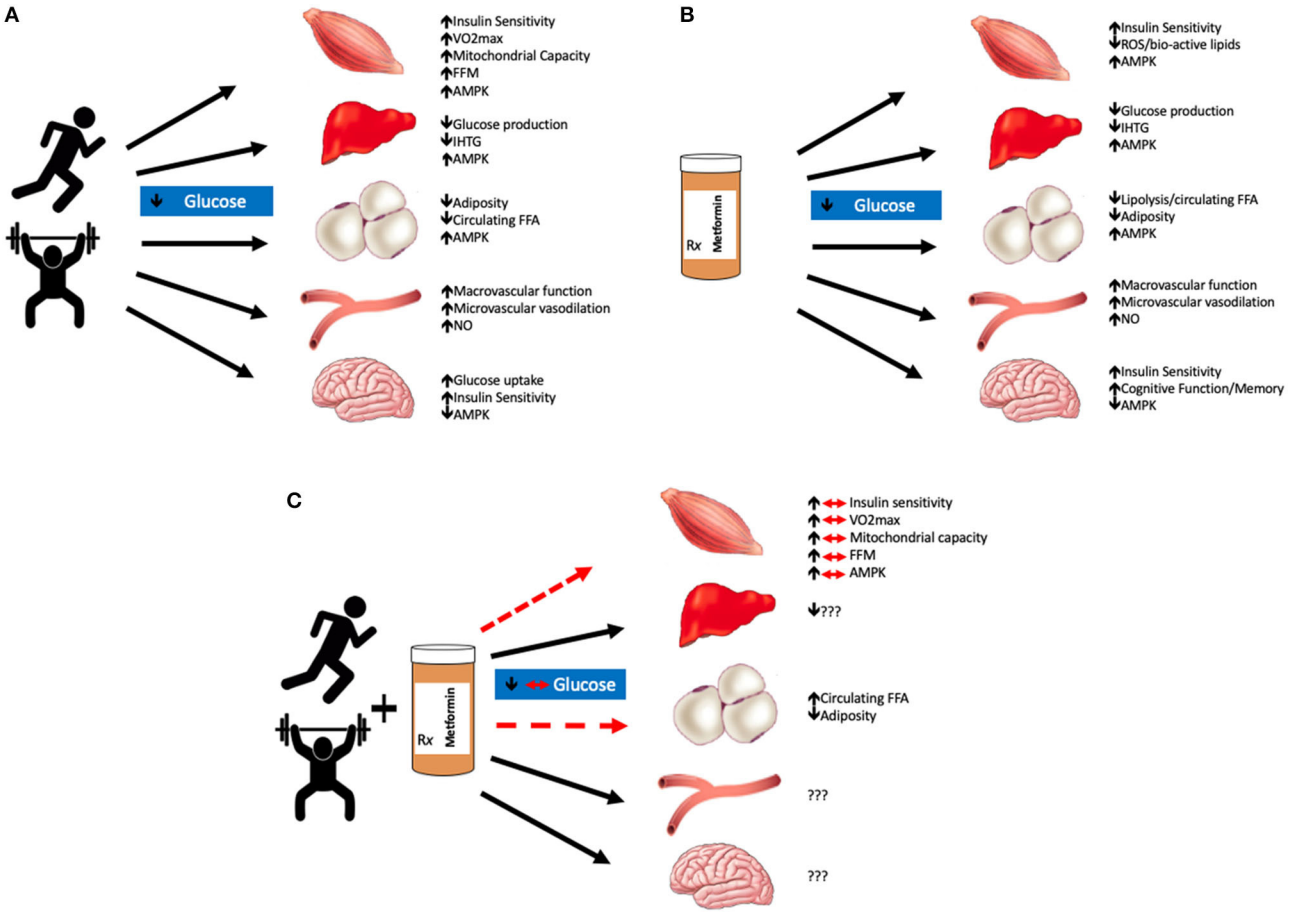


FIGURE 1 | Summary of exercise and/or metformin interactions. Exercise lowers blood glucose mainly through increases in AMPK production in most organs excluding the brain, where production is decreased; and the vasculature, where adaptations are largely driven by nitric oxide (NO) **(A)**. Metformin alone also improves glycemic control through similar mechanisms, primarily by decreasing hepatic glucose production. Metformin also decreases reactive oxygen species (ROS) production which is suspected to improve tissue glycemic control as well as memory and cognitive function in the brain **(B)**. The combination of metformin with exercise has blunted effects on skeletal muscle glucose uptake and visceral adiposity. The effects of metformin with exercise on the liver, vasculature, and brain are still largely unknown **(C)**. We hypothesize that the combination of metformin and exercise are not necessarily additive in terms of glycemic control. Metformin blunts the beneficial adaptations that are typically seen with aerobic and/or resistance training in skeletal muscle tissue. VO2max, maximal oxygen consumption; FFM, fat-free mass; AMPK, adenosine monophosphate kinase; IHTG, intrahepatic triglycerides; FFA, free fatty acids; NO, nitic oxide.

to a greater extent than exercising 90 min after ingestion (28). The Diabetes Aerobic and Resistance Exercise (DARE) trial, however, showed that people with T2D on metformin plus lifestyle modification had similar HbA1c improvements when compared with individuals on lifestyle modification only (29). This is consistent with the IDDP since it was shown that the combination therapy of metformin and lifestyle modification had equivalent effects to reduce the progression from prediabetes to T2D (24). Notwithstanding this, a retrospective analysis of the Look AHEAD study demonstrated that people with type 2 diabetes treated with metformin prior to and during intensive lifestyle therapy had smaller improvements in fasting plasma glucose and HbA1c compared with those undergoing lifestyle therapy only (30). Further, the work by Boulé et al. (31) tested the effect of metformin on glycemic control in response to a single bout of submaximal aerobic exercise at ∼33, 67, and 79% of VO_2peak and resistance exercise (i.e., leg extension and flexion) in individuals with T2D. Their results implied that metformin blunted reductions in post-prandial blood glucose concentrations during a standardized meal. Together, most (26, 27) but not all (24, 31) studies showing an additive effect of metformin plus exercise studied individuals who were already prescribed metformin. In contrast, we showed previously (32) that 12 weeks of metformin plus exercise training prospectively in naïve users had no effect on fasting plasma glucose in adults with prediabetes. This is consistent with newer work (33, 34) whereby in normoglycemic insulin-resistant adults, fasting or postprandial glucose levels did not appear to be negatively affected by metformin. Somewhat surprisingly, however, is the observation that no randomized clinical trial has been designed to date to test the effectiveness of exercise plus metformin on glycemic control. Given that some work shows opposing (31), additive (26, 27), or null findings (32–34), it is reasonable to suggest that metformin

TABLE 1 | Summary of clinical trials examining the impact of metformin in combination with exercise on glycemic control compared to exercise alone.

Study details	Prescription/Population	FPG	2-h Postprandial	HbA1c
Ramachandran et al. (24)	Walk or cycle >30 min/d + 1,000 mg/d metformin for ∼3 years in overweight/obese adults	↔	↔ during OGTT	N/A
Sharoff et al. (23)	An acute bout of cycle ergometry for 30 min @ 65% VO_2Peak + 2–3 weeks of 2,000 mg/d metformin treatment prior in overweight/obese adults	↔	N/A	N/A
Love-Osborne et al. (25)	Self-chosen life style change + 1,700 mg/d metformin for 6 months in overweight/obese adolescents	↔	↔ during OGTT	N/A
Erickson et al. (26)	Postmeal exercise (5 x 10-min bouts of treadmill walking at 60% VO_2Peak) + 1,000–2,000 mg/d metformin in overweight/obese adults	N/A	↓ during MMTT	N/A
Ortega et al. (27)	An acute bout of exercise + physician prescribed dose of metformin in insulin-resistant adults	N/A	↔ during OGTT	N/A
Boulé et al. (31)	∼33 submaximal exercise bouts lasting 3–15 min @ 67–79% VO_2Peak + 28 d of 2,000 mg/d metformin treatment prior in adults with T2D.	↑	↑ during MMTT	N/A
Terada and Boulé (30)	Nutrition intervention/with ≥175 min exercise/wk + metformin therapy in overweight adults with T2D	↑	N/A	↑
Malin et al. (32)	60–75 min of moderate-high intensity concurrent training 3 d/wk + of 2,000 mg/d metformin administration for 12 weeks in adults with prediabetes	↔	N/A	N/A
Konopka et al. (33)	45 min of moderate-high intensity cycle ergometry 3 d/wk + 2,000 mg/d metformin for 12 weeks in older adults with prediabetes	↔	N/A	↔
Walton et al. (34)	PRT + 1,700 mg/d metformin for 14 weeks in healthy older adults	↔	N/A	N/A

FPG, fasting plasma glucose; OGTT, oral glucose tolerance test; MMTT, mixed meal tolerance test; N/A, Not applicable because the measurement was either not reported or measured; ↑, higher; ↓, lower; ↔, no difference.

contributes to inter-individual glycemic response differences (**Table 1**).

EFFECT OF METFORMIN ON EXERCISE-MEDIATED SKELETAL MUSCLE INSULIN SENSITIZING EFFECTS

Exercise improves glycemic control through both skeletal muscle insulin-dependent and insulin-independent mechanisms (35). Subsequently, contraction mediated mechanisms favoring glucose uptake last for ∼3–6 h following a single bout of exercise. In time, insulin-sensitizing effects take over to explain improved glucose control (36). Habitual exercise (i.e., lifestyle change) is recommended to reduce T2D risk in part by maintaining skeletal muscle glucose disposal.

Metformin is suggested to stimulate skeletal muscle glucose uptake and oxidation (37). Moreover, metformin has been shown to lower intramuscular triglyceride content and bioactive acyl-chain bioactive lipids (38, 39) through in part elevations in fat oxidation. Together, these observations indicate that metformin has effects on skeletal muscle energy metabolism that favor glucose homeostasis.

Because metformin is advised as a first-line pharmacological agent, we conducted a double-blind, randomized control trial to test the effect of exercise training with and without metformin on insulin sensitivity in people with prediabetes (32). For 12 weeks, individuals were randomized to either: placebo, metformin, exercise training with placebo, or exercise training with metformin. All people were provided metformin at 2,000 mg/d or a placebo, while those randomized to exercise underwent a progressive aerobic and resistance training program at 70% of their individual heart rate peak and 1-repetition max, respectively. Insulin sensitivity was determined about 28 h post-exercise via the euglycemic-hyperinsulinemic clamp with glucose isotope tracers. Tracers were utilized to determine the effects of metformin on skeletal muscle insulin sensitivity as well as hepatic glucose production. The primary results showed that metformin blunted exercise mediated increases in insulin-stimulated skeletal muscle glucose uptake by ∼30%, suggesting that metformin diminishes both single and repeated bouts of exercise benefit on glucose metabolism (23, 31, 32). Although to date no follow up studies have been conducted using stable isotopes to understand skeletal muscle insulin-stimulated glucose disposal, recent work has tested the effect of metformin on aerobic or resistance exercise skeletal muscle cellular adaptation (33, 34). The results of these studies collectively show that metformin opposes skeletal muscle mitochondrial adaptations as well as inhibits fat-free mass accretion (see below *Cell Mechanisms* for further discussion), which were directly correlated with attenuated gains in aerobic fitness as well as strength. Together, these findings highlight that blunted fitness adaptation may relate to the reduced skeletal muscle insulin sensitivity response. In either case, this smaller gain in insulin sensitivity following the combination of exercise and metformin treatment does not apparently lead to stark blood glucose elevations (23, 31, 32). Further work is warranted

to better understand how the combination of drug-exercise therapies contributes to glycemic control across exercise doses, particularly in people with T2D. For instance, recent work demonstrated that metformin increased carbohydrate utilization during high intensity interval exercise in insulin resistant adults when compared to exercise alone (27). This may be of clinical relevance since carbohydrate use during exercise was related to insulin sensitivity as measured by the intravenous glucose tolerance test. The findings of Ortega et al. (27) also suggest that exercise intensity may interact with metformin to positively influence insulin-stimulated glucose uptake when compared with moderate intensity exercise (31, 40). Whether exercise intensity interacts with metformin to affect skeletal muscle insulin sensitivity in clinical populations remains to be tested to help understand if muscle is the primary driver of glycemic variation responses.

EFFECT OF METFORMIN ON EXERCISE-MEDIATED LIVER INSULIN SENSITIZING EFFECTS

Hepatic glucose production results from gluconeogenesis and/or glycogenolysis, and people with impaired fasting glucose display elevated hepatic glucose production (25, 41), or inappropriately normal levels given the prevailing hyperinsulinemia (40). Indeed, people at risk for or with T2D, in particular, have impaired responses to insulin (42). This highlights that the liver becomes insulin resistant and plays roles in both fasting and fed states. While fasting glucose (and insulin) may serve as a proxy for hepatic glucose production, and study of hepatokines, liver fat, or liver enzymes (43) may provide indirect estimates of hepatic function, use of stable isotopes along with hyperinsulinemic-clamps represent ideal methodologies to depict the role of the liver on glycemic control.

The exercise impact on hepatic glucose production is generally positive. One to seven days of aerobic exercise has been shown in people with T2D to increase hepatic insulin sensitivity (44, 45). Exercise training studies of ∼12 weeks have also demonstrated favorable effects on hepatic insulin sensitivity (46), with at least some of the effect being related to improved hepatokines (i.e., fetuin-A) (46). However, it is worth noting that others have suggested that re-feeding calories expended from exercise negates these liver insulin-sensitizing benefits of exercise in adults with excess weight/insulin resistance (47). It cannot be ruled out though that discrepancies between short-term training studies may relate to exercise intensity, as higher intensity exercise activates AMPK in hepatocytes (48). As a result, it seems that energy deficit, at least partially, created by exercise is an important mechanism improving hepatic insulin sensitivity.

Metformin improves hepatic insulin sensitivity. The mechanism by which metformin lowers hepatic glucose production is mainly thought to be through activation of AMPK and reduction in gluconeogenic enzymes (49), although some suggest antagonism of glucagon may be important (50). In addition, metformin is considered to increase fat oxidation in hepatocytes, thereby reducing the potential delirious effects of lipids on insulin signaling (51). Recent work has suggested that metformin may benefit conditions of hepatic steatosis. In particular, although metformin-induced similar reductions in the hepatic triglyceride content of Otsuka Long-Evans Tokushima Fatty (OLETF) rats under caloric restriction, compared to caloric restriction alone, the combined treatment lowered hepatic-derived inflammation more (52). Additionally, metformin augmented the benefits of caloric restriction on lowering post-prandial circulating glucose in rodents, suggesting that metformin may impact the liver during energy deficit reduce diabetes and non-alcoholic fatty liver disease risk (52). This observation of greater glycemic benefit was in parallel to greater beta-oxidation and mitochondrial mitophagy (i.e., BNIP3).

To date, we are aware of only one study in humans that has systematically tested the effect of combining metformin with exercise on hepatic glucose production (32). In this study, we showed that 12 weeks of metformin, exercise, or the combination of therapies maintained hepatic glucose production as measured by stable isotopes despite reductions in fasting plasma insulin. This highlights that all treatments improved hepatic insulin sensitivity in middle-aged adults with prediabetes. Thus, it would seem the liver is unlikely to explain glycemic variation post-exercise. Further work in humans is required to understand, nevertheless, how exercise and metformin interact to affect hepatic function given that fatty liver disease is prominent in people with obesity and T2D, and fatty liver disease plays a critical role in the development of CVD.

INFLUENCE OF METFORMIN AND EXERCISE ADIPOSE TISSUE INSULIN ACTION

Adipose tissue is the primary supplier of plasma free fatty acids (FFA). FFAs provide energy to working tissues, including skeletal muscle and liver primarily during fasting states. In response to mixed meals (i.e., carbohydrate, protein, and fat), insulin suppresses lipolysis due to a feedback loop with the pancreas (53), and lowers circulating FFA to enable insulin action on the peripheral for glycemic control. However, when adipose tissue becomes resistant to the action of insulin, FFA concentrations rise in circulation and play an important role in the development of insulin resistance (54). In fact, the release of FFAs from adipose tissue contributes, not only to declines in skeletal muscle and hepatic insulin sensitivity but also to endothelial dysfunction and reduced β-cell function in obesity, prediabetes, and T2D (55–57). The reason FFAs contribute to this multi-tissue insulin resistance is beyond the scope of this review, but likely relates to elevated plasma FFA concentrations being linked with reduced mitochondrial function and metabolic flexibility (58), Therefore, it would be reasonable to expect aerobic exercise interventions designed to improve oxidative capacity to not only protect against FFA-induced insulin resistance but also improve adipose insulin action.

Exercise confers several benefits to adipose tissue that include reductions in not only total fat mass but also visceral adiposity (59). A consequence of this improved body fat mass has been proposed to decrease circulating FFAs as well as inflammatory mediators referred to as adipokines. Indeed, we have shown that

changes in circulating FFAs following moderate intensity training are directly related to improved peripheral insulin sensitivity (32) and short-term interval or continuous exercise increases adipose insulin sensitivity in adults with prediabetes (19). While reductions in body fat following exercise training may be a key explanation for reducing circulating FFAs (60) in relation to improved peripheral insulin sensitivity and CVD risk reduction, fat loss is not required for improved adipocyte function. In fact, we recently showed that energy deficit, but not fat mass reduction, is important for improving adipokine profiles during caloric restriction (61). Moreover, Heiston et al. demonstrated that just 2 weeks of aerobic interval or continuous exercise increased adiponectin and lowered leptin prior to clinically meaningful weight loss or reductions in fat mass in older adults with prediabetes (62). Regardless, prior work (63) showed that hepatic insulin sensitivity was increased more following exercise training with a hypocaloric diet than when compared with a eucaloric diet during lipid-infusion. This suggests that in addition to exercise, calorie restriction may protect the liver from obesity-driven insulin resistance more so than training alone, despite comparable peripheral insulin sensitivity (64). Taken together, exercise, with or without caloric restriction, is an effective treatment for improving adipose tissue function.

Metformin is known to induce weight loss in adults with obesity, prediabetes, and T2D (65). Metformin reduces circulating FFA in part through inhibiting lipolysis (66). In fact, in murine adipocytes, metformin activated AMPK and blunted ANP as well as catecholamine-stimulated lipolysis (67, 68). Interestingly, elevated and/or blunted reductions in circulating FFAs have been reported after metformin plus exercise treatment during rest, exercise, or insulin-stimulated conditions compared to exercise alone (23, 31, 32, 69). While recent work suggests that oral metformin administration does not impact subcutaneous adipose tissue lipolysis during submaximal exercise in young lean men (70), it remains possible that in clinical populations alterations in either adipose lipolysis or reduced clearance as well as esterification may contribute to higher plasma FFAs. In either case, the elevated FFAs have been correlated attenuated gains in insulin sensitivity following metformin plus exercise therapy (23, 32). This may be clinically important as intrahepatic fat accumulation was lowered more after a diet and exercise than when lifestyle therapy was combined with metformin in obese adolescents (71). The blunted improvement in hepatic steatosis in these adolescents is consistent with the view that elevated FFAs from adipose tissue travel through the portal vein to the liver for increasing hepatic lipid storage. Collectively, this work highlights that adipose-derived metabolism may play a role in CVD risk following the co-prescription of metformin and exercise.

INFLUENCE OF METFORMIN AND EXERCISE VASCULATURE FUNCTION

Insulin promotes vasodilation in large conduit arteries and resistance arterioles as well as microvasculature perfusion (72). Conduit and resistance arteries are important for the delivery of nutrients and oxygen to metabolically active tissues, whereas the microvasculature provides a critical role in the exchange of these substances. In turn, adequate insulin-stimulated blood flow and endothelial function are essential for glucose regulation. However, during periods of physical inactivity and/or nutrient excess, hyperinsulinemia develops and has been related to elevated endothelin-1 (ET-1) mediated vasoconstriction. This impaired glucose delivery may not only increase risk for T2D but also contribute to endothelial dysfunction through lower nitric oxide bioavailability. Interestingly, people with insulin resistance have been noted to have normal fasting vascular function, but impaired conduit or microvascular insulin action (73). This demonstrates that mechanisms underlying disease states may be unique in the fasted vs. insulin-stimulated state.

Habitual physical activity elevated insulin-mediated skeletal muscle glucose disposal and limb blood flow (65, 74). The dose at which exercise impacts vascular insulin sensitivity, however, is less clear. Although recent work suggests that interval exercise improves flow-mediated dilation (FMD), which measures large conduit arteries, more than continuous exercise in sedentary people (11, 12, 75) not all studies agree (76). Interestingly, we recently studied the effect of interval vs. continuous exercise on fasting and post-prandial arterial stiffness as well as endothelial function as measured by FMD in older adults with prediabetes (77, 78). We found that 2 weeks of high intensity interval or moderate continuous exercise reduced post-prandial arterial stiffness but had no overall effect on fasting or post-prandial FMD. Nonetheless, when examination of responder compared with non-responder analysis was performed, it was shown that continuous exercise elicited a 57% response rate to raise FMD compared with only 42% with interval exercise (78). This latter finding is consistent with work showing that either a single bout or short-term exercise training at moderate continuous intensity can promote vasodilation after glucose-induced insulin stimulation in adults with and without T2D (79–82). Therefore, exercise appears to exert unique effects on the vasculature in fasted compared with fed (or insulin-stimulated) states based on the intensity at which exercise is performed in clinical populations. While these studies tested vascular function under a glucose load, no study to date has investigated the effect of lipid infusion on endothelial function before or during insulin-stimulation following training. However, aerobic fitness has been directly correlated with the preservation of insulin-stimulated microcirculatory function in healthy young adults (83). Moreover, in healthy inactive young adults, 12 weeks of interval exercise was shown to increase brachial artery conduit artery function more so than continuous training alone during a high fat meal (84). Together, fitness mediated mechanisms may be important for opposing FFA-induced vs. glucose-induced skeletal muscle vascular insulin resistance.

Metformin improves brachial artery FMD in people with type 1 diabetes (85) and polycystic ovarian syndrome (86). Moreover, metformin treatment for 4 weeks increases forearm blood flow and glucose uptake following a 75 g glucose load in people with T2D (87). Interestingly, this improvement in forearm blood flow corresponded with improved glucose tolerance and lower FFA levels, suggesting lower gluco-lipid toxicity may contribute to improved endothelial function. Given that

insulin-mediated glucose uptake is more closely associated with microvascular blood flow than total flow (88), it is important to understand the role of metformin on microvasculature function. To date, no data exist in humans studying the impact of metformin on microcirculatory function. Recently, Bradley et al. though showed that 2 weeks of metformin treatment improved microvascular responses during a euglycemic-hyperinsulinemic clamp in the muscle of high-fat fed rat (89). In particular, metformin lowered body weight and FFAs as well as improved insulin-stimulated muscle Akt phosphorylation, which confirms improved insulin signaling. Although there was no change in muscle AMPK phosphorylation, these findings suggest that metformin impacts nutrient exchange with skeletal muscle for glucose uptake. This is consistent with the notion that metformin increases eNOS phosphorylation in cultured endothelial cells (90). While work in human microvasculature insulin sensitivity awaits further investigation, metformin appears to have a direct effect on vasculature insulin action in skeletal muscle.

Traditionally, chronic exercise reduces CVD risk by decreasing blood pressure, triglycerides (TG), and inflammation (91). Metformin is not only used to treat T2D but also it is suggested to lower CVD risk (11). Indeed, the UK Prospective Diabetes Study (UKPDS) was a multi-center trial demonstrating that using pharmacological agents like metformin reduced HbA1c by ∼11% over 10 years as well as lowered microvasculature endpoints (e.g., retinal photocoagulation) (10, 92, 93). However, there are few data from randomized trials examining if metformin alters the vasculature adaptation to exercise. From our observations of blunted insulin sensitivity following the combined treatment (32), we studied the impact metformin would have on exercise-mediated improvements in CVD risk factors (i.e., blood pressure, inflammation, and blood lipids) (94). Interestingly, metformin or exercise training monotherapies lowered systolic blood pressure and C-reactive protein (CRP) by ∼7–8 and 20–25%, respectively, in people with prediabetes. When metformin and exercise were combined though, blunted reductions in systolic blood pressure and CRP were observed. These data were in line with others reporting that combining metformin with a low-fat diet and increase physical activity program had no further improvement in blood pressure (54). Furthermore, our observations were confirmed in obese insulin resistant adolescents whereby the metformin plus lifestyle modification blunted reductions in CRP as well as fibrinogen (71). Taken together, the metformin plus exercise therapy has strong clinical potential to oppose the reversal of chronic disease, including hypertension and metabolic syndrome. Further work is required for elucidating the vascularture mechanism(s) (e.g., FMD or angiogenesis) by which metformin interacts with exercise to lower or prevent CVD risk in people at risk for T2D.

METFORMIN AND EXERCISE ON BRAIN INSULIN SENSITIVITY

Insulin impacts the central nervous system by regulating hepatic glucose production, food intake and adipose metabolism, vasodilation/vasoconstriction of blood vessels as well as pancreatic insulin secretion and skeletal muscle insulin sensitivity (95, 96). Although these effects of insulin are clearly important for systemic glucose control, more recent work highlights that insulin also impacts memory, mood, and cognition (97, 98). Interestingly, Williams et al. (99) demonstrated direct effects of insulin on memory using intravenous insulin administration via a hyperinsulinemic-euglycemic clamp in 12 healthy older adults. In particular, this improvement in memory was related to increased blood oxygen level-dependent (BOLD) signaling as measured by functional MRI (fMRI) during the clamp (99). Furthermore, improved memory was best in those individuals with the highest systemic insulin sensitivity. This suggests that declines in insulin sensitivity may contribute to brain pathology in the hypothalamus (95). Not surprisingly, this may relate to cognitive decline (100), cerebral atrophy (101) as well as low brain blood flow and metabolism across aging (102). Additionally, this altered brain insulin action may be a key pathological factor in regulating glycemic control in individuals with obesity, T2D, aging, and Alzheimer's disease (103, 104).

During exercise brain glucose uptake declines in an intensity-based manner (105). This is likely the result of increased substrate availability (e.g., lactate) that allows glucose to be used by other tissues, such as skeletal muscle and red blood cells, for energy production. Conversely, aerobic interval exercise (4 x 4 min > 90% VO_2peak) for 3 d/wk combined with moderate intensity exercise (70% VO_2peak) for 2d/wk training has been demonstrated to increase basal glucose uptake in brain regions critical to cognitive function in young and older adults (106). Interestingly, the latter findings were observed in the parietal-temporal and caudate regions, which are linked to Alzheimer's disease. In either case, there remains limited data in humans with obesity or T2D confirming the effects of exercise on brain insulin sensitivity in relation to glucose metabolism. It was shown that lifestyle modification inducing weight loss, including increased physical activity and low-fat diet, increased brain insulin sensitivity in people with obesity as assessed by intranasal insulin spray (107). Moreover, Honkala et al. (108) demonstrated in sedentary middle-aged adults with insulin resistance sprint interval training for 2 weeks lowered insulin-stimulated glucose uptake in the temporal cortex, cingulate gyrus, cerebellum as well as global regions when compared with moderate continuous training. This intensity-based effect was observed despite both exercise intensities raising whole-body insulin sensitivity. This later finding of discordance with brain and periphery insulin action following high intensity exercise on tissue-specific glucose uptake, is consistent with the observation that people with increased brain glucose uptake in response to insulin have decreased insulin-stimulated skeletal muscle glucose disposal (108). Because exercise is known to increase skeletal muscle insulin sensitivity, it is paramount to understand the role exercise dose on affecting insulin-mediated brain glucose metabolism. Recently, wheel running in obese rats with T2D indicated that exercise was capable of improving insulin-stimulated posterior cerebral artery vasodilation in association with nitric oxide and reduced ET-1 signaling (109). Moreover, Ruegsegger et al. reported that

exercise improved brain insulin sensitivity of rodents fed a high-fat diet (110). The mechanism by which exercise increased brain insulin sensitivity appears related to increased ATP and reduced ROS generation by mitochondria. Additional work is warranted to understand this brain-skeletal muscle "cross-talk" in order to better understand glycemic control responses to exercise.

Metformin has been suggested as a potential treatment for cognitive impairment (111). Because metformin has been shown to promote peripheral insulin sensitivity, it would be reasonable to expect an impact on the brain. A recent pilot trial was conducted whereby metformin was administered in patients with Alzheimer's disease (112). It was reported that metformin was linked to improved learning, memory, and attention in individuals with mild cognitive impairment. The reason metformin may improve this cognitive function in humans remains to be elucidated, but work in high-fat-fed rodents suggests that increased brain insulin sensitivity, as well as cerebral and hippocampal mitochondrial function, may play a role (113). In addition, metformin is capable of crossing the blood-brain barrier and regulating tau phosphorylation in mouse models, thereby minimizing risk for Alzheimer's disease (114).

To date, no studies have examined how metformin in combination with exercise affects brain regulation of glycemic control. This may be important given the collective body of literature demonstrates that metformin attenuates skeletal muscle insulin sensitivity (23, 32, 54), and skeletal muscle is a key tissue proposed to secrete myokines that affect brain function and cognition (115). Further work in this area is warranted to provide an improved understanding of how exercise and/or metformin benefit not only glycemic control but also reduce T2D and dementia risk in aging adults.

CELLULAR MECHANISM BY WHICH METFORMIN IMPACTS EXERCISE ADAPTATION

Most agree that exercise or metformin therapy alone confer favorable effects on cellular pathways that regulate glycemic control across tissues for T2D and CVD risk reduction. The major concern at hand is the notion that $1 + 1 = 2$ when considering exercise and metformin for cardiometabolic health. It now appears clear that the mechanism(s) by which exercise and metformin act to affect health interact on some yet to be determined pathway(s) that influences adaptation.

Aerobic fitness (i.e., VO_2peak) is related to reduced risk for developing T2D independent of age and family diabetes history (76). Not surprisingly, elevations in VO_2peak have been implicated in metabolic adaptations (e.g., mitochondrial biogenesis, oxidative enzymes) that are strongly associated with elevated insulin sensitivity (91). A reason metformin could constrain gains in aerobic fitness relates to the observation that metformin partially inhibits Complex 1 of the mitochondrial electron transport system (116). In turn, we examined the impact metformin has on VO_2peak 10 weeks of exercise training in individuals with prediabetes (69). Exercise training alone significantly enhanced VO_2peak by nearly 20%, while metformin plus exercise only increased by ∼10%. This attenuated aerobic fitness adaptation has public health relevance since the combined treatment resulted in people exercising at a higher percentage of their post-training VO_2peak of roughly 5% and consequently, people reported a higher perception of effort (via the Borg Scale) (69). This observation is consistent with new work highlighting that even acute administration of metformin raised perceptions of effort during exercise (117). Interestingly, new work highlights in older adults that 12 weeks of metformin treatment blunted the improvement in aerobic fitness by ∼50% (33), which is consistent with our work in middle-aged adults with prediabetes (32). The implication of these findings is important as an increased perception of effort could lead to possibly a decrease in either long-term exercise adherence and/or changes in non-exercise physical activity behavior, thereby independently or collectively negatively influencing cardiometabolic health. However, it is worth acknowledging that not all studies confirm that metformin decreases VO_2peak. In fact, some have shown metformin to raise exercise tolerance in people with coronary artery disease (118).

A possible reason metformin interacts with exercise-mediated skeletal muscle adaptation relates to lowering mitochondrial ROS generation (119). We previously hypothesized that skeletal muscle contraction induced ROS generation is an important mediator of glucose and insulin metabolism adaptation, in part based on literature showing anti-oxidants blunt exercise health benefit (120). Newer literature supports this idea suggesting that blunting NADPH oxidase 2 (NOX2)-mediated ROS, which is responsible for GLUT-4 translocation, blunts glucose uptake during muscle contraction in both human and mouse models (121). But, because metformin counters ROS signaling (119) in muscle, it is possible that the post-exercise cellular signals important for mitochondrial capacity (e.g., PGC-1a), blood flow (e.g., nitric oxide mediated endothelial function), glucose uptake (GLUT-4 translocation), as well as brain glucose metabolism that contribute to multi-organ insulin sensitivity, are blunted. This hypothesis was somewhat supported by prior work, whereby Sharoff et al. showed that metformin blunted the rise in AMPK activity during the immediate post-exercise period in insulin resistant adults, and this skeletal muscle observation directly correlated with attenuated insulin sensitivity (23). However, new work suggests that acute metformin treatment for 4 days did not affect AMPK activity during exercise in skeletal muscle or adipose tissue of lean healthy men. However, a novel observation was that metformin concentrations were detected in skeletal muscle, and it was proposed that longer duration (e.g., 5 days vs. 12 weeks) may be needed to elicit change in AMPK and/or mitochondrial content (117). We recognize though that not all studies support the action of metformin to reduce complex I of the mitochondria and impact indirectly AMPK, and this is an area of much debate (122). Indeed, recent work highlights that metformin may impede both the malate-aspartate as well as the glycerol-phosphate shuttle, thereby together increasing the cytosolic NADH:NAD+ ratio and allosterically inhibiting energetic processes that would support tissue function (49). Interestingly, it was proposed that metformin may impact immune function in older adults following resistance training, and alleviate inflammatory mediated processes that may hinder muscle accretion in response to resistance exercise (34). This is consistent

with the notion that metformin promotes polarization from M1 pro-inflammatory macrophages to M2 anti-inflammatory macrophages (49) as well as induces autophagy to attenuate Th2 immune cell activation and inflammation (123). However, the results of the recent MASTERS trial showed no effect of metformin on resistance training-induced inflammation in skeletal muscle, despite the observation that lean body mass gains were blunted in relation to strength following the combined therapy compared with resistance exercise training alone. This was shown to parallel AMPK activation as well as inhibition of p70S6K1 phosphorylation (an immediate target of mTOR) (34). An additional or alternative explanation for the blunted muscle accretion post-training in the latter study may result from newer work showing that metformin reduces skeletal muscle autophagy and/or cell proliferation in C2C12 myotubes (124, 125), although data in humans following exercise training is unknown. Taken together, with possible influences of gastrointestinal adaptations with metformin of gut microbiota, bile acids, and/or GLP-1 (65), additional work is required to understand the exact cellular mechanisms by which metformin interacts with exercise across tissues for optimization of glycemic control. In fact, it is important to acknowledge that there are no suggestions for altered fasting glucose or liver insulin action in response to exercise plus metformin. Moreover, although elevated FFA levels have been detected following the combined therapy, no studies have been specifically designed to understand adipose insulin sensitivity following exercise plus metformin treatment. Nor has there been work examining the interaction of exercise and metformin on vasculature or brain insulin sensitivity to understand the importance of blood delivery and neural control of glucose metabolism. At this time, skeletal muscle appears to be a primary tissue regulating blood glucose, and additional cellular work is warranted to understand if these combined therapies lead to over-taxation of bioenergetic pathways that result in mal-adaptation. This may be particularly important since new work suggests that exercise may alter the pharmacokinetics and increase the bio-availability of metformin in circulation (126).

CLINICAL CONSIDERATIONS AND CONCLUSIONS

Developing precise exercise programs for maximal glycemic control remains to be identified. The collective literature suggests that, if anything, metformin attenuates the effects of exercise at improving insulin sensitivity at the level of skeletal muscle. Moreover, alterations in blood glucose, hypertension as well as inflammation have been noted. While no study to date has shown blood glucose to worsen as reflected by higher blood glucose concentrations relative to the start of the combined treatment, the literature highlights that there are either null, additive, or blunted effects on glycemia. The reason for this variability is not entirely clear but may relate to studies whereby people are habitual vs. naive metformin users or the outcome of interest. In either case, it is clear the magnitude of benefit will vary based on what tissue or outcome is of interest. Systemic studies determining the benefit of different exercise doses as well as risk factors of people (age, hypertension, dementia, T2D, etc.) co-prescribed metformin would enable individualized treatments that favor glycemic control. For instance, to date a basic biologic question is whether men or women respond differently to exercise plus metformin therapy based on underlying differences in aerobic fitness as well as muscle mass/fiber composition. Further, these gains in aerobic fitness and muscle mass are not only relevant to aging men and women with or without chronic disease, but also children and adolescents. It is well accepted that peak fitness/bone/muscle occur near the 3rd decade of life. But the effect of prescribing metformin with exercise in children and adolescents have on the rate of gain in these fitness outcomes is largely unknown in boys and girls. With emerging literature suggesting that off label or prophylactic use of metformin may be effective for weight management and obesity prevention in adolescents (54, 71, 127) more children may be provided metformin and recommended to exercise. This raises potential concern toward altered maturation growth rates and cardiometabolic risk during youth as well as then for later in life health risk compared with youth advised to exercise only with proper nutrition (54, 71). Thus, health care providers should be aware of these potential interactions to strike balance between current disease risk with long-term well-being. We also recognize that people are not often prescribed only one medication, and further work is warranted to tease out the effects of multiple pharmacological agents or even dietary supplements (e.g., metformin with GLP-1 agonists, SGLT-2 inhibitors, statins, antioxidants, etc.) in combination with exercise to gain a better understanding on glucose metabolism. However, it is important to acknowledge that recent work has suggested that other glycemic medications, including GLP-1 agonists and SGLT-2 inhibitors, have been shown to interact with exercise (128–130). This highlights the potential for medications to interfere or add with exercise-mediated glycemic benefit. Thus, there is potential for people to be at risk for developing T2D or cardiovascular abnormalities when co-prescribed treatments compared with those treated with exercise alone over time. Large-randomized clinical trials are critically needed to determine the effects combining exercise, with or without diet, and medications for improved evidenced-based practice.

AUTHOR CONTRIBUTIONS

SM wrote the majority of the review with NS providing edits. SM and NS collaborated on writing on the metformin and exercise on brain insulin sensitivity section. NS drafted the figure with SM providing edits.

FUNDING

SM was supported by National Institutes of Health RO1-HL130296.

ACKNOWLEDGMENTS

We would like to thank Emily M. Heiston, Udeyvir Cheema and Anna Ballanytne for helpful discussions related to topics herein.

REFERENCES

1. National Diabetes Statistics Report, 2020 | CDC. Available online at: https://www.cdc.gov/diabetes/library/features/diabetes-stat-report.html (accessed April 23, 2020)
2. Church TS, Blair SN, Cocreham S, Johannsen N, Johnson W, Kramer K, et al. Effects of aerobic and resistance training on hemoglobin A1c levels in patients with type 2 diabetes: a randomized controlled trial. *J Am Med Assoc.* (2010) 304:2253–62. doi: 10.1001/jama.2010.1710
3. Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hamäläinen H, Ianne-Parikka P, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med.* (2001) 344:1343–50. doi: 10.1056/NEJM200105033441801
4. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med.* (2002) 346:393–403. doi: 10.1056/NEJMoa012512
5. Houmard JA, Tanner CJ, Slentz CA, Duscha BD, McCartney JS, Kraus WE. Effect of the volume and intensity of exercise training on insulin sensitivity. *J Appl Physiol.* (2004) 96:101–6. doi: 10.1152/japplphysiol.00707.2003
6. Dubé JJ, Allison KF, Rousson V, Goodpaster BH, Amati F. Exercise dose and insulin sensitivity: relevance for diabetes prevention. *Med Sci Sports Exerc.* (2012) 44:793–9. doi: 10.1249/MSS.0b013e31823f679f
7. Malin SK, Solomon TPJ, Blaszczak A, Finnegan S, Filion J, Kirwan JP. Pancreatic β-cell function increases in a linear dose-response manner following exercise training in adults with prediabetes. *Am J Physiol Endocrinol Metab.* (2013) 305:E1248–54. doi: 10.1152/ajpendo.00260.2013
8. Regensteiner JG, Sippel J, McFarling ET, Wolfel EE, Hiatt WR. Effects of non-insulin-dependent diabetes on oxygen consumption during treadmill exercise. *Med Sci Sports Exerc.* (1995) 27:875–81. doi: 10.1249/00005768-199506000-00012
9. Wahl MP, Scalzo RL, Regensteiner JG, Reusch JEB. Mechanisms of aerobic exercise impairment in diabetes: a narrative review. *Front Endocrinol.* (2018) 9:181. doi: 10.3389/fendo.2018.00181
10. American Diabetes Association. 9. Pharmacologic approaches to glycemic treatment: Standards of medical care in diabetes 2019. *Diabetes Care.* (2019) 42:S90–102. doi: 10.2337/dc19-S009
11. Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. *Clin Sci.* (2012) 122:253–70. doi: 10.1042/CS20110386
12. Pollak M. Metformin and pancreatic cancer: a clue requiring investigation. *Clin Cancer Res.* (2012) 18:2723–5. doi: 10.1158/1078-0432.CCR-12-0694
13. Novelle MG, Ali A, Diéguez C, Bernier M, de Cabo R. Metformin: a hopeful promise in aging research. *Cold Spring Harb Perspect Med.* (2016) 6:a025932. doi: 10.1101/cshperspect.a025932
14. Jenkins NT, Padilla J, Arce-Esquivel AA, Bayless DS, Martin JS, Leidy HJ, et al. Effects of endurance exercise training, metformin, and their combination on Adipose tissue leptin and IL-10 secretion in OLETF rats. *J Appl Physiol.* (2012) 113:1873–83. doi: 10.1152/japplphysiol.00936.2012
15. Baron AD. Hemodynamic actions of insulin. *Am J Physiol Endocrinol Metab.* (1994) 267:E187–202. doi: 10.1152/ajpendo.1994.267.2.e187
16. Ahima RS, Antwi DA. Brain regulation of appetite and satiety. *Endocrinol Metab Clin North Am.* (2008) 37:811–23. doi: 10.1016/j.ecl.2008.08.005
17. Ryan D, Espeland M, Foster G, Haffner S, Hubbard V, Johnson K, et al. Look AHEAD (Action for Health in Diabetes): design and methods for a clinical trial of weight loss for the prevention of cardiovascular disease in type 2 diabetes. *Control Clin Trials.* (2003) 24:610–28. doi: 10.1016/S0197-2456(03)00064-3
18. Solomon TPJ, Eves FF, Laye MJ. Targeting postprandial hyperglycemia with physical activity may reduce cardiovascular disease risk. But what should we do, and when is the right time to move? *Front Cardiovasc Med.* (2018) 5:99. doi: 10.3389/fcvm.2018.00099
19. Gilbertson NM, Eichner NZM, Francois M, Gaitan JM, Heiston EM, Weltman A, et al. Glucose tolerance is linked to postprandial fuel use independent of exercise dose. *Med Sci Sports Exerc.* (2018) 50:2058–66. doi: 10.1249/MSS.0000000000001667
20. Slentz CA, Bateman LA, Willis LH, Granville EO, Piner LW, Samsa GP, et al. Effects of exercise training alone vs a combined exercise and nutritional lifestyle intervention on glucose homeostasis in prediabetic individuals: a randomised controlled trial. *Diabetologia.* (2016) 59:2088–98. doi: 10.1007/s00125-016-4051-z
21. Mikus CR, Boyle LJ, Borengasser SJ, Oberlin DJ, Naples SP, Fletcher J, et al. Simvastatin impairs exercise training adaptations. *J Am Coll Cardiol.* (2013) 62:709–14. doi: 10.1016/j.jacc.2013.02.074
22. Lehtovirta M, Forsén B, Gullström M, Häggblom M, Eriksson JG, Taskinen MR, et al. Metabolic effects of metformin in patients with impaired glucose tolerance. *Diabet Med.* (2001) 18:578–83. doi: 10.1046/j.1464-5491.2001.00539.x
23. Sharoff CG, Hagobian TA, Malin SK, Chipkin SR, Yu H, Hirshman MF, et al. Combining short-term metformin treatment and one bout of exercise does not increase insulin action in insulin-resistant individuals. *Am J Physiol Endocrinol Metab.* (2010) 298:E815. doi: 10.1152/ajpendo.00517.2009
24. Ramachandran A, Snehalatha C, Mary S, Mukesh B, Bhaskar AD, Vijay V. The Indian Diabetes Prevention Programme shows that lifestyle modification and metformin prevent type 2 diabetes in Asian Indian subjects with impaired glucose tolerance (IDPP-1). *Diabetologia.* (2006) 49:289–97. doi: 10.1007/s00125-005-0097-z
25. Love-Osborne K, Sheeder J, Zeitler P. Addition of metformin to a lifestyle modification program in adolescents with insulin resistance. *J Pediatr.* (2008) 152:817–22. doi: 10.1016/j.jpeds.2008.01.018
26. Erickson ML, Little JP, Gay JL, McCully KK, Jenkins NT. Postmeal exercise blunts postprandial glucose excursions in people on metformin monotherapy. *J Appl Physiol.* (2017) 123:444–50. doi: 10.1152/japplphysiol.00213.2017
27. Ortega JF, Hamouti N, Fernández-Elías VE, de Prada MVG, Martínez-Vizcaíno V, Mora-Rodríguez R. Metformin does not attenuate the acute insulin-sensitizing effect of a single bout of exercise in individuals with insulin resistance. *Acta Diabetol.* (2014) 51:749–55. doi: 10.1007/s00592-014-0580-4
28. Huang T, Lu C, Schumann M, Le S, Yang Y, Zhuang H, et al. Timing of exercise affects glycemic control in type 2 diabetes patients treated with metformin. *J Diabetes Res.* (2018) 2018:2483273. doi: 10.1155/2018/2483273
29. Boulé NG, Kenny GP, Larose J, Khandwala F, Kuzik N, Sigal RJ. Does metformin modify the effect on glycaemic control of aerobic exercise, resistance exercise or both? *Diabetologia.* (2013) 56:2378–82. doi: 10.1007/s00125-013-3026-6
30. Terada T, Boulé NG. Does metformin therapy influence the effects of intensive lifestyle intervention? Exploring the interaction between first line therapies in the Look AHEAD trial. *Metabolism.* (2019) 94:39–46. doi: 10.1016/j.metabol.2019.01.004
31. Boulé NG, Robert C, Bell GJ, Johnson ST, Bell RC, Lewanczuk RZ, et al. Metformin and exercise in type 2 diabetes. *Diabetes Care.* (2011) 34:1469–74. doi: 10.2337/DC10-2207
32. Malin SK, Gerber R, Chipkin SR, Braun B. Independent and combined effects of exercise training and metformin on insulin sensitivity in individuals with prediabetes. *Diabetes Care.* (2012) 35:131–6. doi: 10.2337/dc11-0925
33. Konopka AR, Laurin JL, Schoenberg HM, Reid JJ, Castor WM, Wolff CA, et al. Metformin inhibits mitochondrial adaptations to aerobic exercise training in older adults. *Aging Cell.* (2019) 18:e12880. doi: 10.1111/acel.12880
34. Walton RG, Dungan CM, Long DE, Tuggle SC, Kosmac K, Peck BD, et al. Metformin blunts muscle hypertrophy in response to progressive resistance exercise training in older adults: a randomized, double-blind, placebo-controlled, multicenter trial: the MASTERS trial. *Aging Cell.* (2019) 18:e13039. doi: 10.1111/acel.13039
35. Hawley JA, Lessard SJ. Exercise training-induced improvements in insulin action. *Acta Physiol.* (2008) 192:127–35. doi: 10.1111/j.1748-1716.2007.01783.x
36. Holloszy JO. Invited review: exercise-induced increase in muscle insulin sensitivity. *J Appl Physiol.* (2005) 99:338–43. doi: 10.1152/japplphysiol.00123.2005
37. Stumvoll M, Häring HU, Matthaei S. Metformin. *Endocr Res.* (2006) 32:39–57. doi: 10.1080/07435800701743828
38. Wang C, Liu F, Yuan Y, Wu J, Wang H, Zhang L, et al. Metformin suppresses lipid accumulation in skeletal muscle by promoting fatty

acid oxidation. *Clin Lab.* (2014) 60:887–96. doi: 10.7754/Clin.Lab.2013.130531
39. Zabielski P, Chacinska M, Charkiewicz K, Baranowski M, Gorski J, Blachnio-Zabielska AU. Effect of metformin on bioactive lipid metabolism in insulin-resistant muscle. *J Endocrinol.* (2017) 233:329–40. doi: 10.1530/JOE-16-0381
40. Malin SK, Stephens BR, Sharoff CG, Hagobian TA, Chipkin SR, Braun B. Metformin's effect on exercise and postexercise substrate oxidation. *Int J Sport Nutr Exerc Metab.* (2010) 20:63–71. doi: 10.1123/ijsnem.20.1.63
41. Linden MA, Fletcher JA, Matthew Morris E, Meers GM, Kearney ML, Crissey JM, et al. Combining metformin and aerobic exercise training in the treatment of type 2 diabetes and NAFLD in OLETF rats. *Am J Physiol Endocrinol Metab.* (2014) 306:E300–10. doi: 10.1152/ajpendo.00427.2013
42. Basu R, Chandramouli V, Dicke B, Landau B, Rizza R. Obesity and type 2 diabetes impair insulin-induced suppression of glycogenolysis as well as gluconeogenesis. *Diabetes.* (2005) 54:1942–8. doi: 10.2337/diabetes.54.7.1942
43. Winnick JJ, Sherman WM, Habash DL, Stout MB, Failla ML, Belury MA, et al. Short-term aerobic exercise training in obese humans with type 2 diabetes mellitus improves whole-body insulin sensitivity through gains in peripheral, not hepatic insulin sensitivity. *J Clin Endocrinol Metab.* (2008) 93:771–8. doi: 10.1210/jc.2007-1524
44. Devlin JT, Hirshman M, Horton ED, Horton ES. Enhanced peripheral and splanchnic insulin sensitivity in NIDDM men after single bout of exercise. *Diabetes.* (1987) 36:434–9. doi: 10.2337/diab.36.4.434
45. Kirwan JP, Solomon TPJ, Wojta DM, Staten MA, Holloszy JO. Effects of 7 days of exercise training on insulin sensitivity and responsiveness in type 2 diabetes mellitus. *Am J Physiol Endocrinol Metab.* (2009) 297:E151–6. doi: 10.1152/ajpendo.00210.2009
46. Malin SK, Del Rincon JP, Huang H, Kirwan JP. Exercise-induced lowering of fetuin-A may increase hepatic insulin sensitivity. *Med Sci Sports Exerc.* (2014) 46:2085–90. doi: 10.1249/MSS.0000000000000338
47. Black SE, Mitchell E, Freedson PS, Chipkin SR, Braun B. Improved insulin action following short-term exercise training: role of energy and carbohydrate balance. *J Appl Physiol.* (2005) 99:2285–93. doi: 10.1152/japplphysiol.00291.2005
48. Camacho RC, Donahue EP, James FD, Berglund ED, Wasserman DH. Energy state of the liver during short-term and exhaustive exercise in C57BL/6J mice. *Am J Physiol Endocrinol Metab.* (2006) 290:E405–8. doi: 10.1152/ajpendo.00385.2005
49. Foretz M, Guigas B, Viollet B. Understanding the glucoregulatory mechanisms of metformin in type 2 diabetes mellitus. *Nat Rev Endocrinol.* (2019) 15:569–89. doi: 10.1038/s41574-019-0242-2
50. Miller RA, Chu Q, Xie J, Foretz M, Viollet B, Birnbaum MJ. Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. *Nature.* (2013) 494:256–60. doi: 10.1038/nature11808
51. Mazza A, Fruci B, Garinis GA, Giuliano S, Malaguarnera R, Belfiore A. The role of metformin in the management of NAFLD. *Exp Diabetes Res.* (2012) 2012:716404. doi: 10.1155/2012/716404
52. Linden MA, Lopez KT, Fletcher JA, Morris EM, Meers GM, Siddique S, et al. Combining metformin therapy with caloric restriction for the management of type 2 diabetes and nonalcoholic fatty liver disease in obese rats. *Appl Physiol Nutr Metab.* (2015) 40:1038–47. doi: 10.1139/apnm-2015-0236
53. Jensen MD, Nielsen S. Insulin dose response analysis of free fatty acid kinetics. *Metabolism.* (2007) 56:68–76. doi: 10.1016/j.metabol.2006.08.022
54. Clarson CL, Mahmud FH, Baker JE, Clark HE, Mckay WM, Schauteet VD, et al. Metformin in combination with structured lifestyle intervention improved body mass index in obese adolescents, but did not improve insulin resistance. *Endocrine.* (2009) 36:141–6. doi: 10.1007/s12020-009-9196-9
55. Denis McGarry J. Dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes.* (2002) 51:7–18. doi: 10.2337/diabetes.51.1.7
56. Cusi K, Kashyap S, Gastaldelli A, Bajaj M, Cersosimo E. Effects on insulin secretion and insulin action of a 48-h reduction of plasma free fatty acids with acipimox in nondiabetic subjects genetically predisposed to type 2 diabetes. *Am J Physiol Endocrinol Metab.* (2007) 292:E1775–81. doi: 10.1152/ajpendo.00624.2006
57. Tönjes A, Fasshauer M, Kratzsch J, Stumvoll M, Bluher M. Adipokine pattern in subjects with impaired fasting glucose and impaired glucose tolerance in comparison to normal glucose tolerance and diabetes. *PLoS ONE.* (2010) 5:e13911. doi: 10.1371/journal.pone.0013911
58. van de Weijer T, Sparks LM, Phielix E, Meex RC, van Herpen NA, Hesselink MKC, et al. Relationships between mitochondrial function and metabolic flexibility in type 2 diabetes mellitus. *PLoS ONE.* (2013) 8:e51648. doi: 10.1371/journal.pone.0051648
59. Brennan AM, Day AG, Cowan TE, Clarke GJ, Lamarche B, Ross R. Individual response to standardized exercise: total and abdominal adipose tissue. *Med. Sci. Sports Exerc.* (2019) 52:490–7 doi: 10.1249/MSS.0000000000001930
60. Solomon TPJ, Sistrun SN, Krishnan RK, Del Aguila LF, Marchetti CM, O'Carroll SM, et al. Exercise and diet enhance fat oxidation and reduce insulin resistance in older obese adults. *J Appl Physiol.* (2008) 104:1313–9. doi: 10.1152/japplphysiol.00890.2007
61. Gilbertson NM, Eichner NZM, Heiston EM, Gaitán JM, Francois ME, Mehaffey JH, et al. A low-calorie diet with or without interval exercise training improves adiposopathy in obese women. *Appl Physiol Nutr Metab.* (2019) 44:1057–64. doi: 10.1139/apnm-2018-0717
62. Heiston EM, Eichner NZ, Gilbertson NM, Malin SK. Exercise improves adiposopathy, insulin sensitivity and metabolic syndrome severity independent of intensity. *Exp Physiol.* (2020) 105:632–40. doi: 10.1113/EP088158
63. Haus JM, Solomon TPJ, Marchetti CM, Edmison JM, González F, Kirwan JP. Free fatty acid-induced hepatic insulin resistance is attenuated following lifestyle intervention in obese individuals with impaired glucose tolerance. *J Clin Endocrinol Metab.* (2010) 95:323–7. doi: 10.1210/jc.2009-1101
64. Solomon TPJ, Haus JM, Marchetti CM, Stanley WC, Kirwan JP. Effects of exercise training and diet on lipid kinetics during free fatty acid-induced insulin resistance in older obese humans with impaired glucose tolerance. *Am J Physiol Metab.* (2009) 297:E552–9. doi: 10.1152/ajpendo.00220.2009
65. Malin SK, Kashyap SR. Effects of metformin on weight loss: potential mechanisms. *Curr Opin Endocrinol Diabetes Obes.* (2014) 21:323–9. doi: 10.1097/MED.0000000000000095
66. Flechtner-Mors M, Ditschuneit HH, Jenkinson CP, Alt A, Adler G. Metformin inhibits catecholamine-stimulated lipolysis in obese, hyperinsulinemic, hypertensive subjects in subcutaneous adipose tissue: an *in situ* microdialysis study. *Diabet Med.* (1999) 16:1000–6. doi: 10.1046/j.1464-5491.1999.00189.x
67. Zhang T, He J, Xu C, Zu L, Jiang H, Pu S, et al. Mechanisms of metformin inhibiting lipolytic response to isoproterenol in primary rat adipocytes. *J Mol Endocrinol.* (2009) 42:57–66. doi: 10.1677/JME-08-0130
68. Bourron O, Daval M, Hainault I, Hajduch E, Servant JM, Gautier JF, et al. Biguanides and thiazolidinediones inhibit stimulated lipolysis in human adipocytes through activation of AMP-activated protein kinase. *Diabetologia.* (2010) 53:768–78. doi: 10.1007/s00125-009-1639-6
69. Malin SK, Braun B. Effect of metformin on substrate utilization after exercise training in adults with impaired glucose tolerance. *Appl Physiol Nutr Metab.* (2013) 38:427–30. doi: 10.1139/apnm-2012-0433
70. Krauzová E, Tuma P, de Glisezinski I, Štich V, Šiklová M. Metformin does not inhibit exercise-induced lipolysis in adipose tissue in young healthy lean men. *Front Physiol.* (2018) 9:604. doi: 10.3389/fphys.2018.00604
71. Mauras N, DelGiorno C, Hossain J, Bird K, Killen K, Merinbaum D, et al. Metformin use in children with obesity and normal glucose tolerance-effects on cardiovascular markers and intrahepatic fat. *J Pediatr Endocrinol Metab.* (2012) 25:33–40. doi: 10.1515/jpem-2011-0450
72. Barrett EJ, Wang H, Upchurch CT, Liu Z. Insulin regulates its own delivery to skeletal muscle by feed-forward actions on the vasculature. *Am J Physiol Endocrinol Metab.* (2011) 301:E252. doi: 10.1152/ajpendo.00186.2011
73. Jahn LA, Hartline L, Rao N, Logan B, Kim JJ, Aylor K, et al. Insulin enhances endothelial function throughout the arterial tree in healthy but not metabolic syndrome subjects. *J Clin Endocrinol Metab.* (2016) 101:1198–206. doi: 10.1210/jc.2015-3293
74. De Filippis E, Cusi K, Ocampo G, Berria R, Buck S, Consoli A, et al. Exercise-induced improvement in vasodilatory function accompanies increased insulin sensitivity in obesity and type 2 diabetes mellitus. *J Clin Endocrinol Metab.* (2006) 91:4903–10. doi: 10.1210/jc.2006-1142
75. Paulsen G, Cumming KT, Holden G, Hallén J, Rønnestad BR, Sveen O, et al. Vitamin C and E supplementation hampers cellular adaptation to endurance training in humans: a double-blind, randomised, controlled trial. *J Physiol.* (2014) 592:1887–901. doi: 10.1113/jphysiol.2013.267419

76. Wei M, Gibbons LW, Mitchell TL, Kampert JB, Lee CD, Blair SN. The association between cardiorespiratory fitness and impaired fasting glucose and type 2 diabetes mellitus in men. *Ann Intern Med.* (1999) 130:89–96. doi: 10.7326/0003-4819-130-2-199901190-00002
77. Eichner NZM, Gaitán JM, Gilbertson NM, Khurshid M, Weltman A, Malin SK. Postprandial augmentation index is reduced in adults with prediabetes following continuous and interval exercise training. *Exp Physiol.* (2019) 104:264–71. doi: 10.1113/EP087305
78. Malin SK, Gilbertson NM, Eichner NZM, Heiston E, Miller S, Weltman A. Impact of short-term continuous and interval exercise training on endothelial function and glucose metabolism in prediabetes. *J Diabetes Res.* (2019) 2019:4912174. doi: 10.1155/2019/4912174
79. Weiss EP, Arif H, Villareal DT, Marzetti E, Holloszy JO. Endothelial function after high-sugar food ingestion is improved by endurance exercise performed on the previous day. *Am J Clin Nutr.* (2008) 88:51–7. doi: 10.1093/ajcn/88.1.51
80. Fujita S, Rasmussen BB, Cadenas JG, Drummond MJ, Glynn EL, Sattler FR, et al. Aerobic exercise overcomes the age-related insulin resistance of muscle protein metabolism by improving endothelial function and Akt/mammalian target of rapamycin signaling. *Diabetes.* (2007) 56:1615–22. doi: 10.2337/db06-1566
81. Zhu W, Zhong C, Yu Y, Li K. Acute effects of hyperglycaemia with and without exercise on endothelial function in healthy young men. *Eur J Appl Physiol.* (2007) 99:585–91. doi: 10.1007/s00421-006-0378-3
82. Mikus CR, Fairfax ST, Libla JL, Boyle LJ, Vianna LC, Oberlin DJ, et al. Seven days of aerobic exercise training improves conduit artery blood flow following glucose ingestion in patients with type 2 diabetes. *J Appl Physiol.* (2011) 111:657–64. doi: 10.1152/japplphysiol.00489.2011
83. Eggleston EM, Jahn LA, Barrett EJ. Hyperinsulinemia rapidly increases human muscle microvascular perfusion but fails to increase muscle insulin clearance: evidence that a saturable process mediates muscle insulin uptake. *Diabetes.* (2007) 56:2958–63. doi: 10.2337/db07-0670
84. Ramírez-Vélez R, Correa-Rodríguez M, Tordecilla-Sanders A, Aya-Aldana V, Izquierdo M, Correa-Bautista JE, et al. Exercise and postprandial lipemia: effects on vascular health in inactive adults. *Lipids Health Dis.* (2018) 17:69. doi: 10.1186/s12944-018-0719-3
85. Pitocco D, Zaccardi F, Tarzia P, Milo M, Scavone G, Rizzo P, et al. Metformin improves endothelial function in type 1 diabetic subjects: a pilot, placebo-controlled randomized study. *Diabetes, Obes Metab.* (2013) 15:427–31. doi: 10.1111/dom.12041
86. Jensterle M, Weber M, Pfeifer M, Prezelj J, Pfutzner A, Janez A. Assessment of insulin resistance in young women with polycystic ovary syndrome. *Int J Gynecol Obstet.* (2008) 102:137–40. doi: 10.1016/j.ijgo.2008.03.017
87. Magalhães FO, Gouveia LMB, Torquato MTCG, Paccola GMGF, Piccinato CE, Foss MC. Metformin increases blood flow and forearm glucose uptake in a group of non-obese type 2 diabetes patients. *Horm Metab Res.* (2006) 38:513–7. doi: 10.1055/s-2006-949522
88. Vincent MA, Clerk LH, Lindner JR, Klibanov AL, Clark MG, Rattigan S, et al. Microvascular recruitment is an early insulin effect that regulates skeletal muscle glucose uptake *in vivo*. *Diabetes.* (2004) 53:1418–23. doi: 10.2337/diabetes.53.6.1418
89. Bradley EA, Premilovac D, Betik AC, Hu D, Attrill E, Richards SM, et al. Metformin improves vascular and metabolic insulin action in insulin-resistant muscle. *J Endocrinol.* (2019) 243:85–96. doi: 10.1530/JOE-19-0067
90. An H, Wei R, Ke J, Yang J, Liu Y, Wang X, et al. Metformin attenuates fluctuating glucose-induced endothelial dysfunction through enhancing GTPCH1-mediated eNOS recoupling and inhibiting NADPH oxidase. *J Diabetes Complic.* (2016) 30:1017–24. doi: 10.1016/j.jdiacomp.2016.04.018
91. Kraus WE, Slentz CA. Exercise training, lipid regulation, and insulin action: a tangled web of cause and effect. *Obesity.* (2009) 17:S21–6. doi: 10.1038/oby.2009.384
92. King P, Peacock I, Donnelly R. The UK Prospective Diabetes Study (UKPDS): clinical and therapeutic implications for type 2 diabetes. *Br J Clin Pharmacol.* (1999) 48:643–8. doi: 10.1046/j.1365-2125.1999.00092.x
93. Wilding JPH. The importance of weight management in type 2 diabetes mellitus. *Int J Clin Pract.* (2014) 68:682–91. doi: 10.1111/ijcp.12384
94. Malin SK, Nightingale J, Choi S-E, Chipkin SR, Braun B. Metformin modifies the exercise training effects on risk factors for cardiovascular disease in impaired glucose tolerant adults. *Obesity.* (2013) 21:93–100. doi: 10.1002/oby.20235
95. Cetinkalp S, Simsir I, Ertek S. Insulin resistance in brain and possible therapeutic approaches. *Curr Vasc Pharmacol.* (2014) 12:553–64. doi: 10.2174/15701611129991402061304 26
96. Kullmann S, Heni M, Hallschmid M, Fritsche A, Preissl H, Häring HU. Brain insulin resistance at the crossroads of metabolic and cognitive disorders in humans. *Physiol Rev.* (2016) 96:1169–209. doi: 10.1152/physrev.00032.2015
97. Benedict C, Hallschmid M, Hatke A, Schultes B, Fehm HL, Born J, et al. Intranasal insulin improves memory in humans. *Psychoneuroendocrinology.* (2004) 29:1326–34. doi: 10.1016/j.psyneuen.2004.04.003
98. Malin SK, Kirwan JP. Fasting hyperglycaemia blunts the reversal of impaired glucose tolerance after exercise training in obese older adults. *Diabetes, Obes Metab.* (2012) 14:835–41. doi: 10.1111/j.1463-1326.2012.01608.x
99. Williams VJ, Trombetta BA, Jafri RZ, Koenig AM, Wennick CD, Carlyle BC, et al. Task-related fMRI BOLD response to hyperinsulinemia in healthy older adults. *JCI Insight.* (2019) 4:e129700. doi: 10.1172/jci.insight.129700
100. Burns JM, Honea RA, Vidoni ED, Hutfles LJ, Brooks WM, Swerdlow RH. Insulin is differentially related to cognitive decline and atrophy in Alzheimer's disease and aging. *Biochim Biophys Acta.* (2012) 1822:333–9. doi: 10.1016/j.bbadis.2011.06.011
101. Willette AA, Xu G, Johnson SC, Birdsill AC, Jonaitis EM, Sager MA, et al. Insulin resistance, brain atrophy, and cognitive performance in late middle-aged adults. *Diabetes Care.* (2013) 36:443–9. doi: 10.2337/dc12-0922
102. Willette AA, Modanlo N, Kapogiannis D. Insulin resistance predicts medial temporal hypermetabolism in mild cognitive impairment conversion to Alzheimer disease. *Diabetes.* (2015) 64:1933–40. doi: 10.2337/db14-1507
103. Chiti F, Dobson CM. Protein misfolding, amyloid formation, and human disease: a summary of progress over the last decade. *Annu Rev Biochem.* (2017) 86:27–68. doi: 10.1146/annurev-biochem-061516-045115
104. Arnold SE, Arvanitakis Z, Macauley-Rambach SL, Koenig AM, Wang HY, Ahima RS, et al. Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums. *Nat Rev Neurol.* (2018) 14:168–81. doi: 10.1038/nrneurol.2017.185
105. Kemppainen J, Aalto S, Fujimoto T, Kalliokoski KK, Långsjö J, Oikonen V, et al. High intensity exercise decreases global brain glucose uptake in humans. *J Physiol.* (2005) 568:323–32. doi: 10.1113/jphysiol.2005.091355
106. Robinson MM, Lowe VJ, Nair KS. Increased brain glucose uptake after 12 weeks of aerobic high-intensity interval training in young and older adults. *J Clin Endocrinol Metab.* (2018) 103:221–7. doi: 10.1210/jc.2017-01571
107. Kullmann S, Valenta V, Wagner R, Tschritter O, Machann J, Häring H-U, et al. Brain insulin sensitivity is linked to adiposity and body fat distribution. *Nat Commun.* (2020) 11:1841. doi: 10.1038/s41467-020-15686-y
108. Honkala SM, Johansson J, Motiani KK, Eskelinen JJ, Virtanen KA, Löyttyniemi E, et al. Short-term interval training alters brain glucose metabolism in subjects with insulin resistance. *J Cereb Blood Flow Metab.* (2018) 38:1828–38. doi: 10.1177/0271678X17734998
109. Olver TD, Mcdonald MW, Klakotskaia D, Richardson RA, Jasperse JL, Melling CWJ, et al. A chronic physical activity treatment in obese rats normalizes the contributions of ET-1 and NO to insulin-mediated posterior cerebral artery vasodilation. *J Appl Physiol.* (2017) 122:1040–50. doi: 10.1152/japplphysiol.00811.2016
110. Ruegsegger GN, Creo AL, Cortes TM, Dasari S, Nair KS. Altered mitochondrial function in insulin-deficient and insulin-resistant states. *J Clin Invest.* (2018) 128:3671–81. doi: 10.1172/JCI120843
111. Yarchoan M, Arnold SE. Repurposing diabetes drugs for brain insulin resistance in Alzheimer disease. *Diabetes.* (2014) 63:2253–61. doi: 10.2337/db14-0287
112. Koenig AM, Mechanic-Hamilton D, Xie SX, Combs MF, Cappola AR, Xie L, et al. Effects of the insulin sensitizer metformin in alzheimer disease: pilot data from a randomized placebo-controlled crossover study. *Alzheimer Dis Assoc Disord.* (2017) 31:107–13. doi: 10.1097/WAD.0000000000000202
113. Ruegsegger GN, Vanderboom PM, Dasari S, Klaus KA, Kabiraj P, McCarthy CB, et al. Exercise and metformin counteract altered mitochondrial function in the insulin resistant brain. *JCI Insight.* (2019) 4:e130681. doi: 10.1172/jci.insight.130681

114. Kickstein E, Krauss S, Thornhill P, Rutschow D, Zeller R, Sharkey J, et al. Biguanide metformin acts on tau phosphorylation via mTOR/protein phosphatase 2A (PP2A) signaling. *Proc Natl Acad Sci USA*. (2010) 107:21830–5. doi: 10.1073/pnas.0912793107
115. Wrann CD, White JP, Salogiannnis J, Laznik-Bogoslavski D, Wu J, Ma D, et al. Exercise induces hippocampal BDNF through a PGC-1α/FNDC5 pathway. *Cell Metab*. (2013) 18:649–59. doi: 10.1016/j.cmet.2013.09.008
116. Brunmair B, Staniek K, Gras F, Scharf N, Althaym A, Clara R, et al. Thiazolidinediones, like metformin, inhibit respiratory complex I: a common mechanism contributing to their antidiabetic actions? *Diabetes*. (2004) 53:1052–9. doi: 10.2337/diabetes.53.4.1052
117. Kristensen JM, Lillelund C, Kjøbsted R, Birk JB, Andersen NR, Nybo L, et al. Metformin does not compromise energy status in human skeletal muscle at rest or during acute exercise: a randomised, crossover trial. *Physiol Rep*. (2019) 7:e14307. doi: 10.14814/phy2.14307
118. Jadhav S, Ferrell W, Greer IA, Petrie JR, Cobbe SM, Sattar N. Effects of metformin on microvascular function and exercise tolerance in women with angina and normal coronary arteries. A Randomized, Double-Blind, Placebo-Controlled Study. *J Am Coll Cardiol*. (2006) 48:956–63. doi: 10.1016/j.jacc.2006.04.088
119. Kane DA, Anderson EJ, Price JW, Woodlief TL, Lin C, Te Bikman BT, et al. Metformin selectively attenuates mitochondrial H2O2 emission without affecting respiratory capacity in skeletal muscle of obese rats. *Free Radic Biol Med*. (2010) 49:1082–7. doi: 10.1016/j.freeradbiomed.2010.06.022
120. Gliemann L, Schmidt JF, Olesen J, Biensø RS, Peronard SL, Grandjean SU, et al. Resveratrol blunts the positive effects of exercise training on cardiovascular health in aged men. *J Physiol*. (2013) 591:5047–59. doi: 10.1113/jphysiol.2013.258061
121. Henríquez-Olguin C, Knudsen JR, Raun SH, Li Z, Dalbram E, Treebak JT, et al. Cytosolic ROS production by NADPH oxidase 2 regulates muscle glucose uptake during exercise. *Nat Commun*. (2019) 10:4623. doi: 10.1038/s41467-019-12523-9
122. Foretz M, Hébrard S, Leclerc J, Zarrinpashneh E, Soty M, Mithieux G, et al. Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. *J Clin Invest*. (2010) 120:2355–69. doi: 10.1172/JCI40671
123. Park MJ, Lee SY, Moon SJ, Son HJ, Lee SH, Kim EK, et al. Metformin attenuates graft-versus-host disease via restricting mammalian target of rapamycin/signal transducer and activator of transcription 3 and promoting adenosine monophosphate–activated protein kinase-autophagy for the balance between T helper 17 and Tregs. *Transl Res*. (2016) 173:115–30. doi: 10.1016/j.trsl.2016.03.006
124. Crocker CL, Baumgarner BL, Kinsey ST. β-guanidinopropionic acid and metformin differentially impact autophagy, mitochondria and cellular morphology in developing C2C12 muscle cells. *J. Muscle Res. Cell Motil*. (2019). doi: 10.1007/s10974-019-09568-0. [Epub ahead of print].
125. Wolff CA, Reid JJ, Musci RV, Bruns DR, Linden MA, Konopka AR, et al. Differential effects of rapamycin and metformin in combination with rapamycin on mechanisms of proteostasis in cultured skeletal myotubes. *J Gerontol Ser A Biol Sci Med Sci*. (2020) 75:32–9. doi: 10.1093/gerona/glz058
126. Nikolaidis S, Virgiliou C, Vekiou M, Skari A, Kechagidou A, Gika H, et al. Effect of exercise on key pharmacokinetic parameters related to metformin absorption in healthy humans: A pilot study. *Scand J Med Sci Sport*. (2020) 30:858–64. doi: 10.1111/sms.13628
127. Czepiel KS, Perez NP, Campoverde Reyes KJ, Sabharwal S, Stanford FC. Pharmacotherapy for the treatment of overweight and obesity in children, adolescents, and young adults in a large health system in the US. *Front Endocrinol*. (2020) 11:290. doi: 10.3389/fendo.2020.00290
128. Jørgensen PG, Jensen MT, Mensberg P, Storgaard H, Nyby S, Jensen JS, et al. Effect of exercise combined with glucagon-like peptide-1 receptor agonist treatment on cardiac function: a randomized double-blind placebo-controlled clinical trial. *Diabetes Obes Metab*. (2017) 19:1040–4. doi: 10.1111/dom.12900
129. Mensberg P, Nyby S, Jørgensen PG, Storgaard H, Jensen MT, Sivertsen J, et al. Near-normalization of glycaemic control with glucagon-like peptide-1 receptor agonist treatment combined with exercise in patients with type 2 diabetes. *Diabetes, Obes Metab*. (2017) 19:172–80. doi: 10.1111/dom.12797
130. Newman AA, Grimm NC, Wilburn JR, Schoenberg HM, Raj Trikha SJ, Luckasen GJ, et al. Influence of sodium glucose cotransporter 2 inhibition on physiological adaptation to endurance exercise training. *J Clin Endocrinol Metab*. (2019) 104:1953–66. doi: 10.1210/jc.2018-01741

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 Endocrinology

REVIEW
published: 28 August 2020
doi: 10.3389/fendo.2020.00567

Exercise-Induced Improvements to Whole Body Glucose Metabolism in Type 2 Diabetes: The Essential Role of the Liver

Shana O. Warner[1], *Michael V. Yao*[2], *Rebecca L. Cason*[1] *and Jason J. Winnick*[1]*

[1] *Division of Endocrinology, Diabetes and Metabolism, Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, OH, United States,* [2] *Division of Endocrinology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States*

Type 2 diabetes (T2D) is a metabolic disease characterized by obesity, insulin resistance, and the dysfunction of several key glucoregulatory organs. Among these organs, impaired liver function is recognized as one of the earliest contributors to impaired whole-body glucose homeostasis, with well-characterized hepatic insulin resistance resulting in elevated rates of hepatic glucose production (HGP) and fasting hyperglycemia. One portion of this review will provide an overview of how HGP is regulated during the fasted state in healthy humans and how this process becomes dysregulated in patients with T2D. Less well-appreciated is the liver's role in post-prandial glucose metabolism, where it takes up and metabolizes one-third of orally ingested glucose. An abundance of literature has shown that the process of hepatic glucose uptake is impaired in patients with T2D, thereby contributing to glucose intolerance. A second portion of this review will outline how hepatic glucose uptake is regulated during the post-prandial state, and how it becomes dysfunctional in patients with T2D. Finally, it is well-known that exercise training has an insulin-sensitizing effect on the liver, which contributes to improved whole-body glucose metabolism in patients with T2D, thereby making it a cornerstone in the management of the disease. To this end, the impact of exercise on hepatic glucose metabolism will be thoroughly discussed, referencing key findings in the literature. At the same time, sources of heterogeneity that contribute to inconsistent findings in the field will be pointed out, as will important topics for future investigation.

Keywords: glucagon, aerobic exercise, resistance training, hepatic glucose production, fasting blood glucose levels, post-prandial glucose

OPEN ACCESS

Edited by:
Jacob Haus,
University of Michigan, United States

Reviewed by:
R. Scott Rector,
University of Missouri, United States
David Williamson,
Penn State Harrisburg, United States

***Correspondence:**
Jason J. Winnick
jason.winnick@uc.edu

Specialty section:
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Endocrinology

Received: *29 April 2020*
Accepted: *13 July 2020*
Published: *28 August 2020*

Citation:
Warner SO, Yao MV, Cason RL and Winnick JJ (2020) Exercise-Induced Improvements to Whole Body Glucose Metabolism in Type 2 Diabetes: The Essential Role of the Liver. Front. Endocrinol. 11:567. doi: 10.3389/fendo.2020.00567

INTRODUCTION

In the United States, type 2 diabetes (T2D) impacts the lives of ∼10% of the population (1) by increasing their risk of developing severe health complications associated with micro- and macro-vascular disease. Some risk factors for the development of T2D are non-modifiable, such as age, gender and race (where increasing age, male gender, and Hispanic- and African-American race increase the risk for developing T2D). However, the presence of modifiable risk factors, such as obesity and leading a sedentary lifestyle, can hasten the development of the disease and its debilitating complications. Type 2 diabetes is insidious in nature, but its clinical manifestations

are well-known, ranging from elevated plasma insulin and/or slightly elevated glycemia initially (pre-diabetes), to overt diabetes characterized by fasting glucose levels ≥126 mg/dl or glucose levels >199 mg/dl 2 h after a 75-gram oral glucose challenge. While the etiology of T2D is often complex and multi-factorial, it is almost universally characterized by whole-body insulin resistance and the dysregulation of a number of key glucoregulatory organs (2). Among these organs, impaired function of the liver is one of the earliest contributors to impaired blood glucose homeostasis in patients with T2D due to its central role in regulating blood sugar levels during fasting and in response to glucose ingestion. It is for these reasons that it continues to be one of the most thoroughly investigated organs as we develop therapies for T2D.

As recent as the mid-nineteenth century, it was accepted that humans could not make their own metabolic substrates to fuel life. This dogma was finally rejected through the pioneering work of Claude Bernard who, among others, discovered that one of the liver's most important responsibilities is to make glucose and release it into the blood during fasting [for review see reference (3)]. Since that time, the scientific community has worked earnestly to understand the liver's role in the regulation of blood glucose homeostasis, which can vary on a daily basis from eating a meal rich in carbohydrate, where glucose is taken up by the liver and stored as glycogen, to intense exercise, where the liver needs to make glucose at an accelerated rate and release it into the blood to maintain euglycemia. As our knowledge of how the liver responds to such stimuli has greatly expanded over the years, so too has our understanding of the pathology of type 2 diabetes, which is characterized by impaired hepatic regulation of blood glucose homeostasis. Two focal points of this review will be to (1) outline the liver's role in the regulation of blood glucose homeostasis during fasting and feeding and; (2) describe how impaired hepatic responses to these physiological conditions contribute to T2D. Fortunately, ample evidence tells us that exercise can improve hepatic glucose metabolism in patients with T2D, which is undoubtedly part of why it has become a cornerstone of the disease's management. Accordingly, the main emphasis of this review will be to discuss the positive impact that exercise has on liver glucose metabolism in this population. While doing so, studies that directly measured hepatic glucose metabolism (e.g., A-V sampling and isotopic dilution studies) will be highlighted, with a reliance on human subject studies, and periodic references to more mechanistic studies using large and small animal models when necessary.

REGULATION OF HEPATIC GLUCOSE PRODUCTION DURING FASTING

Endogenous glucose production (EGP) includes all of the glucose released into the blood per unit of time, no matter the tissue of origin. In order for any organ to contribute to EGP, two things are required. The first is the capability to enzymatically produce the precursor of glucose, glucose-6-phosphate (G6P), which occurs primarily through two pathways. The most easily accessed source of G6P in the liver is the breakdown of glycogen stores by enzymes (e.g., glycogen phosphorylase and debranching enzyme) through a process called glycogenolysis. The second source of G6P comes from the synthesis of six-carbon G6P by enzymatically combining pairs of three-carbon metabolites (e.g., lactate, glycerol, amino acids, and TCA cycle intermediates) through a process called gluconeogenesis. Once G6P is produced, though, there is no mechanism whereby it can exit most tissues, because it is not a substrate for the glucose transporter proteins (Glut). For this reason, the second requirement to contribute to EGP is the presence of the enzyme glucose-6-phosphatase (G6Pase). G6Pase is found in the endoplasmic reticulum of primarily hepatic and renal cells, and dephosphorylates G6P to make free glucose. This glucose can then move freely out of the cell and into the blood via membrane-indigenous Glut proteins such as Glut-2 (not to be confused with sodium-glucose transporters involved in glucose reabsorption in renal tubules). The liver and kidney are the only two organs that express G6Pase in appreciable amounts, thereby making them the only organs that contribute to EGP. While renal glucose production accounts for ∼10% of total EGP, the kidney utilizes as much glucose as it produces, resulting in a net contribution to EGP of zero (4–7). In contrast, the liver accounts for 90% of fasting EGP and undergoes much larger fluctuations in glucose metabolism throughout the course of a day. For example, during high intensity exercise, it is not uncommon for the liver to produce glucose at a rate of 6 mg/kg/min or more to maintain euglycemia in the face of increased metabolic demands of working muscle (8, 9). On the other hand, after the ingestion of carbohydrate, the liver transitions from a glucose production mode, to one that takes up glucose at a rate of ∼4–6 mg/kg/min (10, 11). Fasting overnight or in between meals falls near the middle of this metabolic demand continuum, requiring the liver to produce glucose at the modest rate of ∼2.2 mg/kg/min so the rest of the body's glucose needs can be met (12, 13). This unparalleled level of metabolic flexibility makes the liver ideally suited to sit at the crossroads of normal blood glucose homeostasis, and why impairments in its function are considered a "canary in the coalmine" for T2D development.

During periods of modest fasting which, for the purposes of this review refers to periods between normally spaced meals, healthy humans are remarkably adept at maintaining their blood glucose at a very stable level of ∼90 mg/dL. While the absence of change may seem unremarkable, it is no small achievement, requiring coordination among multiple glucoregulatory organs. Of the organs purported to regulate fasting blood glucose homeostasis (2), the pancreas and liver interact most closely, precisely matching hepatic glucose production (HGP) with systemic glucose utilization such that euglycemia and normal brain function [which accounts for over one half of fasting glucose utilization (14–17); **Figure 1**] can be sustained. In healthy humans, this is achieved by subtle, minute to minute changes in the levels of insulin and glucagon at the liver. For example, it is known that when insulin secretion rises in response to a meal, HGP is markedly suppressed, resulting in a conservation of carbon as glucose enters the blood from the small intestine (18). Likewise, increased secretion of glucagon, such as occurs

during exercise, can markedly stimulate HGP, preventing a fall in blood glucose as its utilization by skeletal muscle is greatly enhanced (19). While these are extreme challenges to blood glucose homeostasis, their fundamental principles also hold true during the fasting state, when fluctuations in hormones and HGP are less apparent. A good illustration of this comes from the work of Flattem et al. who, in dogs, used a hepatic glycogen phosphorylase inhibitor to modestly reduce endogenous HGP and thereby reduce plasma glucose, or increase glucose entry by intravenous (IV) glucose infusion so as to increase the glucose level (20). The changes in insulin and glucagon that accompanied these fluctuations in blood glucose were carefully assessed by sampling from the animals' hepatic portal vein, which is the vessel that delivers islet hormones to the liver first, followed by their delivery to the rest of the body via venous blood. Sampling from the hepatic portal vein is of particular importance to the accurate measurement of insulin at the liver, because insulin extraction by the liver is ∼50–60% in healthy individuals (21), making hepatic portal vein blood insulin levels 2–3 times higher than what is seen by the rest of the body's tissues. Results revealed that the secretion of insulin and glucagon paralleled increases or decreases in plasma glucose levels, respectively, even with a change in glucose of only 10 mg/dL. In fact, the glucose-induced rise in insulin secretion was hardly subtle, with a 50% increase in insulin levels detected in the hepatic portal vein; a change that was undetectable in venous blood (due to hepatic insulin extraction). Notably, an equal fall in glucose inhibited insulin secretion and reduced hepatic portal vein insulin concentrations by ∼50%. At the same time, the rise in glucose had little effect on glucagon, but its fall increased glucagon levels in the hepatic portal vein by 50%. These innovative studies sum up perfectly the widely accepted model of how blood glucose homeostasis is normally regulated during fasting; namely that euglycemia is maintained by plasma glucose-induced minute-to-minute changes in insulin and glucagon secretion which, in turn, modify HGP so that it equals the rate of whole-body glucose utilization.

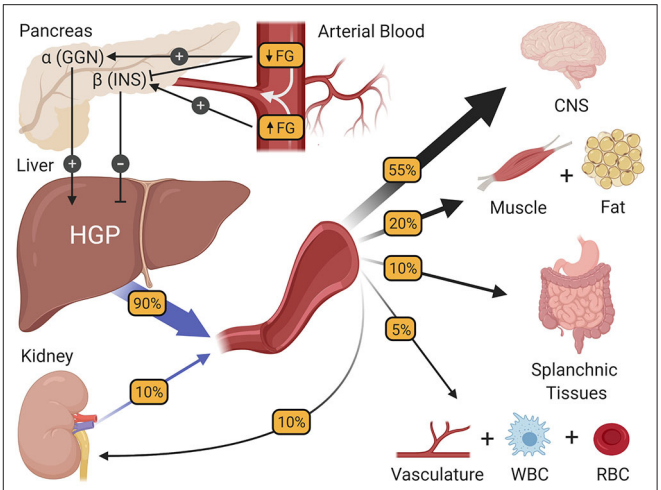


FIGURE 1 | The regulation of fasting blood glucose homeostasis. Subtle changes in fasting glucose levels (FG) entering the pancreas regulate the release of the islet hormones insulin (INS) and glucagon (GGN). In turn, these hormones control the rate of hepatic glucose production (HGP), making HGP equal to the rate at which all other tissues of the body utilize glucose, thereby preserving fasting glucose levels at a steady state. CNS, central nervous system; WBC, white blood cells; RBC, red blood cells.

DYSREGULATION OF FASTING HEPATIC GLUCOSE PRODUCTION IN T2D

One criterion for the diagnosis of T2D is a fasting glucose level of 126 mg/dL (7 mmol/L) or higher (22). Previous studies have shown a close association between HGP and elevated fasting glucose, thereby making the former an early contributor to metabolic dysregulation (23). In healthy humans, fasting HGP is ∼2.2 mg/kg/min, with one-half of this glucose coming from glycogenolysis and the other half from gluconeogenesis (12, 13). In people who are obese but do not have T2D, chronically elevated insulin secretion allows for the needed suppression of fasting HGP to maintain normal fasting glucose levels, despite the presence of hepatic insulin resistance (24). Predictably, however, as insulin resistance progresses, even hyperinsulinemia is unable to adequately suppress HGP, causing hyperglycemia and T2D. Not to be outdone, hyperglucagonemia is often present in T2D and has also received consideration as a sine qua non of elevated fasting HGP in T2D because of its role in the liver to stimulate HGP and signal in opposition to insulin (25).

Given that hepatic glycogenolysis is more responsive to insulin and glucagon than gluconeogenesis *in vivo* (26, 27), it would stand to reason that an elevation in its rate would be a focal point in the pathology of T2D. Interestingly, however, while some have ascribed the increase in EGP observed in patients with T2D to an increase in G6P production via both metabolic pathways (12), others have observed that increased gluconeogenesis is the primary contributor to elevated EGP (13, 28). The mechanism responsible for increased gluconeogenesis in T2D remains controversial. On the one hand, gluconeogenesis could be enhanced in T2D patients as a result of insulin resistance-induced increases in PEPCK expression. In support of this, Satapati et al. reported that the knockdown of PEPCK, to 40% of normal, protected high-fat fed mice from elevated EGP and the contribution of gluconeogenesis to EGP (29). However, this manipulation of PEPCK also lowered G6Pase expression in the liver, making their relative contributions to the reduction in EGP unclear. On the other hand, Burgess et al. observed that when PEPCK protein was reduced to as little as 30% of normal, gluconeogenesis was not impacted. This led the authors to conclude that the enzyme has a low control coefficient on gluconeogenesis, and that substrate flux is more tightly linked with gluconeogenesis (30). These latter results were further corroborated by Ramnanan et al. who, in dogs, also observed that changes in gluconeogenic substrate flux are not closely tethered to hepatic PEPCK levels, but rather substrate availability (31). In either event, it should be noted that the most proximal cause of elevated HGP is the relative activities of the rate limiting enzymes for HGP; glucokinase (GK) and G6Pase. Because the

expression of both enzymes are reciprocally regulated by insulin and glucagon [where insulin increases and decreases GK and G6Pase expression, respectively, and glucagon does the opposite (32)], insulin resistance or hyperglucagonemia can impact the relative activities of these enzymes, favoring the production of glucose over G6P in the liver. Accordingly, this last step of HGP is known to be dysregulated in T2D, thereby leading to excessive HGP (33–35), and making it an important contributor to the etiology of T2D.

A consistent positive energy balance and lack of physical activity are lifestyle factors that track closely with the rise in obesity and T2D, resulting in ectopic fat accumulation and insulin resistance in tissues such as skeletal muscle and liver. Non-alcoholic fatty liver disease (NAFLD) is a common chronic condition that can be caused by excess fat accumulation in the liver (36). NAFLD is a spectrum of diseases, including steatohepatitis which can progress to fibrosis, and potentially more serious liver diseases like cirrhosis and hepatocarcinomas (37, 38), and is estimated to be present in 25–30% of the general population in the United States (39, 40). While NAFLD can be present in normal weight individuals, its prevalence in patients with T2D is closely associated with obesity and increasing BMI (41), with the prevalence among those with T2D being estimated to be as high as 50–60% (37, 41). While consideration of the numerous cellular pathways by which ectopic liver lipid accumulation can contribute to hepatic insulin resistance is beyond the scope of this review, they are thoroughly discussed by Petersen and Shulman (42).

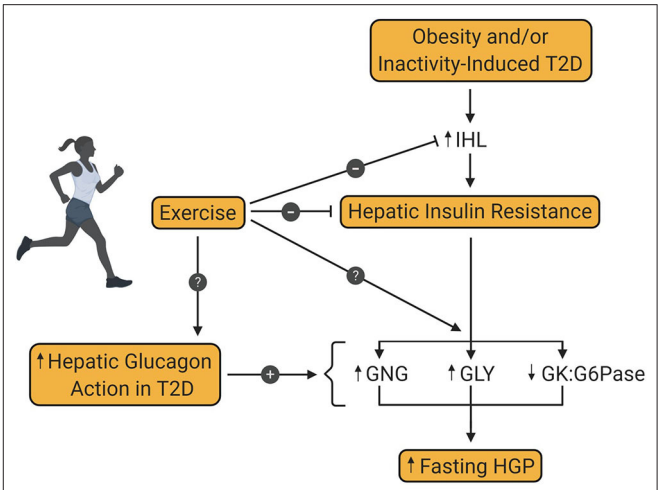


FIGURE 2 | Effect of exercise training on hepatic glucose production in type 2 diabetes. Obesity and/or inactivity are known to be risk factors for the development of type 2 diabetes (T2D). T2D is associated with increased intrahepatic lipid content (IHL) and resultant insulin resistance, which increases fasting hepatic glucose production (HGP). Increased hepatic glucagon action is also a key contributor to excessive HGP in patients with T2D. Exercise training is known to diminish IHL and hepatic insulin resistance in patients with T2D, thereby enhancing insulin mediated suppression of HGP. However, the metabolic pathway(s) impacted by this enhanced suppression is not yet known. Also unknown is the impact of exercise training on glucagon-mediated HGP in patients with T2D. GNG, gluconeogenesis; GLY, glycogenolysis; GK, glucokinase; G6Pase, glucose-6-phosphatase.

IMPACT OF EXERCISE ON FASTING HEPATIC GLUCOSE METABOLISM

Nearly four decades have passed since Bogardus et al. first (to our knowledge) reported that 12 weeks of lifestyle intervention could lower fasting EGP and improve hepatic insulin sensitivity in patients with T2D (43). Since then, considerable effort has gone into improving our understanding of how regular exercise confers its beneficial effects on hepatic glucose metabolism during the fasting state and in response to oral glucose, contributing to the adoption of regular exercise training as a cornerstone in the treatment of T2D.

In 1984, Bogardus et al. demonstrated the beneficial effect of lifestyle intervention on hepatic glucose metabolism in patients with T2D (43). The results of this study revealed, on the one hand, that exercise-induced weight loss over 12 weeks reduced fasting glucose and insulin, lowered fasting EGP, and improved insulin-mediated suppression of EGP. On the other hand, similar effects on EGP were seen in response to weight loss alone, thereby suggesting that exercise was of no additional benefit to hepatic glucose metabolism. The absence of a no-intervention control group, or a group that performed exercise without weight loss complicated the data interpretation, leaving the question of the independent effect of exercise on hepatic glucose metabolism unanswered. But since then, numerous additional investigations have been conducted, showing the positive impact exercise training *per se*, has on hepatic glucose metabolism, even in the absence of weight loss (**Figure 2**).

Hepatic Responses to Aerobic Exercise in People Without T2D

Some of the earlier studies in the field examined the effect of exercise on hepatic glucose metabolism in lean, healthy, non-T2D subjects. In one cross-sectional study, Rodnick et al. (44) observed that healthy, physically trained men had lower rates of EGP at each of two insulin concentrations (10 and 50 μU/ml) compared to untrained counterparts. To the contrary, however, Segal et al. noted that in response to 12 weeks of cycling for 4 h/week at 70% VO_2 max, fasting EGP was not lowered in lean male control subjects, nor was insulin-induced suppression of EGP enhanced (45). While the 12-week training duration may not have been sufficient to bring about the metabolic benefit that lifetime exercise adherence does, it is also plausible that exercise training in healthy adults would not reasonably be expected to alter liver function that is normal to begin with. In contrast, it is known that hepatic insulin resistance is frequently present in people with obesity (non-T2D). While fasting EGP is not usually elevated in this population, this can be ascribed to increased insulin secretion required to suppress EGP such that hyperglycemia does not occur. Meex et al. (46) showed that 12 weeks of cycling (2x/week for 30 min at 55% of workload max, plus 1x/week of resistance training) by non-T2D subjects with obesity lowered fasting insulin and glucose without impacting EGP, and improved the suppression of EGP

in response to hyperinsulinemia. Similar results were published by Shojaee-Moradie et al. (47), who showed that after only 6 weeks of exercise (≥3x/week for 20 min at 60–85% VO_2 max), insulin-mediated suppression of EGP was improved in a similar population, although no changes in glucose or insulin were seen during fasting. Notably, beneficial alterations in hepatic glucose metabolism as a result of the exercise training in the above-mentioned studies (46, 47) were obtained in the absence of weight loss. In summary, while the effect of exercise training on fasting hepatic glucose metabolism is likely to be negligible in healthy humans, it can clearly be of therapeutic value in people with obesity by improving insulin sensitivity, thereby correcting modest derangements in hepatic glucose metabolism that accompany pre-diabetes.

Hepatic Responses to Aerobic Exercise in People With T2D

It is also clear that regular aerobic exercise training can improve hepatic glucose metabolism in patients with T2D, although the manifestation of this improvement can vary. In addition to the previously noted results by Bogardus et al. (43), Segal et al. (45) observed that fasting HGP was lower in patients with T2D after 12 weeks of aerobic exercise training (4x/week, 1 h/day, 70% VO_2 max) without weight loss. On the other hand, most other studies in patients with T2D that used aerobic exercise training durations of 6 weeks or more report that fasting EGP does not change (46, 48–50), even those that incorporate significant weight loss into their study design (48, 49). The reason for this discrepancy is not entirely clear, but this is not to say that subjects with T2D from these latter studies did not demonstrate improved hepatic function as a result of exercise training. In fact, all but one of these studies [which was the only one that showed a lowering of fasting EGP (45)], utilizing an exercise training duration >6 weeks showed that after training, the suppression of EGP by insulin was improved (43, 46, 48–50). Thus, although the manifestations may differ slightly between studies, it is clear that sufficient training durations result in improved hepatic glucose metabolism in patients with T2D, with the most common effect being enhanced suppression of EGP by physiological levels of insulin.

To better understand the nuances about exercise that contribute to improved hepatic glucose metabolism, creative study designs have been employed. For example, it is important to know if the length of a training program is responsible for improved liver function, or if this is a function of the last exercise bout the previous day. Vendelbo et al. (51) did not observe any change in fasting EGP or improved insulin-mediated suppression of EGP 4 h after a single bout of exercise in healthy men (1 h cycling at 65% VO_2 peak). In contrast, Devlin et al. (52) showed that a single bout of exhaustive, high intensity interval exercise can lower fasting plasma glucose values in subjects with T2D. Moreover, this reduction was accompanied by a 20% decrease in fasting EGP the morning after exercise, as well as enhanced suppression of EGP in response to hyperinsulinemia. While the latter study suggests that improved hepatic glucose metabolism after a single bout of exercise is on par with a 12-week training program, caution should also be applied to the interpretation of this robust response. First, the subjects were not fed after the exercise bout that took place the evening prior, thereby making it possible that the lengthy fast duration after exercise (∼12 h) contributed to improved hepatic insulin sensitivity. Second, the exercise bout was exhaustive, which has been shown to reduce the hepatic energy charge in rodents compared to non-exhaustive exercise (53), and most likely had a greater impact on hepatic glucose metabolism than a non-exhaustive bout would have. To our knowledge, these are the only studies that have examined the effect of a single bout of endurance exercise on hepatic glucose metabolism in people with T2D, highlighting the need for future studies to address this question.

Another important question relates to the independent effect of exercise training on hepatic glucose metabolism in patients with T2D. An example of how this can be done, is through the use of a 7-day training protocol, which can improve whole body insulin sensitivity (54), but in the absence of additional insulin-sensitizing metabolic changes that can accompany exercise training (e.g., weight loss). In one such study (55), it was observed that 7-days of aerobic exercise training in patients with T2D (50 min/day at 70% of VO_2 max) did not lower fasting EGP, nor did it improve insulin mediated suppression of EGP when the expended calories from exercise were replaced with additional food. To the contrary, Kirwan et al. (56) observed that a similar 7-day exercise program (60 min/day at 70% of VO_2 max), where calories expended during exercise were not replaced, resulted in lower fasting EGP and improved suppression of EGP by insulin. In a study that appears to reconcile these contrasting results, Black et al. (57) observed that in response to 6-days of aerobic exercise consisting of treadmill walking (60–65% of estimated VO_2 peak for ∼60–65 min per day), not replacing the calories expended through exercise (∼500 kcal/session) led to improved hepatic function in the form of greater suppression of EGP in response to an IV infusion of glucose, while replacing the expended calories negated this response. To the extent that the different outcomes are regulated similarly (e.g., hyperinsulinemia-induced suppression of EGP and IV glucose-induced suppression of EGP) these studies allow us to infer that in the absence of weight loss and increased aerobic power, a modest, acute energy deficit is required for short term aerobic exercise training to improve hepatic glucose metabolism in patients with T2D.

Consistent with the observation that short-term exercise-induced energy deficits can improve hepatic glucose metabolism in T2D, weight loss can also have a beneficial effect. Bogardus et al. (43) and Coker et al. (49) both showed that weight loss can improve hepatic glucose metabolism in patients with T2D, while Petersen et al. (58) also observed that a body weight loss of 8 kg lowered fasting glucose and insulin concentrations in patients with T2D, in addition to lowering fasting EGP and improving insulin-mediated suppression of EGP. Similar results were presented by Viljanen et al. (59), who showed that a 6-week weight loss program (where 11 kg were lost) in subjects with obesity, lowered fasting EGP. In the latter two studies, it was also observed that hepatic fat content was lowered by 60–80%, thereby giving credence to the hypothesis that lowering liver fat can markedly improve hepatic insulin sensitivity.

As the studies above highlighted, weight loss (58, 59) can significantly reduce intrahepatic lipid content (IHL). Not surprisingly, aerobic exercise training has also been shown to reduce IHL in both patients with T2D and non-diabetic individuals with obesity. A 3 month aerobic exercise intervention (60 min/session at 60–75% VO_2 peak 3x/week) significantly reduced visceral adipose tissue (VAT) and IHL in adolescents with obesity (60) and 4 weeks of aerobic exercise training (30–45 min/session at 50–70% VO_2 peak 3x/week) decreased VAT and hepatic TG concentration in men and women who were obese (61). Importantly, changes in IHL content in both studies were achieved in the absence of body weight loss, suggesting an independent effect of exercise in these populations. Likewise, in a study in which patients with T2D and NAFLD were randomized to either an aerobic (60–65% of heart rate reserve) or resistance (70–80% of 1 repetition maximum) exercise training program (3x/week; 60 min/session for 4 months), hepatic fat content was markedly reduced (62). Notably, hepatic fat was decreased comparably with both types of exercise and although dietary intake was not altered and the reduction in BMI was minimal, the decrease in hepatic fat was remarkable (ranging from −26 to −33% from baseline). Given the well-known relationship between IHL and hepatic insulin resistance, the reversal of ectopic hepatic fat accumulation is likely to be a key mechanism by which exercise training exerts its therapeutic effect on hepatic glucose metabolism.

Hepatic Responses to Resistance Training

While the effect of resistance training on peripheral insulin sensitivity has been extensively studied, showing improved insulin action (63–66), much less is known about its effects on the liver. van der Heijden et al. showed that both aerobic (67) and resistance (68) exercise training improves hepatic insulin sensitivity in adolescents with obesity. In the latter study, it was shown that 12 weeks of strength training (2x/week, 1 h/day, progressive training ranging from ∼50% to ∼80–85% 3RM, 2–3 sets, 8–20 repetitions) increased lean body mass and strength, without having a significant impact on overall body weight, body fat, or peripheral insulin sensitivity (68). Interestingly, the authors did observe improved hepatic glucose metabolism in response to training, in the form of a significant reduction in fasting EGP. Moreover, it was also noted that this reduction was accounted for entirely by reduced glycogenolysis, with rates of fasting gluconeogenesis remaining stable over time. Improvements in hepatic insulin action have also been found in response to progressive resistance training in various older-adult populations. Honka et al. showed that 4 months of resistance training (3x/week, moderate intensity) in elderly women improved the suppression of EGP by 28% under insulin-stimulated conditions, despite no change in hepatic fat content (69). On the other hand, Croymans et al. (70) found in sedentary, young men with obesity, that while 12 weeks of resistance training (3x/week, 1 h/day, moderate intensity) increased lean body mass, relative strength and skeletal muscle insulin sensitivity, there was no improvement in the hepatic insulin resistance index. A difference in methodology could explain the discrepancy in findings as the latter study calculated hepatic insulin resistance from glucose and insulin data taken during the first 30 min of an oral glucose tolerance test (OGTT), while the former employed the isotopic dilution technique to measure EGP. Thus, resistance training appears to be efficacious in improving hepatic glucose metabolism in certain special populations (e.g., obese, elderly, and children). At this time, we are not aware of any studies that have examined its impact on hepatic glucose metabolism in patients with T2D, but there is reason to believe that it could be efficacious. In addition to the previously described study showing that resistance training can reduce IHL in patients with T2D (62), Pereira et al. (71) showed similar results in rodents. In that study, it was shown that a high fat diet, combined with 14 weeks of strength training, significantly reduced hepatic TG content and improved hepatic insulin sensitivity despite no changes in body mass or adiposity. Notably, resistance training also protected these animals from the diet induced increases in lipogenic enzymes such as acetyl-CoA carboxylase and fatty acid synthase. In summary, there have not yet been any studies examining the impact of resistance training on hepatic glucose metabolism in patients with T2D. On the other hand, the evidence that it can benefit other populations warrants future studies on this topic. If it does confer an additional benefit on hepatic glucose metabolism in T2D, resistance training could be used in conjunction with aerobic exercise training and/or different medications to maximize the therapeutic benefit of exercise. Moreover, many individuals with T2D may prefer resistance over aerobic exercise training, especially when obesity is a comorbidity, which could have the added benefit of improving compliance.

Hepatic Responses to Glucagon After Aerobic Exercise Training

The assessment of how exercise training improves hepatic glucose metabolism in people with T2D almost invariably centers around how processes like EGP are regulated during the fasting state and in response to hyperinsulinemia. However, this assumes that alterations in glucagon action do not change over the same period. In fact, there are only a handful of studies that have looked at the interaction between exercise training and glucagon action in the liver, the majority of which were performed in rodents. In the only study of its kind in humans, Drouin et al. (72) performed a cross sectional study using trained and untrained male subjects. The metabolic studies were initiated with a pancreatic clamp (the use of somatostatin to inhibit endogenous insulin and glucagon secretion), accompanied by IV-delivered hyperinsulinemia (65 ± 12 and 82 ± 11 pmol/L in trained and untrained, respectively; p = NS), and the plasma glucose level was maintained at euglycemia by an IV infusion of dextrose (4.9 mmol/L in both groups). After a 2-h equilibration period under these conditions, an infusion of glucagon (1.5 ng/kg/min) was added and metabolic responses were monitored. In response to hyperglucagonemia, EGP was twice as high in trained individuals, leading to a markedly higher plasma glucose level. In follow up studies (73), the same group demonstrated

that when perfused with glucagon, the livers of trained rats had greater glucose output compared to those that were untrained. Another important observation was that the nutritional status (fed vs. fasted), and thus liver glycogen availability, made no difference between groups. This suggests that trained rats had greater glucose output in response to glucagon whether it was after a short fast (when glucagon-induced mobilization of liver glycogen is the primary source of HGP) or a more prolonged fast (when liver glycogen is nearly depleted, leaving gluconeogenesis as the predominant pathway). Similar results have been shown to occur in rats in response to a single bout of exercise (74) and, from a mechanistic basis, this increase in hepatic glucagon sensitivity has been ascribed to an increase in the density of glucagon receptors on the liver after training (75). When considering this from the perspective of health and metabolic dysregulation in patients with T2D, the benefit of training-induced sensitization of the liver to glucagon is not obvious. One possibility, as the authors hypothesized, is that this enhances the capability to rapidly stimulate HGP at the outset of exercise, thereby improving performance. Nevertheless, if exercise training has the same glucagon-sensitizing effect in patients with T2D, then it would be antagonistic to the responses to insulin, thereby making gains in insulin action even greater than what is currently believed. Like resistance training, we are not aware of any studies that have examined how regular exercise training impacts hepatic glucagon action in patients with T2D, which is unfortunate given the increasingly prominent recognition that hyperglucagonemia is being given in the causation of fasting hyperglycemia (25).

In summary, because of its importance in the regulation of whole-body glucose metabolism, the study of hepatic responses to insulin sensitizing exercise training should remain a priority in the scientific community. On the one hand, it is clear that regular aerobic exercise training improves hepatic glucose metabolism in patients with T2D, as long as the structure includes sufficient frequency (≥4 days/week), intensity (≥50–60% of VO_2 max) and duration (≥6 weeks). On the other hand, important questions linger, such as the mechanism of action by which exercise training improves hepatic glucose metabolism (e.g., effects of IHL levels and glucagon action) and the impact that other exercise modalities (e.g., resistance training) have on the liver. In addition, it will be important to identify sources of heterogeneity in hepatic responses to exercise training, as these can potentially be exploited to maximize exercise's therapeutic potential. Currently, factors such as study design (e.g., training duration length) and methodologies (e.g., what hepatic responses are being measured) are the most obvious contributors to response heterogeneity, but underappreciated sources include weight loss and/or acute energy balance status, both of which are capable of improving hepatic insulin sensitivity independent of exercise. In spite of these confounding factors, the evidence that exercise training with or without weight loss can have a positive impact on hepatic glucose metabolism in patients with obesity and T2D is consistent and overwhelming, thereby cementing its recognition as an important contributor to exercise-induced improvements in whole body glucose metabolism.

REGULATION OF HEPATIC GLUCOSE METABOLISM IN RESPONSE TO GLUCOSE INGESTION

During the post-prandial state, which for the purpose of this review means in response to ingestion of a modest glucose load, the liver shifts from producing glucose at a rate of ∼2.2 mg/kg/min, to glucose uptake at a rate of ∼4–6 mg/kg/min, with ∼70% of this being stored as glycogen for later use during fasting (76–78). This shift is accomplished by the interaction of hyperinsulinemia with a pair of important metabolic cues from incoming glucose which, when present at the same time, maximize hepatic glucose uptake, and glycogen synthesis (**Figure 3**).

Under euglycemic clamp conditions (95 mg/dL), raising the plasma insulin level to 125 μU/mL via an intravenous (IV) infusion of the hormone, leads to a marked increase in skeletal muscle glucose uptake (accounting for ∼85% of whole-body glucose utilization) and very little glucose uptake by the liver [∼5% (79)]. However, under these metabolic conditions, hyperinsulinemia is the only significant post-prandial metabolic cue present, while important glycemic changes that accompany glucose ingestion are absent (80). One such glycemic cue is hyperglycemia *per se*, which increases the glucose load to the liver, resulting in greater transport of glucose into hepatocytes. While hyperglycemia and hyperinsulinemia are each capable of suppressing HGP, neither can stimulate liver glucose uptake by itself (11, 81). On the other hand, when both are present

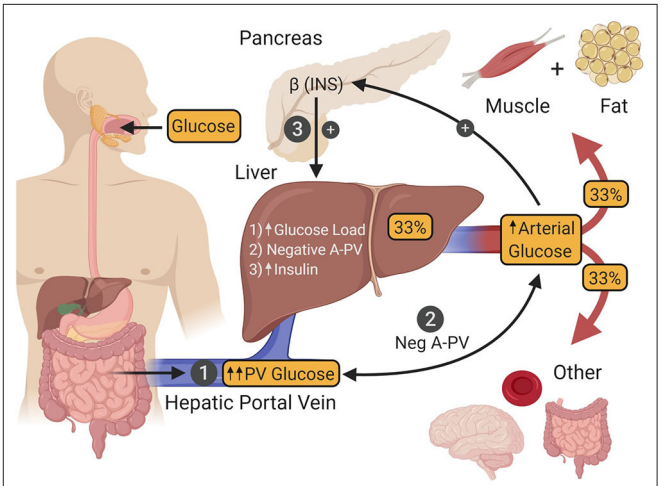


FIGURE 3 | After ingestion, glucose is absorbed into the hepatic portal vein (PV) from the intestine (1). One third of the glucose in the PV is taken up by the liver, after which the remaining fraction is delivered to other tissues of the body by arterial blood. Hepatic glucose uptake is regulated by three factors. The first (1) is an increase in the glucose load to the liver as a result of intestinal glucose absorption-induced hyperglycemia. The second (2) is a negative arterial-PV glucose gradient generated by the absorption of glucose from the intestine into the PV, thereby making its glucose levels higher than that of arterial blood. The third (3) is hyperinsulinemia that occurs as a result of hyperglycemia-induced insulin secretion by islet β-cells. The presence of all three of these signals is required to maximize hepatic glucose uptake. INS, insulin.

simultaneously, they are able to stimulate net hepatic glucose uptake to a limited extent [∼2 mg/kg/min (11, 82)]. The second glycemic cue required to maximize post-prandial rates of hepatic glucose uptake is a negative glucose gradient between the arterial blood and that of the hepatic portal vein (83, 84) which occurs when glucose is being absorbed into the body from the intestine. This neurally mediated "feeding signal" works synergistically with hyperglycemia and hyperinsulinemia such that hepatic glucose uptake reaches a post-prandial rate of ∼4–6 mg/kg/min [**Figure 3**; (10, 11)]. By juxtaposing this rate of hepatic glucose uptake with the suppression of the pre-meal HGP (∼2.2 mg/kg/min), the net change in hepatic glucose metabolism in response to glucose ingestion is actually ∼6–8 mg/kg/min, putting it on par with skeletal muscle, where each organ is responsible for taking up approximately one-third of ingested glucose (85–92).

At the cellular level, glucokinase (GK) is the rate limiting enzyme for hepatic glucose uptake and glycogen synthesis. Given its high Km for glucose (8–10 mM) compared to muscle hexokinase-1 (0.1 mM), GK is ideally suited to respond to physiological changes in the plasma glucose level (93). Compared to the concentration dependent inward diffusion of glucose through Glut2 in hepatocytes, the activation of GK is much more complex. GK expression is directly regulated by insulin, thereby making protein levels their lowest toward the end of a fasting period (93), with the remaining GK bound to its regulatory protein (GKRP) in the nucleus of hepatocytes (94). This sequestration allows G6Pase activity and the production of glucose to proceed unhindered between meals so euglycemia can be preserved. However, when glucose is being absorbed from the small intestine after a meal, the transition of the liver to glucose uptake is facilitated by an insulin-induced and neurally triggered increase in GK expression, and a release of existing GK from GKRP in the nucleus, allowing it to translocate out to the cytosol where it can phosphorylate incoming glucose (95, 96). Once the incoming glucose is phosphorylated, ∼70% is stored as glycogen for later use during fasting and the remaining fraction proceeds through the glycolytic pathway and is either oxidized (∼10%) or released as lactate (∼20%) (76–78).

DYSREGULATION OF POST-PRANDIAL HEPATIC GLUCOSE METABOLISM IN T2D

A second criterion for the diagnosis of T2D is a plasma glucose level >199 mg/dL 2 h after an oral glucose challenge (22). In mammals, the liver has two sources of blood; the hepatic artery, which accounts for ∼20% of total flow, and the hepatic portal vein, which accounts for the remaining 80%. In animal models, such as the dog, net hepatic glucose balance can be easily calculated using the Fick principle. In the human, catheterization of the hepatic portal vein is not permitted for research purposes, leading to an inability to directly measure net hepatic glucose uptake. Instead, arterial—hepatic vein sampling is used to measure net splanchnic glucose balance (NSGB). The primary limitation of this method is that NSGB is influenced by any glucose produced by or taken up by the intestines and spleen.

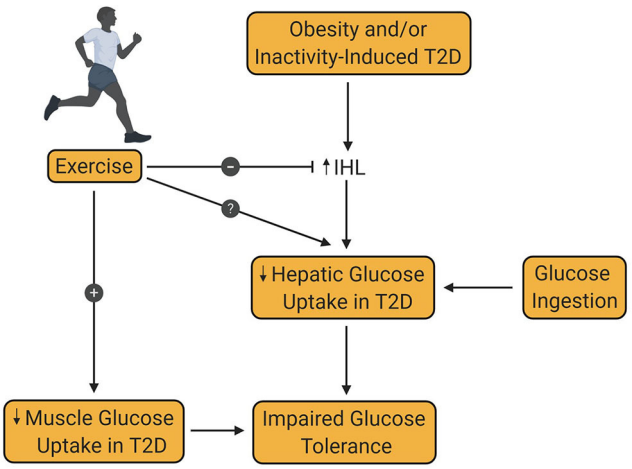


FIGURE 4 | Effect of exercise training on hepatic glucose uptake in type 2 diabetes. Obesity and/or inactivity are known to be risk factors for the development of type 2 diabetes (T2D). T2D is associated with increased intrahepatic lipid content (IHL), which decreases post-prandial hepatic glucose uptake, thereby contributing to glucose intolerance. Decreased muscle glucose uptake in patients with T2D is also an important contributor to glucose intolerance in T2D. Exercise training is known to increase muscle glucose uptake in patients with T2D. At the same time, exercise is known to reduce IHL, although the impact of this reduction on hepatic glucose uptake remains controversial and requires further investigation.

Nevertheless, glucose utilization by these tissues is known to be low and not impacted by insulin. The use of such techniques has led to ample evidence that splanchnic glucose uptake (SGU) and hepatic glycogen synthesis are impaired in patients with T2D [**Figure 4**; (86, 97–100)].

In a particularly comprehensive study, Krssak et al. recruited patients with obesity and T2D to study their hepatic responses to a mixed meal and hyperglycemic/ hyperinsulinemia (97). It was demonstrated that hepatic glycogen synthesis was 45% lower in patients with T2D compared to lean controls in response to either a mixed meal test or hyperglycemic-hyperinsulinemia. Given that liver glucose uptake and glycogen synthesis correspond well with one another (78), it is not surprising that a number of studies have demonstrated diminished SGU in patients with T2D. In a series of particularly elegant studies, Basu et al. showed that SGU was lower in patients with T2D during hyperglycemic/hyperinsulinemic conditions and in response to intraduodenal glucose infusions compared to non-diabetic controls (99). To more closely examine hepatic glycogen metabolism in these studies, they used a ^{14}C-galactose tracer to show that UDP-glucose flux is also lower in patients with T2D, pointing toward diminished GK activity as the cause of reduced SGU (98). While further exploring the mechanistic basis for reductions in SGU associated with T2D, Coate et al. observed that diet induced glucose intolerance impairs net hepatic glucose uptake and lowers liver glycogen synthesis in dogs (101–105). Moreover, the stimulatory effect of the feeding signal generated by a negative arterial-portal vein glucose gradient on net hepatic glucose uptake was

completely ablated (102, 105). At the cellular level, this reduction in glucose uptake was associated with diminished GK protein and activity, which is also consistent with significant hepatic insulin resistance (101, 103, 104). These results are in agreement with the previously described human data (98, 99), demonstrating that post-prandial GK activity is impaired in patients with T2D.

IMPACT OF EXERCISE ON POST-PRANDIAL HEPATIC GLUCOSE METABOLISM

Hepatic Responses to Aerobic Exercise in People Without T2D

Compared to our knowledge of how regular exercise training can improve hepatic insulin sensitivity during fasting, relatively little is known about how exercise impacts hepatic glucose metabolism during the post-prandial state. Maehlum et al. (106) used arterial and hepatic vein sampling in healthy young men to examine splanchnic glucose metabolism in response to a 100 gram oral glucose load ingested either 15 min or 14–15 h after a single session of exercise to exhaustion (cycling at 70% VO_2 max). Results of that study demonstrated that prior exercise, no matter the timing interval, doubled splanchnic glucose output compared to non-exercise controls. Furthermore, the authors also observed that this increase in glucose escape to the periphery accounted for up to 66% of muscle glycogen repletion. Although it is tempting to ascribe the increase in splanchnic glucose output to a reduction in hepatic glucose uptake, alternate explanations (e.g., increased intestinal absorption of glucose or incomplete glucose absorption) prevent a clear interpretation of these data. Similar results were published by Rose et al. (107), who observed that splanchnic glucose output was 30% greater in endurance trained healthy men 30 min after exercise and that this is associated with enhanced whole-body glucose utilization. Together, these data suggest that in healthy people, more of the ingested glucose escapes the splanchnic bed in favor of delivery to peripheral tissues during the post-exercise recovery period.

The disposition of an oral glucose load after exercise was looked at more closely in elegant canine studies conducted by Wasserman and colleagues. Hamilton et al. (108) observed that an intraduodenal glucose infusion (8 mg/kg/min) after 150 min of moderate intensity exercise resulted in higher arterial glucose levels due to enhanced intestinal glucose absorption. However, it was also observed that net hepatic glucose uptake was unchanged after exercise, thereby suggesting that it is the increase in intestinal absorption that increases glucose delivery to the periphery after exercise, not a decrease in *the rate* of hepatic glucose uptake. These data, along with those of Rose et al. (107) and Maehlum et al. (106), suggest that the rate of hepatic glucose uptake after exercise in young males and healthy canines is not diminished; in fact, it is unchanged. Instead, it is the accelerated intestinal absorption of glucose after exercise that facilitates the escape of more glucose from the splanchnic bed, where it is metabolized in the periphery by primarily skeletal muscle.

Hepatic Responses to Aerobic Exercise in People With T2D

Given that hepatic insulin sensitivity is known to be enhanced after exercise training in T2D, it stands to reason that impaired SGU seen in this population would also be improved as a result of lifestyle modification. However, to date, only a handful of studies have been conducted which relate to this topic. Kawamori et al. (109) studied six patients with T2D twice; once 3 h after having performed a single bout of cycle ergometer exercise (90% of measured anaerobic threshold for a total of 200 kcal) and another after having remained sedentary. In response to the exercise bout, it was reported that the percent of the ingested glucose disposed of by the splanchnic bed doubled from 23.4 to 50.5%, thereby supporting the notion that a single bout of insulin-sensitizing exercise can improve SGU in patients with T2D. In another study, but of longer duration (2 weeks), Tamura et al. (88) had two groups of non-obese (BMI of ∼27 kg/m^2) patients with T2D undergo caloric restriction resulting in weight loss, with one of the two groups also performing regular exercise (2–3 sessions per day of 30 min walking at 50–60% of VO_2 max, 5–6 days per week). The data from these experiments show that intrahepatic lipid content was lowered by ∼25–30% in both groups and that the loss was not different between them. Then, in a secondary analysis, they measured SGU in a total of six subjects, three from each group (where the data from the two groups were combined, instead of analyzing them separately). The results showed that the percent of the oral glucose taken up by the splanchnic tissues increased from 38 to 50% within the collapsed group. In a more recent study, Gregory et al. (50) studied the effect of 15 weeks of aerobic exercise training without weight loss (70% VO_2 max, 4–5 days/week, 50 min/day) on SGU in patients with T2D. In addition to observing that insulin-mediated suppression of EGP was improved in the same group of patients after exercise training, it was reported that SGU in response to a 75-gram oral glucose load decreased from 23 grams to 9 grams. Of interest, however, was that the increment in muscle glucose uptake after exercise (22 grams), more than made up for the 14-gram reduction in SGU, thereby leading to a net improvement in whole body glucose metabolism. At this time, the limited number of studies on the topic of exercise and SGU makes it difficult to draw definitive conclusions. However, discrepancies in results may be attributable to the exercise performed (e.g., mode, duration, intensity, etc.), and/or weight loss effects, thereby highlighting the need for future studies to better understand the effect of exercise training on SGU.

In summary, while there are a number of comprehensive studies that have examined the impact of exercise on oral glucose disposition in healthy human subjects and animals, we know relatively little about the responses of patients with T2D. At the current time, it appears that weight loss and/or reduced IHL may improve diminished SGU in this population, whereas exercise training in the absence of weight loss has not been shown to increase SGU in healthy subjects or patients with T2D. Given the liver's prominence in whole-body glucose tolerance and the known dysregulation of this process in T2D, more attention should be placed on discovering the way in

which exercise training impacts post-prandial hepatic glucose metabolism. Most notably, it would be of particular benefit to know the individual and interactive effects of exercise training and weight loss on SGU. Thereafter, the study of variables such as exercise intensity and resistance training should be considered. The low number of relevant studies in the field at this time also contributes to the heterogeneity of responses, making it difficult to make side by side comparisons. Adding to this heterogeneity is the considerable between-study methodological differences (e.g., weight loss, exercise intensity, training duration, low sample size, etc.), which also contributes to differing responses. Moving forward, it will be important to minimize these methodological differences until the key tenets of SGU that are impacted by exercise training in T2D patients are distilled. Such knowledge can then be used to optimize individual treatment strategies for these people.

CONCLUSION

Nearly 40 years ago, it was shown for the first time that impaired hepatic glucose metabolism in patients with T2D can be improved by regular exercise training. Since that time, our knowledge about how this is achieved has greatly expanded from studies ranging from rodents to the human. Despite these developments, fundamental questions remain about how exercise impacts liver glucose metabolism, such as the interaction between lowered IHL and hepatic responses to insulin, the way in which the antagonistic relationship between insulin and glucagon on hepatic glucose metabolism is affected, hepatic responses to different exercise stimuli (e.g., resistance training, intensity-dependent effects) and the nature of the benefit of exercise training on SGU. Furthering our knowledge in this field could lead to the emergence of novel treatment strategies and reduce the negative impact of debilitating vascular complications on the lives of patients with T2D.

AUTHOR CONTRIBUTIONS

SW, MY, and JW wrote and edited the manuscript. RC prepared the figures and provided feedback on the manuscript. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

JW was supported by the NIDDK (R01-DK-106364). Figures were created with BioRender.com.

REFERENCES

1. American Diabetes Association. Economic costs of diabetes in the U.S. in 2017. *Diabetes Care.* (2018) 41:917–28. doi: 10.2337/dci18-0007
2. Defronzo RA. Banting lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes.* (2009) 58:773–95. doi: 10.2337/db09-9028
3. Young FG. Claude Bernard and the discovery of glycogen; a century of retrospect. *Br Med J.* (1957) 1:1431–7. doi: 10.1136/bmj.1.5033.1431
4. Ekberg K, Landau BR, Wajngot A, Chandramouli V, Efendic S, Brunengraber H, et al. Contributions by kidney and liver to glucose production in the postabsorptive state and after 60 h of fasting. *Diabetes.* (1999) 48:292–8. doi: 10.2337/diabetes.48.2.292
5. Cersosimo E, Garlick P, Ferretti J. Renal glucose production during insulin-induced hypoglycemia in humans. *Diabetes.* (1999) 48:261–6. doi: 10.2337/diabetes.48.2.261
6. Meyer C, Dostou JM, Welle SL, Gerich JE. Role of human liver, kidney, and skeletal muscle in postprandial glucose homeostasis. *Am J Physiol Endocrinol Metab.* (2002) 282:E419–27. doi: 10.1152/ajpendo.00032.2001
7. Meyer C, Dostou J, Nadkarni V, Gerich J. Effects of physiological hyperinsulinemia on systemic, renal, and hepatic substrate metabolism. *Am J Physiol.* (1998) 275:F915–21. doi: 10.1152/ajprenal.1998.275.6.F915
8. Bergeron R, Kjaer M, Simonsen L, Bulow J, Galbo H. Glucose production during exercise in humans: a-hv balance and isotopic-tracer measurements compared. *J Appl Physiol.* (1999) 87:111–5. doi: 10.1152/jappl.1999.87.1.111
9. Wahren J, Felig P, Ahlborg G, Jorfeldt L. Glucose metabolism during leg exercise in man. *J Clin Invest.* (1971) 50:2715–25. doi: 10.1172/JCI106772
10. DeFronzo RA, Ferrannini E, Hendler R, Wahren J, Felig P. Influence of hyperinsulinemia, hyperglycemia, and the route of glucose administration on splanchnic glucose exchange. *Proc Natl Acad Sci USA.* (1978) 75:5173–7. doi: 10.1073/pnas.75.10.5173
11. Pagliassotti MJ, Holste LC, Moore MC, Neal DW, Cherrington AD. Comparison of the time courses of insulin and the portal signal on hepatic glucose and glycogen metabolism in the dog. *J Clin Invest.* (1996) 97:81–91. doi: 10.1172/JCI118410
12. Basu R, Chandramouli V, Dicke B, Landau B, Rizza R. Obesity and type 2 diabetes impair insulin-induced suppression of glycogenolysis as well as gluconeogenesis. *Diabetes.* (2005) 54:1942–8. doi: 10.2337/diabetes.54.7.1942
13. Gastaldelli A, Baldi S, Pettiti M, Toschi E, Camastra S, Natali A, et al. Influence of obesity and type 2 diabetes on gluconeogenesis and glucose output in humans: a quantitative study. *Diabetes.* (2000) 49:1367–73. doi: 10.2337/diabetes.49.8.1367
14. Reinmuth OM, Scheinberg P, Bourne B. Total cerebral blood flow and metabolism: a new method for the repeated serial measurement of total cerbral blood flow using Iodoantipyrine (1131) with a report of determination in normal human beings of blood flow, oxygen consumption, glucose utilization and respiratory quotient of the whole brain. *Arch Neurol.* (1965) 12:49–66. doi: 10.1001/archneur.1965.00460250053007
15. Sherwin RS. Role of the liver in glucose homeostasis. *Diabetes care.* (1980) 3:261–5. doi: 10.2337/diacare.3.2.261
16. Boyle PJ, Scott JC, Krentz AJ, Nagy RJ, Comstock E, Hoffman C. Diminished brain glucose metabolism is a significant determinant for falling rates of systemic glucose utilization during sleep in normal humans. *J Clin Invest.* (1994) 93:529–35. doi: 10.1172/JCI117003
17. Dinneen S, Gerich J, Rizza R. Carbohydrate metabolism in non-insulin-dependent diabetes mellitus. *N Engl J Med.* (1992) 327:707–13. doi: 10.1056/NEJM199209033271007
18. Singhal P, Caumo A, Carey PE, Cobelli C, Taylor R. Regulation of endogenous glucose production after a mixed meal in type 2 diabetes. *Am J Physiol Endocrinol Metab.* (2002) 283:E275–83. doi: 10.1152/ajpendo.00424.2001
19. Wasserman DH, Spalding JA, Lacy DB, Colburn CA, Goldstein RE, Cherrington AD. Glucagon is a primary controller of hepatic glycogenolysis and gluconeogenesis during muscular work. *Am J Physiol.* (1989) 257(1 Pt 1):E108–17. doi: 10.1152/ajpendo.1989.257.1.E108
20. Flattem N, Igawa K, Shiota M, Emshwiller MG, Neal DW, Cherrington AD. Alpha- and beta-cell responses to small changes in plasma glucose in the conscious dog. *Diabetes.* (2001) 50:367–75. doi: 10.2337/diabetes.50.2.367
21. Ferrannini E, Wahren J, Faber OK, Felig P, Binder C, DeFronzo RA. Splanchnic and renal metabolism of insulin in human subjects: a dose-response study. *Am J Physiol.* (1983) 244:E517–27. doi: 10.1152/ajpendo.1983.244.6.E517

22. American Diabetes A. Diagnosis and classification of diabetes mellitus. *Diabetes Care.* (2010) 33(Suppl. 1):S62–9. doi: 10.2337/dc10-S062
23. Bogardus C, Lillioja S, Howard BV, Reaven G, Mott D. Relationships between insulin secretion, insulin action, and fasting plasma glucose concentration in nondiabetic and noninsulin-dependent diabetic subjects. *J Clin Invest.* (1984) 74:1238–46. doi: 10.1172/JCI111533
24. DeFronzo RA, Sherwin RS, Kraemer N. Effect of physical training on insulin action in obesity. *Diabetes.* (1987) 36:1379–85. doi: 10.2337/diabetes.36.12.1379
25. Unger RH, Cherrington AD. Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. *J Clin Invest.* (2012) 122:4–12. doi: 10.1172/JCI60016
26. Adkins A, Basu R, Persson M, Dicke B, Shah P, Vella A, et al. Higher insulin concentrations are required to suppress gluconeogenesis than glycogenolysis in nondiabetic humans. *Diabetes.* (2003) 52:2213–20. doi: 10.2337/diabetes.52.9.2213
27. Gastaldelli A, Toschi E, Pettiti M, Frascerra S, Quinones-Galvan A, Sironi AM, et al. Effect of physiological hyperinsulinemia on gluconeogenesis in nondiabetic subjects and in type 2 diabetic patients. *Diabetes.* (2001) 50:1807–12. doi: 10.2337/diabetes.50.8.1807
28. Magnusson I, Rothman DL, Katz LD, Shulman RG, Shulman GI. Increased rate of gluconeogenesis in type II diabetes mellitus. A 13C nuclear magnetic resonance study. *J Clin Invest.* (1992) 90:1323–7. doi: 10.1172/JCI115997
29. Satapati S, Kucejova B, Duarte JA, Fletcher JA, Reynolds L, Sunny NE, et al. Mitochondrial metabolism mediates oxidative stress and inflammation in fatty liver. *J Clin Invest.* (2015) 125:4447–62. doi: 10.1172/JCI82204
30. Burgess SC, He T, Yan Z, Lindner J, Sherry AD, Malloy CR, et al. Cytosolic phosphoenolpyruvate carboxykinase does not solely control the rate of hepatic gluconeogenesis in the intact mouse liver. *Cell Metab.* (2007) 5:313–20. doi: 10.1016/j.cmet.2007.03.004
31. Ramnanan CJ, Edgerton DS, Rivera N, Irimia-Dominguez J, Farmer B, Neal DW, et al. Molecular characterization of insulin-mediated suppression of hepatic glucose production *in vivo*. *Diabetes.* (2010) 59:1302–11. doi: 10.2337/db09-1625
32. Iynedjian PB, Jotterand D, Nouspikel T, Asfari M, Pilot PR. Transcriptional induction of glucokinase gene by insulin in cultured liver cells and its repression by the glucagon-cAMP system. *J Biol Chem.* (1989) 264:21824–9.
33. Caro JF, Triester S, Patel VK, Tapscott EB, Frazier NL, Dohm GL. Liver glucokinase: decreased activity in patients with type II diabetes. *Horm Metab Res.* (1995) 27:19–22. doi: 10.1055/s-2007-979899
34. Clore JN, Stillman J, Sugerman H. Glucose-6-phosphatase flux in vitro is increased in type 2 diabetes. *Diabetes.* (2000) 49:969–74. doi: 10.2337/diabetes.49.6.969
35. Barzilai N, Rossetti L. Role of glucokinase and glucose-6-phosphatase in the acute and chronic regulation of hepatic glucose fluxes by insulin. *J Biol Chem.* (1993) 268:25019–25.
36. Valenti L, Bugianesi E, Pajvani U, Targher G. Nonalcoholic fatty liver disease: cause or consequence of type 2 diabetes? *Liver Int.* (2016) 36:1563–79. doi: 10.1111/liv.13185
37. Dai W, Ye L, Liu A, Wen SW, Deng J, Wu X, et al. Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus: a meta-analysis. *Medicine.* (2017) 96:e8179. doi: 10.1097/MD.0000000000008179
38. Thyfault JP, Rector RS. Exercise combats hepatic steatosis: potential mechanisms and clinical implications. *Diabetes.* (2020) 69:517–24. doi: 10.2337/dbi18-0043
39. Saponaro C, Gaggini M, Gastaldelli A. Nonalcoholic fatty liver disease and type 2 diabetes: common pathophysiologic mechanisms. *Curr Diabetes Rep.* (2015) 15:607. doi: 10.1007/s11892-015-0607-4
40. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol.* (2018) 15:11–20. doi: 10.1038/nrgastro.2017.109
41. Portillo-Sanchez P, Bril F, Maximos M, Lomonaco R, Biernacki D, Orsak B, et al. High prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus and normal plasma aminotransferase levels. *J Clin Endocrinol Metab.* (2015) 100:2231–8. doi: 10.1210/jc.2015-1966
42. Petersen MC, Shulman GI. Mechanisms of insulin action and insulin resistance. *Physiol Rev.* (2018) 98:2133–3. doi: 10.1152/physrev.00063.2017
43. Bogardus C, Ravussin E, Robbins DC, Wolfe RR, Horton ES, Sims EA. Effects of physical training and diet therapy on carbohydrate metabolism in patients with glucose intolerance and non-insulin-dependent diabetes mellitus. *Diabetes.* (1984) 33:311–8. doi: 10.2337/diabetes.33.4.311
44. Rodnick KJ, Haskell WL, Swislocki AL, Foley JE, Reaven GM. Improved insulin action in muscle, liver, and adipose tissue in physically trained human subjects. *Am J Physiol.* (1987) 253(5 Pt 1):E489–95. doi: 10.1152/ajpendo.1987.253.5.E489
45. Segal KR, Edano A, Abalos A, Albu J, Blando L, Tomas MB, et al. Effect of exercise training on insulin sensitivity and glucose metabolism in lean, obese, and diabetic men. *J Appl Physiol.* (1991) 71:2402–11. doi: 10.1152/jappl.1991.71.6.2402
46. Meex RC, Schrauwen-Hinderling VB, Moonen-Kornips E, Schaart G, Mensink M, Phielix E, et al. Restoration of muscle mitochondrial function and metabolic flexibility in type 2 diabetes by exercise training is paralleled by increased myocellular fat storage and improved insulin sensitivity. *Diabetes.* (2010) 59:572–9. doi: 10.2337/db09-1322
47. Shojaee-Moradie F, Baynes KC, Pentecost C, Bell JD, Thomas EL, Jackson NC, et al. Exercise training reduces fatty acid availability and improves the insulin sensitivity of glucose metabolism. *Diabetologia.* (2007) 50:404–13. doi: 10.1007/s00125-006-0498-7
48. Haus JM, Solomon TP, Marchetti CM, Edmison JM, Gonzalez F, Kirwan JP. Free fatty acid-induced hepatic insulin resistance is attenuated following lifestyle intervention in obese individuals with impaired glucose tolerance. *J Clin Endocrinol Metab.* (2010) 95:323–7. doi: 10.1210/jc.2009-1101
49. Coker RH, Williams RH, Yeo SE, Kortebein PM, Bodenner DL, Kern PA, et al. The impact of exercise training compared to caloric restriction on hepatic and peripheral insulin resistance in obesity. *J Clin Endocrinol Metab.* (2009) 94:4258–66. doi: 10.1210/jc.2008-2033
50. Gregory JM, Muldowney JA, Engelhardt BG, Tyree R, Marks-Shulman P, Silver HJ, et al. Aerobic exercise training improves hepatic and muscle insulin sensitivity, but reduces splanchnic glucose uptake in obese humans with type 2 diabetes. *Nutr Diabetes.* (2019) 9:25. doi: 10.1038/s41387-019-0090-0
51. Vendelbo MH, Moller AB, Treebak JT, Gormsen LC, Goodyear LJ, Wojtaszewski JF, et al. Sustained AS160 and TBC1D1 phosphorylations in human skeletal muscle 30 min after a single bout of exercise. *J Appl Physiol.* (2014) 117:289–96. doi: 10.1152/japplphysiol.00044.2014
52. Devlin JT, Hirshman M, Horton ED, Horton ES. Enhanced peripheral and splanchnic insulin sensitivity in NIDDM men after single bout of exercise. *Diabetes.* (1987) 36:434–9. doi: 10.2337/diabetes.36.4.434
53. Camacho RC, Donahue EP, James FD, Berglund ED, Wasserman DH. Energy state of the liver during short-term and exhaustive exercise in C57BL/6J mice. *Am J Physiol Endocrinol Metab.* (2006) 290:E405–8. doi: 10.1152/ajpendo.00385.2005
54. Rogers MA, Yamamoto C, King DS, Hagberg JM, Ehsani AA, Holloszy JO. Improvement in glucose tolerance after 1 wk of exercise in patients with mild NIDDM. *Diabetes Care.* (1988) 11:613–8. doi: 10.2337/diacare.11.8.613
55. Winnick JJ, Sherman WM, Habash DL, Stout MB, Failla ML, Belury MA, et al. Short-term aerobic exercise training in obese humans with type 2 diabetes mellitus improves whole-body insulin sensitivity through gains in peripheral, not hepatic insulin sensitivity. *J Clin Endocrinol Metab.* (2008) 93:771–8. doi: 10.1210/jc.2007-1524
56. Kirwan JP, Solomon TP, Wojta DM, Staten MA, Holloszy JO. Effects of 7 days of exercise training on insulin sensitivity and responsiveness in type 2 diabetes mellitus. *Am J Physiol Endocrinol Metab.* (2009) 297:E151–6. doi: 10.1152/ajpendo.00210.2009
57. Black SE, Mitchell E, Freedson PS, Chipkin SR, Braun B. Improved insulin action following short-term exercise training: role of energy and carbohydrate balance. *J Appl Physiol.* (2005) 99:2285–93. doi: 10.1152/japplphysiol.00291.2005
58. Petersen KF, Dufour S, Befroy D, Lehrke M, Hendler RE, Shulman GI. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes.* (2005) 54:603–8. doi: 10.2337/diabetes.54.3.603
59. Viljanen AP, Iozzo P, Borra R, Kankaanpaa M, Karmi A, Lautamaki R, et al. Effect of weight loss on liver free fatty acid uptake and hepatic insulin resistance. *J Clin Endocrinol Metab.* (2009) 94:50–5. doi: 10.1210/jc.2008-1689

60. Lee S, Bacha F, Hannon T, Kuk JL, Boesch C, Arslanian S. Effects of aerobic versus resistance exercise without caloric restriction on abdominal fat, intrahepatic lipid, and insulin sensitivity in obese adolescent boys: a randomized, controlled trial. *Diabetes.* (2012) 61:2787–95. doi: 10.2337/db12-0214
61. Johnson NA, Sachinwalla T, Walton DW, Smith K, Armstrong A, Thompson MW, et al. Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. *Hepatology.* (2009) 50:1105–12. doi: 10.1002/hep.23129
62. Bacchi E, Negri C, Targher G, Faccioli N, Lanza M, Zoppini G, et al. Both resistance training and aerobic training reduce hepatic fat content in type 2 diabetic subjects with nonalcoholic fatty liver disease (the RAED2 randomized trial). *Hepatology.* (2013) 58:1287–95. doi: 10.1002/hep.26393
63. Holten MK, Zacho M, Gaster M, Juel C, Wojtaszewski JF, Dela F. Strength training increases insulin-mediated glucose uptake, GLUT4 content, and insulin signaling in skeletal muscle in patients with type 2 diabetes. *Diabetes.* (2004) 53:294–305. doi: 10.2337/diabetes.53.2.294
64. Ishii T, Yamakita T, Sato T, Tanaka S, Fujii S. Resistance training improves insulin sensitivity in NIDDM subjects without altering maximal oxygen uptake. *Diabetes Care.* (1998) 21:1353–5. doi: 10.2337/diacare.21.8.1353
65. Holloszy JO. Exercise-induced increase in muscle insulin sensitivity. *J Appl Physiol.* (2005) 99:338–43. doi: 10.1152/japplphysiol.00123.2005
66. Bacchi E, Negri C, Zanolin ME, Milanese C, Faccioli N, Trombetta M, et al. Metabolic effects of aerobic training and resistance training in type 2 diabetic subjects: a randomized controlled trial (the RAED2 study). *Diabetes Care.* (2012) 35:676–82. doi: 10.2337/dc11-1655
67. van der Heijden GJ, Toffolo G, Manesso E, Sauer PJ, Sunehag AL. Aerobic exercise increases peripheral and hepatic insulin sensitivity in sedentary adolescents. *J Clin Endocrinol Metab.* (2009) 94:4292–9. doi: 10.1210/jc.2009-1379
68. van Der Heijden GJ, Wang ZJ, Chu Z, Toffolo G, Manesso E, Sauer PJ, et al. Strength exercise improves muscle mass and hepatic insulin sensitivity in obese youth. *Med Sci Sports Exerc.* (2010) 42:1973–80. doi: 10.1249/MSS.0b013e3181df16d9
69. Honka MJ, Bucci M, Andersson J, Huovinen V, Guzzardi MA, Sandboge S, et al. Resistance training enhances insulin suppression of endogenous glucose production in elderly women. *J Appl Physiol.* (2016) 120:633–9. doi: 10.1152/japplphysiol.00950.2015
70. Croymans DM, Paparisto E, Lee MM, Brandt N, Le BK, Lohan D, et al. Resistance training improves indices of muscle insulin sensitivity and beta-cell function in overweight/obese, sedentary young men. *J Appl Physiol.* (2013) 115:1245–53. doi: 10.1152/japplphysiol.00485.2013
71. Pereira RM, Rodrigues K, Anaruma CP, Sant'Ana MR, de Campos TDP, Gaspar RS, et al. Short-term strength training reduces gluconeogenesis and NAFLD in obese mice. *J Endocrinol.* (2019) 241:59–70. doi: 10.1530/JOE-18-0567
72. Drouin R, Lavoie C, Bourque J, Ducros F, Poisson D, Chiasson JL. Increased hepatic glucose production response to glucagon in trained subjects. *Am J Physiol.* (1998) 274:E23–8. doi: 10.1152/ajpendo.1998.274.1.E23
73. Drouin R, Robert G, Milot M, Massicotte D, Peronnet F, Lavoie C. Swim training increases glucose output from liver perfused *in situ* with glucagon in fed and fasted rats. *Metabolism.* (2004) 53:1027–31. doi: 10.1016/j.metabol.2004.03.010
74. Bonjorn VM, Latour MG, Belanger P, Lavoie JM. Influence of prior exercise and liver glycogen content on the sensitivity of the liver to glucagon. *J Appl Physiol.* (2002) 92:188–94. doi: 10.1152/jappl.2002.92.1.188
75. Legare A, Drouin R, Milot M, Massicotte D, Peronnet F, Massicotte G, et al. Increased density of glucagon receptors in liver from endurance-trained rats. *Am J Physiol Endocrinol Metab.* (2001) 280:E193–6. doi: 10.1152/ajpendo.2001.280.1.E193
76. Winnick JJ, An Z, Ramnanan CJ, Smith M, Irimia JM, Neal DW, et al. Hepatic glycogen supercompensation activates AMP-activated protein kinase, impairs insulin signaling, and reduces glycogen deposition in the liver. *Diabetes.* (2011) 60:398–407. doi: 10.2337/db10-0592
77. Moore MC, Cherrington AD, Cline G, Pagliassotti MJ, Jones EM, Neal DW, et al. Sources of carbon for hepatic glycogen synthesis in the conscious dog. *J Clin Invest.* (1991) 88:578–87. doi: 10.1172/JCI115342
78. Winnick JJ, An Z, Moore MC, Ramnanan CJ, Farmer B, Shiota M, et al. A physiological increase in the hepatic glycogen level does not affect the response of net hepatic glucose uptake to insulin. *Am J Physiol Endocrinol Metab.* (2009) 297:E358–66. doi: 10.1152/ajpendo.00043.2009
79. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes.* (1981) 30:1000–7. doi: 10.2337/diab.30.12.1000
80. Kowalski GM, Moore SM, Hamley S, Selathurai A, Bruce CR. The effect of ingested glucose dose on the suppression of endogenous glucose production in humans. *Diabetes.* (2017) 66:2400–6. doi: 10.2337/db17-0433
81. McGuinness OP, Myers SR, Neal D, Cherrington AD. Chronic hyperinsulinemia decreases insulin action but not insulin sensitivity. *Metabolism.* (1990) 39:931–7. doi: 10.1016/0026-0495(90)90303-T
82. Myers SR, Biggers DW, Neal DW, Cherrington AD. Intraportal glucose delivery enhances the effects of hepatic glucose load on net hepatic glucose uptake *in vivo*. *J Clin Invest.* (1991) 88:158–67. doi: 10.1172/JCI115273
83. Adkins BA, Myers SR, Hendrick GK, Stevenson RW, Williams PE, Cherrington AD. Importance of the route of intravenous glucose delivery to hepatic glucose balance in the conscious dog. *J Clin Invest.* (1987) 79:557–65. doi: 10.1172/JCI112847
84. Pagliassotti MJ, Myers SR, Moore MC, Neal DW, Cherrington AD. Magnitude of negative arterial-portal glucose gradient alters net hepatic glucose balance in conscious dogs. *Diabetes.* (1991) 40:1659–68. doi: 10.2337/diabetes.40.12.1659
85. Capaldo B, Gastaldelli A, Antoniello S, Auletta M, Pardo F, Ciociaro D, et al. Splanchnic and leg substrate exchange after ingestion of a natural mixed meal in humans. *Diabetes.* (1999) 48:958–66. doi: 10.2337/diabetes.48.5.958
86. Ludvik B, Nolan JJ, Roberts A, Baloga J, Joyce M, Bell JM, et al. Evidence for decreased splanchnic glucose uptake after oral glucose administration in non-insulin-dependent diabetes. *J Clin Invest.* (1997) 100:2354–61. doi: 10.1172/JCI119775
87. Ludvik B, Nolan JJ, Roberts A, Baloga J, Joyce M, Bell JM, et al. A noninvasive method to measure splanchnic glucose uptake after oral glucose administration. *J Clin Invest.* (1995) 95:2232–8. doi: 10.1172/JCI117913
88. Tamura Y, Tanaka Y, Sato F, Choi JB, Watada H, Niwa M, et al. Effects of diet and exercise on muscle and liver intracellular lipid contents and insulin sensitivity in type 2 diabetic patients. *J Clin Endocrinol Metab.* (2005) 90:3191–6. doi: 10.1210/jc.2004-1959
89. Bajaj M, Suraamornkul S, Pratipanawatr T, Hardies LJ, Pratipanawatr W, Glass L, et al. Pioglitazone reduces hepatic fat content and augments splanchnic glucose uptake in patients with type 2 diabetes. *Diabetes.* (2003) 52:1364–70. doi: 10.2337/diabetes.52.6.1364
90. Kawamori R, Matsuhisa M, Kinoshita J, Mochizuki K, Niwa M, Arisaka T, et al. Pioglitazone enhances splanchnic glucose uptake as well as peripheral glucose uptake in non-insulin-dependent diabetes mellitus. AD-4833 clamp-OGL study group. *Diabetes Res Clin Pract.* (1998) 41:35–43. doi: 10.1016/S0168-8227(98)00056-4
91. Bajaj M, Pratipanawatr T, Berria R, Pratipanawatr W, Kashyap S, Cusi K, et al. Free fatty acids reduce splanchnic and peripheral glucose uptake in patients with type 2 diabetes. *Diabetes.* (2002) 51:3043–8. doi: 10.2337/diabetes.51.10.3043
92. Kelley D, Mitrakou A, Marsh H, Schwenk F, Benn J, Sonnenberg G, et al. Skeletal muscle glycolysis, oxidation, and storage of an oral glucose load. *J Clin Invest.* (1988) 81:1563–71. doi: 10.1172/JCI113489
93. Vinuela E, Salas M, Sols A. Glucokinase and hexokinase in liver in relation to glycogen synthesis. *J Biol Chem.* (1963) 238:1175–7.
94. van Schaftingen E. A protein from rat liver confers to glucokinase the property of being antagonistically regulated by fructose 6-phosphate and fructose 1-phosphate. *Eur J Biochem.* (1989) 179:179–84. doi: 10.1111/j.1432-1033.1989.tb14538.x
95. de la Iglesia N, Mukhtar M, Seoane J, Guinovart JJ, Agius L. The role of the regulatory protein of glucokinase in the glucose sensory mechanism of the hepatocyte. *J Biol Chem.* (2000) 275:10597–603. doi: 10.1074/jbc.275.14.10597

96. Agius L, Peak M. Intracellular binding of glucokinase in hepatocytes and translocation by glucose, fructose and insulin. *Biochem J.* (1993) 296 (Pt 3):785–96. doi: 10.1042/bj2960785
97. Krssak M, Brehm A, Bernroider E, Anderwald C, Nowotny P, Dalla Man C, et al. Alterations in postprandial hepatic glycogen metabolism in type 2 diabetes. *Diabetes.* (2004) 53:3048–56. doi: 10.2337/diabetes.53.12.3048
98. Basu A, Basu R, Shah P, Vella A, Johnson CM, Jensen M, et al. Type 2 diabetes impairs splanchnic uptake of glucose but does not alter intestinal glucose absorption during enteral glucose feeding: additional evidence for a defect in hepatic glucokinase activity. *Diabetes.* (2001) 50:1351–62. doi: 10.2337/diabetes.50.6.1351
99. Basu A, Basu R, Shah P, Vella A, Johnson CM, Nair KS, et al. Effects of type 2 diabetes on the ability of insulin and glucose to regulate splanchnic and muscle glucose metabolism: evidence for a defect in hepatic glucokinase activity. *Diabetes.* (2000) 49:272–83. doi: 10.2337/diabetes.49.2.272
100. Basu R, Basu A, Johnson CM, Schwenk WF, Rizza RA. Insulin dose-response curves for stimulation of splanchnic glucose uptake and suppression of endogenous glucose production differ in nondiabetic humans and are abnormal in people with type 2 diabetes. *Diabetes.* (2004) 53:2042–50. doi: 10.2337/diabetes.53.8.2042
101. Coate KC, Kraft G, Irimia JM, Smith MS, Farmer B, Neal DW, et al. Portal vein glucose entry triggers a coordinated cellular response that potentiates hepatic glucose uptake and storage in normal but not high-fat/high-fructose-fed dogs. *Diabetes.* (2013) 62:392–400. doi: 10.2337/db12-0417
102. Coate KC, Kraft G, Lautz M, Smith M, Neal DW, Cherrington AD. A high-fat, high-fructose diet accelerates nutrient absorption and impairs net hepatic glucose uptake in response to a mixed meal in partially pancreatectomized dogs. *J Nutr.* (2011) 141:1643–51. doi: 10.3945/jn.111.145359
103. Coate KC, Kraft G, Moore MC, Smith MS, Ramnanan C, Irimia JM, et al. Hepatic glucose uptake and disposition during short-term high-fat vs high-fructose feeding. *Am J Physiol Endocrinol Metab.* (2014) 307:E151–60. doi: 10.1152/ajpendo.00083.2014
104. Coate KC, Kraft G, Shiota M, Smith MS, Farmer B, Neal DW, et al. Chronic overeating impairs hepatic glucose uptake and disposition. *Am J Physiol Endocrinol Metab.* (2015) 308:E860–7. doi: 10.1152/ajpendo.00069.2015
105. Coate KC, Scott M, Farmer B, Moore MC, Smith M, Roop J, et al. Chronic consumption of a high-fat/high-fructose diet renders the liver incapable of net hepatic glucose uptake. *Am J Physiol Endocrinol Metab.* (2010) 299:E887–98. doi: 10.1152/ajpendo.00372.2010
106. Maehlum S, Felig P, Wahren J. Splanchnic glucose and muscle glycogen metabolism after glucose feeding during postexercise recovery. *Am J Physiol.* (1978) 235:E255–60. doi: 10.1152/ajpendo.1978.235.3.E255
107. Rose AJ, Howlett K, King DS, Hargreaves M. Effect of prior exercise on glucose metabolism in trained men. *Am J Physiol Endocrinol Metab.* (2001) 281:E766–71. doi: 10.1152/ajpendo.2001.281.4.E766
108. Hamilton KS, Gibbons FK, Bracy DP, Lacy DB, Cherrington AD, Wasserman DH. Effect of prior exercise on the partitioning of an intestinal glucose load between splanchnic bed and skeletal muscle. *J Clin Invest.* (1996) 98:125–35. doi: 10.1172/JCI118756
109. Kawamori R, Kubota M, Ikeda M, Matsuhisa M, Kubota M, Morishima T, et al. Quantitative determination of hepatic glucose uptake using an innovative approach: effect of strict glycemic regulation and exercise in diabetic subjects. *J Nutr Sci Vitaminol.* (1991) 37(Suppl.):S35–42. doi: 10.3177/jnsv.37.Supplement_S35

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 Endocrinology

BRIEF RESEARCH REPORT
published: 02 September 2020
doi: 10.3389/fendo.2020.00578

Post Meal Exercise May Lead to Transient Hypoglycemia Irrespective of Glycemic Status in Humans

*Jay W. Porter[1], Ryan J. Pettit-Mee[1], Sean T. Ready[1], Ying Liu[1], Guido Lastra[2], Anand Chockalingam[3], Nathan C. Winn[4], Laura Clart[1] and Jill A. Kanaley[1]**

[1] Department of Nutrition and Exercise Physiology, University of Missouri, Columbia, MO, United States, [2] Department of Endocrinology, Internal Medicine, University of Missouri, Columbia, MO, United States, [3] Department of Cardiology, University of Missouri, Columbia, MO, United States, [4] Department of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN, United States

During exercise, there is coordination between various hormonal systems to ensure glucoregulation. This study examined if hypoglycemia occurs during moderate-intensity exercise in non-obese and obese individuals with and without type 2 diabetes (T2D). Eighteen non-obese, 18 obese, and 10 obese with T2D completed 2 study days that included a meal at 1,800 h followed by rest (NOEX) or exercise (PMEX; 45 min/55% of VO_2 max 2 h post meal). Glucose, insulin, and glucagon concentrations were measured throughout this 5.5 h period. Subjects with T2D had elevated glucose responses to the meal on both study days, compared to non-obese and obese subjects ($P < 0.05$). During evening exercise (PMEX), subjects with T2D had a greater drop in glucose concentration (−98.4 ± 13.3 mg/dL) compared to obese (−44.8 ± 7.1 mg/dL) and non-obese (−39.3 ± 6.1 mg/dL; $P < 0.01$) subjects. Glucose levels decreased more so in females than males in both conditions ($P < 0.01$). Nadir glucose levels <70 mg/dL were observed in 33 subjects during NOEX and 39 subjects during PMEX. Obese males had a larger exercise-induced insulin drop than obese females ($P = 0.01$). During PMEX, peak glucagon concentrations were elevated compared to NOEX ($P < 0.001$). Male participants with T2D had an increased glucagon response during NOEX and PMEX compared to females ($P < 0.01$). In conclusion, in individuals with varying glucose tolerance, there is a dramatic drop in glucose levels during moderate-intensity exercise, despite appropriate insulin concentrations prior to exercise, and glucagon levels rising during exercise. Moderate-intensity exercise can result in low glucose concentrations (<60 mg/dL), and yet many of these individuals will be asymptomatic.

Keywords: hypoglycemia, exercise, type 2 diabetes, glucagon, glucose

OPEN ACCESS

Edited by:
Kristian Karstoft, Centre for Physical Activity Research, Rigshospitalet, Denmark

Reviewed by:
David Berrigan, National Cancer Institute (NCI), United States
Carmine Grieco, Colorado Mesa University, United States

****Correspondence:***
Jill A. Kanaley
kanaleyj@missouri.edu

Specialty section:
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Endocrinology

Received: *30 May 2020*
Accepted: *16 July 2020*
Published: *02 September 2020*

Citation:
Porter JW, Pettit-Mee RJ, Ready ST, Liu Y, Lastra G, Chockalingam A, Winn NC, Clart L and Kanaley JA (2020) Post Meal Exercise May Lead to Transient Hypoglycemia Irrespective of Glycemic Status in Humans. Front. Endocrinol. 11:578. doi: 10.3389/fendo.2020.00578

INTRODUCTION

During exercise, as carbohydrate utilization increases and muscle glycogen stores begin to decline, potentially blood glucose levels can begin to drop. Low blood glucose levels can result in dizziness, confusion, nausea, headache, blurred vision, etc., all of which could impact exercise performance. Mammals, however, have evolved to prevent a precipitous decline in glucose concentrations via the counterregulatory hormones to keep the levels in a very tight range during rest and exercise (1).

If hypoglycemia does occur during exercise, it manifests when intense exercise is initiated shortly after the consumption of a high carbohydrate food or during prolonged exercise as fuel sources become depleted (2, 3). In the 1970's and 80's, considerable research was conducted examining the effects of exercise on glucose levels, particularly in prolonged exercise. Much of this work (4–6) focused on the impact of the pre-exercise meal prior to prolonged exercise to prevent hypoglycemia in moderate to well-trained individuals. Additionally, many of these studies were conducted following a 6–12 h fast, and studied effects of feeding 15–90 min before exercise. Few people, however, truly fast prior to exercise resulting in a postprandial state when initiating exercise. Early literature suggests that there is a risk of rebound hypoglycemia if exercise follows the meal too closely. Recently, Kondo et al. (6) demonstrated that transient hypoglycemia after pre-exercise carbohydrate ingestion (30 min prior) occurred in 7/16 subjects with values below 72 mg/dL, and 3 subjects were below 54 mg/dL. Moreover, they noted that individuals with an enhanced insulin response to the pre-exercise meal tended to be more prone to transient hypoglycemia in the fasted state.

Much of the previous work (5, 6) has shown hypoglycemia in well-trained individuals, usually in males, and in response to prolonged exercise. It is unclear if untrained individuals experience hypoglycemia, or if hypoglycemia is impacted by metabolic health. This project examined if hypoglycemia occurs during moderate-intensity exercise in the evening in non-obese and obese individuals with and without type 2 diabetes (T2D). Previous work by our group (7) showed that resistance exercise 45 min post dinner meal reduced glucose in T2Ds but had a rebound glucose response following exercise. Delaying an exercise session until after the dinner meal may provide an optimal time to improve glycemia prior to bedtime. We hypothesized that non-obese and obese individuals without T2D would have better counterregulatory control and thus would have tighter glucose control.

METHODS

Subjects

Eighteen non-obese, 18 obese, and 10 obese+T2D, males and females (25–65 yrs. of age) were recruited and signed an informed consent approved by the University of Missouri Institutional Review Board. Body mass index (BMI) was between 30 and 45 kg/m^2 for obese subjects and <25 kg/m^2 for non-obese subjects. Subjects were weight stable for at least the prior 6 months, and non-smokers. All subjects also had a screening oral glucose tolerance test (OGTT). Non-obese subjects had fasting glucose <100 mg/dL and a 2 h glucose level <140 mg/dL. Subjects also had a screening exercise stress test. Women on oral contraceptives were tested in the pill phase. Pregnant women were excluded.

The T2D subjects were either diagnosed as T2D by their physician or documented fasting glucose levels >110 mg/dL for 5 of 7 days (8). Diabetic participants withheld medications for glucose control the night prior to and during the study day.

Experimental Design

This study is part of a larger project in progress (clinicaltrials.gov, #NCT03019510). Following initial screening, subjects completed two study nights in a counter-balanced design; (1) no exercise (NOEX), and (2) evening exercise−2 h post dinner (PMEX). Study day arrival was ∼1,700 h and standardized dinner meal consumption began at 1,800 h. Blood sampling was initiated from ∼1,740 h until 0700 h, however this paper will focus on the first 5 h of sampling. On an exercise day, subjects exercised for 45 min at ∼55% VO_2 peak on the treadmill.

Screening Day: Anthropometrics and Questionnaires

Height, weight, and waist circumference were measured. Fat mass, fat-free mass, and percent body fat were assessed using a Bod Pod (Life Measurements, Concord, CA) or DEXA (Horizon A, Hologic, Marlborough, MA). All subjects had a screening OGTT, and hematocrit (Hct) was measured. Further, questionnaires were completed on the screening day to determine their inclusion in the study, and included a health inventory (9) and sleep apnea (Berlin questionnaire) (10).

All subjects completed a peak oxygen consumption test (VO_2 peak) on a treadmill. Prior to starting the test, electrodes were placed for EKG and heart rate measurements during the test. The ventilation, and percent oxygen and carbon dioxide were measured by True One 240 Metabolic Measurement Cart; ParvoMedics (Sandy, UT) and VO_2 was calculated. The test began with 2 min of very slow walking (2 mph). Speed and incline of the treadmill were increased every 2 min until the subject reached volitional exhaustion (11). Following the exercise test, subjects actively cooled down and were monitored for 5–10 min until HR and BP returned to near baseline.

Study Day

The evening prior to the study night, subjects were provided with a dinner meal (∼600 kcal; 56.1% Carbohydrate, 21.5% Fat, and 22.4% Protein) as well as breakfast, snack, and lunch for the study day (**Table 1**). Subjects consumed the breakfast at ∼0700 h and lunch at 1,200 h. Subjects then fasted, except for water for the remainder of the day. There was no exercise or alcohol consumption for the 24 h prior to the study day.

At 1,630 h, subjects reported to the lab ∼5 h fasted. An intravenous catheter was placed into a forearm vein. Baseline blood samples began at ∼1,740 h. At 1,800 h, subjects consumed a mixed meal providing 10 kcal/kg of body weight with 1 gram/kg of carbohydrate (target macronutrient composition: 40% Carbohydrate, 35% Fat, 25% Protein; Actual kcal and macronutrients for each group listed in **Table 1**). Carbohydrates were capped at 90 grams for 7 obese and 6 T2D subjects, except for 5 obese and 1 T2D who received >90 grams of carbohydrate prior to the decision to cap the total amount of carbohydrates for participants due to gastrointestinal distress. Following the meal, subjects sat quietly until bedtime. In the PMEX condition, subjects exercised at 55% of VO_2 peak for 45 min on the treadmill at 2,000 h. Blood samples were collected at the following time points: −20, 0, 5, 10, 15, 20, 30, 40, 50, 60, and then every 15 min until the completion of the study. Blood was collected in EDTA

TABLE 1 | Subject characteristics and meal composition for the non-obese, obese, and type 2 diabetic subjects.

	Non-obese	Obese	T2D
N (Male, Female)	18 (9,9)	18 (5,13)	10 (2,8)
Age (years)	45.7 ± 3.6	42.6 ± 3.5	53.9 ± 1.9
Height (cm)	168.5 ± 2.1	168.0 ± 2.2	168.9 ± 3.4
Weight (kg)	69.6 ± 2.3	95.8 ± 4.0*	96.3 ± 9.4*
BMI (kg/m^2)	24.4 ± 0.5	33.8 ± 1.0*	33.9 ± 1.8*
Body Fat (%)	27.9 ± 2.3	44.3 ± 1.8*	42.7 ± 3.0*
Fasting Glucose (mg/dL)	83.8 ± 2.5†	84.5 ± 1.8†	131.9 ± 9.4
VO_2 peak (mL/kg/min)	36.5 ± 1.9	26.8 ± 1.1*	23.7 ± 1.7*
Breakfast, snack, and lunch			
Calories (kcal)	914.7 ± 40.5	1048.6 ± 70.7	962.2 ± 96.4
Carbohydrate (%)	52.1 ± 0.0	55.0 ± 0.1	50.7 ± 0.0
Fat (%)	28.3 ± 0.1	29.9 ± 0.0	27.7 ± 0.0
Protein (%)	14.3 ± 0.0	15.1 ± 0.0	13.9 ± 0.0
Study dinner			
Calories (kcal)	718.6 ± 72.2	919.6 ± 38.3	794.0 ± 28.5
Carbohydrate (%)	37.9 ± 0.4	40.4 ± 0.3	36.9 ± 0.0
Fat (%)	33.1 ± 0.1	35.0 ± 0.1	32.3 ± 0.0
Protein (%)	23.7 ± 0.6	24.6 ± 0.5	23.1 ± 0.0

*Mean ± SE; *p < 0.05 different than Non-Ob; †p < 0.05 different than type 2 diabetic (T2D).*

tubes with and without DPP-IV inhibitor and aprotinin, placed on ice, centrifuged, aliquoted, and stored at −80°C until analysis. Blood samples were analyzed for glucose (YSI 2300 STAT PLUS, YSI Incorporated, Yellow Springs, OH), and insulin and glucagon concentrations (Human Metabolic Hormone Panel, Milliplex, Millipore Sigma), as well as other hormones not reported here. There was a minimum of 3 weeks between study days.

Statistics and Data Analysis

For this study, we have defined low blood glucose as 70 mg/dL according to the American Diabetes Association (12, 13), but also examined the data below 50 and 60 mg/dL. Calculations were made for the Insulinogenic Index [insulin (30–0 min)/glucose (30–0 min)] and Matsuda Index (14). Baseline characteristics were analyzed by one-way ANOVA for statistical differences between groups. Repeated measure ANOVA (RMANOVA) tested interactions between study days (NOEX, PMEX), with a between-subject factor group (non-obese, obese, obese+T2D) and sex (Male, Female). Responses to the meal over time were tested with RMANOVA with study day by time (25 time points between −20 and 300 min) with a between-subject factors of group and sex. *Post-hoc* analysis were performed with a one-way ANOVA and Tukey comparison to explore group differences within dependent variables. Significance set at $P < 0.05$, and data are presented as mean ± SEM.

RESULTS

By design, the non-obese group was significantly lighter, had a lower percent body fat, and a lower BMI than the obese subjects with and without T2D ($P < 0.05$) (**Table 1**). The mean age for all subjects was 46.3 ± 2.1 yrs. Fasting glucose levels were in the normal range for both the non-obese and obese subjects. The non-obese subjects were more aerobically fit than the obese subjects with and without T2D ($P < 0.05$, **Table 1**), yet were still considered sedentary.

Glucose

Baseline glucose levels (∼1,800 h) were similar between study days, but the individuals with T2D (99.4 ± 6.6 mg/dL) had higher baseline glucose levels than the obese and non-obese subjects (79.8 ± 5.4 mg/dL; 76.6 ± 4.0 mg/dL, respectively, group effect: $P < 0.05$). The meal responses revealed a study day by time by group interaction ($P < 0.001$), such that during NOEX, glucose concentrations were elevated for T2D compared to non-obese adults from time −20 min until 15 min post meal consumption ($P < 0.05$), and from 30 to 300 min ($P < 0.05$) compared to both non-obese and obese subjects (**Figure 1A**). During PMEX in individuals with T2D, glucose concentrations were elevated compared to the obese and non-obese groups at all time points ($P < 0.05$, **Figure 1C**).

Peak glucose responses to the meal were similar between study days, but individuals with T2D had peak values of 248.5 ± 11.1 mg/dL, which was significantly greater than both obese and non-obese individuals (164.8 ± 8.7 mg/dL; 170.6 ± 8.2 mg/dL, respectively, $P < 0.001$). The time of the glucose peak was not different between study days or groups.

Glucose concentrations 2 h post meal consumption (start time of PMEX) were elevated during NOEX (145.9 ± 6.5 mg/dL) compared to PMEX (134.1 ± 6.7 mg/dL; $P = 0.01$, **Figures 1B,D**). Subjects with T2D had higher glucose concentrations (191.2 ± 12.7 mg/dL) at time 120 than both obese (122.6 ± 5.0 mg/dL) and non-obese subjects (106.2 ± 9.5 mg/dL; $P < 0.001$). Glucose concentrations at the start of exercise had a study day by group interaction ($P < 0.01$). *Post-hoc* analysis revealed that subjects with T2D had a greater drop in glucose concentration during PMEX (−94.8 ± 13.3 mg/dL) compared to both obese (−44.8 ± 7.1 mg/dL; $P < 0.01$) and non-obese (−39.3 ± 8.7 mg/dL; $P < 0.01$) subjects. Further examination of the change in glucose levels from the beginning to the end of exercise showed a trend for a study day by group by gender interaction ($P = 0.08$), where significant differences were observed by gender ($P < 0.01$). From time 120 to 165, glucose levels decreased more so in females than males in both conditions (NOEX, −55.0 ± 4.1 vs. −29.5 ± 6.8 mg/dL, $P < 0.01$; PMEX, −66.0 ± 6.3 vs. −38.9 ± 10.4 mg/dL, $P = 0.03$). Additionally, females with T2D had a larger drop in glucose levels from time 120 to 165 than males in both conditions (NOEX, −66.8 ± 7.8 vs. −23.5 ± 15.5 mg/dL, respectively, $P = 0.02$; PMEX, −109.9 ± 11.9 vs. −34.5 ± 23.7 mg/dL, respectively, $P < 0.01$).

The blood glucose nadir was different between study day and groups, with a nadir of 80.1 ± 5.0 mg/dL in subjects with T2D, 60.2 ± 4.0 mg/dL in obese subjects, and 53.3 ± 3.7 mg/dL in non-obese subjects; T2D had a higher nadir than both other groups ($P < 0.01$, **Figures 1B,D**). However, hypoglycemia (<70 mg/dL) was prevalent amongst all study days for at least some individuals in all groups. During NOEX, 17 non-obese, 12 obese, and 4 T2D

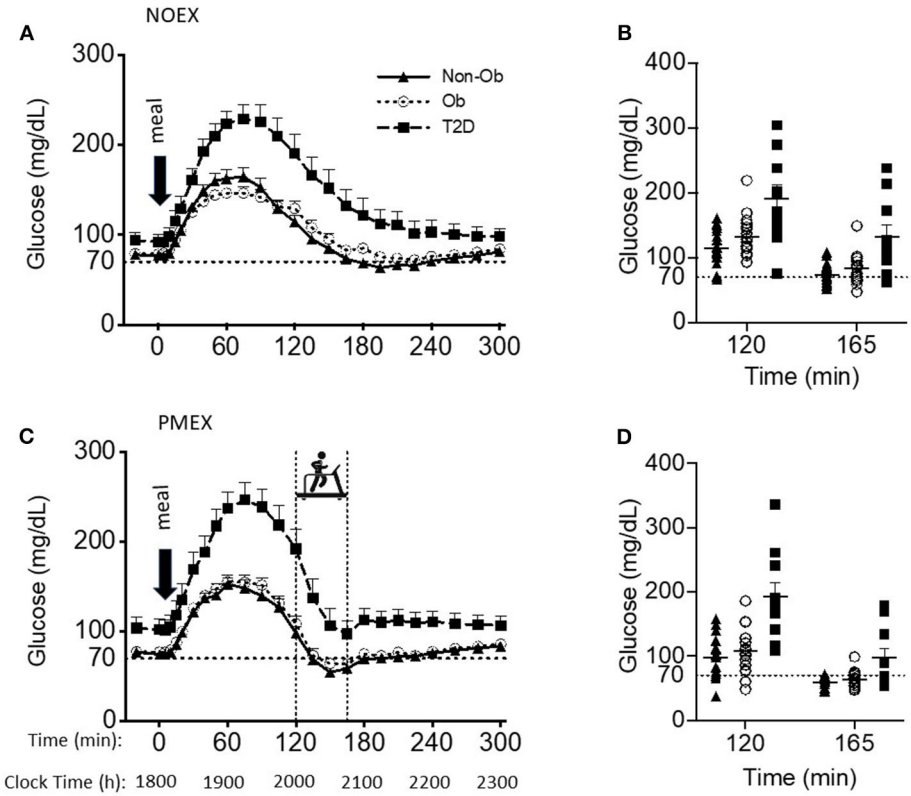


FIGURE 1 | Glucose responses to a dinner meal in non-obese and obese individuals with and without type 2 diabetes (T2D) on a study day **(A)** with no post dinner exercise or a study day **(C)** with exercise (PMEX- 45 min, at 55%VO_2 max). Individual glucose values at time point 120 and time point 165 in each group on the NOEX day **(B)** or PMEX day **(D)**. Mean ± SE.

experienced hypoglycemia, while during PMEX, 17 non-obese, 17 obese, and 5 T2D experienced hypoglycemia. Collectively, 25 participants (15 Non-obese, 10 obese, and 2 obese+T2D) experienced hypoglycemia throughout both conditions. More severe hypoglycemia 60 mg/dL (and 50 mg/dL) was also noted in our subjects: during NOEX, 12 (3) non-obese, 7 (2) obese, and 2 (0) obese+T2D; during PMEX 16 (14) non-obese, 14 (6) obese, and 4 (1) obese+T2D. Only 2 obese+T2D were symptomatic, and this occurred at glucose levels above 70 mg/dL.

Insulin

Baseline insulin concentrations had a main effect of study day ($P < 0.05$), where NOEX was elevated compared to PMEX ($P = 0.001$). Insulin responses to the dinner meal had a study day by time by group interaction ($P < 0.001$), and *post-hoc* analysis revealed that individuals with T2D had elevated insulin concentrations compared to non-obese participants during NOEX from 135 to 225 min post meal consumption ($P < 0.05$). During NOEX, obese participants were elevated compared to non-obese at times 60, 75, and 135 ($P < 0.05$, **Figure 2A**). During PMEX, insulin concentrations in obese individuals were elevated compared to non-obese participants from 20 to 120 min post meal, and insulin concentrations in subjects with T2D were elevated compared to non-obese subjects at times 120, 195, 210, 225, and 240 (**Figure 2C**). Five hours after the dinner meal, insulin concentrations were similar between all groups in all conditions.

Peak insulin responses to the dinner meal were not different between study day, but non-obese participants had lower peak insulin concentrations (95.9 ± 21.9 μU/mL) compared to obese (187.2 ± 23.3 μU/mL; $P < 0.01$) and lower than T2D (183.8 ± 28.5 μU/mL; $P = 0.02$, **Figure 2B**). Time of peak insulin was not different between study days or groups.

Two hours post meal, insulin concentrations were not different by study day, but insulin concentrations were lower in non-obese subjects (70.6 ± 20.1 μU/mL) compared to both other groups (obese 145.5 ± 21.4μU/mL, $P = 0.02$; T2D 153.7 ± 26.3 μU/mL, $P = 0.02$). The change in insulin levels from the beginning of exercise (time 120) to the end of exercise (time 165) had a study day by group interaction ($P = 0.03$), such that non-obese individuals (39.2 ± 8.1 μU/mL) had less of a drop during PMEX compared to T2D (109.7 ± 30.1 μU/mL; $P < 0.05$) and tended to have less of a drop compared to obese (102.3 ± 22.6 μU/mL; $P = 0.06$). The insulin response to exercise had a gender by group interaction ($P < 0.05$), where obese males had a larger drop in insulin compared to obese females (160.8 ± 30.1 vs. 69.9 ± 14.5 μU/mL; $P < 0.01$). Nadir insulin concentrations were different by study day ($P < 0.001$) but not between subject group (**Figures 2B,D**). Participants experienced the lowest insulin concentrations during PMEX

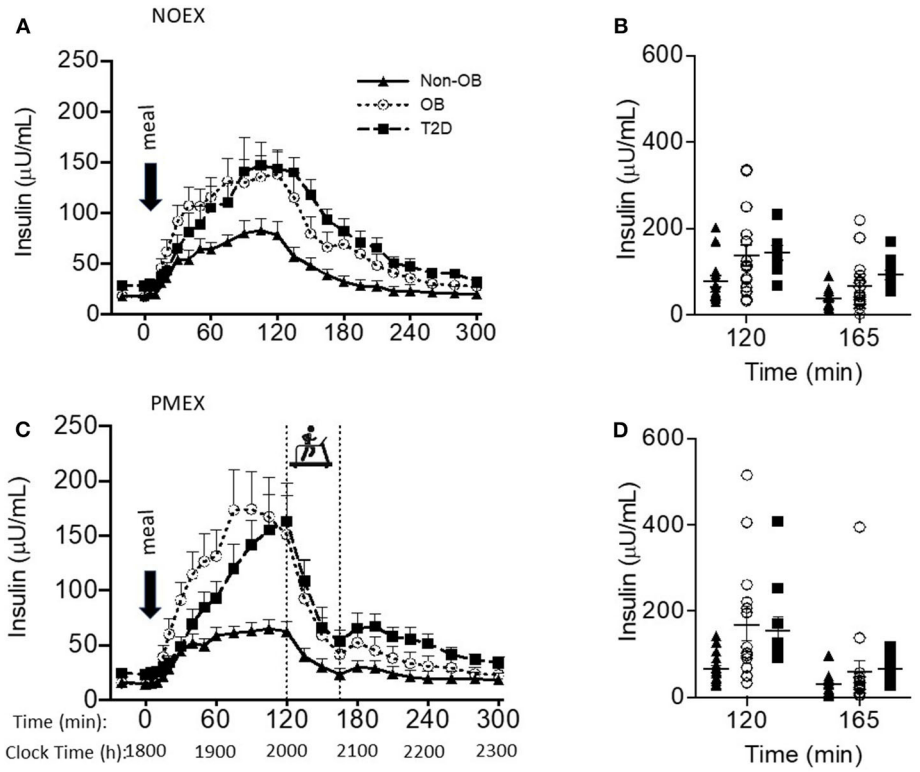


FIGURE 2 | Insulin responses to a dinner meal in non-obese and obese individuals with and without type 2 diabetes (T2D) on a study day **(A)** with no post dinner exercise or a study day **(C)** with exercise (PMEX- 45 min, at 55%VO_2 max). Individual glucose values at time point 120 and time point 165 in each group on the NOEX day **(B)** or PMEX day **(D)**. Mean ± SE.

(18.8 ± 3.1 μU/mL) compared to NOEX (23.6 ± 3.4 μU/mL, $P < 0.001$).

Insulin Sensitivity

Matsuda calculations were different between study day ($P < 0.05$) and group ($P < 0.01$). Non-obese participants had a higher Matsuda index than participants with T2D (7.8 ± 1.2 vs. 1.9 ± 1.5, respectively; $P < 0.01$); obese participants had a Matsuda index (5.3 ± 1.2) that was similar to the T2D. No gender differences were noted for the Matsuda index.

Insulinogenic index to the dinner meal was not different between study days but was significantly different between groups. Obese subject's insulinogenic index (1.6 ± 0.2) was highest compared to all other groups (non-obese, 0.6 ± 0.2; T2D, 0.5 ± 0.3; $P < 0.01$). We found no relationship between insulin sensitivity (Matsuda and insulin index) and the change in glucose levels during exercise, but the glucose nadir and insulin nadir during PMEX was negatively correlated with the Matsuda Index ($r = -0.331$, $P < 0.05$ and $r = -0.476$, $P < 0.001$, respectively).

The insulinogenic index was negatively correlated with the exercise-induced change in glucose on both study days (NOEX, $r = -0.362$, $P < 0.05$; PMEX, $r = -0.399$, $P < 0.01$) and positively with exercise-induced changes in insulin on both study days (NOEX, $r = 0.586$, $P = 0.001$; PMEX, $r = 0.394$, $P < 0.03$).

Glucagon

Baseline glucagon concentrations were different by study day ($P < 0.05$), with higher levels on the NOEX study day (53.4 ± 5.3 ng/L) compared to PMEX (47.7 ± 5.6 ng/L; $P < 0.01$). A group by gender interaction was shown for baseline glucagon concentrations ($P < 0.05$), where T2D males had higher concentrations compared to females (98.8 ± 22.7 ng/L vs. 40.4 ± 11.3 ng/L; $P < 0.05$). Although the response to the dinner meal had a study day by time interaction ($P < 0.001$), with NOEX having elevated glucagon levels compared to PMEX at times 0, 5, 10, 40, and 60, but lower than PMEX at times 150, 165, 180, 195, and 210 ($P < 0.05$ for all time points, **Figures 3A,C**). Glucagon concentrations were similar after 5 h. During PMEX, peak glucagon concentrations were elevated (180.1 ± 13.0 ng/L) compared to NOEX (126.2 ± 9.8 ng/L; $P < 0.001$). A gender by group interaction was also seen for peak glucagon concentrations with T2D males having higher peak values than females (257.5 ± 42.5 ng/L vs. 123.4 ± 21.2 ng/L; $P < 0.01$). The time of peak glucagon concentrations occurred earlier with PMEX (183.8 ± 5.6 min) than the NOEX condition (239.8 ± 5.4 min, $P < 0.001$) (**Figures 3B,D**). Additionally, obese males peak later than females (237.5 ± 12.9 min vs. 208.1 ± 6.5 min; gender by group interaction, $P < 0.05$).

Two hours post meal consumption, no differences in glucagon concentrations were observed by study days. In response to

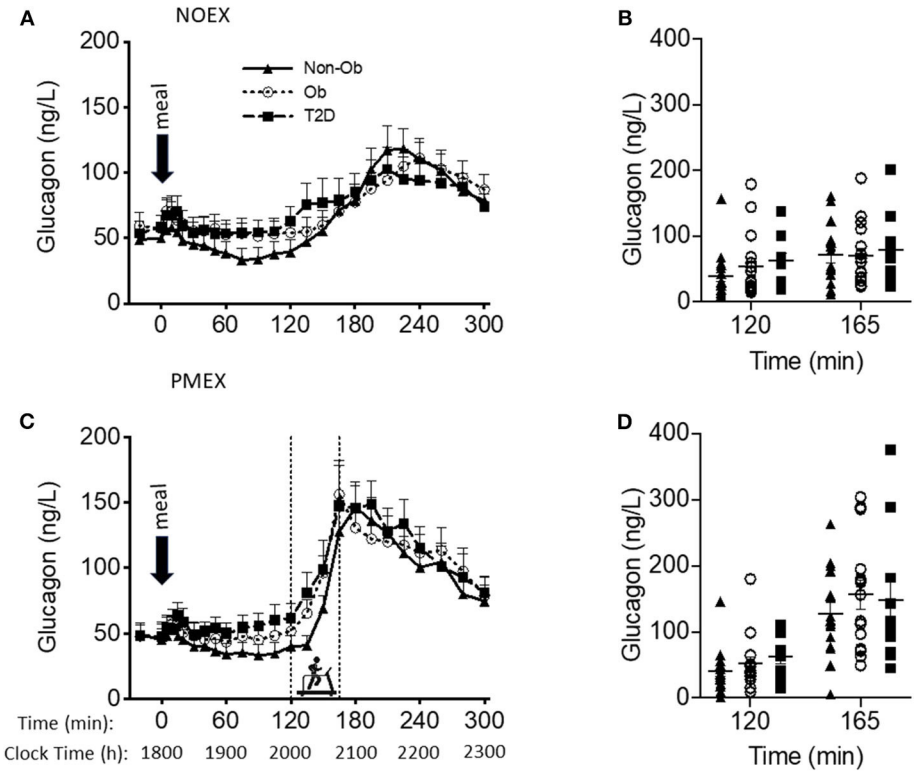


FIGURE 3 | Glucagon responses to a dinner meal in non-obese and obese individuals with and without type 2 diabetes (T2D) on a study day **(A)** with no post dinner exercise or a study day **(C)** with exercise (PMEX- 45 min, at 55%VO_2 max). Individual glucose values at time point 120 and time point 165 in each group on the NOEX day **(B)** or PMEX day **(D)**. Mean ± SE.

PMEX (time 120 to 165), glucagon levels increased by 95.2 ± 11.5 ng/L compared to NOEX at 22.3 ± 3.6 ng/L, $P < 0.001$. There was a study day by group by gender interaction ($P = 0.02$), such that male participants with T2D had an increased glucagon response during NOEX and PMEX compared to females (NOEX, 46.0 ± 15.5 vs. 8.2 ± 7.8 ng/L, $P = 0.04$; PMEX, 228.2 ± 45.0 vs. 50.4 ± 22.5 ng/L, $P = 0.001$).

DISCUSSION

This is one of the first studies to examine the occurrence of moderate-intensity exercise related hypoglycemia in untrained individuals following a dinner meal. We demonstrate here that in a group of relatively sedentary individuals, a dinner meal itself resulted in low glucose levels ∼2 h post dinner, and in many individuals, there was a further exercise-induced decline, increasing the likelihood of hypoglycemia. This occurred in light of the fact that insulin concentrations were not exceedingly high prior to exercise, and glucagon levels were rising. Additionally, this large drop in glucose levels occurred in all subjects despite very different metabolic profiles. Upon completion of exercise, glucose concentrations in all subjects rebounded back to normoglycemic levels, an appropriate counterregulatory response. Further females with T2D experienced a larger drop in glucose levels with moderate-intensity exercise than seen in men.

It is well-known that exercise results in a dramatic increase in glucose turnover, which is a function of increased muscle utilization and increased production by the liver (15, 16), and this occurs in an intensity-dependent manner. If glucose production fails to stay abreast of the pace of glucose uptake, blood glucose concentrations fall. Changes in the hormonal milieu occur, with a fall in insulin levels, and increased catecholamines and glucagon levels in an effort to maintain blood glucose levels. An early study (17) showed that hypoglycemia occurs in moderately active men during prolonged exercise with an exaggerated rise in plasma epinephrine. Simultaneously others (18) have noted that maintaining glucoregulation during moderate-intensity exercise can result in decrements in insulin, increments in glucagon, or both may occur, particularly if catecholamine release is not adequate. Adrenergic means are primarily involved in the prevention of hypoglycemia during exercise. Although we did not measure catecholamines, our subjects showed appropriate hormonal responses during PMEX with a 67% decrease in insulin levels and ∼3.8-fold increase in glucagon levels. Despite an appropriate hormonal response in our subjects, glucose levels declined considerably in most individuals. This is in agreement with an early study (19) that also showed decreases in glucose levels with low intensity walking and similarly demonstrated a 4-fold increase in glucagon levels. We had anticipated that the non-obese and obese subjects would not display the same degree of

hypoglycemia as individuals with T2D, because we hypothesized they would have better counterregulatory control, due to their enhanced metabolic flexibility.

Surprisingly almost all of our non-obese and obese subjects had glucose levels below 70 mg/dL during moderate-intensity exercise, as well as 50% of the individuals with T2D. Even more noteworthy is that ∼89% of the non-obese had glucose concentrations below 60 mg/dL, with 11 of them with low values on both study days, and 14 individuals with values below 50 mg/dL during exercise. Likewise, 39% of the obese subjects had glucose values below 60 mg/dL on the NOEX condition, and 78% on the PMEX condition. In individuals with T2D, 20% had glucose values below 60 mg/dL during NOEX, but 40% went below 60 mg/dL during PMEX. These low glucose levels have been reported before (4) but in response to prolonged exercise. Surprisingly only on two occasions did subjects become symptomatic, and in both conditions, this occurred in individuals with T2D in the absence of true hypoglycemia. This suggests that during exercise, there is not a distinct threshold for hypoglycemia and symptoms (20), and despite low circulating glucose levels, the brain must be sensing adequate glucose levels (20).

The susceptibility to hypoglycemia may be linked to the degree of insulin sensitivity of the individual. We found that insulin sensitivity (Matsuda) correlated modestly negative with the change in insulin during exercise. Further, we found no relationship between insulin sensitivity (Matsuda and insulin index) and the change in glucose levels during exercise, but the glucose nadir and insulin nadir during PMEX was negatively correlated the Matsuda Index indicating that the more insulin sensitive an individual was, the lower the glucose and insulin concentration went during exercise. This alludes that more insulin sensitive individuals may be more prone to these low glucose concentrations and remain asymptomatic despite very low glucose levels across adults of various glycemic control. It should also be considered that the lack of relationship with Matsuda may be due to the fact that insulin is not necessary for glucose disposal during exercise. However, Jentjens et al. found no relationship between hypoglycemia and insulin sensitivity (21). Recently, Kondo et al. (6) showed a transient exercise-induced hypoglycemia (at min 15 of exercise) in those individuals who were fasted overnight and ingested carbohydrates 30 min prior to exercise. These individuals had higher insulin concentrations at the start of exercise when compared to their counterparts that did not display hypoglycemia. Kuipers et al. (3) suggested that the occurrence of hypoglycemia may be due to a combination of enhanced insulin sensitivity, the quantity of glucose ingested, and low sympathetic activity.

Most of the previous reports on hypoglycemia have primarily focused on men. Earlier research has reported that blood glucose levels decline to a greater extent in premenopausal women than in men during prolonged fasting (22). Here we found that females demonstrated a greater drop in glucose levels 2–2.5 h post meal regardless of exercise. The females with T2D decreased glucose levels by 30.7% while the men decreased by 19.9% during rest and even larger decrease with exercise, 51.8% for females, and 32.0% for men. The smaller decrease in men may be attributed to a greater response in glucagon concentrations, thus maintaining their blood glucose levels. In response to exercise, T2D men had 4.5-fold higher glucagon responses during exercise compared to female T2D, with an even larger fold increase (5.6-fold) during the same time period but with rest. Potentially women with T2D may be more susceptible to hypoglycemia as glucose substantially drops 2 h after an evening meal without a compensatory glucagon response.

Human studies have shown circadian rhythms for glucose tolerance and energy metabolism, displaying enhanced glucose/meal tolerance in the morning over the nighttime hours (23). Much of the prior research examining transient hypoglycemia follows a 10 h overnight fast or with a small meal prior to exercise. Due to the circadian variations in glucose tolerance/insulin sensitivity, it may be possible that post dinner exercise may lower glucose levels more dramatically than seen in the morning hours. We have previously shown that glucose integrated area under the curve was reduced by ∼18% when exercise preceded the dinner meal, while it decreased by ∼30% when exercise was after the dinner meal (7). Considerably more research needs to be conducted examining the effect of exercise timing during the day, particularly in individuals with insulin resistance and/or impaired fasting glucose levels.

Most studies have focused on exercise-induced hypoglycemia in the early morning period after prolonged fasting or having a small pre-exercise meal (3, 5, 6, 21). Further, much of this research only studied young healthy men, thus a strength of this project is that both men and women were included, subjects were sedentary to moderately active, and female participants were both pre- and post-menopausal. An additional strength of this study is the large sample size, which included individuals with varying degrees of insulin sensitivity. A limitation of this study is that unlike previous work, this study only examined the glucose response beyond the evening meal and did not compare to a similar meal earlier in the day, thus it is unclear if we would have seen the same degree of hypoglycemia following a breakfast meal. However, we do not think this could explain our higher incidence of hypoglycemia as Van Cauter et al. (24) reported declines in the glucose tolerance from morning to evening in healthy individuals.

In conclusion, moderate-intensity post-meal exercise in the evening results in transient hypoglycemia in many individuals across a spectrum of glycemic status. More work needs to be conducted examining exercise at different times of the day to establish if the same phenomena is occurring in the morning or early afternoon.

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 studies involving human participants were reviewed and approved by University of Missouri Institutional Review

Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR'S NOTE

Exercise-induced hypoglycemia has primarily been investigated by the effects on performance by examining meal timing, quantity of carbohydrate, and fasting vs. fed states, in an attempt to establish the best protocol to minimize hypoglycemia. What is unclear is whether moderate-intensity exercise causes hypoglycemia, and is it related to metabolic health, such as insulin sensitivity. In this study, 46 non-obese, obese and obese+type 2 diabetes (T2D) men and women completed two conditions: (1) in the evening, following a dinner meal over 5 h (NOEX) and (2) preforming 45 min of moderate-intensity exercise 2 h post dinner meal (PMEX). We observed a similar glucose, insulin, and glucagon response to the meal on both occasions, with obese+T2D having the highest glucose responses. Glucose levels 70 mg/dL was prevalent in the evenings of both conditions, with 33 subjects during NOEX and 39 during PMEX. Severe hypoglycemia (50 mg/dL) was also seen, with 5 subjects during NOEX and 21 during PMEX (all but on 2 occasions were asymptomatic). The postmeal glucose decline was greater in females than males. In conclusion, a dinner meal can result in low glucose levels but moderate-intensity exercise can cause hypoglycemia in some individuals, which is usually asymptomatic.

AUTHOR CONTRIBUTIONS

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

FUNDING

This study was funded by NIH R01DK101513. This grant supported the project and some of the co-authors.

ACKNOWLEDGMENTS

We would like to thank the nurses at the Clinical Research Center for all of their support in the blood drawing.

REFERENCES

1. Sotsky MJ, Shilo S, Shamoon H. Regulation of counterregulatory hormone secretion in man during exercise and hypoglycemia. *J Clin Endocrinol Metab*. (1989) 68:9–16. doi: 10.1210/jcem-68-1-9
2. Koivisto VA, Harkonen M, Karonen SL, Groop PH, Elovainio R, Ferrannini E, et al. Glycogen depletion during prolonged exercise: influence of glucose, fructose, or placebo. *J Appl Physiol. (*1985) 58:731–7. doi: 10.1152/jappl.1985.58.3.731
3. Kuipers H, Fransen EJ, Keizer HA. Pre-exercise ingestion of carbohydrate and transient hypoglycemia during exercise. *Int J Sports Med*. (1999) 20:227–31. doi: 10.1055/s-2007-971122
4. Foster C, Costill DL, Fink WJ. Effects of preexercise feedings on endurance performance. *Med Sci Sports*. (1979) 11:1–5.
5. Koivisto VA, Karonen SL, Nikkila EA. Carbohydrate ingestion before exercise: comparison of glucose, fructose, and sweet placebo. *J Appl Physiol Respir Environ Exerc Physiol*. (1981) 51:783–7. doi: 10.1152/jappl.1981.51.4.783
6. Kondo S, Tanisawa K, Suzuki K, Terada S, Higuchi M. Preexercise carbohydrate ingestion and transient hypoglycemia: fasting versus feeding. *Med Sci Sports Exerc*. (2019) 51:168–73. doi: 10.1249/MSS.0000000000001773
7. Heden TD, Winn NC, Mari A, Booth FW, Rector RS, Thyfault JP, et al. Postdinner resistance exercise improves postprandial risk factors more effectively than predinner resistance exercise in patients with type 2 diabetes. *J Appl Physiol.* (2015) 118:624–34. doi: 10.1152/japplphysiol.00917.2014
8. Cederberg H, Saukkonen T, Laakso M, Jokelainen J, Harkonen P, Timonen M, et al. Postchallenge glucose, A1C, and fasting glucose as predictors of type 2 diabetes and cardiovascular disease: a 10-year prospective cohort study. *Diabetes Care*. (2010) 33:2077–83. doi: 10.2337/dc10-0262
9. Heden TD, Liu Y, Park YM, Nyhoff LM, Winn NC, Kanaley JA. Moderate amounts of fructose- or glucose-sweetened beverages do not differentially alter metabolic health in male and female adolescents. *Am J Clin Nutr*. (2014) 100:796–805. doi: 10.3945/ajcn.113.081232
10. Chung F, Yegneswaran B, Liao P, Chung SA, Vairavanathan S, Islam S, et al. Validation of the Berlin questionnaire and American Society of Anesthesiologists checklist as screening tools for obstructive sleep apnea in surgical patients. *Anesthesiology*. (2008) 108:822–30. doi: 10.1097/ALN.0b013e31816d91b5
11. Kanaley JA, Goulopoulou S, Franklin RM, Baynard T, Holmstrup ME, Carhart R Jr, et al. Plasticity of heart rate signalling and complexity with exercise training in obese individuals with and without type 2 diabetes. *Int J Obes*. (2009) 33:1198–206. doi: 10.1038/ijo.2009.145
12. American Diabetes A. Glycemic targets: standards of medical care in diabetes-2019. *Diabetes Care*. (2019) 42:S61–70. doi: 10.2337/dc19-S006
13. NIH NIDDK. *Low Blood Glucose (Hypoglycemia)*. NIH NIDDK (2020). Available online at: https://www.niddk.nih.gov/health-information/diabetes/overview/preventing-problems/low-blood-glucose-hypoglycemia (accessed May 25, 2020).
14. Matsuda M, Defronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care*. (1999) 22:1462–70. doi: 10.2337/diacare.22.9.1462
15. Vranic M, Berger M. Exercise and diabetes mellitus. *Diabetes*. (1979) 28:147–63. doi: 10.2337/diab.28.2.147
16. Ahlborg G, Felig P. Lactate and glucose exchange across the forearm, legs, and splanchnic bed during and after prolonged leg exercise. *J Clin Invest*. (1982) 69:45–54. doi: 10.1172/JCI110440
17. Felig P, Cherif A, Minagawa A, Wahren J. Hypoglycemia during prolonged exercise in normal men. *N Engl J Med*. (1982) 306:895–900. doi: 10.1056/NEJM198204153061503
18. Tuttle KR, Marker JC, Dalsky GP, Schwartz NS, Shah SD, Clutter WE, et al. Glucagon, not insulin, may play a secondary role in defense against hypoglycemia during exercise. *Am J Physiol*. (1988) 254:E713–19. doi: 10.1152/ajpendo.1988.254.6.E713
19. Ahlborg G, Felig P. Substrate utilization during prolonged exercise preceded by ingestion of glucose. *Am J Physiol*. (1977) 233:E188–94. doi: 10.1152/ajpendo.1977.233.3.E188
20. Jeukendrup AE, Killer SC. The myths surrounding pre-exercise carbohydrate feeding. *Ann Nutr Metab.* (2010) 57(Suppl. 2):18–25. doi: 10.1159/000322698
21. Jentjens RL, Jeukendrup AE. Prevalence of hypoglycemia following pre-exercise carbohydrate ingestion is not accompanied By higher insulin sensitivity. *Int J Sport Nutr Exerc Metab*. (2002) 12:398–413. doi: 10.1123/ijsnem.12.4.398
22. Fajans SS, Floyd JC Jr. Fasting hypoglycemia in adults. *N Engl J Med*. (1976) 294:766–72. doi: 10.1056/NEJM197604012941408
23. Poggiogalle E, Jamshed H, Peterson CM. Circadian regulation of glucose, lipid, and energy metabolism in humans. *Metabolism*. (2018) 84:11–27. doi: 10.1016/j.metabol.2017.11.017

24. Van Cauter E, Shapiro ET, Tillil H, Polonsky KS. Circadian modulation of glucose and insulin responses to meals: relationship to cortisol rhythm. *Am J Physiol*. (1992) 262:E467–75. doi: 10.1152/ajpendo.1992.262.4.E467

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 Endocrinology

ORIGINAL RESEARCH
published: 10 September 2020
doi: 10.3389/fendo.2020.00632

Individual Response Variation in the Effects of Weight Loss and Exercise on Insulin Sensitivity and Cardiometabolic Risk in Older Adults

*Andrea M. Brennan, Robert A. Standley, Fanchao Yi, Elvis A. Carnero, Lauren M. Sparks and Bret H. Goodpaster**

Translational Research Institute, AdventHealth Research Institute, Orlando, FL, United States

OPEN ACCESS

Edited by:
John P. Thyfault, University of Kansas Medical Center, United States

Reviewed by:
Jill Kanaley, University of Missouri, United States
Sudip Bajpeyi, The University of Texas at El Paso, United States

***Correspondence:**
Bret H. Goodpaster
bret.goodpaster@adventhealth.com

Specialty section:
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Endocrinology

Received: *28 May 2020*
Accepted: *04 August 2020*
Published: *10 September 2020*

Citation:
Brennan AM, Standley RA, Yi F, Carnero EA, Sparks LM and Goodpaster BH (2020) Individual Response Variation in the Effects of Weight Loss and Exercise on Insulin Sensitivity and Cardiometabolic Risk in Older Adults. Front. Endocrinol. 11:632. doi: 10.3389/fendo.2020.00632

Weight loss induced by decreased energy intake (diet) or exercise generally has favorable effects on insulin sensitivity and cardiometabolic risk. The variation in these responses to diet-induced weight loss with or without exercise, particularly in older obese adults, is less clear. The objectives of our study were to (1) examine the effect of weight loss with or without exercise on the variability of responses in insulin sensitivity and cardiometabolic risk factors and (2) to explore whether baseline phenotypic characteristics are associated with response. Sedentary older obese (BMI 36.3 ± 5.0 kg/m^2) adults (68.6 ± 4.7 years) were randomized to one of 3 groups: health education control (HED); diet-induced weight loss (WL); or weight loss and exercise (WL + EX) for 6 months. Composite Z-scores were calculated for changes in insulin sensitivity (C_IS: rate of glucose disposal/insulin at steady state during hyperinsulinemic euglycemic clamp, HOMA-IR, and HbA1C) and cardiometabolic risk (C_CMR: waist circumference, triglycerides, and fasting glucose). Baseline measures included body composition (MRI), cardiorespiratory fitness, *in vivo* mitochondrial function (ATPmax; P-MRS), and muscle fiber type. WL + EX groups had a greater proportion of High Responders in both C_IS and C_CMR compared to HED and WL only (all $p < 0.05$). Pre-intervention measures of insulin ($r = 0.60$) and HOMA-IR ($r = 0.56$) were associated with change in insulin sensitivity (C_IS) in the WL group ($p < 0.05$). Pre-intervention measures of glucose ($r = 0.55$), triglycerides ($r = 0.53$), and VLDL ($r = 0.53$) were associated with change in cardiometabolic risk (C_CMR) in the WL group ($p < 0.05$), whereas triglycerides ($r = 0.59$) and VLDL ($r = 0.59$) were associated with C_CMR (all $p < 0.05$) in WL + EX. Thus, the addition of exercise to diet-induced weight loss increases the proportion of older obese adults who improve insulin sensitivity and cardiometabolic risk. Additionally, individuals with poorer metabolic status are more likely to experience greater improvements in cardiometabolic risk during weight loss with or without exercise.

Keywords: individual variability, weight loss, exercise, insulin sensitivity, response, cardiometabolic risk, older adults

INTRODUCTION

Aging is associated with increased adiposity, insulin resistance and a higher prevalence of cardiometabolic disease (1). Current evidence suggests that weight loss induced by decreased energy intake or increased energy expenditure improves insulin sensitivity and cardiometabolic risk factors in older adults (2). While limited data exists on the effect of diet-induced weight loss alone on individual variability in cardiometabolic and glycemic control outcomes, several groups have observed substantial heterogeneity in individual responses to exercise-induced weight loss with or without dietary changes. The first observations of interindividual variability in glycemic control indices following exercise stem from the HERITAGE Family Study, wherein ∼600 healthy sedentary individuals completed a 20-week supervised training intervention (3). Although there were statistically significant increases with training in insulin sensitivity measured by intravenous glucose tolerance test (IVGTT) at the group level, the authors noted that ∼42% of participants showed no change or an adverse response. Similar response variation in HOMA-IR (4), HbA1C, fasting glucose, and 2-h oral glucose tolerance test (OGTT) glucose (5) was observed after both high intensity interval training (4) and continuous aerobic exercise (5). An important limitation of the preceding study designs is lack of a time-matched control group which abrogates the ability to account for response variation due to technical error and/or biological fluctuations (6, 7). Additionally, whether diet-induced weight loss with or without exercise differentially improves response variation in glycemic control and cardiometabolic risk is unknown, albeit potentially important for clinicians providing personalized lifestyle counsel.

While there is little doubt that response variation to exercise interventions exists, the characteristics that distinguish those who do and do not respond favorably are unclear. Multiple observations have suggested that baseline glycemic control is a key factor that predicts the magnitude of an individual's response for glycemic control outcomes. Evidence from a 3 month aerobic exercise training intervention in older obese adults illustrated an inverse relationship between baseline fasting glucose and change in fasting glucose (8), suggesting that those with higher baseline glycemia had a blunted response to exercise. Similarly, following a 3–4 month exercise training intervention in 105 individuals with prediabetes or type 2 diabetes mellitus (T2DM), Solomon et al. (5) observed a non-linear U-shaped relationship between baseline HbA1c and change in HbA1C, postulating that individuals with relatively controlled hyperglycemia respond well to training, while individuals with poor glycemic control have blunted improvements or even deteriorations. However, not all findings are consistent with the aforementioned results. Recent findings wherein 285 participants aged 18–75 years participated in a 12-week lifestyle intervention including both dietary and exercise guidance suggested that those who experienced greater improvement in glucose tolerance presented with higher baseline weight, visceral fat, fasting glucose, and triglyceride concentration compared to those who did not respond (9). Thus, it is unclear whether and how baseline phenotypes influence insulin sensitivity responses to diet and exercise-induced weight loss, and response likely depends on multiple factors.

Several gaps in our current knowledge exist concerning response variation in weight loss: (1) examination of the independent (and potentially additive) effect of exercise, particularly in higher risk older obese adults; (2) the use of a control group to examine technical and/or biological changes; and (3) a more wholistic classification of the clinically meaningful outcome that includes a cluster of interrelated responses. Thus, the objectives of our study are 2-fold: (1) to examine the effect of weight loss with or without exercise on the range of responses in insulin sensitivity and cardiometabolic risk factors in a vulnerable older obese population at risk for chronic disease; (2) to perform a comprehensive assessment of the relationships between baseline clinical, metabolic, and skeletal muscle traits and changes in response to weight loss with or without exercise. We conducted a randomized controlled trial to examine the effect of energy restriction-induced weight loss with or without exercise on insulin sensitivity and skeletal muscle function in older obese adults, providing a unique opportunity to address these aims. We hypothesize that the addition of exercise to diet-induced weight loss will increase the number of individuals who respond favorably to intervention, based on improvements in insulin sensitivity and cardiometabolic risk. Our findings may provide mechanistic and clinical insight into response variation to weight loss interventions in this vulnerable population.

MATERIALS AND METHODS

Participants

The participants included in this analysis were a subset of participants enrolled in a larger RCT (unpublished; NCT number: NCT02230839). We conducted a single site, 6-month randomized controlled trial with a parallel group design between 2012 and 2017. The trial operations began at the University of Pittsburgh and subsequently moved to AdventHealth Translational Research Institute (AH TRI) upon re-appointment of the Primary Investigator. Eighty-six older (60–80 years of age), sedentary ($\leq$ 1 continuous exercise session/week) men and women with obesity (BMI $\geq$ 30 kg/m^2) were randomized into one of three treatments: Control (HED; health education); energy restriction-induced weight loss (WL; 10% weight loss), and weight loss with exercise (WL + EX; progressive, moderate intensity supervised exercise sessions). All participants provided informed consent prior to participation and the protocols used in the original investigation and this secondary analysis were approved by both University of Pittsburgh Research Ethics Board and Institutional Review Board of AdventHealth. Participants from the original trial were excluded if they did not have both pre- and post- outcome data ($n = 25$) which resulted in a study sample of 61 participants: HED, $n = 20$; WL, $n = 21$; WL + EX, $n = 20$.

Health Education (HED) Group

Participants randomized to the HED group received bi-weekly in-person general health education group sessions for the 6-month study duration, including informational seminars on

medication and type 2 diabetes management. Each session lasted ∼1 h. However, they were not given specific exercise or dietary education/prescription.

Energy Restriction-Induced Weight Loss (WL) Group

The goal of the WL intervention was to produce a weight loss of 10% of baseline body weight. Using the Harris-Benedict equation corrected for the activity factor, a reduction of 500–1,000 kcal/day based on baseline body weight was prescribed in addition to a low-fat (<30% of kilocalories from fat) diet. Participants met individually with the Registered Dietitian and/or designated staff weekly to record body weight and receive dietary prescription (∼1 h). To eliminate the confounding effects of acute caloric restriction on insulin sensitivity, the dietitian aimed to keep participant weights stable during the last 2 weeks of intervention.

Weight Loss and Exercise (WL + EX) Group

Participants completed a progressive 6-month exercise training program, 4–5 days per week, 45 min per session (180 min per week) consisting of mostly walking (both outside and on an indoor treadmill) and the option to include stationary cycling, elliptical and rowing machines. All indoor exercise was supervised by a trained monitor; aerobic exercise performed outdoors was not supervised. Beginning at week 8, participants also performed 2, non-consecutive resistance exercise sessions per week, 30 min per session, focused on major muscle groups using resistance exercise machines. Aerobic exercise was performed at 50–80% $HR_{reserve}$. The resistance exercises were performed at the highest weight the participant could achieve for the given number of reps (10–12) with proper form. When the participant reached 3 × 12 reps, we increased the weight and reduced the reps. Blood pressure and heart rate were measured for participant safety prior to each exercise session, in addition to weekly body weight. Participants in the WL + EX group also met with the Registered Dietitian and/or designated staff and received the same dietary instruction as the WL group.

Outcomes

Body Composition

Weight and height were measured pre- and post- intervention, and BMI was calculated. Waist circumference was measured using the Gulick II tape measure directly on the skin. Fat mass and fat-free mass were determined by dual-energy X-ray absorptiometry (DXA) using a GE Lunar (GE Healthcare, UK).

Additionally, abdominal and thigh adipose tissue (AT) and muscle volume was measured by MRI at baseline and following treatment on a 3 Tesla magnet (Philips Acheiva) at AH TRI. The MRI scan was performed at the mid-point of the femur to quantify thigh muscle cross-sectional area, subcutaneous, and intermuscular AT (IMAT). For abdominal AT images, high resolution axial images were taken of the entire abdomen to quantify abdominal subcutaneous and visceral AT volume. Resultant images were analyzed using Analyze 11.0 (Biomedical Imaging Resource, Mayo Clinic, Rochester, MN) to segment AT and muscle depots and measure volume.

Cardiorespiratory Fitness and *in vivo* Mitochondrial Function

A VO_{2max} graded exercise test was performed by an exercise physiologist on the cycle ergometer using open circuit indirect calorimetry. Following a standardized warm-up, participants exercised at a moderate intensity with the workload (resistance) increased gradually until they reached volitional fatigue.

In vivo muscle mitochondrial function (ATPmax) was calculated using the PCR recovery time constant (τ) and the PCr level in oxygenated muscle at rest in the *vastus lateralis* using phosphorus (^{31}P) magnetic resonance spectroscopy on the 3-T magnet as previously described (10).

Insulin Sensitivity

Insulin sensitivity was measured using the hyperinsulinemic-euglycemic clamp. Participants arrived at the research facility prior to the clamp procedure, consumed a standard American meal, and stayed overnight in the metabolic ward. After an overnight fast, an intravenous catheter was placed in the antecubital vein for subsequent insulin and glucose infusions and for stable isotope infusions to measure insulin sensitivity. A primed constant infusion of [6,6-2H2] ran throughout the clamp procedure. An additional catheter was placed in the heated hand vein in the contralateral arm to attain arterialized blood samples for blood glucose determination and for [6,6-2H2] glucose enrichment during the insulin and glucose infusions. After a 2.5-h baseline period, an insulin infusion was started and continued for 4 h @ 40 mU/m^2-min. Glucose was measured at 5-min intervals and maintained at 90 mg/dl. A 2 ml blood sample was collected at 0, 30, 60, 100, 110, and 120 min as well as every 10 min during the last 30 min of the clamp for GCMS determination of [6,6-2H2] glucose enrichment. Insulin and FFA samples were also drawn at multiple time points throughout the clamp. Skeletal muscle insulin sensitivity (R_d/Insulin) was assessed as the rate of glucose disposal (mg/min) accounting for insulin during steady state. Hepatic insulin sensitivity was assessed as the suppression of endogenous glucose production (EGP) during steady state using the glucose enrichment data.

Blood Analyses

Lipid profiles (total cholesterol, HDL, LDL, VLDL, and triglycerides) and HbA1C were measured by a fasting blood draw and analyzed in the clinical chemistry laboratory at AH TRI using standard assays. Insulin resistance was also quantified using HOMA-I*R* = fasting plasma insulin (mU/L)*fasting plasma glucose (mg/dL)/405.

Muscle Biopsy

During fasting conditions and following 30–45 min after the start of the glucose clamp, a percutaneous muscle biopsy of the *vastus lateralis* was performed using previously published methods (11). A biopsy sample was taken 10–15 cm above the knee under local anesthesia with a 5-mm Bergstrom needle and suction. A portion of the tissue was prepared for immunohistochemistry.

Histochemical analyses were performed on serial sections using established methods in our laboratory (12). Briefly, muscle was placed vertically in mounting medium on cork and frozen

in isopentane cooled with liquid nitrogen. Biopsy samples were sectioned (10 um) using a cryotome and fixed prior to staining. Sections were incubated in a primary antibody cocktail at 4°C overnight [BA-F8 (type I; IgG2b; 1:50); 6H1 (type IIX; IgM; 1:50); and SC-71 (type IIA; IgG1; 1:50)]. All antibodies were obtained from the University of Iowa Hybridoma Bank. Subsequently, slides were incubated in secondary antibody cocktail consisting of DyLight 405 (IgG2b; 1:500), Alexa Fluor 555 (IgM; 1:500), and Alexa Fluor 488 (IgG1; 1:500). AlexaFluor 647-conjugated wheat germ agglutinin (WGA) was used to stain glycoconjugate (N-acetylglucosamine and N-acetylneuraminic acid) residues. Digital images (4X magnification) of one section per skeletal muscle biopsy were captured using a Nikon eclipse Ti microscope (Nikon Technologies, California) and image analysis was performed using NIS elements software 4.20.01.

Statistical Analysis

One-way ANOVAs were performed to evaluate baseline differences between groups. In cases where the assumption of normality (assessed using the Shapiro Wilk test) was not met, baseline comparisons between groups for these specific variables were performed using the non-parametric Kruskal Wallis test.

Two composite scores (C_Score) were developed to assess changes in insulin sensitivity measures and cardiometabolic risk factors using the average of standardized z scores. C_Scores for both outcomes were calculated using the following equation:

$$\text{Composite Score}: \text{C} = \frac{z_1 + z_2 + z_3}{3}$$

$$\text{Where } z_i = \frac{x_i - \overline{x}_i}{s_i},$$

$\overline{x}_i$ is the sample mean of variable i,

s_i is the sample standard deviation of variable i.

The C_Score for insulin sensitivity (C_IS) comprises the % change in R_d/Insulin from the clamp (x = % change), % change in HOMA-IR [x = –(% change)] and change in HbA1C [x = –(% change)]. The C_Score for cardiometabolic risk (C_CMR) comprises the % change in waist circumference [x = –(% change)], % change in triglycerides [x = –(% change)], and % change in fasting glucose [x = –(% change)]. The median of the composite scores was used to categorize participants as either "Low Responders" or "High Responders," where High Responders had C_Score ≥ the median C_Score and conversely, Low Responders had C_Score < the median C_Score.

One-way ANOVAs were performed to assess between-group differences for C_IS and C_CMR. When a significant difference for the overall model was detected, a Tukey's *post-hoc* test for multiple comparisons was performed. To determine the effects of HED, WL and WL + EX on the proportion of individuals who were classified as "High Responder" and "Low Responder," a 2 × 3 contingency table was generated, and group proportions were compared using the chi-square test. Because the WL and WL + EX groups were not matched for weight change, a one-way ANOVA was performed to assess between-group differences in weight change adjusted for baseline body weight. Additionally, regression analyses were run to assess the relationship between change in body weight and the composite scores, collapsed across the WL and WL + EX groups. Relationships between baseline values of participant characteristics and intervention-induced changes reflected by C_Scores were determined using Pearson correlation coefficients in both the WL and WL + EX groups. Statistical analysis was completed using GraphPad Prism version 8.1.2 for Windows (GraphPad Software, San Diego, California USA) and IBM SPSS Statistics for Macintosh, Version 25 (Armonk, NY:IBM Corp).

RESULTS

Participant baseline characteristics and ranges of percent change following intervention are summarized in **Table 1**. There were no significant differences in baseline characteristics between the HED, WL, and WL + EX groups.

To ensure that the C_Scores were capturing a favorable change in insulin sensitivity and cardiometabolic risk, respectively, simple correlations between the C_Score and each of its components were assessed. C_IS was significantly correlated with an increase in R_d/Insulin ($r = 0.66$, $p < 0.0001$), and a decrease in HOMA-IR ($r = -0.82$, $p < 0.0001$) and HbA1C ($r = -0.62$, $p < 0.05$). C_CMR was significantly correlated with a decrease in waist circumference ($r = -0.65$, $p < 0.0001$), fasting glucose ($r = -0.68$, $p < 0.0001$), and fasting triglycerides ($r = -0.56$, $p < 0.001$). Individual responses for each component of the C_Scores are illustrated in **Supplementary Figures 1**, **2**.

Between group comparisons for C_IS and C_CMR in addition to individual responses are shown in **Figures 1**, **2**. The WL + EX group had a greater mean C_IS compared to both the WL and HED groups ($p < 0.05$, **Figure 1A**). The WL + EX group also had a greater mean C_CMR compared to the HED group ($p < 0.05$, **Figure 2A**), but not the WL group.

For C_IS, the WL and WL + EX groups had a greater proportion of High Responders (HR) compared to the HED group (HR proportions: HED = 32%, WL = 46%, WL + EX = 83%). In addition, the WL + EX group had a greater proportion of High Responders compared to the WL only group ($X^2 = 8.54$, $p = 0.014$; **Figure 1B**). Similarly, for C_CMR, the WL + EX group had a greater proportion of High Responders compared to both the WL and HED groups (HR proportions: HED = 39%, WL = 40%, WL + EX = 74%) ($X^2 = 6.12$, $p < 0.05$; **Figure 2B**). The WL and WL + EX groups differed in body weight change (WL vs. WL + EX: −7.1 ± 4.6 kg vs. −10.6 ± 4.9 kg; $p < 0.05$). C_IS ($r = -0.42$; $p < 0.05$), but not C_CMR ($p > 0.05$), was significantly associated with weight change.

Associations between C_Scores and baseline characteristics, including body composition, clinical laboratory measures, aerobic fitness, insulin sensitivity, and fiber type are summarized in **Table 2**. For change in insulin sensitivity (C_IS), pre-intervention measures of insulin ($r = 0.60$) and HOMA-IR ($r = 0.56$) were positively associated with C_IS in the WL group only ($p < 0.05$). For change in cardiometabolic risk (C_CMR) glucose ($r = 0.55$),

TABLE 1 | Baseline participant characteristics (mean ± SD) and range of change following intervention (%).

	HED		WL		WL + EX	
	Baseline	**Range of % change**	**Baseline**	**Range of % change**	**Baseline**	**Range of % change**
n	20		21		20	
Male:Female	7:13		7:14		8:12	
Age (yr)	70.1 ± 4.8		70.0 ± 4.6		66.8 ± 3.4	
Medication use (no. of participants)						
Statins	9		7		7	
Metformin	6		3		4	
Other Anti-hyperglycemic agents	1		2		1	
Body composition						
Weight (kg)	97.8 ± 10.5	−7.7 to 5.8	101.4 ± 20.3	−17.1 to 1.3	102.9 ± 13.2	−21.0 to (–)4.0
Body mass index (kg/m^2)	35.7 ± 4.4	−7.8 to 5.5	36.1 ± 5.1	−16.5 to 1.3	37.3 ± 5.4	−17.4 to (–)3.5
Fat mass (kg)	44.7 ± 9.4	−14.8 to 8.8	46.7 ± 11.2	−36.3 to 5.9	47.1 ± 10.2	−37.5 to (–)4.5
Fat free mass (kg)	53.6 ± 5.9	−9.3 to 5.9	54.3 ± 12.3	−9.0 to 3.0	56.1 ± 9.5	−9.1 to 3.2
Waist circumference (cm)	114.9 ± 9.9	−10.1 to 6.6	116.4 ± 13.6	−16.1 to 20.0	118.5 ± 14.3	−16.7 to 5.4
Abdominal AT (kg)	20.7 ± 3.3	−14.0 to 16.5	21.2 ± 2.1	−20.6 to 0.2	22.7 ± 5.2	−28.4 to to (–)4.5
Abdominal subcutaneous AT (kg)	14.3 ± 2.6	−17.9 to 5.9	12.7 ± 2.6	−19.7 to 7.6	14.7 ± 3.9	−28.1 to (–)4.8
Abdominal visceral AT (kg)	6.4 ± 1.8	−24.8 to 58.8	8.4 ± 3.1	−23.2 to 16.1	8.0 ± 2.8	−37.0 to 4.7
Thigh intermuscular AT (kg)	0.38 ± 0.09	−13.1 to 42.4	0.42 ± 0.17	−14.8 to 17.9	0.48 ± 0.14	−33.0 to 5.2
Clinical measurements						
SBP (mmHg)	140 ± 11	−16.9 to 11.6	135 ± 15	−29.2 to 20.8	135 ± 11	−18.9 to 14.7
DBP (mmHg)	74 ± 8	−25.0 to 26.2	75 ± 11	−23.3 to 26.9	73 ± 12	−23.3 to 24.1
Insulin (pmol/l)	97.9 ± 46.5	−60.9 to 71.1	103.5 ± 68.8	−58.5 to 35.8	109.7 ± 55.6	−54.6 −54.6 to 46.9
Glucose (mmol/l)	6.0 ± 1.0	−28.8 to 78.6	5.5 ± 0.6	−0.8 to 0.2	6.1 ± 1.2	−37.9 to 6.9
HbA1C (%)	6.3 ± 0.8	−1.9 to 1.7	5.9 ± 0.4	−12.1 to 3.3	6.3 ± 0.9	−2.9 to 0.1
HOMA-IR	3.8 ± 2.5	−62.8 to 80.1	4.5 ± 3.5	−63.6 to 29.8	5.0 ± 3.7	−61.6 to 57.1
GIR/I (mg/kgFFM/min/Insulin)	0.08 ± 0.04	−53.2 to 100	0.08 ± 0.05	−40.5 to 196	0.07 ± 0.04	13.5 to 116
Triglycerides (mmol/l)	1.66 ± 0.63	−43.4 to 91.8	1.54 ± 0.76	−76.6 to 83.6	1.80 ± 0.76	−81.2 to 162
Cholesterol (mmol/l)	4.94 ± 0.98	−30.5 to 60.3	4.64 ± 0.95	−24.8 to 80.1	4.76 ± 0.98	−43.3 to 16.8
LDL-Cholesterol (mmol/l)	2.79 ± 0.85	−44.1 to 100	2.63 ± 0.86	−20.9 to 201	2.76 ± 0.85	−52.3 to 52.5
HDL-Cholesterol (mmol/l)	1.38 ± 0.44	−13.2 to 43.6	1.30 ± 0.38	−15 to 51.3	1.17 ± 0.19	−25.0 to 22.7
VLDL-Cholesterol (mmol/l)	0.77 ± 0.29	−44.8 to 90	0.71 ± 0.35	−77.0 to 83.3	0.83 ± 0.35	−81.5 to 170
Plasma free fatty acids (mmol/l)	0.47 ± 0.20	−93.1 to 885	0.53 ± 0.12	-42.4 to 33.1	0.56 ± 0.22	−74.8 to 216
Aerobic fitness						
VO_{2max} (L/min)	1.7 ± 0.5	−26.2 to 34.9	1.5 ± 0.5	−30.9 to 80.3	1.7 ± 0.5	−13.3 to 31.1
VO_{2max} (ml/kgFFM/min)	31.2 ± 7.4	−27.8 to 27.4	27.8 ± 6.9	−28.0 to 80.4	30.8 ± 4.6	−9.2 to 35.4
ATPmax	0.46 ± 0.13	−45.6 to 91.7	0.44 ± 0.09	−33.5 to 76.7	0.56 ± 0.23	−24.3 to 149
One-step clamp (Values during steady state)						
Suppression of FFA (%)	82.8 ± 25.9	−904 to 14.1	93.5 ± 5.8	−92.6 to 16.8	90.4 ± 10.0	−6.2 to 27.1
Suppression of EGP (%)	78.6 ± 20.8	−37.9 to 67.7	73.6 ± 11.6	−77.6 to 34.7	69.8 ± 25.2	−80.6 to 65.5
Rate of glucose disposal (mg/min/Insulin)	4.7 ± 2.1	−46.0 to 236	4.7 ± 2.2	−32.4 to 110	4.7 ± 2.5	−10.2 to 385
Skeletal muscle histology						
Type I fiber proportion (%)	40.4 ± 11.4	−27.1 to 47.7	41.8 ± 15.7	−38.5 to 28.9	38.9 ± 15.3	−27.7 to 36.6
Type IIA fiber proportion (%)	36.2 ± 13.5	−46.3 to 17.9	32.7 ± 13.3	−20.4 to 23.2	33.2 ± 12.0	−9.2 to 24.8
Type IIA/IIX fiber proportion (%)	8.4 ± 5.6	−7.1 to 5.6	6.7 ± 6.2	−10.1 to 11.2	6.9 ± 7.4	−29.8 to 14.1
Type IIX fiber proportion (%)	14.7 ± 13.1	−12.7 to 45.2	18.6 ± 13.5	−28.1 to 28.3	20.6 ± 13.2	−31.9 to 22.2
Type I CSA (μm^2)	4,365 ± 991	−36.3 to 46.8	4,082 ± 939	−57.8 to 77.1	4,511 ± 1,322	−36.8 to 46.3
Type IIA CSA (μm^2)	4,303 ± 1,503	−72.5 to 103	3,470 ± 848	−38.6 to 58.8	4,101 ± 1,276	−48.1 to 28.5
Type IIA/IIX CSA (μm^2)	3,739 ± 1,520	−60.4 to 137	3,585 ± 1,899	−76.9 to 40.5	4,068 ± 2,211	−72.2 to 48.4

(Continued)

TABLE 1 | Continued

	HED		WL		WL + EX	
	Baseline	**Range of % Change**	**Baseline**	**Range of % Change**	**Baseline**	**Range of % Change**
Type IIX CSA (μm^2)	3,363 ± 1,718	−49.7 to 190	3,340 ± 1,339	−63.2 to 63.7	3,131 ± 1,294	−44.5 to 23.7
Capillary density (# capillaries/fiber CSA)	1.2 ± 0.5	−35.7 to 39.2	0.9 ± 0.4	−85.0 to 123	1.2 ± 0.6	−49.0 to 122

*Range of change (%) = post-intervention − pre-intervention/pre-intervention*100%, with the exception of HbA1C, Suppression of FFA and EGP, and fiber type proportions, in which % range of change = post-intervention − pre-intervention.*

AT, adipose tissue; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; TG, triglycerides; LDL, low-density lipoproteins; HDL, high-density lipoproteins; VLDL, very low-density lipoproteins; FFA, free fatty acids; GIR/I, Glucose Infusion Rate/Steady State Insulin; EGP, endogenous glucose production; CSA, cross-sectional area.

Sample size differs for the following characteristics.

Fasting Glucose: HED, n = 18; WL, n = 20; WL + EX, n = 19.

Abdominal AT, SAT, VAT, thigh IMAT: HED, n = 12; WL, n = 7; WL + EX, n = 13.

GIR/I, Fasting Insulin, HOMA-IR: HED, n = 19; WL, n = 13; WL + EX, n = 12.

Plasma FFA: HED, n = 18; WL, n = 17; WL + EX, n = 17.

VO_{2max}: HED, n = 20; WL, n = 19; WL + EX, n = 20.

ATPmax: HED, n = 13; WL, n = 8; WL + EX, n = 11.

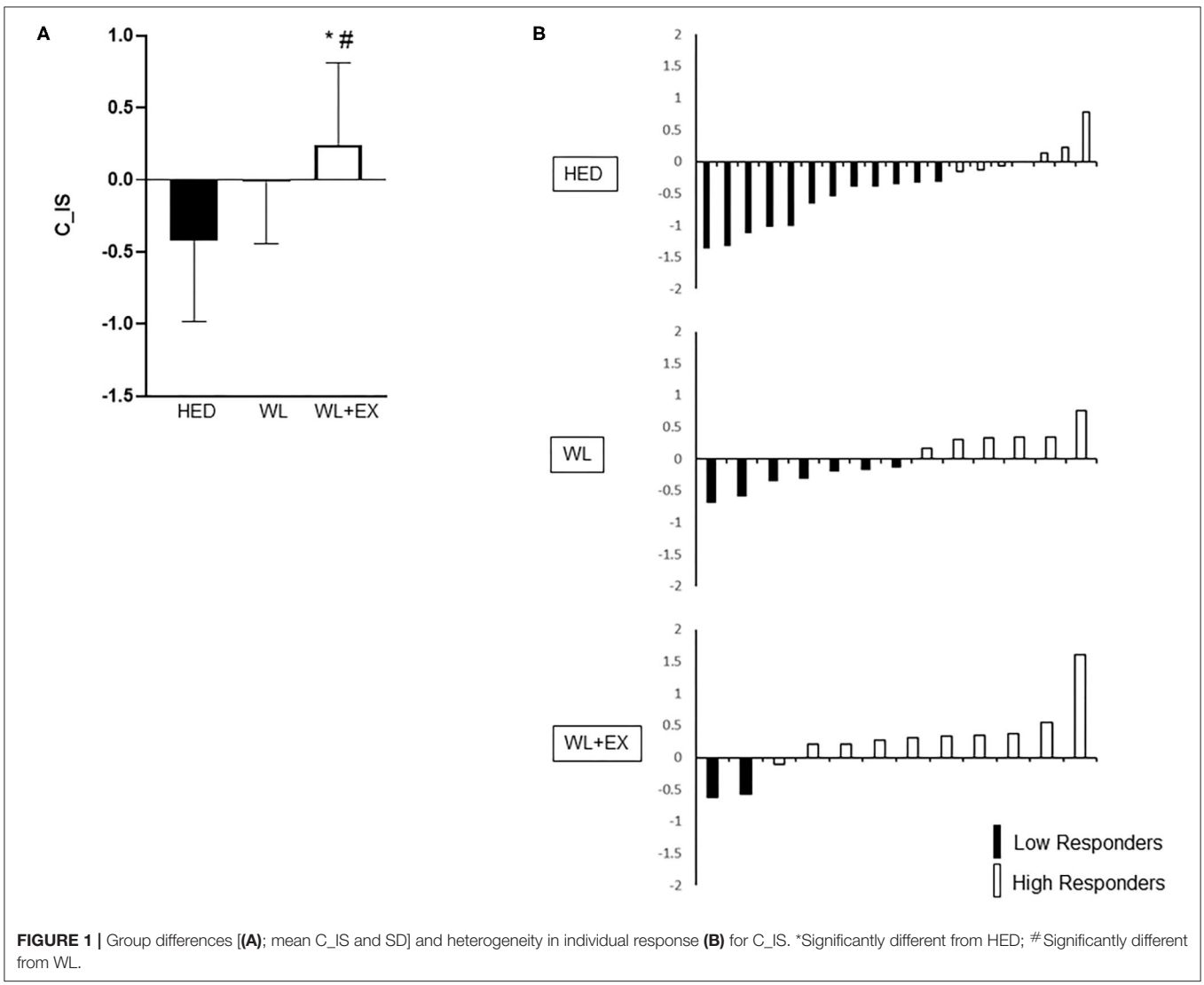


FIGURE 1 | Group differences [**(A)**; mean C_IS and SD] and heterogeneity in individual response **(B)** for C_IS. *Significantly different from HED; #Significantly different from WL.

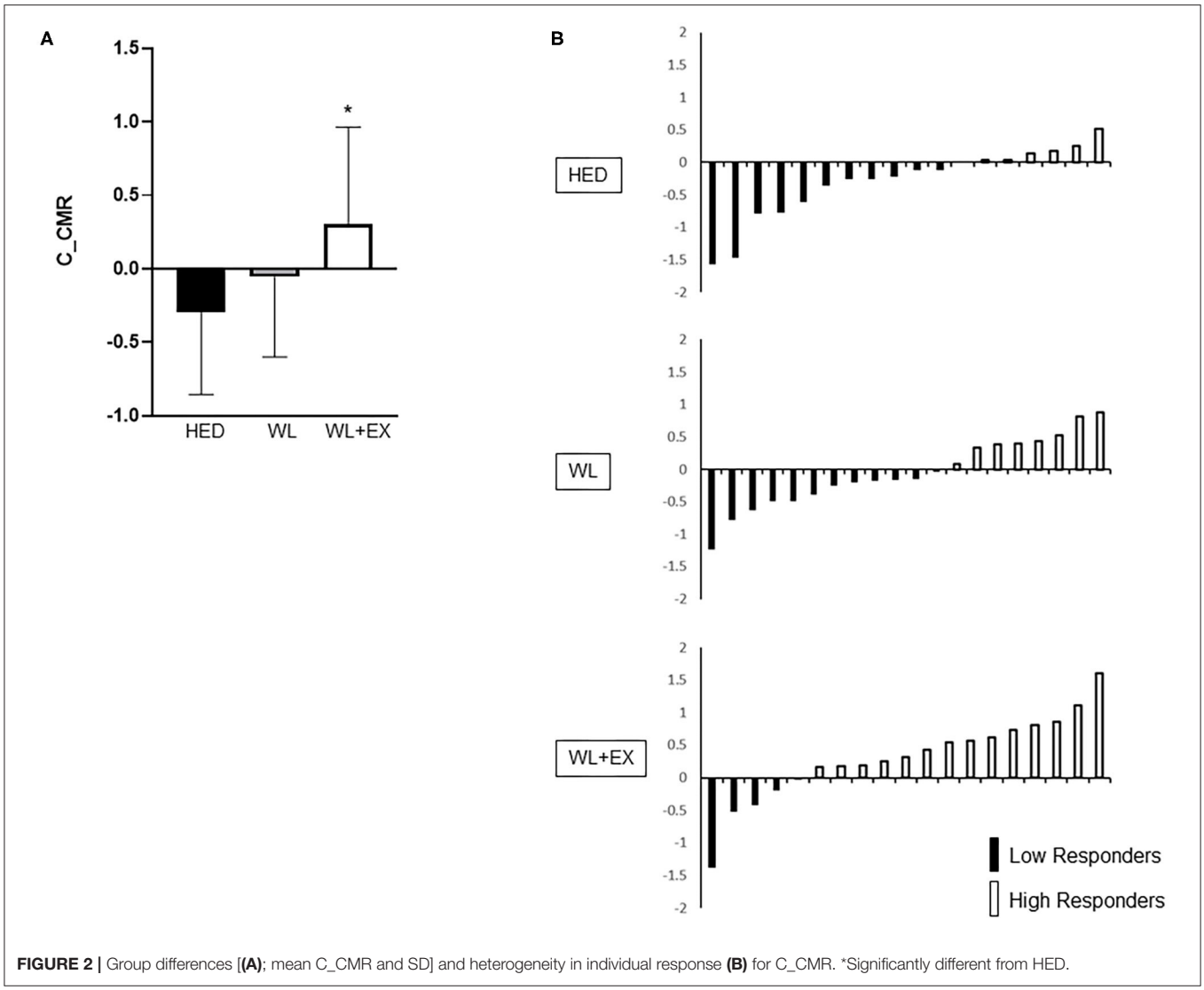


FIGURE 2 | Group differences [**(A)**; mean C_CMR and SD] and heterogeneity in individual response **(B)** for C_CMR. *Significantly different from HED.

triglycerides ($r = 0.53$), and VLDL ($r = 0.53$) were positively associated with C_CMR in the WL only group (all $p < 0.05$). In the WL + EX group, baseline triglycerides ($r = 0.59$) and VLDL ($r = 0.59$) were positively associated with C_CMR (all $p < 0.05$).

DISCUSSION

Recent focus on the application of personalized lifestyle-based medicine in the last decade has stimulated an exponential increase in observations related to response heterogeneity. However, several questions remain including the relative effect of different types of lifestyle-based prescriptions (exercise and/or diet) on interindividual variability, particularly in vulnerable populations such as older obese adults. In the present study, our primary findings indicate that the addition of exercise to energy restriction-induced weight loss improves the proportion of High Responders for glycemic control and cardiometabolic risk compared to weight loss alone and a time-matched control group. Our findings have novel implications for enhancing our understanding of the impact of lifestyle interventions on the variability of important clinical variables in older obese adults that may support future efforts to tailor lifestyle interventions to the individual and optimize treatment outcomes.

To our knowledge no prior studies have assessed the independent contributions of weight loss with or without exercise to the response heterogeneity in insulin sensitivity and cardiometabolic risk, particularly in the older obese population. Additionally, in prior analyses that examine variability, studies have typically been small, and the majority lack a control group, precluding the ability to assess intervention-independent effects on response (4, 5, 13). The current trial includes a time-matched control group that allows assessment of intervention responses beyond both technical error and day-to-day biological fluctuations (6, 7). Using this approach, we observed that exercise combined with energy intake

TABLE 2 | Associations between baseline characteristics and C_Scores.

Characteristic	WL		WL + EX	
	C_IS (r)	C_CMR (r)	C_IS (r)	C_CMR (r)
Age	−0.36	−0.09	0.22	0.11
Body composition				
Weight	−0.27	−0.32	−0.16	−0.19
Body mass index	−0.23	−0.33	0.00	0.04
Waist circumference	−0.37	−0.27	−0.07	0.10
Abdominal AT	−0.04	−0.16	−0.15	−0.26
Abdominal subcutaneous AT	0.12	−0.69	−0.28	−0.13
Abdominal visceral AT	−0.12	0.48	0.08	−0.30
Thigh intermuscular AT	−0.33	0.51	0.66	0.04
Clinical measurements				
SBP	0.25	0.33	−0.22	−0.25
DBP	0.14	0.16	−0.50	−0.39
Insulin	**0.58***	−0.21	0.41	−0.06
Glucose	0.26	**0.55***	0.10	0.36
HbA1C	−0.17	−0.25	0.56	0.37
HOMA-IR	**0.57***	−0.14	0.31	−0.09
Triglycerides	0.33	**0.53***	0.23	**0.59***
Cholesterol	0.46	0.15	−0.13	0.15
LDL-Cholesterol	0.45	0.06	−0.19	−0.07
HDL-Cholesterol	−0.07	−0.27	0.03	0.16
VLDL-Cholesterol	0.33	**0.53***	0.22	**0.59***
Plasma free fatty acids	**0.78***	0.08	0.18	0.07
Aerobic fitness				
VO_{2max} (l/min)	0.16	0.09	−0.08	−0.30
VO_{2max} (ml/kgFFM/min)	0.19	0.25	0.10	−0.06
ATPmax	−0.33	−0.30	−0.14	0.22
One-step clamp (Values during steady state)				
GIR/I	−0.50	−0.15	−0.44	0.06
Suppression of FFA	−0.02	0.05	0.22	−0.29
Suppression of EGP	−0.04	0.41	0.32	0.44
Rate of glucose disposal	**−0.59***	−0.04	**−0.60***	−0.06
Skeletal muscle fiber type				
Type I fiber proportion	−0.28	0.29	−0.52	0.01
Type IIA fiber proportion	−0.03	−0.11	−0.07	0.32
Type IIA/IIX fiber proportion	0.44	−0.24	−0.04	−0.18
Type IIX fiber proportion	0.15	−0.12	0.64	−0.11
Type I CSA	0.17	0.35	0.64	0.18
Type IIA CSA	−0.01	0.16	0.50	−0.15
Type IIA/IIX CSA	−0.42	0.15	0.40	−0.10
Type IIX CSA	0.02	0.27	0.33	−0.21
Capillary density	0.24	0.43	−0.38	−0.08

*AT, adipose tissue; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; TG, triglycerides; LDL, low-density lipoproteins; HDL, high-density lipoproteins; VLDL, very low-density lipoproteins; FFA, free fatty acids; GIR/I, Glucose Infusion Rate/Steady State Insulin; EGP, endogenous glucose production; CSA, cross-sectional area; WL, weight loss; WLEX, weight loss with exercise; C_IS, insulin sensitivity composite score; C_CMR, cardiometabolic risk composite score. *Bold indicates significant association between baseline characteristic value and C_Score at $p < 0.05$.*

restriction-induced weight loss is a superior approach for improving the proportion of individuals who achieve a favorable response for both insulin sensitivity and cardiometabolic risk compared to weight loss alone or no intervention. While others have suggested a similar mean group response to exercise vs. diet-induced weight loss in men for several clinical outcomes (14–17), our findings suggest that more individuals will achieve a greater response magnitude to intervention with the combination of diet-induced weight loss and exercise compared to diet alone. Taken together, our novel findings reinforce and provide support for the inclusion of regular exercise in addition to dietary recommendations to improve the likelihood that an individual responds favorably to treatment.

We completed a comprehensive assessment of relationships between baseline traits and response for glycemic control and cardiometabolic risk, including clinical laboratory outcomes, MRI-derived body composition, aerobic fitness, muscle and hepatic insulin sensitivity, and immunohistochemical analysis of fiber type and capillary density. Overall, while pre-intervention traits were differentially associated with insulin sensitivity and cardiometabolic risk response, in both WL and WL + EX groups a more favorable response to intervention was associated with a higher risk clinical phenotype at baseline. Specifically, in both intervention groups, higher baseline triglycerides and VLDL-cholesterol were associated with greater improvement in cardiometabolic risk while higher plasma insulin and HOMA-IR were associated with increased insulin sensitivity. Consistent with our findings are those from a 12-week diet and exercise intervention in individuals aged 18–75 years who were at risk for type 2 diabetes (9), wherein High Responders for glucose AUC assessed by 2-h OGTT had higher baseline weight, visceral AT, fasting glucose, 2-h OGTT glucose, and triglycerides and lower HDL-cholesterol compared to those who experienced an adverse response or attenuated response to the intervention. However, our findings also contradict many others who observed blunted responses to exercise interventions associated with metabolically unhealthy outcome levels at baseline (4, 5, 18–20). Several factors may explain the discrepant findings, including differences in sample demographics and disease diagnosis, duration of disease, medication use, dissimilar outcome variables, correlation vs. categorical response analysis, intervention characteristics, etc. Thus, further investigation is warranted to evaluate whether response heterogeneity and predictors of response differ across population subtypes and lifestyle modifications to move closer to personalized lifestyle medicine that optimizes changes in clinical outcomes based on individual characteristics.

Numerous mechanisms have been highlighted as potential contributors to an individual's response to lifestyle intervention (21, 22). Prior work from our group demonstrated that skeletal muscle DNA methylation and RNA expression patterns reflective of elevations in antioxidant defense, insulin signaling, and mitochondrial metabolism were present in Non-Responders based on changes in PCR recovery rate (i.e., *in vivo* muscle mitochondrial function) and insulin sensitivity following a 10-week aerobic exercise intervention (23). These molecular characteristics of Non-Responders correlated with higher baseline insulin sensitivity and muscle mitochondrial function *in vivo* (23). Taken together, these mechanistic findings

support the interpretation of our observations that indicate a higher metabolic burden and less healthy skeletal muscle phenotype allows for a greater window of opportunity for improvement. Thus, factors across a range of molecular and metabolic outcomes (genetics, epigenetics, metabolism, physiology, etc.) likely play a role in an individual's response to intervention and should be further exploited in future studies (24).

Given growing interest in the study of individual responses and its implications for personalized exercise and diet prescription, it is important to consider the clinical relevance and interpretation of our findings. This notion is complicated by the range of important health outcomes under interrogation that do not necessarily change in concert. The use of Z-scores to reflect the concurrent change in a collection of predefined outcomes is not a novel concept (25–28). However, we extend this application to the study of interindividual variability. Compared to the interventions described above that focus on a singular outcome, the use of Z-scores appears to reduce the proportion of individuals who respond poorly or do not respond to intervention (24). Classifying an individual as a "non-responder" based on change in a singular outcome without consideration for equally meaningful changes in other outcomes may discourage these individuals from implementing positive exercise and dietary habits into their habitual routines. Furthermore, the use of Z-scores for predefined clusters of variables reduces the biological variability of each component (**Supplementary Figures 1**, **2**), thus reducing the "noise" and more robustly capturing the response to the intervention itself (29). Thus, in this field of response heterogeneity, it may be helpful to consolidate related outcomes to provide an integrative assessment of physiological responses and improve clinical applications and inferences.

There are limitations in our study that should be considered. For some outcome variables, the sample size may not be adequate to assess associations between baseline phenotype and response to WL or WL + EX. This is particularly true for measures of skeletal muscle fiber type and MRI-derived AT. While these are simple associations and do not imply causation, our findings do prompt future work with appropriately powered trials to combine data from molecular, metabolic, physiological and clinical measures to assess predictors of response to weight loss with and without exercise. Our participants reflected a range in diabetes status, from no diabetes to frank type 2 diabetes and thus, differed in medication use. Recent interest in the interaction effects of exercise and medication use on response across a range of outcomes has revealed inconsistent findings. Observations in a large sample ($n = 225$) of men and women with type 2 diabetes suggest that metformin had no effect on HbA1C reduction following aerobic exercise training (30). Contrary to these findings, in both older adults (31) and those with prediabetes (32), the increase in whole-body insulin sensitivity following 12 weeks of aerobic exercise training was attenuated in those taking metformin concurrently. Similar discrepancies are seen with the interaction between statin use and exercise, where evidence from obese elderly males suggests no impact of statins on the beneficial effects of 12 weeks of exercise (33), whereas the addition of statins blunted the increase in cardiorespiratory fitness and citrate synthase activity in overweight or obese adults (34). Thus, just as individuals respond differently to exercise training, the interaction of his/her medication use with exercise may also differ. Taken together, factors associated with medication use (e.g., length of use, sex differences, disease status) may introduce variability in the response to exercise training for cardiometabolic risk and glycemic control. The WL and WL + EX groups were not matched for weight loss and we did not include an EX only group; thus, it is uncertain whether the improved response with exercise is a result of differences in energy balance or exercise *per se*. Additionally, we do not have adherence and compliance records for all participants; both may impact response variability. Future work carefully accounting for energy balance is warranted in order to definitively make these conclusions.

In conclusion, the addition of exercise to energy restriction-induced weight loss improves the number of older obese adults who achieve improvement in insulin sensitivity and cardiometabolic risk. Additionally, individuals with poorer metabolic status at baseline are more likely to experience greater improvements in clinical outcomes with these lifestyle interventions. Our data contributes novel findings with regards to individual response variation to lifestyle interventions, moving us closer to identifying predictors of response and tailoring lifestyle-based treatments to the individual.

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 studies involving human participants were reviewed and approved by Institutional Review Board of AdventHealth. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BG concepted and designed the primary trial and assisted LS and AB in conceptualizing this secondary analysis. RS and EC coordinated the primary trial and organized all data collection. FY provided statistical support for the manuscript. AB completed statistical analysis and data interpretation. BG, LS, and AB were responsible for drafting the manuscript. All authors assisted with manuscript revision.

FUNDING

This study was supported by funding from the National Institutes of Health—National Institute on Aging (RO1 AG021961-09) awarded to BG.

ACKNOWLEDGMENTS

The authors would like to thank the contributions of our study participants and acknowledge the valuable expertise and assistance of the imaging, recruitment, clinic, calorimetry, laboratory, and nutrition staff at TRI, AdventHealth Research Institute.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2020.00632/full#supplementary-material

REFERENCES

1. Zamboni M, Mazzali G, Zoico E, Harris TB, Meigs JB, Di Francesco V, et al. Health consequences of obesity in the elderly: a review of four unresolved questions. *Int J Obes*. (2005) 29:1011–29. doi: 10.1038/sj.ijo.0803005
2. Villareal DT, Apovian CM, Kushner RF, Klein S. Obesity in older adults: technical review and position statement of the American Society for Nutrition and NAASO, the obesity society. *Obes Res*. (2005) 13:1849–63. doi: 10.1038/oby.2005.228
3. Boulé NG, Weisnagel SJ, Lakka TA, Tremblay A, Bergman RN, Rankinen T, et al. Effects of exercise training on glucose homeostasis: the HERITAGE family study. *Diabetes Care*. (2005) 28:108–14. doi: 10.2337/diacare.28.1.108
4. Álvarez C, Ramírez-Campillo R, Ramírez-Vélez R, Izquierdo M. Prevalence of non-responders for glucose control markers after 10 weeks of high-intensity interval training in adult women with higher and lower insulin resistance. *Front Physiol*. (2017) 8:479. doi: 10.3389/fphys.2017.00479
5. Solomon TP, Malin SK, Karstoft K, Haus JM, Kirwan JP. The influence of hyperglycemia on the therapeutic effect of exercise on glycemic control in patients with type 2 diabetes mellitus. *JAMA Intern Med*. (2013) 173:1834–6. doi: 10.1001/jamainternmed.2013.7783
6. Hecksteden A, Kraushaar J, Scharhag-Rosenberger F, Theisen D, Senn S, Meyer T. Individual response to exercise training-a statistical perspective. *J Appl Physiol*. (2015) 118:1450–9. doi: 10.1152/japplphysiol.00714.2014
7. Atkinson G, Batterham AM. True and false interindividual differences in the physiological response to an intervention. *Exp Physiol*. (2015) 100:577–88. doi: 10.1113/EP085070
8. Malin SK, Haus JM, Solomon TP, Blaszczak A, Kashyap SR, Kirwan JP. Insulin sensitivity and metabolic flexibility following exercise training among different obese insulin-resistant phenotypes. *Am J Physiol Endocrinol Metab*. (2013) 305:E1292–8. doi: 10.1152/ajpendo.00441.2013
9. O'Donoghue G, Kennedy A, Andersen GS, Carr B, Cleary S, Durkan E, et al. Phenotypic responses to a lifestyle intervention do not account for inter-individual variability in glucose tolerance for individuals at high risk of type 2 diabetes. *Front Physiol*. (2019) 10:317. doi: 10.3389/fphys.2019.00317
10. Distefano G, Standley RA, Zhang X, Carnero EA, Yi F, Cornnell HH, et al. Physical activity unveils the relationship between mitochondrial energetics, muscle quality, and physical function in older adults. *J Cachexia Sarcopenia Muscle*. (2018) 9:279–94. doi: 10.1002/jcsm.12272
11. Pruchnic R, Katsiaras A, He J, Kelley DE, Winters C, Goodpaster BH. Exercise training increases intramyocellular lipid and oxidative capacity in older adults. *Am J Physiol Endocrinol Metab*. (2004) 287:E857–62. doi: 10.1152/ajpendo.00459.2003
12. Coen P, Hames K, Leachman E, DeLany J, Ritov V, Menshikova E, et al. Reduced skeletal muscle oxidative capacity and elevated ceramide but not diacylglycerol content in severe obesity. *Obesity*. (2013) 21:2362–71. doi: 10.1002/oby.20381
13. Malin SK, Kirwan JP. Fasting hyperglycaemia blunts the reversal of impaired glucose tolerance after exercise training in obese older adults. *Diab Obes Metab*. (2012) 14:835–41. doi: 10.1111/j.1463-1326.2012.01608.x
14. Janssen I, Fortier A, Hudson R, Ross R. Effects of an energy-restrictive diet with or without exercise on abdominal fat, intermuscular fat, and metabolic risk factors in obese women. *Diabetes Care*. (2002) 25:431–8. doi: 10.2337/diacare.25.3.431
15. Ross R, Dagnone D, Jones PJ, Smith H, Paddags A, Hudson R, et al. Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in men: a randomized, controlled trial. *Ann Intern Med*. (2000) 133:92–103. doi: 10.7326/0003-4819-133-2-200007180-00008
16. Williams PT, Krauss RM, Vranizan KM, Wood P. Changes in lipoprotein subfractions during diet-induced and exercise-induced weight loss in moderately overweight men. *Circulation*. (1990) 81:1293–304. doi: 10.1161/01.CIR.81.4.1293
17. Christiansen T, Paulsen SK, Bruun JM, Pedersen SB, Richelsen B. Exercise training versus diet-induced weight-loss on metabolic risk factors and inflammatory markers in obese subjects: a 12-week randomized intervention study. *Am J Physiol Endocrinol Metab*. (2010) 298:E824–31. doi: 10.1152/ajpendo.00574.2009
18. Stefan N, Staiger H, Wagner R, Machann J, Schick F, Häring HU, et al. A high-risk phenotype associates with reduced improvement in glycaemia during a lifestyle intervention in prediabetes. *Diabetologia*. (2015) 58:2877–84. doi: 10.1007/s00125-015-3760-z
19. Thamer C, Machann J, Stefan N, Haap M, Schäfer S, Brenner S, et al. High visceral fat mass and high liver fat are associated with resistance to lifestyle intervention. *Obesity*. (2007) 15:531–8. doi: 10.1038/oby.2007.568
20. Malin SK, Niemi N, Solomon TP, Haus JM, Kelly KR, Filion J, et al. Exercise training with weight loss and either a high-or low-glycemic index diet reduces metabolic syndrome severity in older adults. *Ann Nutr Metab*. (2012) 61:135–41. doi: 10.1159/000342084
21. Böhm A, Weigert C, Staiger H, Häring HU. Exercise and diabetes: relevance and causes for response variability. *Endocrine*. (2016) 51:390–401. doi: 10.1007/s12020-015-0792-6
22. Solomon TP. Sources of inter-individual variability in the therapeutic response of blood glucose control to exercise in type 2 diabetes: going beyond exercise dose. *Front Physiol*. (2018) 9:896. doi: 10.3389/fphys.2018.00896
23. Stephens NA, Brouwers B, Eroshkin AM, Yi F, Cornnell HH, Meyer C, et al. Exercise response variations in skeletal muscle PCr recovery rate and insulin sensitivity relate to muscle epigenomic profiles in individuals with type 2 diabetes. *Diabetes Care*. (2018) 41:2245–54. doi: 10.2337/dc18-0296
24. Bouchard C, Rankinen T. Individual differences in response to regular physical activity. *Med Sci Sports Exerc*. (2001) 33:S446–51. doi: 10.1097/00005768-200106001-00013
25. Yudkin JS, Stehouwer C, Emeis J, Coppack S. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol*. (1999) 19:972–8. doi: 10.1161/01.ATV.19.4.972
26. Dessein PH, Woodiwiss AJ, Norton GR, Tsang L, Solomon A. Independent associations of total and high molecular weight adiponectin with cardiometabolic risk and surrogate markers of enhanced early atherogenesis in black and white patients with rheumatoid arthritis: a cross-sectional study. *Arthritis Res Ther*. (2013) 15:R128. doi: 10.1186/ar4308
27. Puhkala J, Kukkonen-Harjula K, Mansikkamäki K, Aittasalo M, Hublin C, Kärmeniemi P, et al. Lifestyle counseling to reduce body weight and cardiometabolic risk factors among truck and bus drivers–a randomized controlled trial. *Scand J Work Environ Health*. (2015) 41:54–64. doi: 10.5271/sjweh.3463

28. Ramírez-Vélez R, Correa-Bautista JE, Lobelo F, Izquierdo M, Alonso-Martínez A, Rodríguez-Rodríguez F, et al. High muscular fitness has a powerful protective cardiometabolic effect in adults: influence of weight status. *BMC Public Health*. (2016) 16:1012. doi: 10.1186/s12889-016-3678-5
29. O'Brien PC. Procedures for comparing samples with multiple endpoints. *Biometrics*. (1984) 40:1079–87. doi: 10.2307/2531158
30. Boule NG, Kenny GP, Larose J, Khandwala F, Kuzik N, Sigal RJ. Does metformin modify the effect on glycaemic control of aerobic exercise, resistance exercise or both? *Diabetologia*. (2013) 56:2378–82. doi: 10.1007/s00125-013-3026-6
31. Konopka AR, Laurin JL, Schoenberg HM, Reid JJ, Castor WM, Wolff CA, et al. Metformin inhibits mitochondrial adaptations to aerobic exercise training in older adults. *Aging Cell*. (2019) 18:e12880. doi: 10.1111/acel.12880
32. Malin SK, Gerber R, Chipkin SR, Braun B. Independent and combined effects of exercise training and metformin on insulin sensitivity in individuals with prediabetes. *Diabetes Care*. (2012) 35:131–6. doi: 10.2337/dc11-0925
33. Meex RC, Phielix E, Schrauwen-Hinderling VB, Moonen-Kornips E, Schaart G, Schrauwen P, et al. The use of statins potentiates the insulin-sensitizing effect of exercise training in obese males with and without type 2 diabetes. *Clin Sci*. (2010) 119:293–301. doi: 10.1042/CS20100153
34. Mikus CR, Boyle LJ, Borengasser SJ, Oberlin DJ, Naples SP, Fletcher J, et al. Simvastatin impairs exercise training adaptations. *J Am Coll Cardiol*. (2013) 62:709–14. doi: 10.1016/j.jacc.2013.02.074

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 Endocrinology

CLINICAL TRIAL
published: 15 October 2020
doi: 10.3389/fendo.2020.566548

Exercise-Induced Improvements in Postprandial Glucose Response Are Blunted by Pre-Exercise Hyperglycemia: A Randomized Crossover Trial in Healthy Individuals

Steven Carter[1*†] *and Thomas P. J. Solomon*[1,2†]

[1] School of Sport, Exercise, and Rehabilitation Sciences, College of Life and Environmental Sciences, University of Birmingham, Edgbaston, United Kingdom, [2] Institute of Systems and Metabolism Research, College of Medical and Dental Sciences, University of Birmingham, Edgbaston, United Kingdom

OPEN ACCESS

Edited by:
Hans Ulrich Häring,
Tübingen University Hospital,
Germany

Reviewed by:
Asimina Mitrakou-Fanariotou,
National and Kapodistrian University of Athens, Greece
Dominik H. Pesta,
German Center for Diabetes Research (DZD), Germany

***Correspondence:**
Steven Carter
sc2988@bath.ac.uk

†Present address:
Thomas Solomon,
Blazon Scientific, London,
United Kingdom
Steven Carter,
University of Bath,
Bath, United Kingdom

Specialty section:
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Endocrinology

Received: 23 June 2020
Accepted: 15 September 2020
Published: 15 October 2020

Citation:
Carter S and Solomon TPJ (2020) Exercise-Induced Improvements in Postprandial Glucose Response Are Blunted by Pre-Exercise Hyperglycemia: A Randomized Crossover Trial in Healthy Individuals. Front. Endocrinol. 11:566548. doi: 10.3389/fendo.2020.566548

Background: Exercise improves glycemic control but the magnitude, and in some cases, the direction of this effect is variable. Ambient hyperglycemia has been implicated in this exercise response heterogeneity. The current study investigated whether pre-exercise hyperglycemia directly impacts the effect of exercise on glycemic control.

Methods: Twelve healthy normal glucose-tolerant males completed four trials in a randomized, crossover design. Each trial consisted of 24-h pre-intervention monitoring, a 7-h intervention, and 24-h post-intervention monitoring. Glycemic control was measured throughout the study by continuous glucose monitoring. The four interventions were no exercise (CON) or 45 min of cycling exercise (70%HRmax) preceded by 3.5 h of either normoglycemia (NG-Ex), steady-state hyperglycemia induced by constant glucose infusion (HG-Ex) or fluctuating glycemia induced by repeated glucose bolus infusions (FG-Ex).

Results: Physical activity and diet were similar between trials, and energy expenditure during exercise was matched between exercise trials (all $P > 0.05$). Mean glucose during the 3.5 h ± infusion period was higher in HG-Ex (mean ± SEM; 7.2 ± 0.4 mmol/L) and FG-Ex (7.3 ± 0.3 mmol/L) compared to CON (4.8 ± 0.2 mmol/L) and NG-Ex (5.0 ± 0.2 mmol/L) trials ($P < 0.01$). Glycemic variability was greatest in FG-Ex ($P < 0.01$). Following the interventions, the postprandial glucose response (iAUC) was reduced by exercise in NG-Ex compared to CON (321.1 ± 38.6 vs. 445.5 ± 49.7 mmol/L.8h, $P < 0.05$, d=0.81). This benefit was blunted when exercise was preceded by steady-state (HG-Ex, 425.3 ± 45.7 mmol/L.8h) and fluctuating (FG-Ex, 465.5 ± 39.3 mmol/L.8h) hyperglycemia (both $P > 0.05$ vs. CON).

Conclusion: Pre-exercise hyperglycemia blunted the glucoregulatory benefits of acute exercise upon postprandial glucose response, suggesting that exposure to hyperglycemia contributes to exercise response heterogeneity.

Clinical Trial Registration: ClinicalTrials.gov, identifier NCT03284216.

Keywords: exercise, type 2 diabetes, postprandial, glycemic control, hyperglycemia, heterogeneity, variability

INTRODUCTION

The worldwide prevalence of type 2 diabetes mellitus (T2DM) continues to rise, meaning increasing proportions of the global population are at risk of or living with a range of serious microvascular and macrovascular complications (1–3). Consequently, the economic cost of managing T2DM continues to rise and places an increasing burden on healthcare systems. Optimizing interventions to improve glycemic control remains a clinical necessity.

The level of glycemia, and particularly postprandial glucose exposure (4–6), has been implicated with the aforementioned diabetic complications, as well as being predictive of a worsening glycated haemoglobin (HbA1c) level in non-diabetic individuals (7). Therefore, reducing postprandial glucose exposure is particularly important for the prevention and long-term management of glycemic control in individuals with or at risk of T2DM. Exercise training exerts potent glucoregulatory effects in those with and at risk of developing T2DM. For example, exercise training reduces HbA1c and fasting blood glucose (8, 9) as well as increases peripheral insulin sensitivity (10–12) and β-cell insulin secretory function (13, 14). A single exercise bout also potently increases glucose uptake, insulin sensitivity (15) and β-cell insulin secretory function (16) in the hours to days following each exercise bout. Similarly, and of particular relevance to the current study, reduced (i.e., improved) postprandial glucose response is also among the glucoregulatory effects of a single exercise bout (17–21). Many of these benefits are gained by individuals with and without T2DM, but the transient nature of benefits means exercise must be repeated regularly to preserve metabolic health. Accordingly, regular exercise forms the cornerstone in the prevention and management of hyperglycemia-related conditions, including T2DM (22).

While the potent glucoregulatory effects of exercise are unequivocal, the magnitude and direction of change following both acute exercise (16, 23, 24) and exercise training (25, 26) in hyperglycemic individuals vary considerably. Isolating factors contributing to this exercise response heterogeneity is vital to optimizing the glucoregulatory effects of exercise in this population and have been discussed in several recent reviews (27–30). In free-living environments, factors such as diet and exercise characteristics/adherence and exercise-medication interactions (31, 32) likely contribute to variability in this setting (30). Interestingly, evidence from recent well-controlled studies, where free-living sources of heterogeneity (e.g., exercise-drug interaction, exercise adherence, diet) are controlled, implicates the degree of hyperglycemia as one possible contributor to heterogeneity following single exercise bouts as well as exercise training (27, 28, 30). That said, equivocal conclusions have been made in studies to date, with both blunted (16, 33–35) and potentiated (23, 24) exercise-induced glucoregulatory benefits associated with higher baseline fasting plasma glucose and/or HbA1c. Furthermore, the evidence to date is also largely correlational, meaning that the direct effect of hyperglycemia on exercise-mediated improvements in glycemic control remains to be tested experimentally. Accordingly, the current study investigated the impact of pre-exercise hyperglycemia on the response to a single exercise bout in healthy normal glucose-tolerant participants. It was hypothesized that pre-exercise hyperglycemia would blunt the glucoregulatory effects of acute exercise.

MATERIALS AND METHODS

Participants

Healthy, recreationally active males with normal glucose tolerance (n = 12; **Table 1**) were recruited from the local community to participate in the current study. Potential participants underwent an initial screening visit to determine their eligibility for the study. Individuals were excluded from participation if they smoked, had a BMI >30 kg/m^2, and/or had a history of cancer, haematological, pulmonary, cardiac, hepatic, renal, metabolic, or gastrointestinal diseases. All participants provided written informed consent

TABLE 1 | Participant characteristics.

N	12
Age, years	23.6 ± 1.5
Height, cm	175.1 ± 1.7
Weight, kg	69.5 ± 2.3
BMI, kg/m^2	22.7 ± 0.7
Waist circumference, cm	76.5 ± 1.5
HbA1c, %	5.4 ± 0.1
HbA1c, mmol/mol	35.1 ± 1.0
Fasting plasma glucose, mmol/L	4.9 ± 0.2
HOMA-IR	1.1 ± 0.1
Systolic blood pressure, mmHg	121 ± 3
Diastolic blood pressure, mmHg	76 ± 3
$\dot{V}O_2$max, ml/kg/min	41.5 ± 3.3
$\dot{V}O_2$max, L/min	2.8 ± 0.2
W_{max}, W	267.8 ± 13.9
W_{max}, W/kg	3.8 ± 0.4
Habitual step count, steps/day	11452 ± 1578
Habitual energy expenditure, kcals/day	635.1 ± 103.0
Habitual sedentary time, min/day	726.9 ± 47.2
Habitual MVPA time, min/day	72.1 ± 11.4

Data are mean ± SEM.

before participation. The CONSORT diagram in **Supplemental Figure S1** shows an overview of the recruitment/screening/inclusion decisions. Formal sample size calculations were made using G*Power Version 3.1.7 (36). Ethical approval was obtained through the West Midlands - South Birmingham Research Ethics Committee (16/WM/0242) and sponsored by the University of Birmingham Research Governance. The study is registered at ClinicalTrials.gov (NCT03284216).

Screening and Habitual Monitoring

All participants completed a general health questionnaire, as well as had their body composition (height, weight, BMI, waist circumference) and HbA1c analyzed to confirm eligibility for the current study. Subsequently, participants performed a maximal incremental exercise test on a cycle ergometer (Lode Excalibur, Groningen, The Netherlands) to determine their maximum workload capacity (*W*max), maximal oxygen consumption ($\dot{V}O_2$max), and maximal heart rate (HRmax). After 5-min warm-up at 50 W, the workload was increased by 25 W/min until exhaustion. Oxygen consumption was assessed continuously during exercise *via* indirect calorimetry (Vyntus CPX, Jaeger, CareFusion, Germany), with heart rate (Polar Wearlink, Polar Electro Oy, Kempele, Finland), and ratings of perceived exertion (RPE; 6–20 Borg scale) monitored throughout. Following screening, participants completed 7 days of habitual monitoring, during which accelerometry (Actigraph wGT3X-BT, Pensacola, FL) and diet logs were used to assess habitual physical activity and diet, respectively.

Study Design

Participants completed four experimental trials in a randomized, crossover design with trials separated by ~1 week, during which time participants were instructed to maintain habitual physical activity and diet. Each trial consisted of a 24-h pre-trial monitoring period, a 7-h experimental intervention period, and a 24-h post-trial monitoring period (**Figure 1**). The four 7-h interventions were completed in a randomized order (determined using http://www.randomization.com/) and were: normoglycemia, no exercise (CON), normoglycemia plus exercise (NG-Ex), steady-state hyperglycemia plus exercise (HG-Ex), and fluctuating glycemia plus exercise (FG-Ex).

Pre-Trial Standardization and Monitoring Period

In the afternoon of day 0, participants reported to the laboratory for insertion of the continuous glucose monitoring (CGM) glucose sensor (Dexcom G5 mobile, Camberley, UK) into the abdominal subcutaneous adipose tissue, and to receive an accelerometer and diet records. Data was not collected on this day; instead, the purpose of this visit was to allow time for CGM calibration (which required ~2–3 h) in preparation for data collection to commence on day 1. The CGM was calibrated twice daily using a glucose meter (Contour Next One, Ascensia Diabetes Care UK, Berkshire, UK) on fingertip capillary blood samples. Participants were provided with detailed written instructions, including refraining from strenuous physical activity and the consumption of alcohol and caffeine from day 0 to day 3, inclusive.

On day 1 (24-h pre-trial), diet record (food type, amount and time of ingestion), accelerometry-derived physical activity, and CGM data collection commenced and continued until 24-h post-exercise (i.e., days 1–3, inclusive). Participants consumed a self-selected diet before trial 1 and then replicated this chosen diet on day 1 of subsequent trials. Similarly, participants were instructed to replicate as closely as possible activity patterns (e.g., mode of transportation) during the 24 h before each trial. This approach to pre-trial standardization is similar to other studies assessing the acute effects of exercise on glucose metabolism (37–39). Repeated verbal and written reminders of the importance of adhering to these standardizations were provided throughout.

Experimental Trials

On day 2, participants reported to the laboratory at 08:00, after an overnight fast (≥10 h, except water). Bodyweight, height, and waist circumference were measured by standard procedures, and bilateral antecubital venous lines were placed for glucose infusion and blood sampling. The four trials were identical except for the pre-exercise glycemic intervention and the rest vs. exercise

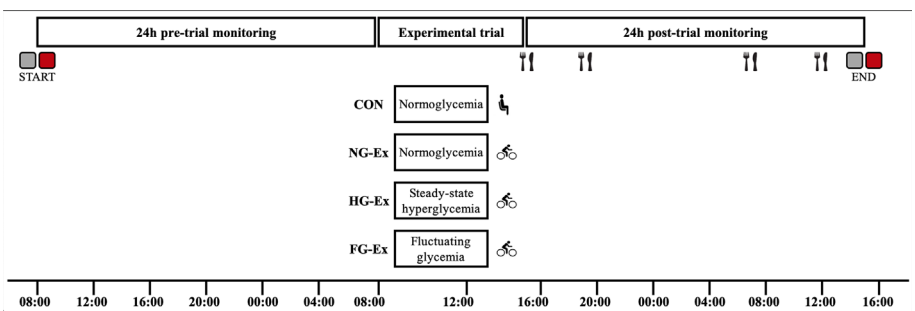


FIGURE 1 | Schematic overview of the study design. Pre-trial monitoring involving physical activity ( ) and CGM-derived glycemic control ( ) began 24 h before each experimental trial. During experimental trials, participants were exposed to differing glycemic profiles followed by 45 min of rest ( ) or exercise ( ). Post-trial glycemic control was assessed under strict dietary control ( ) but otherwise free-living conditions. A full description is provided in the text.

conditions. Specifically, after baseline measurements, one of four 3.5-h pre-exercise glycemic interventions commenced: CON (no infusion); NG-Ex (no infusion); HG-Ex, a constant glucose infusion of 1.2 g/kg for 3.5 h (5.71 mg/kg/min); and FG-Ex, a total of 1.2 g/kg of glucose infused over 3.5 h but divided into 8 equal boluses every 30 min (0.15 g/kg per bolus, infused over 3.5 min at 42.86 mg/kg/min). Glucose (20% w/v glucose monohydrate; Baxter FKB0213B) infusion was administered using a volumetric infusion system (Infusomat Space pump, B. Braun Medical Ltd, Sheffield, UK). Heart rate, blood pressure, oxygen saturation, and body temperature were measured throughout to monitor participant safety and well-being; no within- or between-trial differences were found (data not shown). Sixty minutes after cessation of the respective glycemic intervention (to allow for normalization of glucose levels), participants completed either a 45-min rest period (CON; remain seated) or a 45-min exercise bout (NG-Ex, HG-Ex, and FG-Ex; stationary cycling at 70% HRmax). Indirect calorimetry, heart rate and power output were measured continuously throughout the exercise/rest period. The end of the 45-min ± exercise period marked the start of the 24-h post-trial monitoring period.

Post-Trial Standardization and Monitoring Period

For the remainder of day 2 until ~15:00 on day 3 (i.e., the 24-h post-exercise period), CGM, accelerometry, and diet record monitoring continued in free-living conditions. During this time, participants were instructed to minimise any unnecessary physical activity (e.g., walking around at home was permitted but structured exercise was not) and to eat only the standardized meals provided.

The standardized 24-h diet consisted of four meals ingested at predetermined time points (lunch at 15:00, dinner at 19:00, breakfast at 07:00, lunch at 12:00). The first meal (lunch) was consumed after 30-min seated rest following the cessation of the ± exercise period, after which participants were free to leave the laboratory. The standardized 24-h diet provided 2467.6 ± 4.6 kcals and consisted of a mixed macronutrient composition (54.9 ± 0.1% of energy from carbohydrates, 31.6 ± 0.1% from fat, and 13.5 ± 0.1% from protein). Participants were instructed to consume meals within a 20-min timeframe and recorded the exact meal ingestion start and end times to allow specific data to be extracted from CGM data files. This approach ensured that the dietary intake (food type, amount and timing) was standardized within-subjects across all four trials; an important approach frequently adopted when using CGM to evaluate exercise-induced changes in measures of glycemic control in free-living settings (20, 21, 38, 40–43). In the afternoon of day 3 (≥24 h following exercise cessation), participants reported to the laboratory for removal of CGM, accelerometer and collection of diet records and activity logs, marking the end of the trial.

It should be noted that no additional calories were provided to replace those expended during exercise in the exercise trials (NG-Ex, HG-Ex, FG-Ex) compared to CON (**Table 3**), or to account for extra calories gained from glucose infusions in HG-Ex and FG-Ex (83.5 ± 2.8 g of glucose infused, equating to 333.8 ± 11.2 kcals). Resultant energy balance induced by experimental interventions (i.e., crudely calculated as kcal gained during infusion minus kcal expended during exercise) is as follows: CON = 61.7 ± 3.6 kcals deficit; NG-Ex = 326.1 ± 18.8 kcals deficit; HG-Ex = 10.9 ± 20.5 kcals surplus; FG-Ex = 9.8 ± 17.7 kcals surplus). Although caloric imbalances between trials may be a confounder (44), accounting for imbalances between trials with the provision of food, for example, would result in differences in macronutrient provision between trials, which in itself would also be a confounding variable. Therefore, the meals were kept constant across all conditions.

Blood Sample Analyses

HbA1c was measured in capillary blood samples during the screening visit (HemoCue HbA1c 501, Radiometer, Copenhagen, Denmark). Venous blood samples were collected in pre-chilled EDTA-coated tubes and centrifuged at 2,000 g for 15 min at 4°C, with resulting plasma samples stored at −80°C until analysis. Plasma insulin (DINS00), plasma IL-6 (HS600C), and plasma CRP (DCRP00) concentrations were determined using solid-phase sandwich ELISAs (Quantikine, Biotechne, R&D). Plasma glucose concentrations were measured using a HemoCue Glucose 201+ analyzer (Radiometer, Copenhagen, Denmark).

Calculations

Diet records were analyzed using the UK food database within MyFitnessPal (Under Armour, Baltimore, MD). The 24-h pre-trial glucose and physical activity data were derived from CGM and Actigraph data, respectively, from 08:00 on the day before the trial to 08:00 on the day of the trial. The 24-h post-trial glucose and physical activity data were derived from CGM and Actigraph data, respectively, from the end of exercise until 24-h later. CGM-derived measures of glucose exposure (mean glucose and incremental area under the curve, iAUC) and glycemic variability (standard deviation and coefficient of variation, CV %) were calculated following clinical guidelines (45). The 24-h prevalence of hyperglycemia (time spent above 8 mmol/L) and hypoglycemia (time below 4 mmol/L) were also calculated.

Postprandial glucose control was defined as the iAUC above pre-meal glucose level (average of 30-min pre-meal) over the 2-h postprandial period for four individual meals (lunch, 15:00; dinner, 19:00; breakfast, 07:00; snack, 12:00). Total postprandial glucose iAUC was also calculated as the summation of iAUCs from all four meal periods. Such approach has been used frequently when assessing the effects of exercise on postprandial glucose outside of the laboratory (18, 20, 21). Post-absorptive insulin sensitivity was estimated using the homeostasis model assessment (HOMA-IR) (46). Whole-body substrate oxidation rates were calculated from $\dot{V}O_2$ and $\dot{V}CO_2$ at rest (47) and during exercise (48).

Statistical Analysis

Either one- or two-way repeated-measures ANOVA were used where appropriate, and in the case of significant interaction effects, pairwise comparisons were made with Bonferroni corrections. Effect sizes for *post hoc* pairwise comparisons were calculated using Cohen's d and are presented in the figure

legends. All analyses were performed in GraphPad Prism 7.0 (La Jolla, CA) with a value of $P < 0.05$ considered to be statistically significant. Data are presented as mean ± SEM.

RESULTS

Participant Characteristics and Pre-Trial Standardization

Table 1 presents characteristics and habitual physical activity levels of participants recruited to complete the study. **Table 2** confirms adherence to pre-trial diet and activity standardizations, showing no between-trial differences in dietary intake, physical activity or CGM-derived glycemic control collected during 24-h pre-trial. There were also no significant changes in body composition (body mass, BMI and waist circumference) between trials ($P > 0.05$, data not shown).

Blood Biochemistry During the Experimental Trials

Figure 2A shows that the glucose infusion protocol successfully induced three distinct glycemic profiles: normoglycemic (CON and NG-Ex) and two different hyperglycemic conditions (HG-Ex and FG-Ex). Mean glucose concentration (**Figure 2B**) and glucose iAUC (**Figure 2C**) during the 3.5 h ± infusion period were significantly higher during HG-Ex and FG-Ex compared to CON and NG-Ex (all comparisons $P < 0.05$), with no differences between CON and NG-Ex ($P > 0.05$). **Figure 2D** confirms that the FG-Ex trial had an unstable and fluctuating glucose profile, since CV%, a measure of glycemic variability, was significantly higher than in all other trials (all comparisons $P < 0.05$).

Figure 3A shows that plasma insulin concentrations were significantly increased post-infusion vs. pre-infusion in both HG-Ex and FG-Ex (both $P < 0.0001$ vs. respective baseline), but not NG-Ex and CON ($P > 0.05$). Post-infusion values in HG-Ex and FG-Ex were also significantly higher than time-matched values in NG-Ex and CON ($P < 0.0001$ vs. time-matched values). There was no significant interaction effect of experimental treatments upon plasma IL-6 (**Figure 3B**) or plasma CRP (**Figure 3C**) concentrations ($P > 0.05$).

Physiological Responses to Exercise During the Trials

The exercise/rest period lasted 44.6 ± 0.4 min and characteristics of these periods are shown in **Table 3**. Heart rate, power output, $\dot{V}O_2$, work done, and energy expenditure were similar between the three exercise trials ($P > 0.05$) and significantly higher than during the resting trial ($P < 0.05$). RER was significantly higher during the three exercise trials vs. CON ($P < 0.05$; **Table 3**), and also significantly higher in HG-Ex and FG-Ex compared to NG-Ex ($P < 0.05$; **Table 3**). Furthermore, carbohydrate oxidation rates during exercise/rest were significantly higher in all three exercise trials (NG-Ex, HG-Ex, and FG-Ex) compared to CON ($P < 0.05$), with rates during HG-Ex and FG-Ex also significantly higher than NG-Ex ($P < 0.05$; **Figure 4A**). Fat oxidation rates during exercise in NG-Ex were significantly higher than all other trials ($P < 0.05$), with no further between-condition differences ($P > 0.05$; **Figure 4B**).

Post-Trial Standardization

Table 4 confirms that both dietary intake and physical activity levels during the 24-h post-trial period were similar between trials ($P > 0.05$).

Post-Trial 24-h Glycemic Control

There were no significant between-condition differences in 24-h glucose exposure (i.e., mean 24-h glucose concentrations) during the post-exercise period ($P > 0.05$; **Table 4**). Since the regulatory mechanisms (e.g., changes in glucose uptake and insulin sensitivity) of exercise-induced glucoregulatory benefits follow time-dependent profiles (15), the 24-h post-exercise period was broken down to discreet periods, namely: 0–6, 0–12, 0–18, 6–12, 6–18, 6–24, 12–18, 12–24, and 18–24 h. However, there were no significant between-condition differences in mean glucose during these periods ($P > 0.05$). Measures of 24-h glycemic variability,

TABLE 2 | Physical activity, dietary intake and free-living CGM variables measured for 24 h before each experimental trial.

	Pre-trial standardizations			
	CON	**NG-Ex**	**HG-Ex**	**FG-Ex**
Dietary intake				
Energy intake, kcals	2250.3 ± 164.1	2116.3 ± 141.1	2043.8 ± 155.4	2080.5 ± 169.0
Carbohydrate intake (% of kcals)	47.0 ± 4.0	49.4 ± 4.6	47.0 ± 3.7	48.2 ± 4.3
Fat intake (% of kcals)	35.9 ± 2.0	33.5 ± 2.4	35.7 ± 2.1	34.7 ± 2.2
Protein intake (% of kcals)	17.1 ± 2.5	17.1 ± 2.4	17.3 ± 2.1	17.0 ± 2.4
Physical activity				
Step count, steps	10715 ± 1893	10722 ± 1725	10032 ± 1746	10229 ± 2048
Energy expenditure, kcals	587.9 ± 132.9	554.4 ± 115.1	529.1 ± 109.3	562.4 ± 122.3
Sedentary time, min	726.0 ± 67.6	619.1 ± 86.8	685.8 ± 72.4	753.1 ± 71.7
MVPA time, min	72.3 ± 17.2	70.8 ± 14.7	66.3 ± 13.9	68.9 ± 18.0
CGM-derived variables				
Mean 24 h glucose, mmol/L	5.2 ± 0.3	5.4 ± 0.1	5.5 ± 0.2	5.2 ± 0.1
Glucose variability, SD	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.7 ± 0.1
Glucose variability, CV%	17.0 ± 1.4	14.7 ± 0.8	16.4 ± 1.7	13.7 ± 0.9

Data are mean ± SEM. No significant between-trial differences.

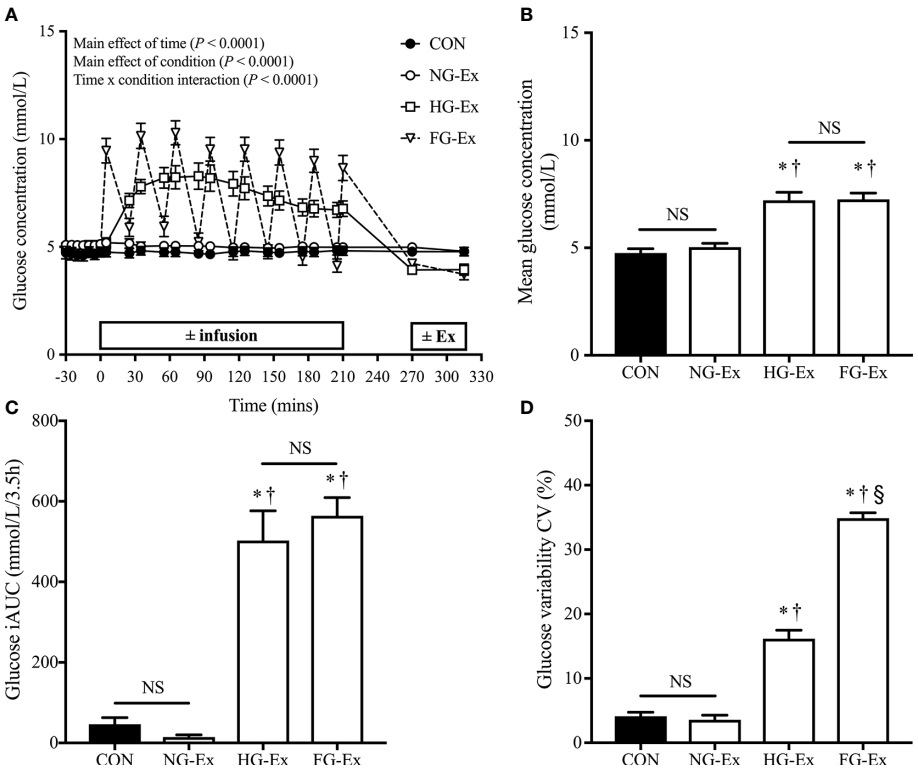


FIGURE 2 | Glucose control during conditions of ± glucose infusion and ± exercise. **(A)** Time course for glucose concentration (mmol/L) during each experimental trial. **(B)** The mean glucose concentration (mmol/L) during each 3.5 h ± infusion period. *$P < 0.05$ (HG-Ex, $d = 2.38$; FG-Ex, $d = 4.02$) vs. CON. †$P < 0.05$ (HG-Ex, $d = 2.17$; FG-Ex, $d = 3.83$) vs. NG-Ex. **(C)** Glucose iAUC during each 3.5 h ± infusion period. *$P < 0.05$ (HG-Ex, $d = 2.47$; FG-Ex, $d = 4.40$) vs. CON. †$P < 0.05$ (HG-Ex, $d = 2.70$; FG-Ex, $d = 4.94$) vs. NG-Ex. **(D)** Glycemic variability (coefficient of variation; %CV) during each 3.5 h ± infusion period. *$P < 0.05$ (HG-Ex, $d = 3.35$; FG-Ex, $d = 12.05$) vs. CON. †$P < 0.05$ (HG-Ex, $d = 3.38$; FG-Ex, $d = 11.45$) vs. NG-Ex. §$P < 0.05$ (FG-Ex, $d = 4.84$) vs. HG-Ex.

namely, SD and %CV, were not significantly affected by the experimental conditions (**Table 4**, both $P > 0.05$). Similarly, there were no significant between-condition differences in the 24-h prevalence of hyperglycemia or hypoglycemia (both $P > 0.05$; data not shown).

Post-Trial Postprandial Glycemic Control

There were no significant between-condition differences in mean glucose or glucose iAUC in the 2-h postprandial period following the four meals provided when analyzed per meal ($P > 0.05$). However, as presented in **Figure 5**, total postprandial glucose response, measured as the sum of post-meal glucose iAUC, was significantly reduced by exercise in NG-Ex compared to CON (321.1 ± 38.6 mmol/L.8h vs. 445.5 ± 49.7 mmol/L.8h, $P < 0.05$). However, such benefits were blunted when exercise was preceded by steady-state hyperglycemia (425.3 ± 45.7 mmol/L.8h) and fluctuating glycemia (465.5 ± 39.3 mmol/L.8h) in HG-Ex and FG-Ex, respectively (both $P > 0.05$ vs. CON).

DISCUSSION

The current study is the first to experimentally investigate the direct effect of hyperglycemia on exercise-induced benefits in glucose control. The findings demonstrate that pre-exercise hyperglycemia blunts the exercise-induced improvement in postprandial glucose response following a single exercise bout in healthy normal glucose-tolerant individuals.

Excessive postprandial glucose exposure contributes to the worsening of HbA1c in non-diabetic individuals (7), as well as exacerbating the risk of developing diabetic complications and premature mortality (4, 6). Accordingly, reducing postprandial glucose is a prime therapeutic target in the prevention and management of hyperglycemia-related conditions, such as T2DM. In this study, when exercise was preceded by normoglycemia, total postprandial glucose iAUC was significantly reduced compared to the non-exercise CON condition ($d = 0.81$; **Figure 5**). While reference ranges for CGM-derived treatment targets are constantly being refined (45, 49), a reference range for what constitutes a clinically important exercise-induced change in a CGM-derived variable does not currently exist. Nevertheless, the improved postprandial glucose response in the current study is in agreement with previous research also demonstrating the potency of exercise in improving this specific CGM-derived measure of glycemic control (18, 20, 21). However, this beneficial effect was blunted when the same individuals were exposed to steady-state

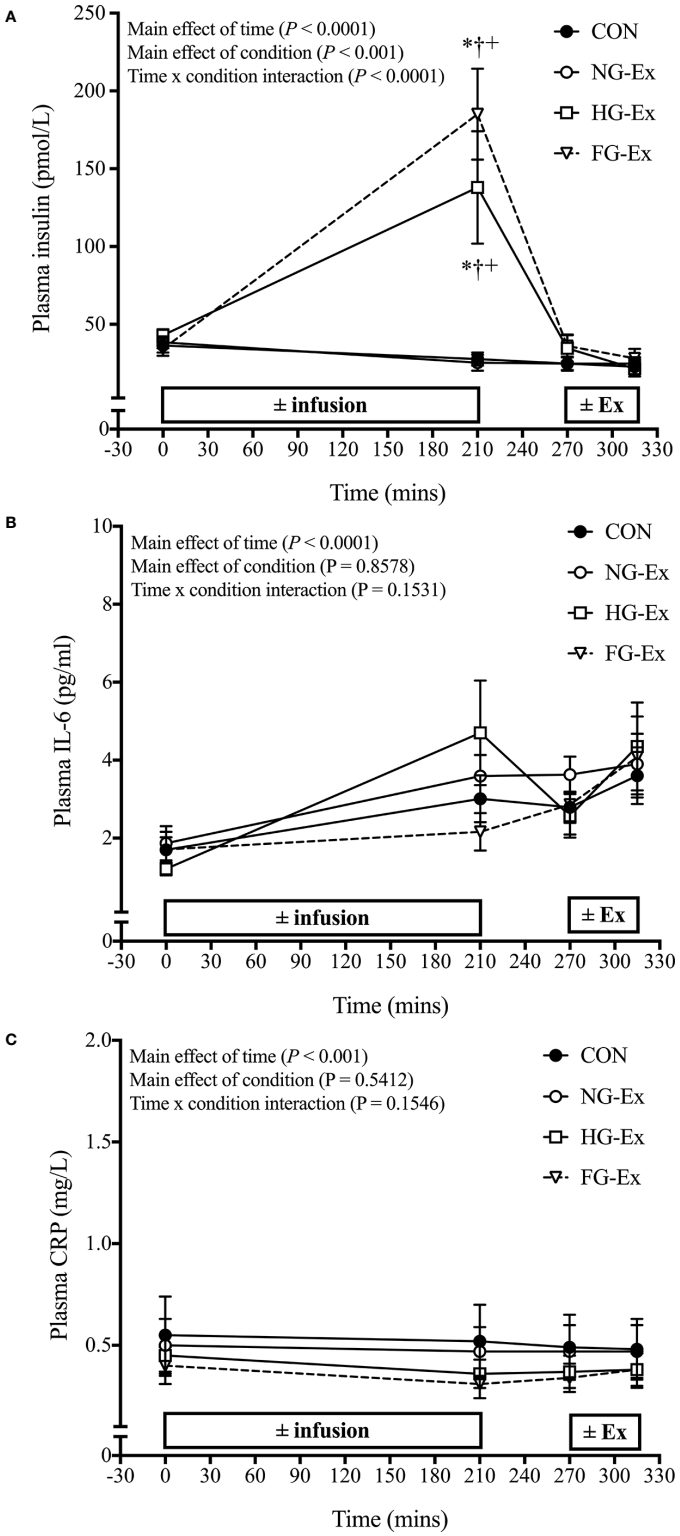


FIGURE 3 | Time course for changes in plasma insulin, IL-6, and CRP. **(A)** Time course for plasma insulin (pmol/L) during each experimental trial. *$P < 0.0001$ (HG-Ex, $d = 1.24$; FG-Ex, $d = 2.18$) vs. time-matched CON; $^{\dagger}P < 0.0001$ (HG-Ex, $d = 1.26$; FG-Ex, $d = 2.20$) vs. time-matched NG-Ex; $^{+}P < 0.0001$ (HG-Ex, $d = 1.07$; FG-Ex, $d = 2.09$) vs. pre-infusion. **(B)** Time course for IL-6 concentration (pg/mL) during each experimental trial. **(C)** Time course for CRP (mg/L) during each experimental trial.

TABLE 3 | Physiological responses to acute exercise intervention.

	CON	NG-Ex	HG-Ex	FG-Ex
Duration, min	44.6 ± 0.4	44.6 ± 0.4	44.6 ± 0.4	44.6 ± 0.4
Heart rate, bpm	61 ± 2	132 ± 2*	131 ± 2*	130 ± 2*
Heart rate, %HRmax	32.2 ± 1.2	69.5 ± 0.4*	68.7 ± 0.7*	68.1 ± 0.7*
Power output, W	0.0 ± 0.0	88.6 ± 4.8*	88.0 ± 5.1*	90.3 ± 4.7*
Power output, %Wmax	0.0 ± 0.0	33.1 ± 0.7*	32.9 ± 1.3*	33.9 ± 1.2*
$\dot{V}O_2$, ml/min/kg	4.0 ± 0.3	22.0 ± 1.6*	21.5 ± 1.2*	21.5 ± 1.1*
$\dot{V}O_2$, %$\dot{V}O_2$max	10.0 ± 0.5	55.0 ± 3.5*	53.3 ± 2.4*	54.7 ± 4.0*
Work done, J	0.0 ± 0.0	3884.7 ± 209.2*	3875.3 ± 223.3*	3950.7 ± 214.6*
Energy expenditure, kcals	61.7 ± 3.6	326.1 ± 18.8*	322.9 ± 15.0*	324.0 ± 14.6*
RER, a.u.	0.82 ± 0.01	0.90 ± 0.01*	0.94 ± 0.01*†	0.94 ± 0.01*†

*Data are mean ± SEM. *P < 0.05 vs. CON. †P < 0.05 vs. NG-Ex.*

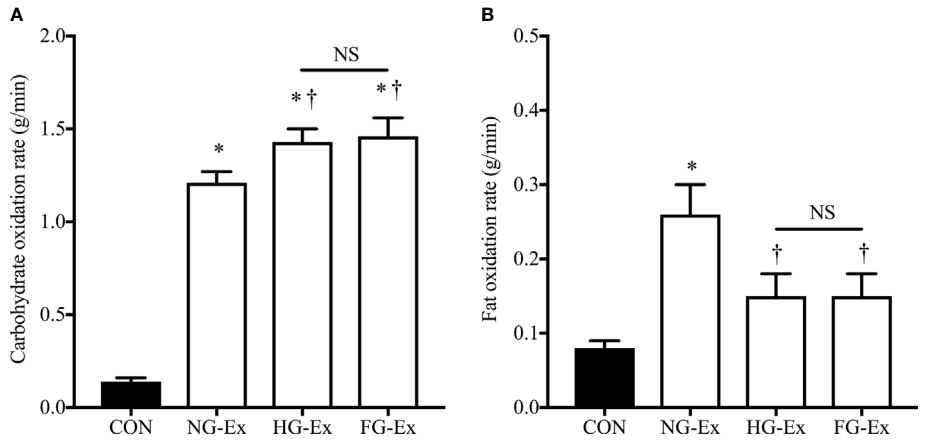


FIGURE 4 | Rates of substrate oxidation during exercise. **(A)** Carbohydrate oxidation rates during the 45-min ± exercise period within the four experimental trials. *$P < 0.05$ (NG-Ex, $d = 6.85$; HG-Ex, $d = 7.06$; FG-Ex, $d = 5.18$) vs. CON. †$P < 0.05$ (HG-Ex, $d = 0.96$; FG-Ex, $d = 0.84$) vs. NG-Ex. **(B)** Fat oxidation rates during the 45-min ± exercise period within the four experimental trials. *$P < 0.05$ (NG-Ex, $d = 1.81$) vs. CON. †$P < 0.05$ (HG-Ex, $d = 0.90$; FG-Ex, $d = 0.89$) vs. NG-Ex.

TABLE 4 | Physical activity, dietary intake, and free-living CGM variables measured for 24 h following each experimental trial.

	Post-trial standardizations			
	CON	NG-Ex	HG-Ex	FG-Ex
Dietary intake				
Energy intake, kcals	2467.6 ± 4.6	2467.6 ± 4.6	2467.6 ± 4.6	2467.6 ± 4.6
Carbohydrate intake (% of kcals)	54.9 ± 0.1	54.9 ± 0.1	54.9 ± 0.1	54.9 ± 0.1
Fat intake (% of kcals)	31.6 ± 0.1	31.6 ± 0.1	31.6 ± 0.1	31.6 ± 0.1
Protein intake (% of kcals)	13.5 ± 0.1	13.5 ± 0.1	13.5 ± 0.1	13.5 ± 0.1
Physical activity				
Step count, steps	8729 ± 1333	8895 ± 853	7355 ± 998	8661 ± 1260
Energy expenditure, kcals	476.4 ± 63.8	474.7 ± 50.1	397.3 ± 60.0	473.6 ± 76.9
Sedentary time, min	711.5 ± 75.3	733.8 ± 66.7	764.7 ± 60.7	709.5 ± 64.5
MVPA time, min	56.8 ± 10.4	55.2 ± 6.5	42.5 ± 7.7	55.6 ± 10.9
CGM-derived variables				
Mean 24-h glucose, mmol/L	5.3 ± 0.2	5.2 ± 0.1	5.3 ± 0.2	5.3 ± 0.2
Glucose variability, SD	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
Glucose variability, CV%	14.1 ± 0.8	14.1 ± 1.1	13.8 ± 0.9	14.3 ± 1.3

Data are mean ± SEM. No significant differences.

hyperglycemia or fluctuating glycemia before exercise (**Figure 5**). This is the first experimental evidence demonstrating a direct effect of pre-exercise hyperglycemia upon the potency of exercise to induce glucoregulatory benefits. The findings, therefore, support that hyperglycemia contributes to the inter-individual heterogeneity of the metabolic response to exercise.

The magnitude and profile of an individual's postprandial glucose response are determined by multiple factors. These

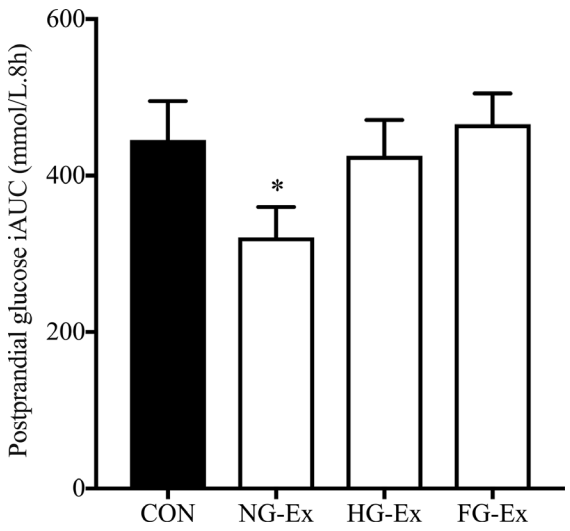


FIGURE 5 | The effects of the four interventions on total postprandial glucose iAUC. Total postprandial glucose response (the sum of post-meal glucose iAUC across four standardized meals) was measured using CGM following four experimental trials. *$P < 0.05$ (NG-Ex, $d = 0.81$) vs. CON.

include meal characteristics such as caloric content and macronutrient composition, but also the nutrient-induced gastrointestinal responses, tissue glucose uptake, insulin sensitivity, pancreatic β-cell insulin secretory function, glucose effectiveness, and hepatic and renal glucose handling (50–53), all of which are regulated by exercise. Therefore, the exercise-induced enhancement in postprandial glucose control seen in the NG-Ex trial is likely attributable to coordinated exercise-mediated enhancements in any of the above factors. As a corollary, if pre-exercise hyperglycemia impairs any of these adaptive processes, this would feasibly blunt exercise-induced improvements in postprandial glucose response. This notion is supported by evidence demonstrating that chronic hyperglycemia is associated with smaller exercise-induced improvements in peripheral insulin sensitivity, which in turn was linked with blunted exercise-induced improvements in postprandial glucose response (54).

Furthermore, hyperglycemia impairs skeletal muscle cell insulin sensitivity (55–58), pancreatic β-cell insulin secretory capacity (59, 60), and endothelial cell function (61, 62) within *in vitro* experimental models. Likewise, skeletal muscle (63, 64) and hepatic (65) insulin sensitivity, insulin secretory capacity (63), and vascular function (66) are impaired by exposure to experimental hyperglycemia *in vivo*, even in healthy individuals with normal glucose tolerance. Such hyperglycemia-induced impairments could also explain the elevated postprandial glucose response following the HG-Ex and FG-Ex trials.

Impaired function of key glucoregulatory tissues, including muscle, liver, and the endocrine pancreas, may also contribute indirectly to poorer postprandial glucose response following HG-Ex and FG-Ex since the impaired function of these tissues at baseline has also been associated with poorer outcomes following exercise. For example, blunted exercise-induced improvements in insulin sensitivity have been documented in individuals with higher baseline insulin resistance (54), and poorer contraction-induced improvements in glucose metabolism and insulin sensitivity has been found in primary myotubes from insulin resistant (e.g., obese and/or T2DM) vs. healthy donors (67, 68). Similarly, poorer baseline pancreatic β-cell function is predictive of poorer exercise responsiveness (33). Collectively, in this study, pre-exercise hyperglycemia may have impaired the function of glucoregulatory tissues (muscle, pancreas, etc.) while also blunting their ability to respond to exercise.

Markers of inflammation and oxidative stress are elevated in T2DM and are increased by experimental hyperglycemia (66). Since excessive levels of inflammation and oxidative stress can impair exercise adaptations (69), pre-exercise hyperglycemia-induced inflammation and oxidative stress may influence exercise-induced benefits. However, we found no significant interaction effect in two markers of systemic inflammation (IL-6 and CRP; **Figure 3**) suggesting that inflammation may not explain our findings. That said, tissue inflammation was not examined and these outcomes may be different in a patient population. Alternatively, pre-exercise hyperglycemia appeared to promote carbohydrate utilization (**Figure 4A**) and suppress fat utilization (**Figure 4B**) during exercise compared to exercise under normoglycemic conditions despite matched exercise stimuli (**Table 3**). Such alterations in substrate use during exercise may contribute to altered glucoregulation during the post-exercise period.

In contrast to the exercise-induced improvements in postprandial glucose, there were no significant effects of exercise upon mean 24-h glucose concentration, glucose SD or glucose CV%. This contradicts the improvements in 24-h glycemic control seen in hyperglycemic participants (20, 38, 40, 70). The smaller margin for improvement in the normal glucose-tolerant participants in the current study (HbA1c = 5.4 ± 0.1%) compared to the aforementioned studies in hyperglycemic individuals may contribute to this discrepancy. Interestingly, there were also no significant effects of HG-Ex or FG-Ex on these markers of 24-h glycemic control compared to either normoglycemic condition, highlighting that the ability of healthy humans to regulate glucose homeostasis under a challenge from distinctly different stimuli (i.e., exercise and hyperglycemia) is remarkably well-preserved.

Given the aim of the current study to investigate possible contributing factors to response heterogeneity in glycemic improvements following exercise, it would be remiss to not discuss variability in outcomes. Inter-individual heterogeneity showed that 9, 7, and 7 out of 12 individuals showed a decrease in 24-h mean glucose, glucose SD, and glucose CV%, respectively, following exercise. This variability in responses likely contributes to the lack of significance seen for these outcomes. A further anecdotal observation is that pre-exercise hyperglycemia in HG-Ex and FG-Ex reduced the participants' enjoyment during exercise when compared to NG-Ex. This is important because reduced enjoyment during exercise could feasibly impact exercise adherence in a free-living setting.

In the present study, healthy, recreationally active, non-diabetic individuals were exposed to blood glucose profiles similar to those seen in T2DM (i.e., elevated and unstable) for 3.5 h. It is important to note that the short-term, acute nature of this likely represents a different metabolic and physiological challenge compared to the chronic state of hyperglycemia and/or glycemic instability in T2DM. Nevertheless, the approach used enabled us to isolate the effect of acute hyperglycemia from other symptoms or comorbidities of T2DM (e.g., chronic low-grade inflammation, dyslipidaemia, impaired insulin sensitivity and/or secretion, etc.), while also avoiding changes in circulating incretin hormones (e.g., GLP-1) otherwise induced by oral glucose ingestion (71), thus justifying the study design used. That said, since pancreatic clamp conditions were not employed during glucose infusion in the current study, the possibility that increased plasma insulin in hyperglycemic (HG-Ex and FG-Ex) vs. normoglycemic (NG-Ex and CON) conditions (**Figure 3A**) contributed to the responses seen cannot be excluded. Additionally, short-term hyperglycemia, such as during a 2-h hyperglycemic clamp, reduces plasma glucagon concentration, and suppresses endogenous glucose production in healthy individuals (63), and such effects may contribute to responses observed in the current study. Furthermore, since exercise in the current study is acute and of moderate-intensity, whether exercise within training regimes and/or of greater intensity elicits the same outcomes remains to be determined. Future studies should also aim to quantify the long-term impact of changes in CGM-derived outcomes on the risk of developing hyperglycemia-related conditions/complications.

Using glucose infusion to induce hyperglycemia introduced caloric and carbohydrate imbalances between trials. Specifically, there was an energy deficit in CON (−61.7 ± 3.6 kcals) and NG-Ex (−326.1 ± 18.8 kcals) and a slight energy surplus in HG-Ex (+10.9 ± 20.5 kcals) and FG-Ex (+9.8 ± 17.7 kcals). Similarly, while more carbohydrate was oxidized during exercise within the infusion trials compared to NG-Ex (**Figure 4A**), this difference does not account for the amount of glucose infused during HG-Ex and FG-Ex. While the thermic effect of glucose infusion itself, which increases resting energy expenditure (by ~5%–11%) (72–74) and carbohydrate oxidation (75), may attenuate these between-trial imbalances to some extent, the possibility that they contributed to findings cannot be excluded. Excess glucose may have increased liver (76) and skeletal muscle (64) glycogen storage which may impact the capacity for postprandial glucose disposal during the post-exercise period in the infusion trials only. That said, while glucose infusion to maintain glucose concentrations at +2.5 mmol/L above fasting for 3 days doubled muscle glycogen (64), the short-term, acute nature of infusion in the current study (3.5 h) is unlikely to induce such a stark increase. Additionally, moderate-intensity exercise still reduces muscle glycogen under conditions of experimental hyperglycemia (via glucose infusion) (77) and when pre-exercise muscle glycogen is elevated (78). Furthermore, post-exercise glycogen storage is not solely reliant on changes at the muscle itself, but also splanchnic bed responses that increase the rate of oral glucose appearance in the circulation (79).

Compensating for the aforementioned imbalances with altered food provision without several additional control trials would introduce other confounding variables (e.g., differences in macronutrient provision), meaning that imbalance of some form is somewhat inevitable. Therefore, in line with many studies evaluating the effects of exercise vs. rest (20, 21, 38) or normo- vs. hyperglycemia (63, 64) upon glucose metabolism, compensation of these caloric and carbohydrate imbalances were not included, with the meals kept constant across all conditions. Moreover, this should not detract from the significance of the findings since the exercise-induced improvements in the function of key glucoregulatory tissues (e.g., muscle, pancreas, liver, gastrointestinal tract, etc.) that contribute to improved postprandial glucose result from more than simply an energy and glycogen deficit.

A key methodological strength of the current study is the balance between having strict control of pre-trial and post-trial standardization, glycemic interventions, exercise interventions, and dietary control, while monitoring clinically relevant outcomes (i.e., CGM-derived glycemic control) in an otherwise free-living, ecologically-valid setting. Additionally, unlike some previous exercise vs. control studies using CGM-derived outcomes (18, 70, 80), trial order was randomized to minimise the risk of bias. The consumption of the same controlled diet and the same physical activity levels across all 24-h post-trial periods increases the confidence that observed effects upon postprandial glucose are likely attributable to differences in the experimental treatments (i.e., ± glucose infusion, ± exercise) during the respective trials.

In the context of ecological validity, this study implicates a direct inhibitory effect of pre-exercise hyperglycemia upon exercise-induced improvements in postprandial glucose control. Therefore, it could be speculated that reducing hyperglycemia before exercise and/or coinciding exercise sessions with periods of improved glucose control (i.e., lower, more stable levels) may be a necessary strategy for optimizing the therapeutic effects of exercise in individuals with hyperglycemia-related conditions, such as T2DM. Hyperglycemia-lowering medication, dietary interventions, or optimizing exercise-meal timing may provide viable options in this regard—that said, both diet and medication have themselves been implicated in variable exercise responsiveness (27, 30). Therefore, an individualized approach to glucose management will likely be best and more work in that area is required. Future work must also resolve how the findings translate to longer-term exercise training interventions in patients with T2DM before extrapolation to disease management. That said, large portions of the worldwide population are non-diabetic meaning these results hold clinical significance in the context of disease prevention.

In conclusion, this study provides the first experimental evidence that pre-exercise hyperglycemia can blunt the glucoregulatory benefits of acute exercise, suggesting that hyperglycemia contributes to exercise response heterogeneity. Resolving hyperglycemia before exercise and/or coinciding exercise sessions with periods of improved glucose control may, therefore, be vital for maximizing the therapeutic effects of exercise in individuals with and at risk of hyperglycemia-related conditions, such as T2DM.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical approval was obtained through the West Midlands - South Birmingham Research Ethics Committee (16/WM/0242) and sponsored by the University of Birmingham Research Governance. All participants provided written informed consent before participation.

AUTHOR CONTRIBUTIONS

SC and TPJS conceived the idea, designed the study, and implemented the trials. SC completed the data analysis and wrote the manuscript. SC and TPJS interpreted the findings and discussed the data. TPJS edited the manuscript. All authors agree to be accountable for the content of the work. All authors contributed to the article and approved the submitted version.

FUNDING

The study was funded in part by the Physiological Society and the European Commission (Marie Skłodowska-Curie Individual Fellowship).

ACKNOWLEDGMENTS

The authors thank the research participants for their time, Ascensia Diabetes Care UK for the provision of Contour Next One blood glucose monitors, and Andy Nicholson at Dexcom UK for his analytical and technical support throughout the study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2020.566548/full#supplementary-material

REFERENCES

1. Rubin J, Matsushita K, Ballantyne CM, Hoogeveen R, Coresh J, Selvin E. Chronic hyperglycemia and subclinical myocardial injury. *J Am Coll Cardiol* (2012) 59:484–9. doi: 10.1016/j.jacc.2011.10.875
2. Sarwar N, Gao P, Kondapally Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: A collaborative meta-analysis of 102 prospective studies. *Lancet* (2010) 375:2215–22. doi: 10.1016/S0140-6736(10)60484-9
3. Ceriello A. Hyperglycemia and the vessel wall: the pathophysiological aspects on the atherosclerotic burden in patients with diabetes. *Eur J Cardiovasc Prev Rehabil* (2010) 17:S15–9. doi: 10.1097/01.hjr.0000368193.24732.66
4. Marini MA, Succurro E, Castaldo E, Cufone S, Arturi F, Sciacqua A, et al. Cardiometabolic risk profiles and carotid atherosclerosis in individuals with prediabetes identified by fasting glucose, postchallenge glucose, and hemoglobin A 1c criteria. *Diabetes Care* (2012) 35:1144–9. doi: 10.2337/dc11-2032
5. Cavalot F. Do data in the literature indicate that glycemic variability is a clinical problem? Glycemic variability and vascular complications of diabetes. *Diabetes Obes Metab* (2013) 15:3–8. doi: 10.1111/dom.12140
6. Cavalot F, Petrelli A, Traversa M, Bonomo K, Fiora E, Conti M, et al. Postprandial blood glucose is a stronger predictor of cardiovascular events than fasting blood glucose in type 2 diabetes mellitus, particularly in women: Lessons from the San Luigi Gonzaga diabetes study. *J Clin Endocrinol Metab* (2006) 91:813–9. doi: 10.1210/jc.2005-1005
7. Færch K, Alssema M, Mela DJ, Borg R, Vistisen D. Relative contributions of preprandial and postprandial glucose exposures, glycemic variability, and non-glycemic factors to HbA1c in individuals with and without diabetes. *Nutr Diabetes* (2018) 8:0–8. doi: 10.1038/s41387-018-0047-8
8. Boniol M, Dragomir M, Autier P, Boyle P. Physical activity and change in fasting glucose and HbA1c: a quantitative meta-analysis of randomized trials. *Acta Diabetol* (2017) 54:983–91. doi: 10.1007/s00592-017-1037-3
9. Colberg SR, Sigal RJ, Yardley JE, Riddell MC, Dunstan DW, Dempsey PC, et al. Physical activity/exercise and diabetes: A position statement of the American Diabetes Association. *Diabetes Care* (2016) 39:2065–79. doi: 10.2337/dc16-1728
10. Kasumov T, Solomon TPJ, Hwang C, Huang H, Haus JM, Zhang R, et al. Improved insulin sensitivity after exercise training is linked to reduced plasma C14:0 ceramide in obesity and type 2 diabetes. *Obesity* (2015) 23:1414–21. doi: 10.1002/oby.21117
11. Meex RCR, Schrauwen-Hinderling VB, Moonen-Kornips E, Schaart G, Mensink M, Phielix E, et al. Restoration of muscle mitochondrial function and metabolic flexibility in type 2 diabetes by exercise training is paralleled by increased myocellular fat storage and improved insulin sensitivity. *Diabetes* (2010) 59:572–9. doi: 10.2337/db09-1322
12. Kirwan JP, Solomon TPJ, Wojta DM, Staten MA, Holloszy JO. Effects of 7 days of exercise training on insulin sensitivity and responsiveness in type 2 diabetes mellitus. *Am J Physiol Metab* (2009) 297:E151–6. doi: 10.1152/ajpendo.00210.2009
13. Dela F, von Linstow ME, Mikines KJ, Galbo H. Physical training may enhance β-cell function in type 2 diabetes. *Am J Physiol Metab* (2004) 287:E1024–31. doi: 10.1152/ajpendo.00056.2004
14. Solomon TPJ, Haus JM, Kelly KR, Rocco M, Kashyap SR, Kirwan JP. Improved pancreatic β-cell function in type 2 diabetic patients after lifestyle-induced weight loss is related to glucose-dependent insulinotropic polypeptide. *Diabetes Care* (2010) 33:1561–6. doi: 10.2337/dc09-2021
15. Sylow L, Richter EA. Current advances in our understanding of exercise as medicine in metabolic disease. *Curr Opin Physiol* (2019) 12:12–9. doi: 10.1016/j.cophys.2019.04.008
16. Knudsen SH, Karstoft K, Winding K, Holst JJ, Pedersen BK, Solomon TPJ. Effects of acute exercise on pancreatic endocrine function in subjects with type 2 diabetes. *Diabetes Obes Metab* (2015) 17:207–10. doi: 10.1111/dom.12413
17. Kearney ML, Thyfault JP. Exercise and postprandial glycemic control in type 2 diabetes. *Curr Diabetes Rev* (2016) 12:199–210. doi: 10.2174/1573399811666150615112441
18. Gillen JB, Little JP, Punthakee Z, Tarnopolsky MA, Riddell MC, Gibala MJ. Acute high-intensity interval exercise reduces the postprandial glucose response and prevalence of hyperglycemia in patients with type 2 diabetes. *Diabetes Obes Metab* (2012) 14:575–7. doi: 10.1111/j.1463-1326.2012.01564.x
19. MacLeod SF, Terada T, Chahal BS, Boulé NG. Exercise lowers postprandial glucose but not fasting glucose in type 2 diabetes: A meta-analysis of studies using continuous glucose monitoring. *Diabetes Metab Res Rev* (2013) 29:593–603. doi: 10.1002/dmrr.2461
20. Oberlin DJ, Mikus CR, Kearney ML, Hinton PS, Manrique C, Leidy HJ, et al. One bout of exercise alters free-living postprandial glycemia in type 2 diabetes. *Med Sci Sports Exerc* (2014) 46:232–8. doi: 10.1249/MSS.0b013e3182a54d85

21. van Dijk JW, Venema M, van Mechelen W, Stehouwer CDA, Hartgens F, van Loon LJC. Effect of moderate-intensity exercise versus activities of daily living on 24-hour blood glucose homeostasis in male patients with type 2 diabetes. *Diabetes Care* (2013) 36:3448–53. doi: 10.2337/dc12-2620
22. ADA. 5. Lifestyle management: Standards of medical care in diabetes — 2019. *Diabetes Care* (2019) 42:S46–60. doi: 10.2337/dc19-S005
23. van Dijk JW, Manders RJF, Canfora EE, Van Mechelen W, Hartgens F, Stehouwer CDA, et al. Exercise and 24-h glycemic control: Equal effects for all type 2 diabetes patients? *Med Sci Sports Exerc* (2013) 45:628–35. doi: 10.1249/MSS.0b013e31827ad8b4
24. Terada T, Friesen A, Chahal BS, Bell GJ, McCargar LJ, Boulé NG. Exploring the variability in acute glycemic responses to exercise in type 2 diabetes. *J Diabetes Res* (2013) 2013:591574. doi: 10.1155/2013/591574
25. Boulé NG, Weisnagel SJ, Lakka TA, Tremblay A, Bergman RN, Rankinen T, et al. Effects of exercise training on glucose homeostasis: the HERITAGE Family Study. *Diabetes Care* (2005) 28:108–14. doi: 10.2337/diacare.28.1.108
26. Bouchard C, Blair SN, Church TS, Earnest CP, Hagberg JM, Häkkinen K, et al. Adverse metabolic response to regular exercise: is it a rare or common occurrence? *PLoS One* (2012) 7:e37887. doi: 10.1371/journal.pone.0037887
27. Solomon TP. Sources of inter-individual variability in the therapeutic response of blood glucose control to exercise in type 2 diabetes: Going beyond exercise dose. *Front Physiol* (2018) 9:896. doi: 10.3389/fphys.2018.00896
28. Sparks LM. Exercise training response heterogeneity: Physiological and molecular insights. *Diabetologia* (2017) 60:2329–36. doi: 10.1007/s00125-017-4461-6
29. Böhm A, Weigert C, Staiger H, Häring HU. Exercise and diabetes: Relevance and causes for response variability. *Endocrine* (2016) 51:390–401. doi: 10.1007/s12020-015-0792-6
30. Færch K, Hulman A PJ. Solomon T. Heterogeneity of pre-diabetes and type 2 diabetes: Implications for prediction, prevention and treatment responsiveness. *Curr Diabetes Rev* (2015) 12:30–41. doi: 10.2174/1573399811666150416122903
31. Malin SK, Gerber R, Chipkin SR, Braun B. Independent and combined effects of exercise training and metformin on insulin sensitivity in individuals with prediabetes. *Diabetes Care* (2012) 35:131–6. doi: 10.2337/dc11-0925
32. Sharoff CG, Hagobian TA, Malin SK, Chipkin SR, Yu H, Hirshman MF, et al. Combining short-term metformin treatment and one bout of exercise does not increase insulin action in insulin-resistant individuals. *Am J Physiol Endocrinol Metab* (2010) 298:E815–23. doi: 10.1152/ajpendo.00517.2009
33. Solomon TPJ, Malin SK, Karstoft K, Kashyap SR, Haus JM, Kirwan JP. Pancreatic β-cell function is a stronger predictor of changes in glycemic control after an aerobic exercise intervention than insulin sensitivity. *J Clin Endocrinol Metab* (2013) 98:4176–86. doi: 10.1210/jc.2013-2232
34. AbouAssi H, Slentz CA, Mikus CR, Tanner CJ, Bateman LA, Willis LH, et al. The effects of aerobic, resistance, and combination training on insulin sensitivity and secretion in overweight adults from STRRIDE AT/RT: a randomized trial. *J Appl Physiol* (2015) 118:1474–82. doi: 10.1152/japplphysiol.00509.2014
35. Malin SK, Kirwan JP. Fasting hyperglycemia blunts the reversal of impaired glucose tolerance after exercise training in obese older adults. *Diabetes Obes Metab* (2012) 14:835–41. doi: 10.1111/j.1463-1326.2012.01608.x
36. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* (2007) 39:175–91. doi: 10.3758/BF03193146
37. Jakobsen I, Solomon TPJ, Karstoft K. The acute effects of interval-type exercise on glycemic control in type 2 diabetes subjects: Importance of interval length. A controlled, counterbalanced, crossover study. *PLoS One* (2016) 11:1–15. doi: 10.1371/journal.pone.0163562
38. Manders RJF, Van Dijk JWM, Van Loon LJC. Low-intensity exercise reduces the prevalence of hyperglycemia in type 2 diabetes. *Med Sci Sports Exerc* (2010) 42:219–25. doi: 10.1249/MSS.0b013e3181b3b16d
39. Karstoft K, Christensen CS, Pedersen BK, Solomon TPJ. The acute effects of interval- vs continuous-walking exercise on glycemic control in subjects with type 2 diabetes: A crossover, controlled study. *J Clin Endocrinol Metab* (2014) 99:3334–42. doi: 10.1210/jc.2014-1837
40. van Dijk JW, Tummers K, Stehouwer CDA, Hartgens F, van Loon LJC. Exercise therapy in type 2 diabetes: Is daily exercise required to optimize glycemic control? *Diabetes Care* (2012) 35:948–54. doi: 10.2337/dc11-2112
41. Karstoft K, Clark MA, Jakobsen I, Müller IA, Pedersen BK, Solomon TPJ, et al. The effects of 2 weeks of interval vs continuous walking training on glycemic control and whole-body oxidative stress in individuals with type 2 diabetes: a controlled, randomised, crossover trial. *Diabetologia* (2017) 60:508–17. doi: 10.1007/s00125-016-4170-6
42. van Dijk JW, Manders RJF, Tummers K, Bonomi AG, Stehouwer CDA, Hartgens F, et al. Both resistance- and endurance-type exercise reduce the prevalence of hyperglycemia in individuals with impaired glucose tolerance and in insulin-treated and non-insulin-treated type 2 diabetic patients. *Diabetologia* (2012) 55:1273–82. doi: 10.1007/s00125-011-2380-5
43. Nygaard H, Rønnestad BR, Hammarström D, Holmboe-Ottesen G, Høstmark AT. Effects of exercise in the fasted and postprandial state on interstitial glucose in hyperglycemic individuals. *J Sports Sci Med* (2017) 16:254–63.
44. Braun B, Brooks GA. Critical importance of controlling energy status to understand the effects of "exercise" on metabolism. *Exerc Sport Sci Rev* (2008) 36:2–4. doi: 10.1097/jes.0b013e31815e42c2
45. Battelino T, Danne T, Bergenstal RM, Amiel SA, Beck R, Biester T, et al. Clinical targets for continuous glucose monitoring data interpretation: Recommendations from the international consensus on time in range. *Diabetes Care* (2019) 42:1593–603. doi: 10.2337/dci19-0028
46. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* (1985) 28:412–9. doi: 10.1007/BF00280883
47. Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol* (1983) 55:628–34. doi: 10.1152/jappl.1983.55.2.628
48. Jeukendrup AE, Wallis GA. Measurement of substrate oxidation during exercise by means of gas exchange measurements. *Int J Sports Med* (2005) 26:S28–37. doi: 10.1055/s-2004-830512
49. Danne T, Nimri R, Battelino T, Bergenstal RM, Close KL, DeVries JH, et al. International consensus on use of continuous glucose monitoring. *Diabetes Care* (2017) 40:1631–40. doi: 10.2337/dc17-1600
50. Solomon TPJ, Eves FF, Laye MJ. Targeting postprandial hyperglycemia with physical activity may reduce cardiovascular disease risk. But what should we do, and when is the right time to move? *Front Cardiovasc Med* (2018) 5:99. doi: 10.3389/fcvm.2018.00099
51. Nathan DM, Davidson MB, DeFronzo RA, Heine RJ, Henry RR, Pratley R, et al. Impaired fasting glucose and impaired glucose tolerance: Implications for care. *Diabetes Care* (2007) 30:753–9. doi: 10.2337/dc07-9920
52. Abdul-Ghani MA, Jenkinson CP, Richardson DK, Tripathy D, DeFronzo RA. Insulin secretion and action in subjects with impaired fasting glucose and impaired glucose tolerance: Results from the veterans administration genetic epidemiology study. *Diabetes* (2006) 55:1430–5. doi: 10.2337/db05-1200
53. van Wijck K, Lenaerts K, Grootjans J, Wijnands KAP, Poeze M, van Loon LJC, et al. Physiology and pathophysiology of splanchnic hypoperfusion and intestinal injury during exercise: Strategies for evaluation and prevention. *Am J Physiol Gastrointest Liver Physiol* (2012) 303:155–68. doi: 10.1152/ajpgi.00066.2012
54. Malin SK, Haus JM, Solomon TPJ, Blaszczak A, Kashyap SR, Kirwan JP. Insulin sensitivity and metabolic flexibility following exercise training among different obese insulin-resistant phenotypes. *Am J Physiol Endocrinol Metab* (2013) 305:E1292–8. doi: 10.1152/ajpendo.00441.2013
55. Aas V, Kase ET, Solberg R, Jensen J, Rustan AC. Chronic hyperglycemia promotes lipogenesis and triacylglycerol accumulation in human skeletal muscle cells. *Diabetologia* (2004) 47:1452–61. doi: 10.1007/s00125-004-1465-9
56. Aas V, Hessvik NP, Wettergreen M, Hvammen AW, Hallén S, Thoresen GH, et al. Chronic hyperglycemia reduces substrate oxidation and impairs metabolic switching of human myotubes. *Biochim Biophys Acta Mol Basis Dis* (2011) 1812:94–105. doi: 10.1016/j.bbadis.2010.09.014
57. Green CJ, Henriksen TI, Pedersen BK, Solomon TPJ. Glucagon like peptide-1-induced glucose metabolism in differentiated human muscle satellite cells is attenuated by hyperglycemia. *PLoS One* (2012) 7:e44284. doi: 10.1371/journal.pone.0044284
58. Zhang W, Liu J, Tian L, Liu Q, Fu Y, Garvey WT. TRIB3 mediates glucose-induced insulin resistance via a mechanism that requires the hexosamine biosynthetic pathway. *Diabetes* (2013) 62:4192–200. doi: 10.2337/db13-0312
59. Vasu S, McClenaghan NH, McCluskey JT, Flatt PR. Cellular responses of novel human pancreatic β-cell line, 1.1B4 to hyperglycemia. *Islets* (2013) 5:170–7. doi: 10.4161/isl.26184

60. Bensellam M, Van Lommel L, Overbergh L, Schuit FC, Jonas JC. Cluster analysis of rat pancreatic islet gene mRNA levels after culture in low-, intermediate- and high-glucose concentrations. *Diabetologia* (2009) 52:463–76. doi: 10.1007/s00125-008-1245-z
61. Quagliaro L, Piconi L, Assaloni R, Martinelli L, Motz E, Ceriello A. Intermittent high glucose enhances apoptosis related to oxidative stress in human umbilical vein endothelial cells: The role of protein kinase C and NAD(P)H-oxidase activation. *Diabetes* (2003) 52:2795–804. doi: 10.2337/diabetes.52.11.2795
62. Quagliaro L, Piconi L, Assaloni R, Da Ros R, Maier A, Zuodar G, et al. Intermittent high glucose enhances ICAM-1, VCAM-1 and E-selectin expression in human umbilical vein endothelial cells in culture: The distinct role of protein kinase C and mitochondrial superoxide production. *Atherosclerosis* (2005) 183:259–67. doi: 10.1016/j.atherosclerosis.2005.03.015
63. Solomon TPJ, Knudsen SH, Karstoft K, Winding K, Holst JJ, Pedersen BK. Examining the effects of hyperglycemia on pancreatic endocrine function in humans: Evidence for in vivo glucotoxicity. *J Clin Endocrinol Metab* (2012) 97:4682–91. doi: 10.1210/jc.2012-2097
64. Shannon C, Merovci A, Xiong J, Tripathy D, Lorenzo F, McClain D, et al. Effect of chronic hyperglycemia on glucose metabolism in subjects with normal glucose tolerance. *Diabetes* (2018) 67:2507–17. doi: 10.2337/db18-0439
65. Tripathy D, Merovci A, Basu R, Abdul-Ghani M, Defronzo RA. Mild physiologic hyperglycemia induces hepatic insulin resistance in healthy normal glucose-tolerant participants. *J Clin Endocrinol Metab* (2019) 104:2842–50. doi: 10.1210/jc.2018-02304
66. Ceriello A, Esposito K, Piconi L, Ihnat MA, Thorpe JE, Testa R, et al. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. *Diabetes* (2008) 57:1349–54. doi: 10.2337/db08-0063
67. Park S, Turner KD, Zheng D, Brault JJ, Zou K, Chaves AB, et al. Electrical pulse stimulation induces differential responses in insulin action in myotubes from severely obese individuals. *J Physiol* (2019) 597:449–66. doi: 10.1113/JP276990
68. Feng YZ, Nikolić N, Bakke SS, Kase ET, Guderud K, Hjelmesæth J, et al. Myotubes from lean and severely obese subjects with and without type 2 diabetes respond differently to an in vitro model of exercise. *Am J Physiol Cell Physiol* (2015) 308:C548–56. doi: 10.1152/ajpcell.00314.2014
69. Radak Z, Chung HY, Koltai E, Taylor AW, Goto S. Exercise, oxidative stress and hormesis. *Ageing Res Rev* (2008) 7:34–42. doi: 10.1016/j.arr.2007.04.004
70. Macdonald AL, Philp A, Harrison M, Bone AJ, Watt PW. Monitoring exercise-induced changes in glycemic control in type 2 diabetes. *Med Sci Sports Exerc* (2006) 38:201–7. doi: 10.1249/01.mss.0000183852.31164.5a
71. Mingrone G, Panunzi S, De Gaetano A, Ahlin S, Spuntarelli V, Bondia-Pons I, et al. Insulin sensitivity depends on the route of glucose administration. *Diabetologia* (2020) 63:1382–95. doi: 10.1007/s00125-020-05157-w
72. Acheson KJ, Ravussin E, Wahren J, Jequier E. Thermic effect of glucose in man. Obligatory and facultative thermogenesis. *J Clin Invest* (1984) 74:1572–80. doi: 10.1172/JCI111573
73. Ravussin E, Acheson KJ, Vernet O, Danforth E, Jéquier E. Evidence that insulin resistance is responsible for the decreased thermic effect of glucose in human obesity. *J Clin Invest* (1985) 76:1268–73. doi: 10.1172/JCI112083
74. Ravussin E, Bogardus C, Schwartz RS, Robbins DC, Wolfe RR, Horton ES, et al. Thermic effect of infused glucose and insulin in man. Decreased response with increased insulin resistance in obesity and noninsulin-dependent diabetes mellitus. *J Clin Invest* (1983) 72:893–902. doi: 10.1172/JCI111060
75. Yki-Jarvinen H, Bogardus C, Howard BV. Hyperglycemia stimulates carbohydrate oxidation in humans. *Am J Physiol Metab* (1987) 253:E376–82. doi: 10.1152/ajpendo.1987.253.4.E376
76. Shulman GI, DeFronzo RA, Rossetti L. Differential effect of hyperglycemia and hyperinsulinemia on pathways of hepatic glycogen repletion. *Am J Physiol Metab* (1991) 260:E731–5. doi: 10.1152/ajpendo.1991.260.5.E731
77. Mohebbi H, Campbell IT, Keegan MA, Malone JJ, Hulton AT, MacLaren DPM. Hyperinsulinaemia and hyperglycemia promote glucose utilization and storage during low- and high-intensity exercise. *Eur J Appl Physiol* (2020) 120:127–35. doi: 10.1007/s00421-019-04257-9
78. Hargreaves M, McConell G, Proietto J. Influence of muscle glycogen on glycogenolysis and glucose uptake during exercise in humans. *J Appl Physiol* (1995) 78:288–92. doi: 10.1152/jappl.1995.78.1.288
79. Hamilton KS, Gibbons FK, Bracy DP, Lacy DB, Cherrington AD, Wasserman DH. Effect of prior exercise on the partitioning of an intestinal glucose load between splanchnic bed and skeletal muscle. *J Clin Invest* (1996) 98:125–35. doi: 10.1172/JCI118756
80. Praet SF, Manders RJ, Lieverse AG, Kuipers H, Stehouwer CD, Keizer HA, et al. Influence of acute exercise on hyperglycemia in insulin-treated type 2 diabetes. *Med Sci Sports Exerc* (2006) 38:2037–44. doi: 10.1249/01.mss.0000235352.09061.1d

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 Endocrinology

MINI REVIEW
published: 21 October 2020
doi: 10.3389/fendo.2020.573275

Factors Influencing Insulin Absorption Around Exercise in Type 1 Diabetes

Jason P. Pitt[1]*, *Olivia M. McCarthy*[1], *Thomas Hoeg-Jensen*[2], *Benjamin M. Wellman*[1] and *Richard M. Bracken*[1]

[1] Applied Sport, Technology, Exercise and Medicine Research Centre (A-STEM), College of Engineering, Swansea University, Swansea, United Kingdom, [2] Diabetes Peptide and Protein Chemistry, Novo Nordisk A/S, Maaloev, Denmark

International charities and health care organizations advocate regular physical activity for health benefit in people with type 1 diabetes. Clinical expert and international diabetes organizations' position statements support the management of good glycemia during acute physical exercise by adjusting exogenous insulin and/or carbohydrate intake. Yet research has detailed, and patients frequently report, variable blood glucose responses following both the same physical exercise session and insulin to carbohydrate alteration. One important source of this variability is insulin delivery to the circulation. With modern insulin analogs, it is important to understand how different insulins, their delivery methods, and inherent physiological factors, influence the reproducibility of insulin absorption from the injection site into circulation. Furthermore, contrary to the adaptive pancreatic response to exercise in the person without diabetes, the physiological and metabolic shifts with exercise may increase circulating insulin concentrations that may contribute to exercise-related hyperinsulinemia and consequent hypoglycemia. Thus, a furthered understanding of factors underpinning insulin delivery may offer more confidence for healthcare professionals and patients when looking to improve management of glycemia around exercise.

Keywords: absorption, insulin, exercise, pharmacokinetics, type 1 diabetes (T1D), subcutaneous tissue, physiology

OPEN ACCESS

Edited by:
Kristian Karstoft, Rigshospitalet, Denmark

Reviewed by:
Andreas Buch Møller, Aarhus University Hospital, Denmark
George L. King, Joslin Diabetes Center and Harvard Medical School, United States

***Correspondence:**
Jason P. Pitt, j.p.pitt@swansea.ac.uk

Specialty section:
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Endocrinology

Received: *16 June 2020*
Accepted: *16 September 2020*
Published: *21 October 2020*

Citation:
Pitt JP, McCarthy OM, Hoeg-Jensen T, Wellman BM and Bracken RM (2020) Factors Influencing Insulin Absorption Around Exercise in Type 1 Diabetes. Front. Endocrinol. 11:573275. doi: 10.3389/fendo.2020.573275

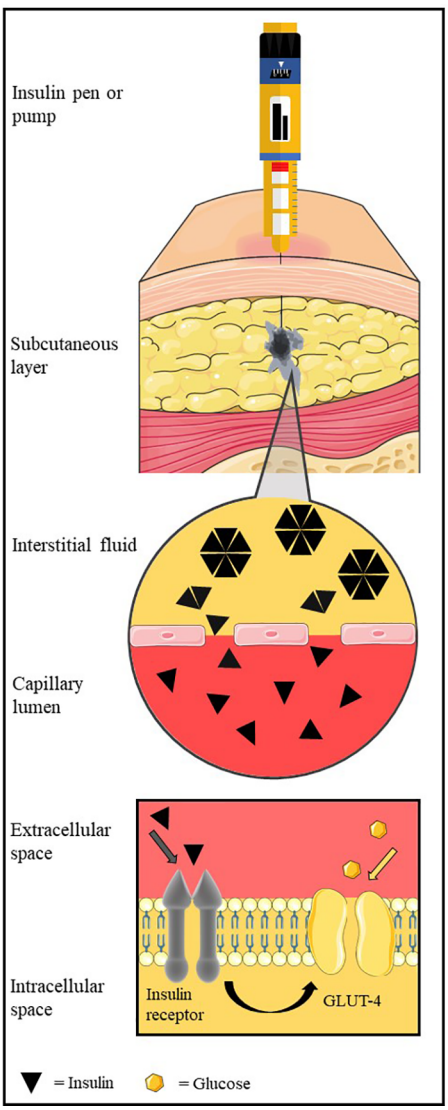
INTRODUCTION

People with type 1 diabetes (T1D) are required to administer exogenous insulin *via* multiple daily insulin (MDI) regimen or automated pump therapy. For both delivery methods, insulin is administered into adipose tissue that lies beneath the dermal layers of the skin and above the musculoskeletal compartment. Upon entering this subcutaneous layer, the injected insulin forms a depot, where it is at its highest concentration in the interstitium. Insulin spreads in the subcutaneous layer, following the path of least resistance around adipocytes and along loose connective tissue, towards the capillary system (1) (**Figure 1**). Separating the interstitium and the capillary lumen is the vascular endothelium which is poorly penetrated by hexameric insulin—the typical state in which it is stored. Insulin molecules must dissociate into smaller monomer units to move across the capillary endothelium and enter the circulation. The rate at which insulin molecules can move from

the initial depot to being physiologically available in the bloodstream determines the insulin's profile of metabolic activity.

It is of clinical concern when the *same* insulin regimen exhibits a varied glucose-lowering effect for the patient injecting insulin. Intraindividual variability in an insulin's absorption compromises the patient's ability to predict blood glucose concentrations, thereby increasing the risk of fluctuations outside the physiologic range (i.e. ≤3.9 - ≥10.0 mmol.L^{-1}) (2). This is especially pertinent to the individual with T1D who is engaging in structured exercise or spontaneous bouts of physical activity. The response to acute exercise induces significant changes in physiological systems which have the potential to alter the environment in which exogenous insulin is administered into, affecting its absorption kinetics and subsequent circulatory appearance. To aid in reproducing good glucose control, and mitigate the risk of exercise-induced hypoglycemia, it is important to be aware of the effects of exercise on the rate of insulin absorption (from injection depot into circulation).

This review aims to (i) inform the reader of relevant factors that influence insulin absorption at rest and (ii) critique the



Rest	Acute exercise	Chronic exercise
Insulin analogue type; Needle length; Technique; Region.	Exercise type; Duration; Intensity; Proximity to working muscle.	Potentially reduced total daily dose.
Subcutis thickness; Temperature.	Increased capillary distribution and vasodilation; Temperature; Contractile massage-like effect.	Reduced adiposity; Capillary angiogenesis; Potential efficient temperature response.
Basal or post-prandial adipose tissue blood flow.	Increased blood flow.	Lower cardiac output for given submaximal work.
Insulin sensitivity status.	Exercise-induced insulin-independent GLUT-4 translocation.	Improved insulin sensitivity.

FIGURE 1 | The pathway of subcutaneously administered exogenous insulin. Insulin is injected/released from formulation in the insulin pen/pump cartridge into the subcutaneous tissue. The insulin oligomers disassociate into monomer units before translocating across the capillary endothelium into blood circulation. Insulin circulates before binding to an insulin receptor to facilitate glucose uptake into the cell (e.g. into the myocyte). Factors at rest, acute exercise, and chronic exercise which affect each stage are listed along the row beside each illustrated stage of the pathway. Insulin diffusion in the subcutaneous layer is adapted with permission from digital images of insulin depot formation 15 to 30 s after bolus injection into porcine subcutaneous tissue; authored by Jockel et al. (1). Image is not to scale for illustration purposes. Created using Servier Medical Art (https://smart.servier.com/); Sevier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License.

research investigating exercise effects on insulin absorption, providing an evidence-based explanation of the underlying mechanisms, where possible.

EXOGENOUS INSULIN

At concentrations necessary for subcutaneous injection, human insulin self-associates into hexamer crystals which are too large to pass through capillary fenestrae and reach circulation. The significant time required to allow the hexamers in the subcutis to dissociate to monomer units, that are small enough to diffuse into the capillary lumen, results in serum insulin concentrations out of sync with post-absorption blood glucose concentrations. The development of genetic engineering in recombinant DNA technology enabled modifications of insulin's molecular structure that influence its pharmacokinetic properties. Initial modifications focused on inverting (Lys^{B28}-Pro^{B29}: insulin lispro) (3) and substituting (Pro^{B28} ➔ Asp^{B28}: insulin aspart) (4) the amino acid sequence to weaken the van der Waals forces and hydrogen bonds shared at the C-termini B-chains at the dimer interface to decrease self-association, while avoiding alterations of the insulin receptor binding sites of insulin. Development of long-acting insulin has been based on acylation and pH-dependent subcutaneous precipitation (5). Further to structural modifications, excipients can be added to (or removed from) marketed formulations to effect pharmacokinetics. For example, insulin fiasp includes the excipient niacinamide to induce local vasodilation and counter-act the aggregating effect of Zn^{2+} (6), which promotes faster absorption. Improvements in insulin absorption have also been demonstrated in protease (that prevent enzymatic degradation of insulin) (7) and hyaluronidase (that cleaves hyaluronate polymers at injection site) (8) excipients; however, these are yet to appear in marketed formulations. **Table 1** summarizes different exogenous insulins' chemical composition and their consequent pharmacokinetic properties.

PHYSICAL AND PHYSIOLOGICAL FACTORS AFFECTING THE DELIVERY OF INSULIN

Subcutaneous Tissue

The subcutaneous tissue consists primarily of adipocytes and an extracellular matrix made up of connective tissue and interstitial fluid, which present barriers of different resistances to insulin in its pathway to the vascular system (28). The impedance of insulin movement upon injection is subject to factors influencing the status of the subcutis, namely, the tissue type (injection depth), adipose tissue layer thickness, and temperature (**Figure 1**).

Injection Depth

Several studies demonstrate the importance of subcutaneous injection in tempering, and reducing the variation of, the pharmacokinetic/pharmacodynamic profiles of injected insulin compared to intramuscular injection at rest (29–31). Additionally, the extent to which the subcutaneous and muscular sites of injection are affected by exercise are not identical, which may result in differing rates of insulin absorption in the injection sites during exercise. An exercise-induced increase in insulin absorption was observed after intramuscular injection of ^{125}I-labeled insulin actrapid (human insulin) into the thigh prior to moderate-intensity cycling (intramuscular injection: rest, 0.46 ± 0.08 vs exercise, 1.17 ± 0.14%.min^{-1}; $p < 0.001$), but not after subcutaneous injection (rest, 0.31 ± 0.05 vs exercise, 0.45 ± 0.09%.min^{-1}; $p > 0.05$) in people with T1D (32). Consequently, there was a greater exercise-induced fall in blood glucose during intramuscular injection (intramuscular: −4.6 ± 0.4 vs subcutaneous: −2.8 ± 0.7 mmol.L^{-1}; $p < 0.05$) (32).

Combined with data collected on the thickness of the subcutaneous tissue in patients (33), recent recommendations advocate a transition to shorter needle lengths (such as 4 and 5 mm) to minimize the variability and altered glucodynamic activity of intramuscular injections (34). Further work is needed to determine whether intramuscular injection into the exercising muscle has a different effect on absorption than injection into a non-working muscle.

Sub-section conclusion:
The effects of intramuscular insulin are more rapid and variable than subcutaneous insulin injections at rest and during exercise.

Subcutaneous Tissue Properties

An inverse relationship exists between subcutaneous thickness and the rate of insulin absorption. In healthy participants, weak-moderate negative correlations exist between subcutaneous fat layer thickness and serum insulin appearance rate (35) and a slower time to peak plasma insulin concentrations in those with a higher body mass index (BMI) (> 23.6 kg.m^{-2}) by 31 min (95% CI 13.7–48.5; $p < 0.05$) (36). In two separate studies, Vora and colleagues employed similar methodologies, measuring the rate of absorption of ^{125}I-labeled insulin actrapid from different injection sites in healthy participants (37) and those with T1D (38), with otherwise matched characteristics. The results from individuals with T1D suggested a weaker inverse correlation between the rate of absorption of insulin and the degree of adiposity than those without T1D. This prompts the questions 'what causes this difference?' and 'is there a difference between the rate of absorption of exogenous insulin in T1D and healthy individuals?' The use of a range of rate constants in these studies makes comparison between the two population cohorts difficult and warrants further investigation to answer these questions. Differences in fasted blood flow in abdominal subcutaneous fat tissue may contribute to the impact of tissue thickness in healthy and T1D populations (39), but studies that directly compare the structure and absorption characteristics within adipose tissue are lacking.

TABLE 1 | Synopsis of the pharmacokinetic properties of exogenous insulin.

Insulin action type	Name	Manufacturer	Chemical modifications	Mechanism	Pharmacokinetic profile			References
					Duration	*Onset*	*Peak*	
ULTRA-RAPID	Fiasp (Faster-acting insulin aspart)	NovoNordisk	Niacinamide and L-arginine added to solution (insulin structure is that of insulin aspart)	Niacinamide excipient destabilizes hexamer in subcutis and may mediate local vasodilation	3–5 h	3–5 min	45–60 min	Hövelmann et al. (9)
	Lyumjev (Ultra-rapid lispro)	Eli Lilly and Company	Treprostinil and citrate added to solution (insulin structure is that of lispro)	Citrate increases local vascular permeability and treprostinil increases local vasodilation	5 h	2 min	45–60 min	Leohr et al. (10)
RAPID	Humalog (Lispro)	Eli Lilly and Company	Inverted *Lys* (B29) and *Pro* (B28) sequence	Distortion at the dimer interface destabilizes hexamer	3–5 h	5–20 min	45–60 min	Howey et al. (11)
	Apidra (Glulisine)	Sanofi	*Asn* (B3) replaced with *Lys*, and *Lys* (B29) replaced with *Glu*. Formulation is zinc free.	Lower isoelectric point improves solubility at physiological pH	3–5 h	10 min	45–60 min	Danne et al. (12)
	Novorapid (Aspart)	NovoNordisk	*Pro* (B28) replaced with *Asp* in B-chain	Removing *Pro* (B28) intermolecular contact to *Glu* (B21) and disrupting hydrogen bonds at dimer interface destabilizes hexamer	3–5 h	10 min	45–60 min	Plank et al. (13)
SHORT	Actrapid	NovoNordisk	Regular human insulin	Hexamer formation in storage delays appearance in circulation	8 h	30 min	1–2.5 h	Mortensen et al. (14)
INTERMEDIATE	Novo NPH	NovoNordisk	Protamine added to insulin solution	Formation of crystals in solution	10–14 h	1.5 h	4 h	Lepore et al. (15)
LONG	Levemir (Detemir)	NovoNordisk	C14 fatty acid is bound to *Lys* (B29) and *Thr* (B30) is removed	Human serum albumin binding and dodecamer formation	20–24 h	2.5 h	None	Porcellati et al. (16)
	Lantus (Glargine)	Sanofi	*Asp* (A21) replaced by *Gly*, and adding two *Arg* amino acids onto the B-chain (B31, B32)	Isoelectric point ~7 leads to precipitation in subcutis	20–24 h	1.5 h	None	Lepore et al. (15)
ULTRA-LONG	Degludec (Tresiba)	NovoNordisk	C16 fatty diacid γ-*Glu* is bound to *Lys* (B29) and *Thr* (B30) is removed	Formation of multi-hexamer units	24–42 h	30–90 min	None	Haahr & Heise (17)
	Glargine U300 (Toujeo)	Sanofi	*Asp* (A21) replaced by *Gly*, and adding two *Arg* amino acids onto the B-chain (B31, B32)	Larger precipitate than U100 glargine delays dissolution	>30 h	30–90 min	None	Becker et al. (18)

Arg, Arginine; Asp, aspartic acid; Glu, glutamic acid; Gly, glycine; Lys, lysine; NPH, neutral protamine Hagedorn; Pro, proline; Thr, threonine.

Sub-section conclusion:
Greater subcutaneous adipose tissue layer thickness is associated with a tempered absorption profile of injected insulin.

Local and Ambient Temperature

The effect of temperature on exogenous insulin absorption has been investigated in those with and without T1D. One study investigated insulin absorption in individuals with T1D who injected insulin actrapid 60 min before two 25-min bouts of sitting in a sauna at 85°C (40). Compared to a control day (22°C), participants experienced a 110% greater disappearance of ^{125}I-labeled actrapid from the site of injection during the whole sauna period, corresponding with a significant blood glucose drop of ≥3 mmol.L^{-1} after the sauna ($p < 0.05$). Later results from a pilot trial (41) and a randomized controlled trial (42) supported these findings, showing that after administering insulin aspart *via* pump the time to peak insulin action was faster using a local skin-warming device (40°C) compared to without its use (77 ± 5 vs 111 ± 7 min, respectively; $p < 0.001$). It is interesting to note here that favorable absorption kinetics have been demonstrated during application of both local and ambient heating, as well as in pump and MDI therapy. Using a long-acting insulin, Bitton and colleagues recently reported a trend of a lower drop in glucose from baseline after 6 h of warming the injection site of insulin glargine U100 (−2.2 ± 0.7 mmol.L^{-1}) compared to a control group (−1.0 ± 0.6 mmol.L^{-1}), yet the difference between the two trials was statistically non-significant ($p = 0.11$) (43).

Sub-section conclusion:
Increased ambient temperature or local warming of the injection site accelerates insulin absorption.

THE IMPACT OF ACUTE PHYSICAL EXERCISE ON INSULIN ABSORPTION

Muscular exercise induces rapid changes in the physiological systems of the person with type 1 diabetes to supply working muscles with oxygen and nutrients. The exercise pressor reflex (i.e. a peripheral neural reflex arising from skeletal muscle

contraction) prompts cardiovascular changes, namely: an increase in cardiac output, blood pressure, and a shunting of blood away from the viscera towards the working muscles, aided by increased concentrations of adrenaline, noradrenaline, and cortisol (44, 45). After rapid adaptation to the exercise intensity, sympatho-adrenal activity is well-regulated relative to the power output during sustained aerobic activities. To maintain normoglycemia, pancreatic insulin secretion decreases and glucagon secretion increases to better match hepatic glucose release to the raised muscular glucose uptake (46).

For the individual with T1D, the physiological changes induced by exercise pose a problem for maintaining glucose control. The synergistic effect of relative hyperinsulinemia (from the previous exogenous injection) and exercise-induced insulin-independent pathways cause the uptake of glucose into myocytes to exceed hepatic glucose release and a decline in blood glucose during continuous steady-state exercise (47). What may further exacerbate the imbalance between glucose uptake and glucose input to the circulation is an exercise-induced acceleration of insulin absorption from the injection site into the blood.

Table 2 overviews the randomized controlled trials that have compared the rate of insulin absorption in individuals during acute exercise compared to rest. There is considerable variation in the findings that can be broadly separated into studies investigating short- and intermediate-acting insulins and studies on long-acting insulins. The agreement that basal insulin glargine U100 does not produce a rise in absorption during exercise opposes the findings that bolus insulins, such as actrapid, become significantly elevated (**Table 2**). Interestingly, it was found that neutral protamine Hagedorn (NPH), an intermediate-acting insulin, was greatly elevated during exercise compared to resting conditions (24); a rise even more marked than those exhibited by some short-acting insulins in the literature (19, 21, 23, 48). This may, in part, be explained by the longer needle length of 12.7 mm being used, likely creating an insulin depot that is closer to the capillary-dense muscle tissue (see 2.1 *Needle Length* section). In contrast, Kemmer et al. (20) demonstrated no change in insulin absorption rate during continuous exercise using actrapid, a short-acting insulin. It is initially unclear what caused Kemmer and colleagues' investigation to evidence no change in insulin mobilization during exercise, but it is noteworthy that the methods employed in the study detailed a large (20 U) volume of injected insulin. Larger insulin volumes may result in altered absorption times due to a smaller surface area: volume ratio and slower diffusion rate (49). The reasons for the overall discrepancy between the effects of exercise on short- and intermediate-acting insulin against long-acting insulins are still unclear and warrant further investigation. However, it should be noted that many of the articles included in this review are >30 years old, during which time new insulin analogs have been developed. This is somewhat reflected by the wide use of ^{125}I-labeled insulin disappearance measurements. While there is evidence iodo-radioactively labeling insulin analogs slows absorption kinetics, its use is valid when comparing absorption rates within studies (50).

Increasing exercise workload to a high intensity, such as heavy weightlifting in resistance exercise, is associated with large increases in adrenaline and noradrenaline levels, in addition to high rates of H^+ generation and efflux from muscle cells. In people with T1D, elevations in catecholamine concentrations stimulate hepatic glucose release to a degree which exceeds muscular glucose uptake, contributing to an increase in blood glucose that contrasts the decline typically observed at lower intensities. Turner and colleagues conducted two studies from which point-concentrations of plasma insulin can be compared between resistance exercise protocols and a rested control session (26, 27). It can be assumed from these studies that the total insulin measurements obtained reflected glargine concentrations, as participants were c-peptide negative and omitted their prior bolus insulin dose on the morning of the trial. Both studies found no exercise-induced change in insulin concentrations (**Table 2**). This finding is pertinent to the basal insulin glargine (U100) being measured, as its long-acting profile is dependent on its precipitation at the higher pH (~7.4) of human subcutis compared to marketed formulation (pH ~4.0) (51). Despite a resistance exercise-induced nadir in venous blood pH of ~7.2, the pharmacokinetics of insulin glargine were unaltered compared to control group.

Sub-section conclusion:
Physical exercise increases the rate of insulin absorption in intermediate-, short-, and rapid-acting insulins but not in older long-acting insulins. There remains a dearth in the literature studying this effect in modern insulins and with exercise modalities other than sub-maximal endurance activities.

The protracted mechanism of action of intermediate- and long-acting insulins is primarily dependent on the slowed movement and delayed dissociation of insulin oligomers into monomeric form to cross the endothelial layer into systemic circulation. Exercise has limited influence on the rate of insulin dissociation, and consequent availability for absorption, as its initial location is confined to the subcutaneous interstitium (48). Hence, the molecular structure of insulin oligomers is the initial rate-limiting factor in its translocation across the capillary membrane, preceding any influence of exercise. Furthermore, the influence of exercise on insulin absorption is likely negatively correlated with the specific insulin analog duration of action (i.e. a lesser effect on long-acting insulins). For example, a bout of exercise lasting for a guideline-recommended time of 30 min (52), overlaps with, and accelerates the rate of absorption during, a greater segment of insulin action in a rapid-acting insulin (e.g. insulin aspart: time until peak onset of action = 31–70 min, time of duration of action = 3–5 h (53)) compared to a long-acting insulin (e.g. insulin degludec: peakless, time of duration of action > 24 h (54)). The more rapid shift to monomer units in short-acting insulins transfers the rate limitation of insulin absorption to other influencing factors, such as blood flow and diffusion distance to the vasculature, which are more readily influenced by acute exercise (55).

The decision to inject into a specific injection site around exercise may be hampered by logistical reasons (e.g. a rugby

TABLE 2 | Randomized controlled trials investigating the effect of exercise compared to rest on insulin absorption in people with type 1 diabetes or healthy individuals.

Authors and date (arrow indicating exercise-induced change in insulin absorption)	Investigated insulin (units injected)	Site of injection	Insulin absorption measurement	Exercise methodology	Insulin absorption outcome
Short- and intermediate-acting insulins investigated					
Ferrannini et al. (19) ↑	Actrapid (8 U)	Thigh and abdomen	^{125}I-labeled actrapid (radioactivity count)	Healthy participants (n = 8; undefined M/F) performed 20 min of moderate-intensity continuous exercise (ending in 170 bpm HR) on cycle ergometer	Increased RIA during exercise in leg injection (exercise 1.12 ± 0.12 vs Rest 0.68 ± 0.15%.min^{-1}; p < 0.05). No change in abdomen (exercise 0.87 ± 0.18 vs Rest 0.75 ± 011%.min^{-1}; p>0.05)
Kemmer et al. (20) ↔	Actrapid (20 U)	Leg and arm (undefined)	^{125}I-labeled actrapid (radioactivity count)	Participants with T1D (n=9; M 8/F 1) performed 10 min bouts separated by 5 min rest, for 30 min total exercising, continuous low-to-moderate intensity exercise (125 ± 5 bpm) on cycle ergometer	Increased RIA after exercise in leg injection compared to same time period at rest (undefined, statistically significant); however, no change during exercise. No change in RIA at any timepoint in arm injection compared to rest
Kolendorf et al. (1979) (21) ↑	Actrapid (8 U)	Thigh	^{131}I-labeled actrapid insulin (radioactivity count)	Participants with T1D (n = 5; undefined M/F) performed four 10-min periods, with 400-sec intervals, of moderate-intensity continuous exercise (120 ± 10 bpm) on cycle ergometer	Increased RIA during exercise compared to rest (Exercise 0.71 ± 0.18 vs Rest 0.41 ± 0.15%.min^{-1}; p < 0.05)
McAuley et al. (22) ↑	Aspart (pump) (TDD 0.55 ± 0.10 U.kg^{-1}.day^{-1})	Abdomen	Venous blood sampling (radioimmunoassay)	Participants with type 1 diabetes (n = 14; M 7/F 7) performed 30 min, including a 5 min warm up, of moderate-intensity continuous exercise (65–70% age-predicted maximal heart rate on a cycle ergometer)	Significant increase of mean free insulin concentration during exercise by 6 ± 2 pmol.L^{-1} compared to rest (p < 0.001)
Ronnemaa & Koivisto (23) ↑	Actrapid (5 ± 1 U)	Thigh	Venous blood sampling (radioimmunoassay)	Participants with type 1 diabetes (C-peptide negative) (n = 9; M 9/F 0) performed three 15-min periods, with 5-min rest intervals, of moderate-intensity continuous exercise (3-min warm-up, then 12-min at 60% VO_{2max}) on cycle ergometer, in either cold (10°C) or warm (30°C) ambient temperatures	Significant difference in plasma free insulin (average difference over whole exercise bout) between exercise and rest in 10°C, 2.7 mU.L^{-1} (p < 0.01) and 30°C, 3.7 mU.L^{-1} (p < 0.05)
Thow et al. (24) ↑	NPH (0.25 U.kg^{-1})	Thigh	Venous blood sampling (radioimmunoassay)	Healthy participants (n=7; M 7/F 0) performed 60 min low-to-moderate-intensity continuous exercise (5 km.h^{-1} at 5° gradient) on treadmill	Increased serum insulin concentration from pre-exercise rest to average peak in exercise (13.7 ± 1.2 vs 27.3 ± 3.2 mU.L^{-1}; NSR)
Susstrunk et al. (25) (undefined) ↑	Actrapid (0.12 U.kg^{-1})	Abdomen or Thigh	Venous blood sampling (radioimmunoassay)	Healthy volunteers (n = 4; undefined M/F) performed three 15-min bouts exercise, separated by 5-min rest periods, of continuous exercise at low-to-moderate-intensity (50% maximum power capacity) on a cycle ergometer	Rate of insulin absorption was higher upon injecting into the abdomen (0.039 U.min^{-1}) than into the thigh (0.027 U.min^{-1}; p<0.05). Both sites experienced marginal enhancements of RIA under exercising conditions compared to rest (NDR+NSR)
Long-acting insulins investigated					
Peter et al. (3) ↔	Glargine (27.2 ± 9.1 U)	Thigh	^{125}I-labeled Glargine	Participants with type 1 diabetes (n = 13; M 12/F 1) performed 30 min of moderate-intensity continuous exercise (65% VO_{2max}) on cycle ergometer	No significant change in RIA between exercise and rest trial days (NDR; p=0.548)
Turner et al. (26) ↔	Glargine (27.5 ± 3.1 U)	NDR	Venous blood samples (immunometric assay)	Participants with type 1 diabetes (n = 8; M 7/F 1) performed either control (rest), 1, 2, or 3 sets of moderate to high intensity (67 ± 3% 1RM) resistance exercise	No significant change in plasma insulin concentrations between or within trials (during exercise = NDR, post exercise p=0.096)
Turner et al. (27) ↔	Glargine (27.5 ± 3.1 U)	NDR	Venous blood samples (immunometric assay)	Participants with type 1 diabetes (n = 8; M 7/F 1) performed either control (rest), 1, 2, or 3 sets of moderate-to-high intensity (60–70% 1RM) resistance exercise	No significant change in plasma insulin concentrations between any exercise trials and control, at any timepoints after exercise (during exercise = NDR)

bpm, beats per minute; F, females; HR, heart rate; M, males; NDR, no data reporting; NPH, neutral protamine Hagedorn insulin; NSR, no statistical reporting; RIA, rate of insulin absorption; RM, repetition maximum; TDD, total daily dose; U, units (of insulin); VO_{2max}, peak rate of oxygen uptake.

player removing their pump prior to a match, or an endurance cyclist unable to inject into the thigh during a ride) and also by a lack of knowledge as to any potential effects that are consequent of choosing one location over another. Few studies have compared the use of different injection sites during exercise. One study demonstrated the rate of absorption increased when injecting ^{125}I-labeled actrapid into the exercising limb (thigh) compared to a non-exercising limb (arm) in people with T1D performing bouts of moderate-intensity bicycle exercise (20). However, this increase was only after exercise had ceased.

Consistent with this finding, another study reported a greater average increase for the first 60 min of insulin absorption from the thigh (exercise 1.12 ± 0.12 vs rest 0.68 ± 0.15%.min^{-1}; $p < 0.05$) than from the abdomen (exercise 0.87 ± 0.18 vs rest 0.75 ± 0.17%.min^{-1}; $p > 0.05$) when healthy participants completed 20 min exercise on a cycle ergometer (19). The results of these studies, however, contrast those of another study using healthy participants in which significantly lower plasma insulin concentrations have been noted following 15 min of cycling after insulin actrapid injection into the thigh compared to the abdomen (25). However, the lack of data on statistical reporting, and the small (n = 4) sample size hinders the interpretation and applicability of this study. There is little data to confirm conclusions pertaining to injecting into the exercising limb and its effect on insulin pharmacokinetics. While injecting insulin into a site local to exercising muscle has been shown to accelerate absorption, injecting into a non-local site may also be subject to increased absorption (22).

The distribution of blood flow to the periphery for the purposes of thermoregulatory heat dissipation has been suggested as the underlying mechanism that explains the influence of temperature (40–43), and exercise (13, 22, 49, 56, 57), on the rise of the rate of insulin absorption. While this may apply to the rested individual, there is debate whether a temperature-induced increase in subcutaneous adipose tissue blood flow can solely account for the elevated rates induced by exercise. Upon starting exercise, blood flow is initially unchanged or shifted away from the skin towards the working muscles until thermoregulatory requirements stimulate the need for increased heat dissipation, due to elevated muscular metabolism, and blood flow to the skin begins to increase (58). As skin blood flow does not increase at the start (or, in hyperthermia, for its full duration (23)) of muscular activity, it is likely other factors contribute to the increased insulin absorption rate, which starts concurrently with the onset of exercise. Indeed, some authors detail an increase in insulin absorption despite no alterations in subcutaneous blood flow (19, 48). As the movement of blood through a section of subcutaneous-based capillary vessel interacts minimally with monomer movement in the interstitial fluid (due to the capillary membrane separating the interstitium and blood compartments), it is likely that blood flow *per se* does not directly impact the rate of insulin absorption. More probable, the rate-limiting step (after hexamer dissociation) is the 'access' insulin monomers have to capillary blood flow, gauged primarily by the diffusion distance from the depot to capillary endothelia. Vasodilation of terminal arterioles in subcutaneous tissue has been demonstrated to increase capillary recruitment, effectively increasing endothelial exchange surface area and potentially reducing monomer diffusion distance (59). However, this phenomenon is not yet fully elucidated in an exercise setting. While the zinc-hexamer association state has been shown to have higher thermic stability than the monomer state (60), to the authors' best knowledge no studies have investigated the potential effects of temperature, separate to the concomitant effects of blood flow, on insulin absorption *in vivo*.

Diffusion of insulin into the circulation is dependent on the concentration gradient (i.e. a smaller concentration in the blood than the depot); hence, greater blood flow that transports insulin away from the vasculature, local to the depot, may indirectly promote the diffusion of insulin monomers away from the injection site by enabling a higher concentration gradient (55). Additionally, monomer movement may be influenced by a flushing effect of plasma volume movement into the local interstitium, or a massage effect from the underlying contracting muscle (61, 62). People with T1D should be aware that inter-individual differences may exist when injecting into exercising limbs, alongside the potential for increased rates of absorption.

Sub-section conclusion:
The exercise-induced increased rate of insulin absorption is likely due to a combination of factors relevant to the changes at injection site during exercise. The dissociation of insulin oligomers into biologically active units remains the initial rate-limiting step.

CONCLUSION

Insulin absorption rate into circulation is influenced by different factors both at rest and during exercise. Compared to the same individual at rest, the exercise-induced increased appearance of insulin in the blood leads to a greater reduction in blood glucose. This phenomenon is often over-looked by individuals performing spontaneous bouts of activity or planning insulin adjustments around structured exercise. There is some evidence to suggest that the choice of location and depth of injection causes additional variability to absorption rates, whereby injections that are deeper and local to the working muscles are susceptible to even higher rates of insulin absorption. Overall, the cause of the increase in absorption during exercise is likely due to a myriad of factors including capillary recruitment, massage-effect, blood flow, temperature, and flushing effect; however, further studies are required to clarify their relative importance. Furthermore, the studies that have investigated the effects of exercise on absorption are now dated, using insulin types that are becoming increasingly less common among the T1D population. Studies using modern ultra-rapid and ultra-long acting insulins are required to determine whether the exercise-induced increase in the rate of absorption is still applicable. Patients and healthcare providers should be aware that the insulin pharmacokinetics around exercise may differ to resting profiles, enabling proactive avoidance of low blood glucose concentrations.

AUTHOR CONTRIBUTIONS

JP—Main investigator. Led literature search, draft composure, and draft review. OM—Aided literature search, draft composure, and draft review. TH-J—Aided draft composure, provided expert knowledge, and draft review. BW—Aided literature search, draft composure, and draft review. RB—Aided literature search, draft composure, and draft review and provided expert knowledge. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Leuenberger Jockel JP, Roebrock P, Shergold OA. Insulin depot formation in subcutaneous tissue. *J Diabetes Sci Technol* (2013) 7(1):227–37. doi: 10.1177/193229681300700128
2. Battelino T, Danne T, Bergenstal RM, Amiel S, Beck R, Biester T, et al. Clinical Targets for Continuous Glucose Monitoring Data Interpretation: Recommendations From the International Consensus on Time in Range. *Diabetes Care* (2019) 42(8):1–11. doi: 10.2337/dci19-0028
3. Peter R, Luzio SD, Dunseath G, Miles A, Hare B, Backx K, et al. Effects of exercise on the absorption of insulin glargine in patients with type 1 diabetes. *Diabetes Care* (2005) 28(3):560–5. doi: 10.2337/diacare.28.3.560
4. Brems DN, Alter LA, Beckage MJ, Chance RE, Dimarchi RD, Green KL, et al. Altering the association properties of insulin by amino acid replacement. *Protein Eng Des Sel* (1992) 5(6):527–33. doi: 10.1093/protein/5.6.527
5. Pettus J, Cavaiola T, Tamborlane W, Edelman S. The past, present, and future of basal insulins. *Diabetes Metab Res Rev* (2015) 32(11):478–96. doi: 10.1002/dmrr
6. Kildegaard J, Buckley ST, Nielsen RH, Povlsen GR, Seested T, Ribel U, et al. Elucidating the Mechanism of Absorption of Fast-Acting Insulin Aspart: The Role of Niacinamide. *Pharm Res* (2019) 36(3):1–14. doi: 10.1007/s11095-019-2578-7
7. Ansari M. Role of protease inhibitors in insulin therapy of diabetes..are these beneficial? *Bull Environ Pharmacol Life Sci* (2015) 4(11):1–8.
8. Garg SK, Buse JB, Skyler JS, Vaughn DE, Muchmore DB. Subcutaneous injection of hyaluronidase with recombinant human insulin compared with insulin lispro in type 1 diabetes. *Diabetes Obes Metab* (2014) 16(11):1065–9. doi: 10.1111/dom.12315
9. Hövelmann U, Heise T, Nosek L, Sassenfeld B, Thomsen KMD, Haahr H. Pharmacokinetic Properties of Fast-Acting Insulin Aspart Administered in Different Subcutaneous Injection Regions. *Clin Drug Investig* (2017) 37(5):503–9. doi: 10.1007/s40261-017-0499-y
10. Leohr J, Dellva MA, Coutant DE, LaBell E, Heise T, Andersen G, et al. Pharmacokinetics and Glucodynamics of Ultra Rapid Lispro (URLi) versus Humalog® (Lispro) in Patients with Type 2 Diabetes Mellitus: A Phase I Randomised, Crossover Study. *Clin Pharmacokinet* (2020). doi: 10.1007/s40262-020-00901-2
11. Howey DC, Bowsher RR, Brunelle RL, Woodworth JR. [Lys(B28), Pro(B29)]-human insulin: A rapidly absorbed analogue of human insulin. *Diabetes* (1994) 43(3):396–402. doi: 10.2337/diab.43.3.396
12. Danne T, Becker RHA, Heise T, Bittner C, Frick AD, Rave K. Pharmacokinetics, prandial glucose control, and safety of insulin glulisine in children and adolescents with type 1 diabetes. *Diabetes Care* (2005) 28(9):2100–5. doi: 10.2337/diacare.28.9.2100
13. Plank J, Wutte A, Brunner G, Siebenhofer A, Semlitsch B, Sommer R, et al. A direct comparison of insulin aspart and insulin lispro in patients with type 1 diabetes. *Diabetes Care* (2002) 25(11):2053–7. doi: 10.2337/diacare.25.11.2053
14. Mortensen HB, Lindholm A, Olsen BS, Hylleberg B. Rapid appearance and onset of action of insulin aspart in paediatric subjects with type 1 diabetes. *Eur J Pediatr* (2000) 159(7):483–8. doi: 10.1007/s004310051315
15. Lepore M, Pampanelli S, Fanelli C, Porcellati F, Bartocci L, Vincenzo AD, et al. Pharmacokinetics and Pharmacodynamics of Subcutaneous Injection of Long-Acting Human Insulin Analog Glargine, NPH Insulin, and Ultralente Human Insulin and Continuous Subcutaneous Infusion of Insulin Lispro. *Diabetes* (2000) 49(12):2142–8. doi: 10.2337/diabetes.49.12.2142
16. Porcellati F, Rossetti P, Bolli GB, Fanelli CG. Comparison of pharmacokinetics and dynamics of the long-acting insulin analogs glargine and detemir at steady state in type 1 diabetes: A double-blind, randomized, crossover study. *Diabetes Care* (2007) 30(3):2447–52. doi: 10.2337/dc07-2234
17. Haahr H, Heise T. Heise T. A Review of the Pharmacological Properties of Insulin Degludec and Their Clinical Relevance. *Clin Pharmacokinet* (2014) 53(9):787–800. doi: 10.1007/s40262-014-0165-y
18. Becker RHA, Dahmen R, Bergmann K, Lehmann A, Jax T, Heise T. New insulin glargine 300 units/mL provides a more even activity profile and prolonged glycemic control at steady state compared with insulin Glargine 100 units/mL. *Diabetes Care* (2015) 38(4):637–43. doi: 10.2337/dc14-0006
19. Ferrannini E, Linde B, Faber O. Effect of bicycle exercise on insulin absorption and subcutaneous blood flow in the normal subject. *Clin Physiol* (1982) 2(1):59–70. doi: 10.1111/j.1475-097X.1982.tb00007.x
20. Kemmer F, Berchtold P, Berger M, Starke A, Cupper HJ, Gries FA, et al. Exercise-induced Fall of Blood Glucose in Insulin-treated Diabetics unrelated to Alteration of Insulin Mobilization. *Diabetes* (1979) 28(12):1131–7. doi: 10.2337/diab.28.12.1131
21. Kolendorf K, Bojsen J, Nielsen SL. Adipose tissue blood flow and insulin disappearance from subcutaneous tissue. *Clin Pharmacol Theapeut* (1979) 25(5):598–603. doi: 10.1002/cpt1979255part1598
22. McAuley SA, Horsburgh JC, Ward GM, La Gerche A, Gooley JL, Jenkins AL, et al. Insulin pump basal adjustment for exercise in type 1 diabetes: a randomised crossover study. *Diabetologia* (2016) 59(8):1636–44. doi: 10.1007/s00125-016-3981-9
23. Ronnemaa T, Koivisto VA. Combined effect of exercise and ambient temperature on insulin absorption and postprandial glycemia in type I patients. *Diabetes Care* (1988) 11(10):769–73. doi: 10.2337/diacare.11.10.769
24. Thow JC, Johnson AB, Antsiferov M, Home PD. Exercise Augments the Absorption of Isophane (NPH) Insulin. *Diabetes Med* (1989) 6:342–5. doi: 10.1111/j.1464-5491.1989.tb01177.x
25. Süsstrunk H, Morell B, Ziegler WH, Froesch ER. Insulin absorption from the abdomen and the thigh in healthy subjects during rest and exercise: Blood glucose, plasma insulin, growth hormone, adrenaline and noradrenaline levels. *Diabetologia* (1982) 22(3):171–4. doi: 10.1007/BF00283747
26. Turner D, Luzio S, Kilduff LP, Gray BJ, Dunseath G, Bain SC, et al. Reductions in resistance exercise-induced hyperglycaemic episodes are associated with circulating interleukin-6 in Type 1 diabetes. *Diabetes Med* (2014) 31(8):1009–13. doi: 10.1111/dme.12462
27. Turner D, Luzio S, Gray BJ, Dunseath G, Rees ED, Kilduff LP, et al. Impact of single and multiple sets of resistance exercise in type 1 diabetes. *Scand J Med Sci Sports* (2015) 25(1):404–12. doi: 10.1111/sms.12202
28. Richter WF, Bhansali SG, Morris ME. Mechanistic determinants of biotherapeutics absorption following SC administration. *AAPS J* (2012) 14(3):559–70. doi: 10.1208/s12248-012-9367-0
29. Frid A, Gunnarsson R, Guntner P, Linde B. Effects of accidental intramuscular injection on insulin absorption in IDDM. *Diabetes Care* (1988) 11(1):41–5. doi: 10.2337/diacare.11.1.41
30. Spraul M, Chantelau E, Koumoulidou J, Berger M. Subcutaneous or Nonsubcutaneous Injection of Insulin. *Diabetes Care* (1988) 11(9):733–6. doi: 10.1080/02699050500110314
31. Vaag A, Handberg A, Lauritzen M, Henriksen JE, Damgaard Pedersen K, Beck-Nielsen H. Variation in absorption of NPH insulin due to intramuscular injection. *Diabetes Care* (1990) 13(1):74–6. doi: 10.2337/diacare.13.1.74
32. Frid A, Ostman J, Linde B. Hypoglycemia Risk During Exercise After Intramuscular Injection of Insulin in Thigh in IDDM. *Diabetes Care* (1990) 13(5):473–7. doi: 10.2337/diacare.13.5.473
33. Gibney MA, Arce CE, Byron KJ, Hirsch LJ. Adult skin and subcutaneous insulin injection site thickness in diabetes. *Curr Med Res Opin* (2010) 26(6):1519–30. doi: 10.1185/03007995.2010.481203
34. Frid A, Kreugel G, Grassi G, Halimi S, Hicks D, Hirsch LJ, et al. New Insulin Delivery Recommendations. *Mayo Clin Proc* (2016) 91(9):1231–55. doi: 10.1016/j.mayocp.2016.06.010
35. Sindelka G, Heinemann L, Berger M, Frenck W, Chantelau E. Effect of insulin concentration, subcutaneous fat thickness and skin temperature on subcutaneous insulin absorption in healthy subjects. *Diabetologia* (1994) 37(4):377–80. doi: 10.1007/BF00408474
36. De Galan BE, Engwerda EEC, Abbink EJ, Tack CJ. Body mass index and the efficacy of needle-free jet injection for the administration of rapid-acting insulin analogs, a post hoc analysis. *Diabetes Obes Metab* (2013) 15(1):84–6. doi: 10.1111/j.1463-1326.2012.01666.x
37. Vora JP, Peters JR, Burch A, Owens D. Relationship Between Absorption of Radiolabeled Soluble Insulin Subcutaneous Blood Flow and Anthropometry. *Diabetes Care* (1992) 15(11):1484–93. doi: 10.2337/diacare.15.11.1484
38. Vora JP, Burch A, Peters JR, Owens DR. Absorption of Radiolabelled Soluble Insulin in Type 1 (Insulin-dependent) Diabetes: Influence of Subcutaneous Blood Flow and Anthropometry. *Diabetes Med* (1993) 10:736–43. doi: 10.1111/j.1464-5491.1993.tb00157.x

39. Haggendal E, Steen B, Svanborg A. Blood flow in subcutaneous fat tissue in patients with diabetes mellitus. *Acta Med Scand* (1970) 187:49–53. doi: 10.1111/j.0954-6820.1970.tb02905.x
40. Koivisto VA. Sauna-induced acceleration in insulin absorption. *Br Med J* (1980) 280:1411–3. doi: 10.1136/bmj.281.6240.621-a
41. Raz I, Weiss R, Yegorchikov Y, Bitton G, Nagar R, Pesach B. Effect of a local heating device on insulin and glucose pharmacokinetic profiles in an open-label, randomized, two-period, one-way crossover study in patients with type 1 diabetes using continuous subcutaneous insulin infusion. *Clin Ther* (2009) 31(5):980–7. doi: 10.1016/j.clinthera.2009.05.010
42. Cengiz E, Weinzimer SA, Sherr JL, Tichy EM, Carria L, Cappiello D, et al. Faster in and faster out: Accelerating insulin absorption and action by insulin infusion site warming. *Diabetes Technol Ther* (2014) 16(1):20–5. doi: 10.1089/dia.2013.0187
43. Bitton G, Rom V, Hadelsberg U, Raz I, Cengiz E, Weinzimer S, et al. Effect of Injection Site Cooling and Warming on Insulin Glargine Pharmacokinetics and Pharmacodynamics. *J Diabetes Sci Technol* (2019) 13(6):1123–8. doi: 10.1177/1932296819842151
44. Saltin B, Boushel R, Secher N, Mitchell J. *Exercise and Circulation in Health and Disease*. Champaign, IL: Human Kinetics (2000).
45. Ball D. Metabolic and endocrine response to exercise: Sympathoadrenal integration with skeletal muscle. *J Endocrinol* (2015) 224(2):R79–95. doi: 10.1530/JOE-14-0408
46. Camacho RC, Galassetti P, Davis SN, Wasserman DH. Glucoregulation during and after exercise in health and insulin-dependent diabetes. *Exerc Sport Sci Rev* (2005) 33(1):17–23.
47. DeFronzo RA, Ferrannini E, Sato Y, Felig P, Wahren J. Synergistic interaction between exercise and insulin on peripheral glucose uptake. *J Clin Invest* (1981) 68(6):1468–74. doi: 10.1172/JCI110399
48. Fernqvist E, Linde B, Ostman J, Gunnarsson R. Effects of physical exercise on insulin absorption in inuslin-dependent diabetics. A comparison between human and porcine insulin. *Clin Physiol* (1986) 6:489–98. doi: 10.1111/j.1475-097X.1986.tb00782.x
49. Søeborg T, Rasmussen CH, Mosekilde E, Colding-Jørgensen M. Absorption kinetics of insulin after subcutaneous administration. *Eur J Pharm Sci* (2009) 36(1):78–90. doi: 10.1016/j.ejps.2008.10.018
50. Sohoel A, Plum A, Frokjaer S, Thygesen P. 125I used for labelling of proteins in an absorption model changes the absorption rate of insulin aspart. *Int J Pharm* (2007) 330(1-2):114–20. doi: 10.1016/j.ijpharm.2006.09.004
51. Heinemann L, Linkeschova R, Rave K, Hompesch B, Sedlak M, Heise T. Time-Action Profile of the Long-Acting Insulin Analog Insulin Glargine (HOE901) in Comparison With Those of NPH Insulin and Placebo. *Diabetes Care* (2000) 23(5):644–9. doi: 10.2337/diacare.23.5.644
52. Colberg SR, Sigal RJ, Yardley JE, Riddell MC, Dunstan DW, Dempsey PC, et al. Physical activity/exercise and diabetes: A position statement of the American Diabetes Association. *Diabetes Care* (2016) 39(11):2065–79. doi: 10.2337/dc16-1728
53. Lindholm A, Jacobsen L. Clinical Pharmacokinetics and Pharmacodynamics of Insulin Aspart. *Clin Pharmacokinet* (2001) 40(9):641–59. doi: 10.2165/00003088-199937020-00002
54. Nasrallah SN, Reynolds LR. Insulin Degludec, The New Generation Basal Insulin or Just another Basal Insulin? *Clin Med Insights Endocrinol Diabetes* (2012) 5:31–7. doi: 10.4137/CMED.S9494
55. Hildebrandt P, Sejrsen P, Nielsen SL, Birch K, Sestoft L. Diffusion and polymerization determines the insulin absorption from subcutaneous tissue in diabetic patients. *Scand J Clin Lab Invest* (1985) 45(8):685–90. doi: 10.1080/00365518509155280
56. Gradel AKJ, Porsgaard T, Lykkesfeldt J, Seested T, Gram-Nielsen S, Kristensen NR, et al. Factors Affecting the Absorption of Subcutaneously Administered Insulin: Effect on Variability. *J Diabetes Res* (2018) 2018:1–17. doi: 10.1155/2018/1205121
57. Heinemann L. Variability of Insulin Action: Does It Matter? *Insulin* (2008) 3 (1):37–45. doi: 10.1016/S1557-0843(08)80010-3
58. González-alonso J, Crandall CG, Johnson JM. The cardiovascular challenge of exercising in the heat. *J Physiol* (2008) 586(1):45–53. doi: 10.1113/jphysiol.2007.142158
59. Tobin L, Simonsen L, Bülow J. Real-time contrast-enhanced ultrasound determination of microvascular blood volume in abdominal subcutaneous adipose tissue in man. Evidence for adipose tissue capillary recruitment. *Clin Physiol Funct Imag* (2010) 30(6):447–52. doi: 10.1111/j.1475-097X.2010.00964.x
60. Huus K, Havelund S, Olsen HB, Van De Weert M, Frokjaer S. Thermal dissociation and unfolding of insulin. *Biochemistry* (2005) 44(33):11171–7. doi: 10.1021/bi0507940
61. Berger M, Cüppers HJ, Hegner H, Jörgens V, Berchtold P. Absorption kinetics and biologic effects of subcutaneously injected insulin preparations. *Diabetes Care* (1982) 5(2):77–91. doi: 10.2337/diacare.5.2.77
62. Linde B. Dissociation of insulin absorption and blood flow during massage of subcutaneous site. *Diabetes Care* (1986) 9(6):570–4. doi: 10.2337/diacare.9.6.570

Conflict of Interest: TH-J is an employee of Novo Nordisk A/S.

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.

frontiers in Endocrinology

BRIEF RESEARCH REPORT
published: 25 February 2021
doi: 10.3389/fendo.2021.599164

The Effect of Metformin on Self-Selected Exercise Intensity in Healthy, Lean Males: A Randomized, Crossover, Counterbalanced Trial

*Nanna Skytt Pilmark[1], Christina Petersen-Bønding[1], Nielse Frederich Rose Holm[1], Mette Yun Johansen[1], Bente Klarlund Pedersen[1], Katrine Bagge Hansen[2] and Kristian Karstoft[1,3]**

[1] Centre for Physical Activity Research (CFAS), University of Copenhagen, Rigshospitalet, Copenhagen, Denmark, [2] Steno Diabetes Center Copenhagen, Gentofte, Denmark, [3] Department of Clinical Pharmacology, Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark

OPEN ACCESS

Edited by:
Rainer Lehmann,
Tübingen University Hospital, Germany

Reviewed by:
Steven K. Malin,
Rutgers, The State University of New Jersey, United States
Christian Cadeddu Dessalvi,
University of Cagliari, Italy

***Correspondence:**
Kristian Karstoft
Kristian.karstoft@regionh.dk
orcid.org/0000-0002-6596-4199

Specialty section:
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Endocrinology

Received: 26 August 2020
Accepted: 19 January 2021
Published: 25 February 2021

Citation:
Pilmark NS, Petersen-Bønding C, Holm NFR, Johansen MY, Pedersen BK, Hansen KB and Karstoft K (2021) The Effect of Metformin on Self-Selected Exercise Intensity in Healthy, Lean Males: A Randomized, Crossover, Counterbalanced Trial. Front. Endocrinol. 12:599164. doi: 10.3389/fendo.2021.599164

Introduction: In general, patients with type 2 diabetes have lower cardiorespiratory fitness levels and perform exercise at lower intensities compared to healthy controls. Since metformin (MET) has been shown to increase the rate of perceived exertion (RPE) during exercise with a fixed intensity, MET *per se* may reduce self-selected exercise intensity. The aim of this study was to assess the effect of MET on self-selected exercise intensity.

Methods: Healthy males were eligible for this crossover, counterbalanced study with two treatment periods: MET and placebo (PLA), each lasting 17 days. Treatment dose was gradually increased and reached 2 g/day on treatment day 9, and continued at that level for the rest of the treatment period. The two periods were performed in randomized order. Two experimental days (A+B) were conducted on Day 15 (A) and Day 17 (B) of each period, respectively. Day A consisted of an exercise bout with self-selected exercise intensity (equal to RPE = 14–15 on the Borg Scale). Day B consisted of an exercise bout with fixed intensity (70% of VO_2peak). Oxygen consumption rate was assessed continuously during both exercise bouts.

Results: Fifteen males (age 23.7 ± 0.6 years, BMI 22.3 ± 2.0, VO_{2peak} 3.5 ± 0.6 L/min) were included in the study. On Day B, RPE was higher in MET compared to PLA (14.8 ± 0.4 vs. 14.0 ± 0.3, $P = 0.045$). On Day A, no difference in self-selected exercise intensity measured by oxygen consumption rate (PLA 2.33 ± 0.09 L O_2/min, MET 2.42 ± 0.10 L O_2/min, $P = 0.09$) was seen between treatment periods.

Conclusions: Self-selected exercise intensity was not reduced by MET in healthy males, despite the fact that MET increased RPE during an exercise bout with fixed intensity.

Keywords: exercise, metformin, rate of perceived exertion, type 2 diabetes, self-selected exercise intensity

INTRODUCTION

Physical activity is a cornerstone in the treatment of patients with type 2 diabetes (1). However, most patients with type 2 diabetes are not able to achieve satisfying glycemic control with physical activity alone, which is why pharmacological treatment is often initiated. Metformin is the initial glucose-lowering drug of choice for patients with type 2 diabetes, and most patients are prescribed metformin as a lifelong treatment shortly after the diagnosis (2). Compared to matched, healthy controls, patients with type 2 diabetes have lower cardiorespiratory fitness levels (3) and, perform free-living, unsupervised exercise at lower intensities (4); intensities that are too low to induce robust metabolic improvements (5). The reason for this unclear, but reduced mitochondrial function in skeletal muscle has been suggested as a potential explanation (6). Moreover, and potentially in continuation of this, the rate of perceived exertion (RPE) may play an important role, since patients with type 2 diabetes are known to report higher RPE during exercise with a given intensity, compared to healthy controls (7). Although debatable (8), it has been reported that RPE is associated with blood lactate levels (9), and since metformin treatment has been shown to increase blood lactate levels, heart rate (HR) and RPE in healthy individuals at a given exercise intensity (10), it may be speculated that it is the metformin treatment *per se* and not the diabetes phenotype, which is responsible for the low self-selected exercise intensity in patients with type 2 diabetes. Therefore, the aim of this study was to assess the effect of metformin on self-selected exercise intensity. We hypothesized that metformin, possibly through an increase in blood lactate and heart rate, would increase RPE, and thereby decrease the self-selected exercise intensity. We chose to test this hypothesis in young, healthy, lean males in order to reduce variance due to heterogeneity in the included population and in order to evaluate the effects of metformin treatment *per se*, independent of the diabetes phenotype and prior metformin treatment.

METHODS

Healthy, lean (BMI<25), low-to moderately physically active (≤150 min of structured physical activity/week), male individuals were eligible for the study. Exclusion criteria were: smoking, daily medication, contraindications to increased levels of physical activity (11), liver cell damage (ALT/AST at least 3 times above upper normal level), prior history of lactic acidosis and eGFR<60 ml/min.

To ensure that all inclusion and no exclusion criteria were met, a screening day was performed. The screening day included a medical examination, recording of medical history, a blood chemistry screen, an ECG and a cardiorespiratory fitness (VO_2peak) test on a cycle ergometer (Monark 739E, Varberg, Sweden) using indirect calorimetry (Cosmed Quark, Rome, Italy). The VO_2peak test started with a 5-min warm up at 80 Watt (W), after which the load was gradually increased by 20 W every minute until at least one of the following criteria was met: plateau of HR and VO_2 with incremental workloads, respiratory exchange ratio > 1.1, or volitional exhaustion, as previously described (12). Written informed consent was obtained from all participants. The study was approved by the ethical committee of the Capital Region of Denmark (H-16032037) and registered at ClinicalTrials.org (NCT02951260).

Study Design and Treatment Periods

Participants fulfilling inclusion criteria were included in a double-blinded, crossover, counterbalanced study with two treatment periods performed in a randomized order. The two treatment periods were identical except for the following treatment:

PLA: Placebo treatment (17 days)

MET: Metformin treatment (17 days)

To ensure adherence to the treatment protocol and maintain participant blinding, both metformin (MET) and placebo (PLA) treatments were gradually increased to minimize gastrointestinal discomfort caused by metformin.

- Treatment days 1–4: 500 mg × 2
- Treatment days 4–8: 500 mg + 1000 mg
- Treatment days 9–17: 1000 mg × 2

After completion of the first treatment period (first 17 days of treatment), a 4-day washout period was applied before initiation of the second treatment period.

On day 15 and 17 of each treatment period, participants underwent two experimental days (Experimental day A and B, respectively). Participants were instructed to keep a diet record from day 13 to day 17 in the first treatment period, and subsequently to follow this diet closely during the same days in the 2nd treatment period. **Figure 1** shows the study design.

Randomization and Blinding

Participants were randomly allocated, using a computer-based algorithm (randomizer.org) to one of two arms, MET or PLA, with an allocation ratio 1:1. The random number sequence was created by an endocrinologist (KBH), not involved in any of the study procedures. The sequence was stored and concealed from other staff and participants in a locked cabinet in a locked office at a geographical location different from the place of the study procedures.

All study personnel and participants were blinded to the allocation throughout the study period, until all participants had completed their last test and all outcomes were computed. The two study medications (visually identical pills) were prepared by a clinical pharmacy (Region Hovedstadens Apotek, Herlev Hospital) and delivered to KBH, who dispensed the medication in identical packages according to the allocation number. Following the allocation, KBH delivered the identical pre-packed medications to the test centre (CFAS) prior to the first test day. After the screening day, participants received the pre-packed medications with instructions on how to administer the pills and how to report serious adverse events (SAE) and adverse events (AE) to KBH. Since known side-effects of metformin, e.g., gastrointestinal pain and nausea, could potentially reveal the treatment allocation, all SAE's and AE's were reported to the endocrinologist, who was not involved in the study procedures,

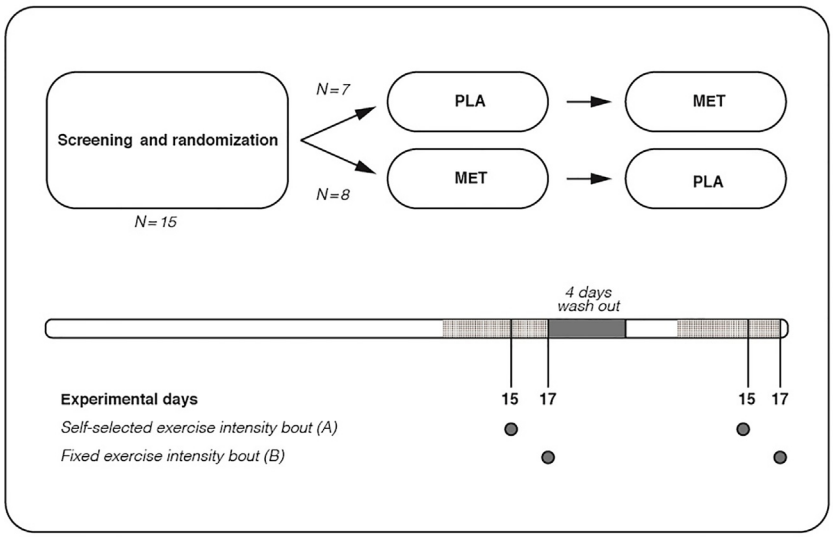


FIGURE 1 | Study design. Shaded area indicates that diet records were kept from day 13 to 17 in the first treatment period, and subsequently this diet was mirrored from day 13 to 17 in the 2nd treatment period.

in order to maintain blinding. Moreover, the participants were instructed not to share information about any SAE's and AE's with the study personnel. A priori, it was decided that the concealment could be revealed upon the endocrinologist's discretion based on the frequency and severity of SAE's and AE's. After completion of the 1st treatment period, participants received the medication for the 2nd treatment period.

Experimental Days

Participants arrived in the laboratory 1 h after having had a standardized breakfast (60 g bun with 20 g cheese (220 kcal: fat 8.8 g, carbohydrates 24.7 g, protein 9.9 g) that was ingested with the morning dose of MET/PLA. Upon arrival, a venous line for blood sampling was placed and 1 h after arrival, a 45-min exercise bout (self-selected intensity on Experimental day A; fixed intensity on Experimental day B) was initiated, see detailed descriptions below. Prior to initiation of the exercise bout, a baseline blood sample was drawn for analyses of blood lactate and glucose (ABL 7 series; Radiometer, Denmark). These analyses were repeated every 15 min during the 45-min exercise bout and results were averaged to provide mean values during the exercise bouts.

Self-Selected Exercise Intensity (Experimental Day A, 15 Days After Treatment Start)

On Experimental day A, participants were instructed to perform a 45-min exercise bout on an cycle ergometer equaling a subjective RPE of 14 to 15 on the Borg Scale (corresponding to "somewhat hard" or "hard"). In order to ensure maintenance of the prespecified RPE, the participants were asked every 5 min (in standardized terms) by the investigator, whether the load should be adjusted. Participants were blinded for the load, which was adjusted by 10 Watts (increased or decreased), if a change was required. In addition to the predefined, standardized 5-min intervals, participants were encouraged to ask the investigator to change the load, if needed, at any time point during the exercise bout. To ensure correct starting intensity, a 15-min warm-up period was completed prior to the actual 45-min exercise bout. During the warm-up period, the load was gradually increased, ensuring that participants would begin the exercise bout at a RPE of 5 to 15.

Three hours after termination of the exercise bout with self-selected exercise intensity, a VO_2peak test was performed. This completed Experimental day A.

Indirect calorimetry (Cosmed Quark, Rome, Italy) and HR (Polar RS400) measurements were performed continuously both during the exercise bout and the VO_2peak test, using a mask and breath-by-breath measurements and a heart rate strap, respectively. Oxygen consumption rate was used as a proxy measure of exercise intensity during the exercise bout with self-selected exercise intensity. Furthermore, external work (kJ) was calculated by multiplying load (Watt) by time (seconds) spent on each load, divided by 1000.

Fixed Intensity (Experimental Day B, 17 Days After Treatment Start)

On Experimental day B, participants underwent a 45-min exercise bout with fixed intensity at 70% of VO_2peak. The correct intensity was calculated from the VO_2peak test performed on the screening day. The exercise bout was initiated by a 5-min warm-up period with gradually increasing intensity, ensuring that the participants would begin the exercise bout at 70% of VO_2peak. RPE for the entire exercise bout was assessed at the termination. Indirect calorimetry and HR measurements were performed continuously during the exercise bout, analogue to Experimental day A.

Outcomes and Sample Size

The primary outcome was differences between MET and PLA in self-selected exercise intensity, measured as oxygen consumption rate (L O_2/min). Secondary outcomes included differences in RPE, mean blood lactate levels and HR levels during the 45-min exercise bout with fixed intensity.

No studies have, to our knowledge, investigated the effects of metformin on self-selected exercise intensity. The impact of short-term metformin treatment on RPE, blood lactate and HR has been investigated in various studies (10, 13, 14). These studies have each included between 9 and 17 participants. In general, metformin treatment has been found to robustly increase RPE, blood lactate, and HR. Based on these studies, and a pragmatic assumption that these variables would directly affect self-selected exercise intensity, we included 15 participants in the current study.

Statistical Methods

Normality was assessed by D'Agostino-Pearson, Shapiro-Wilk, Kolmogorov-Smirnov test. All variables were normal-distributed. Therefore, between-treatment period variables were compared using Student´s paired t-test. All statistical analyses were performed by Prism version 8 (GraphPad, Canada). Statistical significance was accepted with $P<0.05$ (2-sided). Values are presented as mean ± SEM, baseline characteristics as mean ± SD.

TABLE 1 | Baseline characteristics.

N	15
Age (years)	23.7 ± 0.6
Height (m)	1.8 ± 0.1
Body weight (kg)	75.3 ± 9.4
BMI (kg/m^2)	22.3 ± 2.0
VO_2peak Absolute (L/min)	3.5 ± 0.6
VO_2peak Relative (ml/kg/min)	46.5 ± 4.9
Resting heart rate (bpm)	61 ± 8
Peak heart rate (bpm)	197 ± 9
Fasting blood lactate (mmol/L)	1.1 ± 0.4
Fasting glucose (mmol/L)	4.6 ± 0.3
HbA1c (mmol/mol)	32.7 ± 3.0

Baseline characteristics are presented as mean ± SD. BMI, body mass index; VO_2peak, maximal oxygen consumption; HbA1c, hemoglobin A1c.

RESULTS

Fifteen young, lean, healthy males were included and completed the study (**Table 1**). Seven individuals started with the PLA treatment period, whereas eight started with the MET treatment period (**Figure 2**). No difference in body weight was seen between treatment periods at Experimental day 17 (PLA 75.5 ± 9.8 kg, MET 75.2 ± 9.7 kg, $P = 0.9$).

Study procedures took place from October 2016 to April 2017. All data were collected at the Centre for Physical Activity Research, Rigshospitalet, Copenhagen, Denmark.

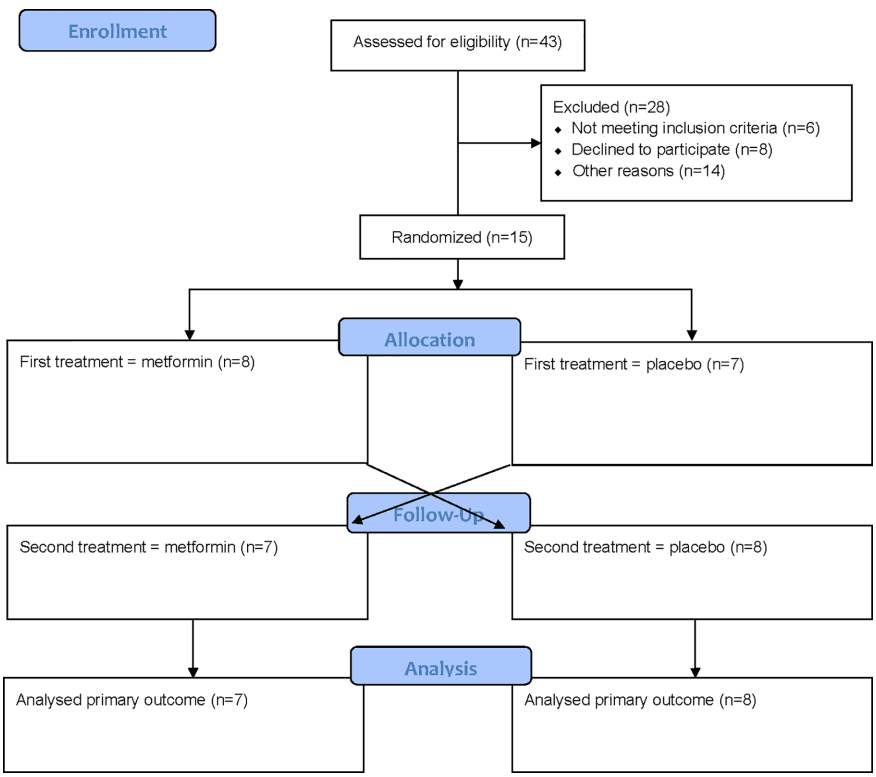


FIGURE 2 | Flowchart.

Exercise Bout With Fixed Intensity

RPE was significantly higher in MET compared to PLA in the exercise bout with fixed intensity (P = 0.045). Furthermore, a tendency towards higher blood lactate levels was seen during MET compared to PLA (P = 0.06). Besides that, no differences were observed between PLA and MET for any variables in the exercise bout with fixed intensity (**Table 2**).

Exercise Bout With Self-Selected Exercise Intensity

During the exercise bout with self-selected exercise intensity, no significant differences between treatment periods were observed in either oxygen consumption rate (PLA 2.33 ± 0.09 L O_2/min, MET 2.42 ± 0.10 L O_2/min, P = 0.09) (**Figures 3A, B**), mean Watt (PLA 154 ± 6, MET 159 ± 6, P = 0.3), external work (PLA 398 ± 19 kJ, MET 415 ± 21 kJ, P = 0.07) (**Figures 3C, D**), RER (PLA 0.90 ± 0.01, MET 0.90 ± 0.01, P = 0.8), HR (PLA 154 ± 4 bpm, MET 159 ± 4 bpm, P = 0.1), blood glucose (PLA 5.1 ± 0.1 mmol/L, MET 4.9 ± 0.1 mmol/L, P = 0.2), or blood lactate (PLA 3.2 ± 0.5 mmol/L MET 3.6 ± 0.4 mmol/L, P = 0.2).

Likewise, no differences between treatment periods were seen in VO_2peak (PLA 3.5 ± 0.1, MET 3.5 ± 0.1, P = 0.5).

DISCUSSION

The main finding of this study is that self-selected exercise intensity was not reduced by metformin treatment, despite the fact that metformin treatment significantly increased RPE during the exercise bout with fixed intensity. Instead, we found a tendency towards a higher self-selected exercise intensity with metformin treatment (P = 0.09).

There are several potential explanations for this unexpected and apparently contradictory result. An obvious speculation is that self-selected exercise intensity is not affected by RPE. In the literature, however, it is well established that RPE and work intensity are linearly and closely associated (15–17).

Another potential explanation for the findings could be that the RPE assessment was inaccurate and/or imprecise. In this context, Morgan et al. demonstrated that RPE has a considerable intra-individual day-to-day variation (18). In continuation of this, RPE is a subjective variable, which may be affected by a variety of factors. As such, various psychological traits may play a role in the perceptual procession of information related to muscular work and thereby RPE (18).

Taking this into account, it may be speculated that the exercise bout with self-selected intensity, where the participants were asked to keep the exercise intensity corresponding to 14 to 15 on the Borg scale, resulted in large noise-to-signal in the oxygen consumption rate. This could be the case, regardless of the great effort that was put into standardizing the exercise bouts to reduce the impact of confounding factors, potentially affecting RPE. The same problem might have been expected in a reverted version during the exercise bout with fixed intensity, but here it may be argued that the RPE was less influenced by external factors, since RPE was assessed after the exercise bout, in resting conditions, which gave the participants time to carefully consider their answer. Nonetheless, the RPE results must be interpreted cautiously. In continuation of this and despite causality has been debated, RPE and lactate have been shown to be linearly associated (19), and metformin-induced increases in RPE and blood lactate have been reported to be associated (20). In the present study, during the exercise bout with fixed intensity, a tendency towards higher blood lactate was seen in MET compared to PLA (P = 0.06), which may support differences in RPE between MET and PLA in the fixed-intensity exercise bout. However, the fact that numerical differences in lactate (and HR) between MET and PLA were comparable between Experimental day A and B speaks against this association between lactate (and HR) and RPE.

When working with a subjective variable such as RPE, another important speculation is whether the order of the treatment periods might have affected the outcomes. To circumvent this, the study was counterbalanced. Moreover, a two-way (treatment*treatment order) repeated-measures ANOVA demonstrated that the treatment order had not influenced the primary outcome (P = 0.2 for interaction). Hence, we do not believe that this affected the interpretation of the findings.

Finally, it may be questioned whether a statistically significant difference of 0.8 RPE points on the 6–20 Borg scale is too low to have any clinical relevance, including an effect on self-selected exercise intensity. In this context, it is important that the effects found of metformin on RPE in the current study are in line with previous observations (10, 13). Furthermore, the fact that not only "no difference", but a tendency towards a higher self-selected exercise intensity with metformin treatment was observed, makes the results convincing, and suggests that clinicians can prescribe metformin without worrying whether self-selected exercise intensity may be negatively affected.

It has been suggested that metformin treatment may reduce mitochondrial respiration and therefore cardiorespiratory fitness *per se* (21), although data are conflicting (22). In this situation, the increased RPE in MET during exercise with fixed intensity could potentially be explained by a lower cardiorespiratory fitness level. However, based on the results from the present study, in which no difference was observed in VO_2peak between treatments, nothing points towards inhibition of mitochondrial respiration to be the explanation for the increased RPE.

TABLE 2 | Exercise bout with fixed intensity.

	PLA	MET	P-value
Mean oxygen consumption rate (L/min)	2.6 ± 0.1	2.6 ± 0.1	>0.9
Mean external work (kJ)	433 ± 13	442 ± 15	0.4
Mean intensity (Watt)	166 ± 5	169 ± 5	0.6
RER	0.89 ± 0.00	0.89 ± 0.01	0.8
Mean HR (bpm)	158 ± 4	164 ± 4	0.2
Percentage of HR peak (%)	86.9 ± 1.5	88.3 ± 1.4	0.4
Mean blood lactate (mmol/L)	3.0 ± 0.3	3.5 ± 0.4	0.06
Mean blood glucose (mmol/L)	4.7 ± 0.1	4.8 ± 0.1	0.4
RPE	14.0 ± 0.3	14.8 ± 0.4	0.045

All variables are presented as mean ± SEM. RER, respiratory exchange ratio; HR, heart rate; bpm, beat per minute; RPE, rate of perceived exertion. P-values were calculated from student's paired t-test.

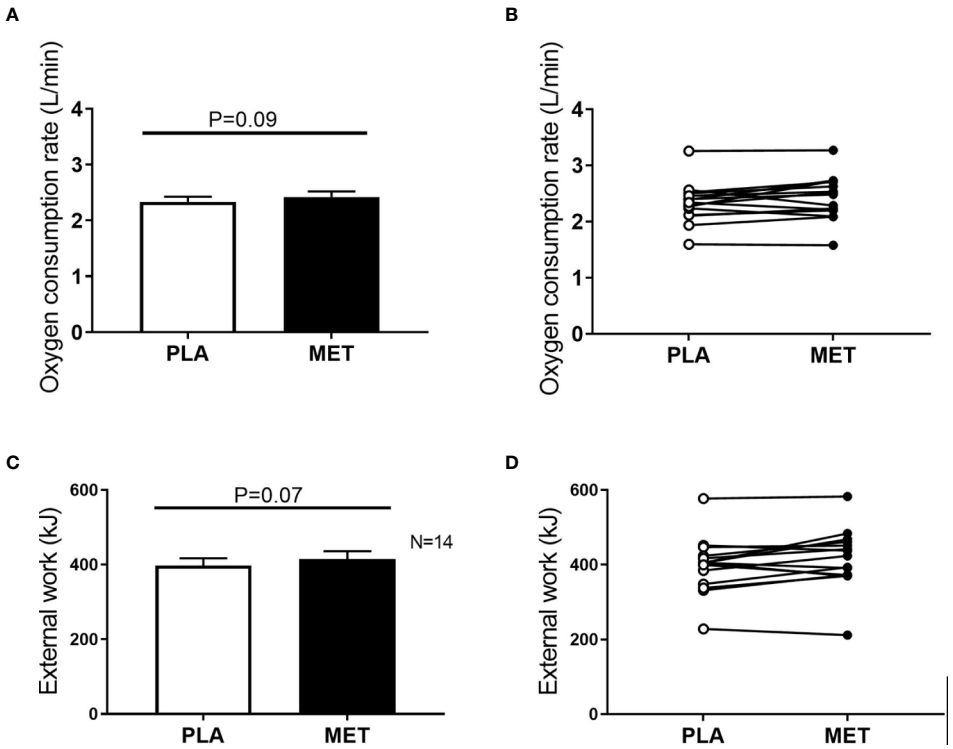


FIGURE 3 | Oxygen consumption rate and external work during an exercise bout with self-selected exercise intensity. Panel **(A)** mean oxygen consumption rate (L/min) during an exercise bout with self-selected intensity. Panel **(B)** individual oxygen consumption rates (L/min) during an exercise bout with self-selected intensity. Panel **(C)** mean external work (kJ) during an exercise bout with self-selected exercise intensity. Panel **(D)** individual values of external work (kJ) during an exercise bout with self-selected exercise intensity. Values are presented as means ± SEM. P-values were calculated from student´s paired *t* test.

Limitations

A potential limitation is that the study was performed in healthy individuals instead of patients with type 2 diabetes. However, to our knowledge, there are no indications that the effect of metformin on RPE, lactate and HR should be different in patients with type 2 diabetes than in healthy individuals (23). Nonetheless, if patients with type 2 diabetes had been included, a larger difference in blood lactate between treatments might have been observed, since patients with type 2 diabetes typically have higher blood lactate levels than non-diabetic individuals (24), and since metformin inhibits lactate uptake by the liver. Following this, it may be speculated that more robust differences in blood lactate between treatments would have influenced self-selected exercise intensity.

Another limitation of the present study is the small number of participants, which may lead to both type 1 and type 2 statistical errors. Moreover, the inclusion of only males limits the external validity of the study results.

Conclusion

In conclusion, this study has shown that RPE is increased by metformin treatment but that this does not lead to lower self-selected exercise intensity in male subjects with normal glucose tolerance. Thus, the clinical importance of the increased RPE during exercise seen with metformin treatment remains unclear.

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 studies involving human participants were reviewed and approved by the ethical committee of the capital region of Denmark. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

NP and KK wrote the manuscript. NP and KK performed the statistical analyses. KK and KH designed the study and conceptualized and designed the analyses with contributions from NP and BP. NP and BP obtained the funding. NP, MJ, NH, and CP-B contributed to data collection, data analysis/processing, and/or data quality control procedures. All authors contributed to drafting the article and/or revising it critically for important intellectual content. All authors approved the final version of the manuscript. All authors accept responsibility for all aspects of the work insofar as ensuring that questions related

to the accuracy or integrity of any part of the article are appropriately investigated and resolved. KK is responsible for the integrity of the work as a whole. All authors contributed to the article and approved the submitted version.

FUNDING

This work was partially supported by a grant from Aase and Ejnar Danielsen Foundation (NP), a grant from the Research Foundation of Rigshospitalet (E-23606-03) (NP), and Christian d. X Foundation (NP). The Centre for Physical Activity Research is supported by grants from TrygFonden (grants ID 101390 and ID 20045) (BP).

ACKNOWLEDGMENTS

The authors would like to thank the participants for their great commitment in this project. Furthermore, the authors want to thank Marc Donath, Basel Universitätshospital, Switzerland for his contribution to the study design.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.599164/full#supplementary-material

REFERENCES

1. Buse JB, Wexler DJ, Tsapas A, Rossing P, Mingrone G, Mathieu C, et al. 2019 Update to: Management of Hyperglycemia in Type 2 Diabetes, 2018. A Consensus Report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* (2020) 43(2):487–93. doi: 10.2337/dci19-0066
2. Cosentino F, Grant PJ, Aboyans V, Bailey CJ, Ceriello A, Delgado V, et al. ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. *Eur Heart J* (2020) 41(2):255–323. doi: 10.1093/eurheartj/ehz486
3. Regensteiner JG, Sippel J, McFarling ET, Wolfel EE, Hiatt WR. Effects of non-insulin-dependent diabetes on oxygen consumption during treadmill exercise. *Med Sci Sports Exerc* (1995) 27(6):875–81. doi: 10.1249/00005768-199506000-00012
4. Johnson ST, Tudor-Locke C, McCargar LJ, Bell RC. Measuring habitual walking speed of people with type 2 diabetes: are they meeting recommendations? *Diabetes Care* (2005) 28(6):1503–4. doi: 10.2337/diacare.28.6.1503
5. Tudor-Locke C, Bell RC, Myers AM, Harris SB, Ecclestone NA, Lauzon N, et al. Controlled outcome evaluation of the First Step Program: a daily physical activity intervention for individuals with type II diabetes. *Int J Obes Relat Metab Disord* (2004) 28(1):113–9. doi: 10.1038/sj.ijo.0802485
6. Mogensen M, Sahlin K, Fernstrom M, Glintborg D, Vind BF, Beck-Nielsen H, et al. Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes. *Diabetes* (2007) 56(6):1592–9. doi: 10.2337/db06-0981
7. Huebschmann AG, Reis EN, Emsermann C, Dickinson LM, Reusch JE, Bauer TA, et al. Women with type 2 diabetes perceive harder effort during exercise than nondiabetic women. *Appl Physiol Nutr Metab* (2009) 34(5):851–7. doi: 10.1139/H09-074
8. Hall MM, Rajasekaran S, Thomsen TW, Peterson AR. Lactate: Friend or Foe. *PM R* (2016) 8(3 Suppl):S8–S15. doi: 10.1016/j.pmrj.2015.10.018
9. Scherr J, Wolfarth B, Christle JW, Pressler A, Wagenpfeil S, Halle M. Associations between Borg's rating of perceived exertion and physiological measures of exercise intensity. *Eur J Appl Physiol* (2013) 113(1):147–55. doi: 10.1007/s00421-012-2421-x
10. Kristensen JM, Lillelund C, Kjobsted R, Birk JB, Andersen NR, Nybo L, et al. Metformin does not compromise energy status in human skeletal muscle at rest or during acute exercise: A randomised, crossover trial. *Physiol Rep* (2019) 7(23):e14307. doi: 10.14814/phy2.14307
11. Pedersen BK, Saltin B. Exercise as medicine - evidence for prescribing exercise as therapy in 26 different chronic diseases. *Scand J Med Sci Sports* (2015) 25 Suppl 3:1–72. doi: 10.1111/sms.12581
12. Pilmark NS LM, Oberholzer L, Elkjær I, Petersen-Bønding C, Kofoed K, Siebenmann C, et al. The interaction between metformin and physical activity on postprandial glucose and glucose kinetics: a randomized, clinical trial. *Diabetologia* (2020) 64(2):397–409. doi: 10.1007/s00125-020-05282-6
13. Boule NG, Robert C, Bell GJ, Johnson ST, Bell RC, Lewanczuk RZ, et al. Metformin and exercise in type 2 diabetes: examining treatment modality interactions. *Diabetes Care* (2011) 34(7):1469–74. doi: 10.2337/dc10-2207
14. Sharoff CG, Hagobian TA, Malin SK, Chipkin SR, Yu H, Hirshman MF, et al. Combining short-term metformin treatment and one bout of exercise does not increase insulin action in insulin-resistant individuals. *Am J Physiol Endocrinol Metab* (2010) 298(4):E815–23. doi: 10.1152/ajpendo.00517.2009
15. Eston RG, Davies BL, Williams JG. Use of perceived effort ratings to control exercise intensity in young healthy adults. *Eur J Appl Physiol Occup Physiol* (1987) 56(2):222–4. doi: 10.1007/BF00640648
16. Alsamir Tibana R, Manuel Frade de Sousa N, Prestes J, da Cunha Nascimento D, Ernesto C, Falk Neto JH, et al. Is Perceived Exertion a Useful Indicator of the Metabolic and Cardiovascular Responses to a Metabolic Conditioning Session of Functional Fitness? *Sports (Basel)* (2019) 7(7). doi: 10.3390/sports7070161
17. Zinoubi B, Zbidi S, Vandewalle H, Chamari K, Driss T. Relationships between rating of perceived exertion, heart rate and blood lactate during continuous and alternated-intensity cycling exercises. *Biol Sport* (2018) 35(1):29–37. doi: 10.5114/biolsport.2018.70749
18. Morgan WP. Psychological factors influencing perceived exertion. *Med Sci Sports* (1973) 5(2):97–103. doi: 10.1249/00005768-197300520-00019
19. Borg G, Ljunggren G, Ceci R. The increase of perceived exertion, aches and pain in the legs, heart rate and blood lactate during exercise on a bicycle ergometer. *Eur J Appl Physiol Occup Physiol* (1985) 54(4):343–9. doi: 10.1007/BF02337176
20. Malin SK, Stephens BR, Sharoff CG, Hagobian TA, Chipkin SR, Braun B. Metformin's effect on exercise and postexercise substrate oxidation. *Int J Sport Nutr Exerc Metab* (2010) 20(1):63–71. doi: 10.1123/ijsnem.20.1.63
21. Braun B, Eze P, Stephens BR, Hagobian TA, Sharoff CG, Chipkin SR, et al. Impact of metformin on peak aerobic capacity. *Appl Physiol Nutr Metab* (2008) 33(1):61–7. doi: 10.1139/H07-144
22. Cadeddu C, Nocco S, Cugusi L, Deidda M, Fabio O, Bandino S, et al. Effects of Metformin and Exercise Training, Alone or in Combination, on Cardiac Function in Individuals with Insulin Resistance. *Cardiol Ther* (2016) 5(1):63–73. doi: 10.1007/s40119-016-0057-3
23. Das S, Behera SK, Srinivasan A, Xavier AS, Selvarajan S, Kamalanathan S, et al. Effect of metformin on exercise capacity: A meta-analysis. *Diabetes Res Clin Pract* (2018) 144:270–8. doi: 10.1016/j.diabres.2018.08.022
24. Brinkmann C, Brixius K. Hyperlactatemia in type 2 diabetes: Can physical training help? *J Diabetes Complications* (2015) 29(7):965–9. doi: 10.1016/j.jdiacomp.2015.05.018

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: 12 May 2021
doi: 10.3389/fphys.2021.656909

Exercising for Insulin Sensitivity – Is There a Mechanistic Relationship With Quantitative Changes in Skeletal Muscle Mass?

Jasmine Paquin[1,2], Jean-Christophe Lagacé[1,2], Martin Brochu[1,2] and Isabelle J. Dionne[1,2]*

[1] Research Centre on Aging, Affiliated With CIUSSS de l'Estrie-CHUS, Sherbrooke, QC, Canada, [2] Faculty of Physical Activity Sciences, University of Sherbrooke, Sherbrooke, QC, Canada

Skeletal muscle (SM) tissue has been repetitively shown to play a major role in whole-body glucose homeostasis and overall metabolic health. Hence, SM hypertrophy through resistance training (RT) has been suggested to be favorable to glucose homeostasis in different populations, from young healthy to type 2 diabetic (T2D) individuals. While RT has been shown to contribute to improved metabolic health, including insulin sensitivity surrogates, in multiple studies, a universal understanding of a mechanistic explanation is currently lacking. Furthermore, exercised-improved glucose homeostasis and quantitative changes of SM mass have been hypothesized to be concurrent but not necessarily causally associated. With a straightforward focus on exercise interventions, this narrative review aims to highlight the current level of evidence of the impact of SM hypertrophy on glucose homeostasis, as well various mechanisms that are likely to explain those effects. These mechanistic insights could provide a strengthened rationale for future research assessing alternative RT strategies to the current classical modalities, such as low-load, high repetition RT or high-volume circuit-style RT, in metabolically impaired populations.

Keywords: insulin sensitivity, muscle mass, muscle quality, resistance training, muscle hypertrophy, muscle capillarization, muscle mitochondrial activity, muscle mitochondrial biogenesis

OPEN ACCESS

Edited by:
Thomas P. J. Solomon, Blazon Scientific, United Kingdom

Reviewed by:
Ciarán Fealy, Maastricht University, Netherlands
Christiano R. R. Alves, Massachusetts General Hospital, Harvard Medical School, United States

****Correspondence:***
Jasmine Paquin
jasmine.paquin2@usherbrooke.ca

Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

Received: *21 January 2021*
Accepted: *14 April 2021*
Published: *12 May 2021*

Citation:
Paquin J, Lagacé J-C, Brochu M and Dionne IJ (2021) Exercising for Insulin Sensitivity – Is There a Mechanistic Relationship With Quantitative Changes in Skeletal Muscle Mass? Front. Physiol. 12:656909. doi: 10.3389/fphys.2021.656909

INTRODUCTION

Skeletal muscle (SM) is the most important component of fat-free mass (FFM) and plays a key role in overall metabolic health. SM accounts for up to 80% of whole-body glucose consumption under insulin-stimulated conditions (DeFronzo and Tripathy, 2009; Honka et al., 2018) and is thought to contribute to as much as 25% of basal metabolic rate (Dulloo et al., 2012). Furthermore, the existing body of research on components of total daily energy expenditure supports a robust correlation between total FFM and resting energy expenditure (Tam et al., 1999; Illner et al., 2000; Blanc et al., 2004). The onset of insulin resistance (IR) at the SM level have been suggested to initiate the development of IR, which if not managed could lead consecutively to glucose intolerance and then type 2 diabetes (T2D) (Petersen and Shulman, 2002; Roden and Shulman, 2019), a multifactorial metabolic and endocrine disease with high individual and public health consequences (Rosenquist and Fox, 2009). Supporting the role of SM in cardiometabolic health,

multiple observational have repetitively demonstrated an association between low relative FFM and cardiometabolic complications (Srikanthan and Karlamangla, 2011; Lee et al., 2018; Zhang et al., 2018). In older individuals, sarcopenia as defined by musculoskeletal decline (Cooper et al., 2012) has shown itself to be associated with altered glucose homeostasis (Yang et al., 2017; Shou et al., 2020) as well as autonomy and overall quality of life (Tsekoura et al., 2017).

Exercise, by inherently enhancing SM metabolism and function, has proven itself to be incredibly efficient for whole-body glucose homeostasis management and overall cardiometabolic health (Umpierre et al., 2011; Bird and Hawley, 2017). Likewise, the exercise physiology community also agrees on the importance of maintaining an optimal degree of SM tissue mass for exercise capacity and overall autonomy (Yang, 2014). Results from seminal studies investigating the impact of resistance training (RT) and concurrent quantitative changes of SM mass on whole-body glucose homeostasis suggested an interplay between SM hypertrophy and SM glucose disposal rates (Yki Jarvinen and Koivisto, 1983; Miller et al., 1984; Eriksson et al., 1997, 1998). These studies, which have been frequently replicated since, have contributed to the hypothesis that RT targeting SM hypertrophy could be as efficient as aerobic exercise performed alone for improving SM insulin sensitivity (IS) (Dela and Kjaer, 2006; Pesta et al., 2017; DiMenna and Arad, 2018). Additionally, according to the role of SM glycogen synthesis in IS through Akt signaling (Russell, 2010; Kleinert et al., 2013; Yang, 2014), it could be inferred that increasing total SM mass will lead to a greater storage capacity (Shepherd et al., 2014). Collectively, it has been suggested that exercising toward SM hypertrophy (or maintenance of it in a context of weight loss) can therefore protect against metabolic syndrome and other metabolic diseases related to energy surplus (Ravussin and Bogardus, 1990; Wood et al., 2012).

However, difficult they are to identify, a certain number of studies have postulated a divergence between SM hypertrophy *per se*, and intrinsic changes in SM IS or have pointed out a lack of clarity around the mechanisms involved (Holten et al., 2004; Bird and Hawley, 2017; Pesta et al., 2017). Furthermore, the relationship between relative or absolute FFM and glucose homeostasis is inconsistent across studies (Gippini et al., 2002; Goulet et al., 2007; Glouzon et al., 2015; Perreault et al., 2016; Stuart et al., 2017). Amongst these studies, Brochu et al. (2008) have shown in non-diabetic postmenopausal women matched for fat mass that those displaying a larger FFM had higher fasting insulin and glucose compared to those with lower FFM (Brochu et al., 2008). Furthermore, the results of Glouzon et al. (2015) show that following a 6-month mixed-exercise intervention, overweight to obese women who had lost FFM had better improvement in HOMA-IR compared to women who had gained muscle mass, independently of visceral adipose tissue and fat mass percentage (Glouzon et al., 2015). Although it is impossible to infer a causal relationship, or lack thereof, between SM hypertrophy and glucose homeostasis improvements, these results are favorable to the idea that exercise-induced muscle metabolic changes in favor of IS and SM hypertrophy might be independent. A recent systematic literature review addressing the comprehensive effects of physical activity on IS conducted by Bird and Hawley (2017) stated the mechanisms that underpin the effects of SM mass quantitative changes on IS are not fully understood, given the disparity in the current body of research on RT and glucose homeostasis in humans (Bird and Hawley, 2017).

Given the plausible independence between SM hypertrophy and whole-body glucose homeostasis improvements, a closer look at the disparities in different populations and at the mechanistic insights behind these discrepancies is warranted. The current review will attempt to identify some of the mechanisms that could be involved with a specific and narrow focus on exercise intervention studies. Hence, the first section will highlight the present level of evidence supporting SM hypertrophy as a way to enhance whole-body glucose homeostasis as. The second and third sections will review some of the plausible mechanistic explanations of the relationship between quantitative changes of SM mass and IS.

NARRATIVE LITERATURE REVIEW

Glucose Homeostasis, Exercise, and Skeletal Muscle Hypertrophy: Previous and Current State-of-the-Art

Miller et al. (1984) pioneered the research domain of exercise-induced SM hypertrophy and concurrent improvement in IS by demonstrating a significant association between RT-induced SM mass increases and a lower insulin area under the curve during a glucose tolerance test in 10 inactive, but otherwise healthy, male college students following a 10-week intervention. A decade later, the same group (Miller et al., 1994) reported similar results in a sample of 11 men in their fifties and early sixties. The 16-week intervention led to an improvement in IS, measured with the gold standard euglycemic-hyperinsulinaemic clamp, together with a concomitant 1.2 kg average SM increase. Another seminal study that has shaped the field is the one by Eriksson et al. (1997) who reported conclusions comparable to those of Miller et al. Their results support a strong and negative association ($r = -0.73$; $p < 0.05$) between increased SM mass and HbA1c in middle-aged men and women diagnosed with T2D in response to 3 months of circuit-style RT (Eriksson et al., 1997). Since, a growing body of evidence support circuit-style RT as an effective training modality to enhance metabolic health components, such as whole-body glucose homeostasis (Kolahdouzi et al., 2019) body composition and physical fitness (Kim et al., 2018) and pro-inflammatory cytokine profile (Kolahdouzi et al., 2019). One clear advantage of such a training protocol is its similarity to aerobic exercise from the perspective of the broad continuum of the energy systems, according to its ability to induce an elevated cardiovascular response (Gotshalk et al., 2004).

Given the irrefutable relevance of increasing peripheral IS in prediabetic and T2D populations, a large number of studies have conducted exercise trials in those populations in an attempt to identify the optimal RT exercise prescription (Praet and Van Loon, 2009). Cuff et al. (2003), compared the impact of 16 weeks of either AT alone, RT alone or a combination of

the two on hyperinsulinemic-euglycemic clamp-derived IS and CT-scan derived SM cross-sectional area in a sample of 28 postmenopausal women with T2D (Cuff et al., 2003). Changes in SM cross-sectional area, as well as SM normal density (therefore fat-free tissue) were both strongly correlated with changes in glucose uptake during the IS assessment. Cauza et al. (2009) robustly investigated the impact of a 4-month RT intervention in untrained men and women who fulfilled diagnosis criteria for T2D (Cauza et al., 2009). CT-scan derived muscle cross-sectional area showed significant increases in quadriceps size after the intervention. Furthermore, there were significant improvements in HbA1C and fasting blood glucose, lowered body fat percentage higher lower-limb strength. Unfortunately, none of these outcomes were significantly correlated with increases in quadriceps cross-sectional area. Findings in line with those of Cauza et al. (2009) have been replicated more than once and are not atypical. Mavros et al. (2013) compared two RT protocols – low intensity without load increase and high intensity with progressive load increase – in a cohort of older adults with T2D (Mavros et al., 2013). Their results revealed that, in the high-intensity group, changes in SM mass were significantly and inversely correlated with HOMA2-IR. Conversely, in the low intensity group, there was no such association. In addition, in the high-intensity group, only participants who had an increase in SM mass had a HbA1c reduction. Surprisingly, participants in the low intensity group whom SM mass increased did not show improvements in any glycemic control outcomes. In T2D individuals, SM quantitative changes in response to RT do not always occur and are presumably unpredictable despite the application of a proper training stimulus according to population studies. A systematic review conducted by Gordon et al. (2009) looking at RT as a metabolic health enhancing tool for individuals with T2D has concluded that the minority of studies analyzed (2 amidst the 24 listed papers) reported significant increases in SM as well as inconsistent improvements in SM intrinsic insulin signaling between trials (Gordon et al., 2009). Nonetheless, the aforementioned review strongly supports a favorable effect of RT on whole-body glycemic control, IS and SM strength in people with T2D, again displaying a discrepancy between SM improvements in IS and whole-body glucose homeostasis in response to RT. In sum, one plausible explanation of a higher efficiency of one exercise protocol over another might be total training load, i.e., the combined effect of intensity and volume, irrespective of the effect on SM quantity.

The association between increases in SM and glucose homeostasis may also be highly relative to inter-population variability. **Table 1** provides a brief overview of highly cited studies investigating FFM quantitative changes and glucose homeostasis in response to either resistance or mixed training intervention, or a comparison of the two, in different population studies. Aside from a few exceptions (Cuff et al., 2003; Bucci et al., 2016), studies that report concurrent improvements in SM mass and IS or its surrogates are often conducted in either young male adults (Roberts et al., 2013; Cocks et al., 2014; Shepherd et al., 2014) or adolescents (Shaibi et al., 2006; Van Der Heijden et al., 2010; Lee et al., 2012). Amongst others, areas where counter-intuitive results have been found include post-menopausal women and women with PCOS. For instance, Kogure et al. (2016), using a 16-week RT intervention, showed a positive association between HOMA-IR and SM mass in middle-aged women with and without polycystic ovary syndrome (PCOS), both at baseline and after the intervention, suggesting worsening glucose homeostasis with increased SM mass (Kogure et al., 2016). Furthermore, the results of Glouzon et al. (2015) show that following a 6-month mixed-exercise intervention, overweight to obese women who had lost FFM had better improvement in HOMA-IR compared to women who had gained muscle mass, independently of visceral adipose tissue and fat mass percentage (Glouzon et al., 2015). Even more, the authors found a positive correlation between exercise-induced changes in SM mass index [lean body mass(kg)/height(m^2)] and HOMA-IR after the intervention. Interestingly, the association was even stronger after controlling for visceral fat and relative fat mass (% of total body weight) changes. Indeed, women and men differ not only in physical attributes but also in their SM substrates metabolism (Ansdell et al., 2020). Accumulating evidence suggests a higher intrinsic IS in women's SM, given a higher relative proportion of type I SM fibers with greater capillary density and fatty acid oxidation rates, as well as higher anti-inflammatory adipokine activity (mainly leptin and adiponectin) (Lundsgaard and Kiens, 2014). Indeed, these different metabolic features are thought to be in part driven by enhanced SM estrogen signaling (Ikeda et al., 2019) and adipose tissue partitioning (Mancuso and Bouchard, 2019). In contrast, Bucci et al. (2016) observed a significant effect of a 4-month RT program on MRI-derived SM mass and thigh muscle glucose uptake assessed with positron emission tomography (^{18}F-FDG) in older frail women offspring of normal weight/lean or overweight/obese mothers (Bucci et al., 2016). Furthermore, there was a significant and positive correlation between increases in glucose uptake and increases in absolute SM mass. While indices of SM quality, such as absolute and relative strength, were not measured in the latter study, these results might suggest that increasing SM in the context of frailty, or pathological losses of SM such as sarcopenia may improve glucose homeostasis, as it has been suggested before (Lexell, 1995; Volpi et al., 2004; Phu et al., 2015).

In weight loss trials, a great deal of attention is directed toward the importance of SM mass maintenance in order to counter any loss in resting metabolic rate and therefore, energy expenditure. It is hypothesized that the reduced energy expenditure due to reduced SM mass will contribute to weight regain and deterioration of body composition, a phenomenon coined fat overshooting. However, this fat overshooting phenomenon appears to be attenuated in obese individuals (Jacquet et al., 2020; Dulloo, 2021) and, accordingly, the risk of developing T2D (Zou et al., 2020) or cardiovascular diseases (Zou et al., 2019) does not appear to be greater with weight cycling in initially obese individuals. This discrepancy between normal weight and overweight to obese individuals could be attributable to the "less essential FFM" theory, which was suggested by Marks and Rippe (1996) as an explanation to the observed benefits of decreasing total FFM after weight loss in severely obese individuals (Marks and Rippe, 1996). The foundation of their theory was that a certain proportion of the high SM mass of obese individuals is

TABLE 1 | Summary of studies that has investigated fat-free mass quantitative changes and glucose homeostasis in response to either resistance or mixed training intervention or a comparison.

Study	Intervention (Modality)	Sample	FFM and FM changes	Glucose homeostasis	Association
Miller et al., 1984	10-week, 3/week (10 exercises, 3 sets of 8 REPS)	10 Young male college students	↑ FFM ↔ FM	↓ OGTT insulin AUC	Positive association: ↑ FFM and ↓ OGTT insulin No association: ↑ FFM and ↓ OGTT glucose
Miller et al., 1994	16-weeks, 3/week (14 exercises, 3 to 15 REPS)	11 Healthy older (58 ± 1 years) men (BMI = 26.9 ± 1.0)	↑ FFM ↓ FM	↔ F-Glucose and Glucose OGTT ↓ F-insulin and insulin AUC ↑ Glucose disposal during clamp	No association shown.
Bucci et al., 2016	4-months, three times/week	Older frail women offspring of normal weight/lean mothers (n = 20) and or overweight/obese mothers (n = 17) Avg age: 72.3 and 71.5 years; Avg BMI: 26.6 and 27.9.	↔ Overall BC ↑ Quadriceps mass and adductor longus mass.	↔ F-Glucose and F-insulin ↑Muscle GU ↑ Whole-body IS (OOM group only)	Positive association: ↑ GU per kg and ↑ FFM
Roberts et al., 2013	12 weeks, 3/week (Progressive overload – from 2 sets of 12–15 to 3 sets of 6–8 REPS)	28 Healthy young (avg 21.5 years) men, overweight/obese (avg BMI 30.9)	RT group: ↑ FFM ↓ Total FM ↓ Trunk FM	RT group: ↔ HbA1c ↑ GT (OGTT)	No association shown.
Shaibi et al., 2006	16-week, 2/week (Multiple sets, moderate intensity/high volume, multiple joint exercises)	11 overweight (Avg BMI 32.5) adolescent males (Avg 15.1 years)	RT group: ↑ FFM ↓ %BF Control group: ↑ FFM	RT group: ↑ IS (FSIGVTT) Control group: ↔	IS changes independent of BC changes.
Dionne et al., 2004	6 months, 3/week (9 exercises targeting major muscle groups, 3 sets of 10 REPS)	33 Young (27.8 ± 3.5 years) and 12 old (66.6 ± 4.9) women (avg BMI: young; 21.9 ± 2.3; old: 25.4 ± 2.6)	Young: ↑ FFM Old: ↑ FFM ↓ FM	Young: ↑ M (+38mg/min) Old: ↔ M	No significant improvement in young when reported relative to FFM.
Lee et al., 2012	3 months, 3/week AT (40–60 min, 50–70% VO_2peak) or RT (10 whole-body exercises, 1–2 sets 8–12 REPS, 60% 1RM)	42 Obese adolescents (avg age: control = 14.8, AT = 15.2, RT = 14.6)	AT group: ↓ FM RT group: ↑ FFM ↓ FM Control group: ↔	AT group: ↔ GT (OGTT) ↔ IS RT group: ↔ GT (OGTT) ↔ ↑ IS Control group: ↔	No association shown.
Kogure et al., 2016	16 weeks, 3/week (Strength and hypertrophy focused)	45 Young (28 ± 5.4 years) [women with (n = 47) or without (n = 52)] PCOS	With PCOS: ↑ FFM Without PCOS: ↔	With PCOS: ↑ HOMA-IR Without PCOS: ↔	Positive association: FFM and HOMA-IR in PCOS and control at baseline and post-intervention. Changes in FFM (LM/height^2) were independent of changes in HOMA-IR after the intervention.
Poehlman et al., 2000	6-months, 3 days/week AT (endurance-based) or RT (target intensity: 80% 1RM)	51 Premenopausal (age range 18–35 years; BMI < 26)	AT group: ↔ FFM and thigh CSA ↑ Muscle attenuation RT group: ↑ FFM and thigh CSA ↑ Muscle attenuation	AT group: ↑ M RT group: ↑ M	When IS was expressed relative to FFM (mg/kg FFM/min), it improved significantly only in the AT group.
Glouzon et al., 2015	6-month, 3/week (Mixed intervention)	48 Post-menopausal (avg age 60 ± 5.0 years) women	↑ FFM ↑ FFMI ↑ Appendicular FFM	↑ F-glucose ↔ F-insulin ↔ HOMA-IR	Baseline: Positive: ↑ FFMI and HOMA-IR Positive: ↑ appendicular FFMI and HOMA-IR Post-intervention: Positive: ΔFFMI and ΔHOMA-IR

(Continued)

TABLE 1 | Continued

Study	Intervention (Modality)	Sample	FFM and FM changes	Glucose homeostasis	Association
Amankwaah et al., 2019	9-month, mixed intervention (RT 2 days/week, RT 1 day/week)	Middle-aged men (n = 69) and women (n = 83) with mild obesity (avg BMI 30.0 ± 2.7 kg/m^2)	↑FFM ↓ FM	↑ F-glucose ↔ HOMA-IR	Increases in FFM did not predict changes in measured cardiometabolic health outcomes. Although the relation between IS and Δ FFM was significant when expressed relative to body weight, it was not when expressed absolutely or relative to baseline FFM.
Fukushima et al., 2016	6 months, 3 days/week (Mixed intervention)	92 Obese (BMI: 33.2 ± 4.6) women (avg age 40.9 ± 10.4 years)	↓ FFMI ↓ FM	↓ HOMA-IR ↓ F-glucose ↓ F-insulin	ΔHOMA-IR was inversely associated with Δ%FFM (relative to weight).
Cuff et al., 2003	16 weeks, RT or mixed intervention (AT: aerobic classes; RT: 5 exercises, 2 sets of 12 REPS)	28 postmenopausal women with T2D (avg age: control: 60 ± 2.9; AT+RT: 63.4 ± 2.2; AT: 59.4 ± 1.9)	Both groups: ↑ FFM (SM CSA) ↓ Low-density SM	AT: ↔ GIR Mixed: ↑ GIR	Positive association: ↑ SM CSA and ↑ GIR.

AT, aerobic training; Avg, average; AUC, area under the curve; BMI, body mass index (kg/m^2); BC, body composition; CSA, cross-sectional area; F-glucose, fasting glucose; F-insulin, fasting insulin; FFM, fat-free mass; FFMI, fat-free mass index (kg/m^2); FM, fat mass; GIR, glucose infusion rate; GT, glucose tolerance; GU, glucose uptake; HOMA-IR, homeostatic model assessment for insulin resistance; M, hyperinsulinemic-euglycemic clamp-derived insulin sensitivity; IS, insulin sensitivity; OGTT, oral glucose tolerance test; REPS, repetitions; RM, maximal repetition; RT, resistance training; SM, skeletal muscle; T2D, type 2 diabetes.

"functionally inessential" due to a lessened relative quality. They also speculated that a high SM mass was associated with a lower density tissue in obese individuals, impaired strength-to-size ratio as well as a lower mitochondrial density and capillarization. Taken together, these factors compromised SM work capacity. Considering those speculations, although SM is a functional physiological reserve in many circumstances, more does not necessarily mean better. This may suggest a "ceiling effect" at a certain level of SM, wherein adding muscle mass does not provide further metabolic advantage. In line with this, a study from Ghachem et al. (2019) has identified a cut-off point of appendicular muscle mass index [7.02 muscle mass (kg)/height (m)^2] in sedentary older women above which IS is significantly reduced(Ghachem et al., 2019). Fukushima et al. (2016) using a 6-month mixed-exercise and nutritional intervention in 92 middle-aged obese women found that fasting insulin and glucose decreased in those whose SM mass index (kg/m^2) also decreased over the course of the intervention (Fukushima et al., 2016). Leon et al. (2013) also illustrated this point in 132 middle-aged women who participated in a 6-month diet and exercise program (Leon et al., 2013). Before the intervention, the authors noted that appendicular SM mass was proportional to the level of obesity and was also proportionally related to fasting insulin levels. Interestingly, changes in appendicular SM mass were moderately correlated (r = 0.337) to changes in fasting insulin after the intervention. In a large intervention study, Amankwaah et al. (2019) investigated the contribution of body composition changes to improvements in cardiometabolic health following a 9-month mixed exercise intervention (RT twice a week and AT once a week) in obese (BMI 30.0 ± 2.7 kg/m^2) middle-aged men (n = 69) and women (n = 83) (Amankwaah et al., 2019). Unexpectedly, SM hypertrophy did not contribute to improvements in glucose homeostasis. Furthermore, the authors mentioned that the relationship between changes in SM mass and some cardiometabolic indices (HDL-Cholesterol and IS index) was inconsistent across different expressions of SM mass. On one hand, the relationship was significant when SM mass changes were expressed as a percentage of total body weight, while it was not the case when changes were expressed relatively to baseline (%) SM mass or in absolute values (kg). This suggests that the observed changes in cardiometabolic health indices were predicted by changes in fat mass rather than quantitative changes in SM.

Given the paucity of findings with regards to the implications of SM hypertrophy in response to exercise in different populations and study design, the exercise physiology community would likely benefit from a more in-depth understanding of the mechanisms involved in such adaptations, as well as their implications. Herein, we thus suggest moving the debate forward by examining if and how the presence or the absence of SM hypertrophy influences glucose delivery and utilization in response to exercise interventions. The reader is directed toward **Table 2**, which provides a brief overview of studies simultaneously reporting insulin-sensitizing SM metabolic properties, FFM quantitative changes and glucose homeostasis parameters changes in the context of exercise interventions.

Skeletal Muscle Hypertrophy, Microvascularization and Glucose Homeostasis – Barrier or Enhancer?

Adequate perfusion is critical for efficient glucose delivery toward SM tissue. For instance, both the architecture of the microvasculature and its adaptability to vasodilation cues orchestrate glucose delivery from the circulation to the cytoplasm

(Schalkwijk and Stehouwer, 2005; Sjøberg et al., 2017). Under those circumstances, the endothelium acts as the main "gatekeeper" for glucose delivery (Richey, 2013). The sophisticated network of mechanisms behind insulin-stimulated vasodilation and recruitment of the vasculature being beyond the scope of the current review, readers are directed toward other excellent reviews for a more extensive discussion of these topics (Cocks and Wagenmakers, 2016; Lenasi and Klonizakis, 2016; Olver and Laughlin, 2016).

Capillary rarefication applies a physical barrier to adequate substrate flow toward SM tissue and is consequently an early indicator of SM-IR (Lillioja et al., 1987). Conversely, exercise-induced increases in capillary density allows for enlargement of the diffusible surface area, which promotes greater IS at the SM level (Akerstrom et al., 2014; Prior et al., 2015). In response to chronic AT, SM capillarity indexes (i.e., capillary ultrastructure, capillary density, capillary-to-fiber ratio, etc.) and IS have been found to both increase in a positive and linear fashion (Prior et al., 2014; Cocks et al., 2016; Mortensen et al., 2019). On another hand, collective evidence on vascular adaptations to exercise suggests a plausible relationship between total SM mass, SM fiber-type characterization and capillary density indexes. Interestingly, the impact of SM hypertrophy *per se* on SM capillary architecture has only been explored by a few trials. In those studies, individuals with lower total SM mass had distinct characterizations of SM fiber type (Trappe et al., 1995). For example, capillary density has been found to be significantly higher in endurance athletes compared to age-matched powerlifting athletes with substantially higher SM mass (Tesch et al., 1984). In a previous study, Green et al. (1999) demonstrated a parallel increase in the number of capillaries in contact with each fiber type and SM fibers area after a 12-week high intensity RT regimen in young college students (Green et al., 1999). More recently, Holloway et al. (2018) investigated the temporal response of SM angiogenesis during RT-induced SM hypertrophy in a sample of 36 young men (Holloway et al., 2018). Not only did they see significant hypertrophy of type I and type II muscle fibers, but they also measured a concomitant increase in capillary-to-fiber ratio in type I muscle fibers. The findings from this study suggest SM hypertrophy may

TABLE 2 | Summary of studies reporting insulin-sensitizing skeletal muscle metabolic properties, fat-free mass quantitative changes, and glucose homeostasis parameters.

Study	Intervention (Modality)	Sample	FFM and FM changes	Glucose homeostasis	SM metabolic properties	Association
Layne et al., 2011	8-week RT (progressive overload)	19 sedentary middle-aged individuals, with MetS (5F/10M) and control (5F/4M)	MetS: ↑ FFM ↔ FM Control: ↑ FFM (1.3kg) ↔ FM	MetS: ↔ Control: ↑ GIR (25%)	Mets: ↑ GLUT4 expression ↑ ATP synthase ↑ AMPK expression Control: ↑ ATP synthase ↑ GLUT4 expression ↑ PGC-1α ↑ AMPK expression	No association shown. Higher absolute FFM and type 2 fiber proportion in Mets participants pre- and post-intervention.
Andersen et al., 2003	3 months of de-training after a 3-month heavy RT	7 young (26 ± 1y) inactive men	↓ Quadriceps CSA after detraining	↔ F-glucose, insulin, c-peptide, [insulin] during clamp ↓ Whole-body M during the last 30min of the clamp (11 ± 4%)	↔ GLUT4 mRNA ↔ CS mRNA ↔ HAD mRNA ↔ GS mRNA ↔ Capillary density ↓ Glycogen content	No significant correlation between changes in leg glucose uptake rates and changes in muscle mas Type IIX fiber proportion increased in the detrained state.
Cocks et al., 2014; Shepherd et al., 2014 (shared sample)	6-week RT focused on hypertrophy and strength (9 exercises, 3 sets of 12 rep) IMTG breakdown during 1-h cycling ≈65% VO_2peak	13 young (20 ± 1 years) lean (avg BMI: 24 ± 0.8) sedentary men	↑ FFM ↓ FM	↑ GH: ↑ Matsuda index ↓ OGTT glucose and insulin	↑ IMTG content and density in type I and type II fibers ↑ IMTG breakdown during 1-h cycling in type I and type II fibers ↑ COX expression ↑ SDH activity ↔ Capillary density, capillary contacts, $eNOS^{ser1177}$ phosphorylation	No association discussed between FFM changes, SM metabolic properties changes and GH.
Iglay et al., 2007	12-weeks RT, 3/week (8 exercises, 2 sets of 8), coupled with either low or high protein diet	36 older (62.2 ± 2 years) men (n = 18) and women (n = 18)	High-protein group: ↑ FFM Low-protein group: ↑ FFM	Both groups: ↔HbA1c, ISI-composite, plasma glucose, insulin, C-peptide and HOMA-IR	Both groups: ↔ IRS-1, Akt ↑ Atypical protein kinase	Significant effect of RT on FFM and FM, independent of protein intake. BC changes were not correlated with changes in GH.

(Continued)

TABLE 2 | Continued

Study	Intervention (Modality)	Sample	FFM and FM changes	Glucose homeostasis	SM metabolic properties	Association
Holten et al., 2004	6-week, 3/week (One-leg RT)	10 men with T2D and 7 healthy controls	Both groups: ↑ FFM	Both groups: ↑ Leg glucose clearance	Both groups: ↑ GLUT4 content ↑ Insulin receptor ↑ GS synthase activity ↑ GS protein content ↑AKT (1/2) ↔ Oxidative enzymes (CS, LDH, HAD)	No association shown. Changes in muscle metabolic properties were likely to be independent of leg FFM quantitative changes.
Ferrara et al., 2006	10 to 12-week weight stabilization, followed by either AT or RT exercise for 6 months, 3 days/week.	21 Overweight or obese (avg BMI: 29.9 ± 0.7) middle aged and older men (50–79 years)	AT: ↑ FFM ↓ Thigh CSA ↓ Subcutaneous FM RT: ↑ FFM	AT: ↔ F-glucose ↔ F-insulin ↑ M (480 pmol/m^2/min insulin infusion clamp) RT: ↔ F-glucose ↔ F-insulin ↑ M (480 pmol/m^2/min insulin infusion clamp) ↑ Non-oxidative carbohydrate metabolism	AT: ↑ GS fractional activity ↔ CS, PI 3-k, glycogen content RT: ↑ GS fractional activity ↔ CS, PI 3-k, glycogen content	The effect of insulin on GS activity was significantly and 2.5-fold greater in the AT group.
Van Der Heijden et al., 2010	12 weeks RT, 2/week (2–3 sets of 8–12 repetitions, 3 sets of 15–20 during week 9–12)	12 obese (BMI: 35.3 ± 0.7) adolescents	↑ FFM ↑ Subcutaneous FM	↑ Hepatic IS; ↓ glucose production rate (SLIVGTT and glucose tracer infusion)	↔IMTG content	Neither FFM at baseline and post-exercise were correlated with peripheral and hepatic IS.

Akt, protein kinase B (PKB); AMPK, AMP-activated protein kinase; AT, aerobic training; ATP, adenosine triphosphate; Avg, average; AUC, area under the curve; BMI, body mass index (kg/m^2); BC, body composition; BF, body fat; C, citrate synthase; COX, cytochrome oxidase; CSA, cross-sectional area; eNOS^{ser1177}, endothelial nitric oxide synthase at phosphorylation site 1177; F-glucose, fasting glucose; F-insulin, fasting insulin; FFM, fat-free mass; FFMI, fat-free mass index (kg/m^2); FM, fat mass; GIR, glucose infusion rate; GLUT4, glucose transporter type 4; GS, glycogen synthase; GT, glucose tolerance; GU, glucose uptake; HOMA-IR, homeostatic model assessment for insulin resistance; HAD, 3-hydroxyacyl-CoA dehydrogenase; IMTG, intramuscular triglycerides; IRS-1, insulin receptor substrate-1; LDH, lactate dehydrogenase; M, hyperinsulinemic-euglycemic clamp-derived insulin sensitivity; OGTT, oral glucose tolerance test; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PI 3-k, phosphatidylinositol 3-kinases; REPS, repetitions; RM, maximal repetition; RT, resistance training; SLIVGTT, stable-label iv glucose tolerance test; SM, skeletal muscle; T2D, Type 2 diabetes.

not be a limiting factor for SM angiogenesis in type I muscle fibers. Noteworthy, these conclusions were not supported by an association between the observed changes in fiber size and capillarization indexes. In the same line, interesting results from Snijders et al. (2017) demonstrated that baseline SM capillarity was a strong driver of SM hypertrophy and increases in satellite cell content after a RT intervention in healthy older men (Snijders et al., 2017). Contrasting with the results of Holloway et al. (2018), the intervention did not elicit any increase in capillary density, neither in type I nor type II muscle fibers, although SM hypertrophy was present. By the same token, a recent study of Moro et al. (2019) has shown that baseline SM capillarization was a strong driver of SM hypertrophy following RT in older adults (Moro et al., 2019). Overall, those findings suggest that improving SM perfusion capacity for glucose, insulin and other growth factors transport may be paramount to SM hypertrophy. The other side of the coin of these findings is that improving muscle quality first, by improving capillarity, could be the most efficient strategy regardless of the ultimate goal being SM hypertrophy, enhanced glucose homeostasis, or both.

Even though the aforementioned findings revealed important insights on how microvascular adaptations may influence SM mass quantitative changes, few investigations showed a comprehensive assessment of the implications for IS. Yet, only a few trials and reviews have previously shed light on potential hypotheses. A study by Cocks et al. (2014) examined the effects of a short-term RT intervention on SM capillary architecture indexes in a sample of eight sedentary young men (Cocks et al., 2014). They found no significant differences in either capillary contacts or number of capillaries per fiber after the intervention, although participants saw significant improvement in post absortive glucose tolerance. Previously, a review by Deschenes and Kraemer (2002) reported that RT-induced SM adaptation generally resulted in a reduction in relative capillarization when SM hypertrophy also occurs (Deschenes and Kraemer, 2002). However, according to a later review from Harris (2005) this interpretation is highly conditional to the ways in which muscle capillarity is expressed (Harris, 2005). When assessing capillarization as a matter of capillary contacts, hence the number of capillaries per myofiber, it rather seems that RT has a positive impact. It is generally accepted that

these adaptations are the direct consequence of an elevated local oxygen (O_2) demand from the SM – that is greater oxidative phosphorylation (OXPHOS) rates (Guyton et al., 2010, Chapter 7). These observations are in favor of the superiority of circuit-type RT above low-rep/high load RT for improving IS-related SM properties. Likely, the ideal RT prescription for improving microvascular function and SM-IS has not been identified yet (Olver and Laughlin, 2016).

In all likelihood, there exist an important interplay between SM angiogenesis and the mitochondrial biogenesis transcription factors. Irrefutably, PGC-1α transcription is likely one of the main drivers of mitochondrial biogenesis (Tiraby and Langin, 2005) and has been shown to play a significant role in microvascular reactivity and adaptations, through eNOS phosphorylation and vascular endothelial growth factor (VEGF) transcription pathways (Arany et al., 2008; Chinsomboon et al., 2009; Baldelli et al., 2014). Moreover, PGC-1α transcription has been shown to reflect Hypoxia Inducible Factor-1α (HIF-1α) activity, hence SM local O_2 demand (Gorski and De Bock, 2019). Indeed, endurance exercise generates continuous blood flow elevation toward SM for adequate O_2 and substrate delivery (Andersen and Saltin, 1985). As a response, the capillary network surface increases in order to enlarge the perfusion surface available. These adaptations seem highly dependent on PGC-1α transcription (Arany et al., 2008). As high-load, low-rep or hypertrophy-driven RT generally elicits short duration muscle contractions and an intermittent elevation of blood flow comparatively to AT, it may be an insufficient physiological stimulus to promote PGC-1α-driven angiogenesis. By the same token, results from Mortensen et al. (2019) suggested that improved vasodilator and insulin signaling mostly occur in SM undergoing a significant exercise volume (i.e., duration of stimuli application) (Mortensen et al., 2019). In their recent review, Olver and Laughlin (2016) provided in-depth analysis of the impact of different exercise modes on microvascular dysfunction in T2D (Olver and Laughlin, 2016) and stated that:

[exercise] program that engages the most skeletal muscle and the most muscle fibers within each skeletal muscle (i.e., greatest increase in fiber recruitment from rest to exercise), given that the stimulus is applied for a sufficient duration of time, will generate the most widespread adaptations, leading to improvements in microvascular function and insulin sensitivity.

Such a statement reinforces the hypothesis that RT solely centered on SM hypertrophy may not be the most appropriate stimulus for microvascular adaptations and SM IS. A unique study of Hansen et al. (2012) provided important insights on the respective impact of endurance-type RT and hypertrophy-focused RT regimens (Hansen et al., 2012). In short, they compared the two exercise modalities with regards to their effect on glucose metabolism in a sample of 18 older men and women with impaired glucose tolerance. Their results revealed that both exercise modalities had positive effects on baseline IS, but the endurance-type modality had an additional effect on pancreas's beta-cell function.

As pointed out earlier, another important feature of efficient glucose delivery is the endothelium's vasodilation capacity in response to either elevated insulin levels or a high metabolic demand induced by SM contraction (Cocks and Wagenmakers, 2016). In insulin resistant states, capillary blood volume and flow do not increase normally in response to insulin, which is likely to contribute to abnormal glucose homeostasis (Lenasi and Klonizakis, 2016). To date, the exact effect of SM hypertrophy on the SM microvasculature response to vasodilation physiological cues remains unclear, although RT has been found to exert significant benefits (Olver and Laughlin, 2016). Using an ultrasound method, Cohen et al. (2008) reported improvements in vasodilation of the forearm skin microvasculature in elderly T2D patients after a 14-month RT period (Cohen et al., 2008). Another recent study from Russell et al. (2017) using a 6-week RT protocol in T2D patients also showed significant improvements in muscle microvascular blood flow and a concomitant significant improvement in IS (Russell et al., 2017). Changes in fasting blood glucose, HbA1c and glucose area under the curve during an oral glucose tolerance test were all induced by a higher microvascular blood flow (adjusted for percent SM, body mass index, brachial blood flow and mean arterial pressure). Importantly, these changes were accompanied by a 44% increase in relative strength and an average of 1.3 kg SM gain. It is therefore legitimate to debate which one of those two improvements is more related to enhanced glucose homeostasis, relative strength being an important feature of muscle quality (Barbat-Artigas et al., 2013).

Additionally, the heterogeneity of blood flow distribution between muscle fiber types is likely to play an important role in the insulin-mediated vasoreactivity. Heinonen et al. (2015) reviewed this topic and reported a study by Behnke et al. (2011) that established that muscle fibers with lower oxidative potential, or known as type II fibers, are also prone to high adrenergic stimulation (Behnke et al., 2011; Heinonen et al., 2015). Thus, it is likely that these SM fibers display less upregulation of vasodilation mediated by endothelial factors, as well as an elevated α-adrenergic-mediated vasoconstriction, compared to type I fibers. Previous data suggested that obese (Krotkiewski et al., 1990), first-degree relatives (Nyholm et al., 1997), and T2D (Mårin et al., 1994) individuals might have a higher proportion of type II muscle fibers and second, usually hypertrophy-driven RT exercise has been hypothesized to mostly target type II muscle fibers (Folland and Williams, 2007; Netreba et al., 2013; Grgic and Schoenfeld, 2018). Admittedly, one could hypothesize that targeting SM hypertrophy might not be ideal in order to improve SM mechanisms of IS.

New Insights in Mitochondrial Adaptations in Skeletal Muscle Hypertrophy

Amongst the vast array of insulin-sensitizing adaptations from chronic exercises, enhanced oxidative capacity is one of the most regarded. Indeed, a high SM mitochondrial density has been relentlessly highlighted as a univocal feature of SM-IS (Gouspillou et al., 2014; Roden and Shulman, 2019; Houzelle et al., 2020). Conversely, mitochondrial dysfunction has been related to loss of SM mass (Gouspillou et al., 2010) Furthermore, peripheral OXPHOS capacity is positively associated with an efficient glucose metabolism (Rimbert et al., 2004). In brief,

elevated peripheral oxidative capacity is thought to be one of the strongest predictors of whole-body-IS in virtue of a higher total substrate utilization (Bruce et al., 2003). A recent study of Zampino et al. (2020) highlighted the influence of SM OXPHOS activity on resting metabolic rate, independently of total FFM, which suggests that intrinsic SM OXPHOS capacity is a stronger driver of energy expenditure than SM quantity (Zampino et al., 2020). Nonetheless, whether exercise-induced SM hypertrophy leads to significant mitochondrial adaptations with regards to improved IS remains unclear. A comprehensive study of St-Jean-Pelletier et al. (2017) has found a significant association between SM mitochondrial content and thigh lean mass and CSA, as well as lower body strength, in a pooled sample of young and older/sedentary or active men. Interestingly, they also found to significant relationship between mitochondrial and lipid content (St-Jean-Pelletier et al., 2017). Sparks et al. (2013) investigated the influence of either AT, RT or their adjunction (ATRT) matched for time in older individuals with T2D and found that ATRT has the most impact on mitochondrial content and substrate oxidation (Sparks et al., 2013). Interestingly, participants in the ATRT group displayed no significant changes in SM mass. This observation echoes Olver and Laughlin's (2016) suggestion that with regards to exercise adaptations related to SM-IS, the combination of the two exercise modalities is more efficient than one of the two in isolation (Olver and Laughlin, 2016). According to the authors, this could be explained by a broader recruitment of the musculoskeletal system and potentially, a greater total exercise workload. Recently, Parry et al. (2020) reviewed the equivocal character of SM mitochondrial adaptations in response to RT regimens. In short, the authors proposed a novel theory, the "mitochondrial dilution" model, wherein SM hypertrophy without an equivalent rate of concurrent mitochondrial biogenesis leads to a decrease in relative mitochondrial content and ultimately, lowered or unchanged total oxidative capacity and mitochondrial respiration (Parry et al., 2020). Although the contribution of SM mitochondrial dilution on IS needs further investigations, one could hypothesize that since lower mitochondrial activity is a well-known feature of IR (Petersen et al., 2004), such a dilution could lead to a decrease in relative SM IS (Petersen et al., 2004). The authors also recommend examining how high-repetition/low-load or "aerobic-like" training, versus high-load/low-repetition or "hypertrophy-driven" training modulates markers of mitochondrial biogenesis. In line with those speculations, a study from Burd et al. (2010) has demonstrated that acute low-load, high volume RT, in terms of absolute number of repetitions has a greater impact on mitochondrial protein synthesis, compared to high-load – low volume RT (Burd et al., 2010).

A reduced turnover rate of intramuscular triglycerides (IMTG) is also a hallmark of SM-IR (Petersen et al., 2004; Roden and Shulman, 2019) and is thought to be the consequence of a low oxidative capacity (Consitt et al., 2009). Conversely, an elevated IMTG turnover rate is associated with greater SM-IS, irrespective of the magnitude of IMTG pools (Goodpaster et al., 2001). Although the relevance of both aerobic and resistance exercises is no longer to be proven regarding enhanced IMTG metabolism, it remains unknown whether quantitative changes in SM play a role in those molecular adaptations. However, it is generally accepted that hypertrophied SM contains proportionally more type IIA and IIB than type I fibers (Karp, 2001). Nonetheless, these types of muscle fibers are thought to display a lower oxidative capacity (Henriksson and Reitman, 1976). A study of Shepherd et al. (2014) provided insightful observation to this issue by revealing that RT has the ability to increase IMTG breakdown in type II fibers (Shepherd et al., 2014). In short, their results demonstrated an increase in lipid droplet-associated proteins 2 and 5 (PLIN2 and PLIN5) covering lipid droplets, as well as an increase in intramuscular triglyceride utilization during a 1-h moderate intensity cycling session after a 6-week RT protocol in lean, healthy young men. Furthermore, there was an increase in the expression of cytochrome oxidase (COX; a marker of SM oxidative capacity) in both type I and II fibers. There was also a slight increase in total SM mass. But most importantly, all adaptations occurred in both type I and type II muscle fibers. In sum, the current body of evidence suggests that even if the importance of AT and RT in glucose metabolism is no longer to be proven regarding IMTG metabolism, it has yet to be confirmed whether quantitative changes in SM mass further drives those adaptations in other populations, such as individuals with metabolic impairments.

A Perspective Approach on the Optimal Strategy

Body weight management and exercise are amongst the most promising strategies to improve and maintain overall metabolic health (Tuomilehto et al., 2001). However, it is still unclear if the current focus on SM hypertrophy, or the prevention of SM losses during weight loss trials is appropriate amongst all populations. In contrast, studies aiming at increasing muscle quality, regardless of quantity, are currently scarce (Barbat-Artigas et al., 2013). This can be achieved with low-load-high repetition RT, which is an overlooked exercise modality in metabolically impaired populations. Further studies that will seek to determine the real contribution of quantitative changes of SM on glucose homeostasis in populations who would benefit from an optimal exercise prescription for this purpose, such as prediabetic, T2D or metabolically impaired individuals, are highly warranted.

CONCLUSION

Regular exercise, be it AT or RT, has repetitively been associated with improved IS through various mechanisms. However, the mechanisms underlying a relationship between SM hypertrophy and whole-body glucose homeostasis have not been demonstrated without doubt. A fundamental mechanism that could contribute to IS improvements is greater SM capillarity (i.e., greater perfusion surface) and its vasodilator response. The associated increase in blood-flow and diffusion surface for O_2 support improved SM OXPHOS capacity and intramuscular lipid

turnover. We argue that RT protocols both involving a high O_2 demand and aiming to improve muscle function have the highest potential to induce SM adaptations in favor to an improved IS. With this in mind, SM hypertrophy ability to equivocally improve whole-body glucose homeostasis might be re-evaluated in specific populations. Moreover, in virtue of better known intersex- and age- related physiological differences in response to acute and chronic exercise (Lundsgaard and Kiens, 2014; Hughes et al., 2015; Snijders et al., 2017; Ansdell et al., 2020), specific recommendations are warranted. Finally, while RT is a highly relevant and proven strategy to prevent T2D (Bird and Hawley, 2017; Pesta et al., 2017), future investigation should seek to determine which modality of RT would maximize such benefits and verify if RT, through improvements of SM quality, can substantially increase IS independently of SM mass changes.

AUTHOR CONTRIBUTIONS

ID and MB had the idea of the article and supervised JP in the writing. JP performed the literature search, article analysis and wrote the whole manuscript and tables. J-CL critically revised the manuscript and tables. All authors contributed to the article and approved the submitted version.

FUNDING

JP has received a doctoral scholarship from *Fonds de recherche en santé du Québec-Santé* (FRQ-S). J-CL has received a doctoral scholarship from *Instituts de recherche en santé du Canada* (IRSC).

REFERENCES

Akerstrom, T., Laub, L., Vedel, K., Brand, C. L., Pedersen, B. K., Lindqvist, A. K., et al. (2014). Increased skeletal muscle capillarization enhances insulin sensitivity. *American Journal of Physiology - Endocrinology and Metabolism* 307, E1105–E1116. doi: 10.1152/ajpendo.00020.2014

Amankwaah, A. F., Hudson, J. L., Kim, J. E., and Campbell, W. W. (2019). Reductions in whole-body fat mass but not increases in lean mass predict changes in cardiometabolic health indices with exercise training among weight-stable adults. *Nutrition Research* 63, 63–69. doi: 10.1016/j.nutres.2018.11.004

Andersen, J. L., Schjerling, P., Andersen, L. L., and Dela, F. (2003). Resistance training and insulin action in humans: effects of de-training. *J. Physiol.* 551, 1049–1058. doi: 10.1111/j.1469-7793.2003.01049.x

Andersen, P., and Saltin, B. (1985). Maximal perfusion of skeletal muscle in man. *The Journal of Physiology* 366, 233–249. doi: 10.1113/jphysiol.1985.sp015794

Ansdell, P., Thomas, K., Hicks, K. M., Hunter, S. K., Howatson, G., and Goodall, S. (2020). Physiological sex differences affect the integrative response to exercise: Acute and chronic implications. *Experimental Physiology* 105, 1–42. doi: 10.1113/EP088548

Arany, Z., Foo, S. Y., Ma, Y., Ruas, J. L., Bommi-Reddy, A., Girnun, G., et al. (2008). HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1α. *Nature* 451, 1008–1012. doi: 10.1038/nature06613

Baldelli, S., Barbato, D. L., Tatulli, G., Aquilano, K., and Ciriolo, M. R. (2014). The role of nNOS and PGC-1α in skeletal muscle cells. *Journal of Cell Science* 127, 4813–4820. doi: 10.1242/jcs.154229

Barbat-Artigas, S., Rolland, Y., Vellas, B., and Aubertin-Leheudre, M. (2013). Muscle quantity is not synonymous with muscle quality. *Journal of the American Medical Directors Association* 14, e1–e7. doi: 10.1016/j.jamda.2013.06.003 * 852.e1-7,

Behnke, B. J., Armstrong, R. B., and Delp, M. D. (2011). Adrenergic control of vascular resistance varies in muscles composed of different fiber types: Influence of the vascular endothelium. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology* 301, R783–R790. doi: 10.1152/ajpregu.00205.2011

Bird, S. R., and Hawley, J. A. (2017). Update on the effects of physical activity on insulin sensitivity in humans. *BMJ Open Sport and Exercise Medicine* 2, 1–26. doi: 10.1136/bmjsem-2016-000143

Blanc, S., Schoeller, D. A., Bauer, D., Danielson, M. E., Tylavsky, F., Simonsick, E. M., et al. (2004). Energy requirements in the eighth decade of life. *American Journal of Clinical Nutrition* 79, 303–310. doi: 10.1093/ajcn/79.2.303

Brochu, M., Mathieu, M., Karelis, A. D., Doucet, É, Lavoie, M., Garrel, D., et al. (2008). Contribution of the lean body mass to insulin resistance in postmenopausal women with visceral obesity: a Monet study. *Obesity* 16, 1085–1093.

Bruce, C. R., Anderson, M. J., Carey, A. L., Newman, D. G., Bonen, A., Kriketos, A. D., et al. (2003). Muscle Oxidative Capacity Is a Better Predictor of Insulin Sensitivity than Lipid Status. *Journal of Clinical Endocrinology and Metabolism* 88, 5444–5451. doi: 10.1210/jc.2003-030791

Bucci, M., Huovinen, V., Guzzardi, M. A., Koskinen, S., Raiko, J. R., Lipponen, H., et al. (2016). Resistance training improves skeletal muscle insulin sensitivity in elderly offspring of overweight and obese mothers. *Diabetologia* 59, 77–86. doi: 10.1007/s00125-015-3780-8

Burd, N. A., West, D. W. D., Staples, A. W., Atherton, P. J., Baker, J. M., Moore, D. R., et al. (2010). Low-load high volume resistance exercise stimulates muscle protein synthesis more than high-load low volume resistance exercise in young men. *PLoS ONE* 5:e12033. doi: 10.1371/journal.pone.0012033

Cauza, E., Strehblow, C., Metz-Schimmerl, S., Strasser, B., Hanusch-Enserer, U., Kostner, K., et al. (2009). Auswirkung von progressivem krafttraining auf die muskelmasse bei patienten mit typ-2-diabetes mellitus: Messung mit der computertomographie. *Wiener Medizinische Wochenschrift* 159, 141–147. doi: 10.1007/s10354-009-0641-4

Chinsomboon, J., Ruas, J., Gupta, R. K., Thom, R., Shoag, J., Rowe, G. C., et al. (2009). The transcriptional coactivator PGC-1α mediates exercise-induced angiogenesis in skeletal muscle. *Proceedings of the National Academy of Sciences of the United States of America* 106, 21401–21406. doi: 10.1073/pnas.0909131106

Cocks, M., Shaw, C. S., Shepherd, S. O., Fisher, J. P., Ranasinghe, A., Barker, T. A., et al. (2016). Sprint interval and moderate-intensity continuous training have equal benefits on aerobic capacity, insulin sensitivity, muscle capillarisation and endothelial eNOS/NAD(P)Hoxidase protein ratio in obese men. *Journal of Physiology* 594, 2307–2321. doi: 10.1113/jphysiol.2014.285254

Cocks, M., Shaw, C. S., Shepherd, S. O., Fisher, J. P., Ranasinghe, A. M., Barker, T. A., et al. (2014). Effect of Resistance Training on Microvascular Density and eNOS Content in Skeletal Muscle of Sedentary Men. *Microcirculation* 21, 738–746. doi: 10.1111/micc.12155

Cocks, M., and Wagenmakers, A. J. M. (2016). The effect of different training modes on skeletal muscle microvascular density and endothelial enzymes controlling NO availability. *Journal of Physiology* 594, 2245–2257. doi: 10.1113/JP270329

Cohen, N. D., Dunstan, D. W., Robinson, C., Vulikh, E., Zimmet, P. Z., and Shaw, J. E. (2008). Improved endothelial function following a 14-month resistance exercise training program in adults with type 2 diabetes. *Diabetes Research and Clinical Practice* 79, 405–411. doi: 10.1016/j.diabres.2007.09.020

Consitt, L. A., Bell, J. A., and Houmard, J. A. (2009). Intramuscular lipid metabolism, insulin action, and obesity. *IUBMB Life* 61, 47–55. doi: 10.1002/iub.142

Cooper, C., Dere, W., Evans, W., Kanis, J. A., Rizzoli, R., Sayer, A. A., et al. (2012). Frailty and sarcopenia: definitions and outcome parameters. *Osteoporosis International* 23, 1839–1848. doi: 10.1007/s00198-012-1913-1

Cuff, D. J., Meneilly, G. S., Martin, A., Ignaszewski, A., Tildesley, H. D., and Frohlich, J. J. (2003). Effective Exercise Modality to Reduce Insulin Resistance in Women With Type 2 Diabetes. *Diabetes Care* 26, 2977–2982. doi: 10.2337/diacare.26.11.2977

DeFronzo, R. A., and Tripathy, D. (2009). Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care* 32(Suppl.), 2. doi: 10.2337/dc09-s302

Dela, F., and Kjaer, M. (2006). Resistance training, insulin sensitivity and muscle function in the elderly. *Essays in Biochemistry* 42, 75–88. doi: 10.1042/bse0420075

Deschenes, M. R., and Kraemer, W. J. (2002). Performance and physiologic adaptations to resistance training. *American Journal of Physical Medicine and Rehabilitation* 81, S3–S16. doi: 10.1097/00002060-200211001-00003

DiMenna, F. J., and Arad, A. D. (2018). Exercise as 'precision medicine' for insulin resistance and its progression to type 2 diabetes: A research review. *BMC Sports Science, Medicine and Rehabilitation* 10:21. doi: 10.1186/s13102-018-0110-8

Dionne, I. J., Mélançon, M. O., Brochu, M., Ades, P. A., and Poelhman, E. T. (2004). Age-related differences in metabolic adaptations following resistance training in women. *Exp. Gerontol.* 39, 133–138. doi: 10.1016/j.exger.2003.10.006

Dulloo, A. G. (2021). Physiology of weight regain: Lessons from the classic Minnesota Starvation Experiment on human body composition regulation. *Obesity Reviews* 22, e13189.

Dulloo, A. G., Jacquet, J., Montani, J. P., and Schutz, Y. (2012). Adaptive thermogenesis in human body weight regulation: More of a concept than a measurable entity? *Obesity Reviews* 13, 105–121. doi: 10.1111/j.1467-789X.2012.01041.x

Eriksson, J., Taimela, S., Eriksson, K., Parviainen, S., Peltonen, J., and Kujala, U. (1997). Resistance training in the treatment of non-insulin-dependent diabetes mellitus. *International Journal of Sports Medicine* 18, 242–246. doi: 10.1055/s-2007-972627

Eriksson, J., Tuominen, J., Valle, T., Sundberg, S., Sovijärvi, A., Lindholm, H., et al. (1998). Aerobic endurance exercise or circuit-type resistance training for individuals with impaired glucose tolerance? *Hormone and Metabolic Research* 30, 37–41. doi: 10.1055/s-2007-978828

Ferrara, C. M., Goldberg, A. P., Ortmeyer, H. K., and Ryan, A. S. (2006). Effects of aerobic and resistive exercise training on glucose disposal and skeletal muscle metabolism in older men. *J Gerontol. A Biol. Sci. Med. Sci.* 61, 480–487. doi: 10.1093/gerona/61.5.480

Folland, J. P., and Williams, A. G. (2007). The adaptations to strength training: Morphological and neurological contributions to increased strength. *Sports Medicine* 37, 145–168. doi: 10.2165/00007256-200737020-00004

Fukushima, Y., Kurose, S., Shinno, H., Thu, H. C., Takao, N., Tsutsumi, H., et al. (2016). Importance of lean muscle maintenance to improve insulin resistance by body weight reduction in female patients with obesity. *Diabetes and Metabolism Journal* 40, 147–153. doi: 10.4093/dmj.2016.40.2.147

Ghachem, A., Lagacé, J. C., Brochu, M., and Dionne, I. J. (2019). Fat-free mass and glucose homeostasis: is greater fat-free mass an independent predictor of insulin resistance? *Aging Clinical and Experimental Research* 31, 447–454. doi: 10.1007/s40520-018-0993-y

Gippini, A., Mato, A., Pazos, R., Suarez, B., Vila, B., Gayoso, P., et al. (2002). Effect of long-term strength training on glucose metabolism. Implications for individual impact of high lean mass and high fat mass on relationship between BMI and insulin sensitivity. *Journal of Endocrinological Investigation* 25, 520–525. doi: 10.1007/bf03345494

Glouzon, B. K. J., Barsalani, R., Lagace, J. C., and Dionne, I. J. (2015). Muscle mass and insulin sensitivity in postmenopausal women after 6-month exercise training. *Climacteric* 18, 846–851. doi: 10.3109/13697137.2015.1083002

Goodpaster, B. H., He, J., Watkins, S., and Kelley, D. E. (2001). Skeletal muscle lipid content and insulin resistance: Evidence for a paradox in endurance-trained athletes. *Journal of Clinical Endocrinology and Metabolism* 86, 5755–5761. doi: 10.1210/jcem.86.12.8075

Gordon, B. A., Benson, A. C., Bird, S. R., and Fraser, S. F. (2009). Resistance training improves metabolic health in type 2 diabetes: A systematic review. *Diabetes Research and Clinical Practice* 83, 157–175. doi: 10.1016/j.diabres.2008.11.024

Gorski, T., and De Bock, K. (2019). Metabolic regulation of exercise-induced angiogenesis. *Vasc Biol* 1, H1–H8.

Gotshalk, L. A., Berger, R. A., and Kraemer, W. J. (2004). Cardiovascular Responses to a High-Volume Continuous Circuit Resistance Training Protocol. *The Journal of Strength and Conditioning Research* 18, 760. doi: 10.1519/14954.1

Goulet, E. D. B., Lord, C., Chaput, J. P., Aubertin-Leheudre, M., Brochu, M., and Dionne, I. J. (2007). No difference in insulin sensitivity between healthy postmenopausal women with or without sarcopenia: A pilot study. *Applied Physiology, Nutrition and Metabolism* 32, 426–433. doi: 10.1139/H07-005

Gouspillou, G., Bourdel-Marchasson, I., Rouland, R., Calmettes, G., Franconi, J.-M., Deschodt-Arsac, V., et al. (2010). Alteration of mitochondrial oxidative phosphorylation in aged skeletal muscle involves modification of adenine nucleotide translocator. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* 1797, 143–151.

Gouspillou, G., Sgarioto, N., Norris, B., Barbat-Artigas, S., Aubertin-Leheudre, M., Morais, J. A., et al. (2014). The relationship between muscle fiber type-specific PGC-1α content and mitochondrial content varies between rodent models and humans. *PLoS One* 9:e103044. doi: 10.1371/journal.pone.0103044

Green, H., Goreham, C., Ouyang, J., Ball-Burnett, M., and Ranney, D. (1999). Regulation of fiber size, oxidative potential, and capillarization in human muscle by resistance exercise. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *276* 2, 591–596. doi: 10.1152/ajpregu.1999.276.2.r591

Grgic, J., and Schoenfeld, B. J. (2018). Are the hypertrophic adaptations to high and low-load resistance training muscle fiber type specific? *Frontiers in Physiology* 9:402. doi: 10.3389/fphys.2018.00402

Guyton, L., Hall, F., Training, O., Training, P., Darin, C., Training, R. O., et al. (2010). *Guyton and Hall Textbook of Medical Physiology*, 12th Edn. 966. https://www.elsevier.com/books/guyton-and-hall-textbook-of-medical-physiology/hall/978-0-8089-2400-5*link q

Hansen, E., Landstad, B. J., Gundersen, K. T., Torjesen, P. A., and Svebak, S. (2012). Insulin sensitivity after maximal and endurance resistance training. *Journal of Strength and Conditioning Research* 26, 327–334. doi: 10.1519/JSC.0b013e318220e70f

Harris, B. A. (2005). The influence of endurance and resistance exercise on muscle capillarization in the elderly: A review. *Acta Physiologica Scandinavica* 185, 89–97. doi: 10.1111/j.1365-201X.2005.01461.x

Heinonen, I., Koga, S., Kalliokoski, K. K., Musch, T. I., and Poole, D. C. (2015). Heterogeneity of Muscle Blood Flow and Metabolism: Influence of Exercise. *Aging, and Disease States. Exercise and Sport Sciences Reviews* 43, 117–124. doi: 10.1249/JES.0000000000000044

Henriksson, J., and Reitman, J. S. (1976). Quantitative Measures of Enzyme Activities in Type I and Type II Muscle Fibres of Man after Training. *Acta Physiologica Scandinavica* 97, 392–397. doi: 10.1111/j.1748-1716.1976.tb10279.x

Holloway, T. M., Snijders, T., Van Kranenburg, J., Van Loon, L. J. C., and Verdijk, L. B. (2018). Temporal Response of Angiogenesis and Hypertrophy to Resistance Training in Young Men. *Medicine and Science in Sports and Exercise* 50, 36–45. doi: 10.1249/MSS.0000000000001409

Holten, M. K., Zacho, M., Gaster, M., Juel, C., Wojtaszewski, J. F. P., and Dela, F. (2004). Strength Training Increases Insulin-Mediated Glucose Uptake, GLUT4 Content, and Insulin Signaling in Skeletal Muscle in Patients with Type 2 Diabetes. *Diabetes* 53, 294–305. doi: 10.2337/diabetes.53.2.294

Honka, M. J., Latva-Rasku, A., Bucci, M., Virtanen, K. A., Hannukainen, J. C., Kalliokoski, K. K., et al. (2018). Insulin-stimulated glucose uptake in skeletal muscle, adipose tissue and liver: A positron emission tomography study. *European Journal of Endocrinology* 178, 523–531. doi: 10.1530/EJE-17-0882

Houzelle, A., Jörgensen, J. A., Schaart, G., Daemen, S., van Polanen, N., Fealy, C. E., et al. (2020). Human skeletal muscle mitochondrial dynamics in relation to oxidative capacity and insulin sensitivity. *Diabetologia* 64, 424–436. doi: 10.1007/s00125-020-05335-w

Hughes, W. E., Ueda, K., Treichler, D. P., and Casey, D. P. (2015). Rapid onset vasodilation with single muscle contractions in the leg: Influence of age. *Physiological Reports* 3, e12516. doi: 10.14814/phy2.12516

Iglay, H. B., Thyfault, J. P., Apolzan, J. W., and Campbell, W. W. (2007). Resistance training and dietary protein: effects on glucose tolerance and contents of skeletal muscle insulin signaling proteins in older persons. *Am. J. Clin. Nutr.* 85, 1005–1013. doi: 10.1093/ajcn/85.4.1005

Ikeda, K., Horie-Inoue, K., and Inoue, S. (2019). Functions of estrogen and estrogen receptor signaling on skeletal muscle. *Journal of Steroid Biochemistry and Molecular Biology* 191, 105375. doi: 10.1016/j.jsbmb.2019.105375

Illner, K., Brinkmann, G., Heller, M., Bosy-Westphal, A., and Müller, M. J. (2000). Metabolically active components of fat free mass and resting energy expenditure in nonobese adults. *American Journal of Physiology-Endocrinology and Metabolism* 278, E308–E315. doi: 10.1152/ajpendo.2000.278.2.E308

Jacquet, P., Schutz, Y., Montani, J.-P., and Dulloo, A. (2020). How dieting might make some fatter: modeling weight cycling toward obesity from a perspective of body composition autoregulation. *International Journal of Obesity* 44, 1243–1253. doi: 10.1038/s41366-020-0547-1

Karp, J. R. (2001). Muscle Fiber Types and Training. *Strength and Conditioning Journal* 23, 21–26. doi: 10.1519/00126548-200110000-00004

Kim, J.-W., Ko, Y.-C., Seo, T.-B., and Kim, Y.-P. (2018). Effect of circuit training on body composition, physical fitness, and metabolic syndrome risk factors in obese female college students. *Journal of Exercise Rehabilitation* 14, 460–465. doi: 10.12965/jer.1836194.097

Kleinert, M., Sylow, L., and Richter, E. A. (2013). Regulation of glycogen synthase in muscle and its role in Type 2 diabetes. *Diabetes Management* 3, 81–90. doi: 10.2217/dmt.12.54

Kogure, G. S., Miranda-Furtado, C. L., Silva, R. C., Melo, A. S., Ferriani, R. A., De Sá, M. F. S., et al. (2016). Resistance exercise impacts lean muscle mass in women with polycystic ovary syndrome. *Medicine and Science in Sports and Exercise* 48, 589–598. doi: 10.1249/MSS.0000000000000822

Kolahdouzi, S., Baghadam, M., Kani-Golzar, F. A., Saeidi, A., Jabbour, G., Ayadi, A., et al. (2019). Progressive circuit resistance training improves inflammatory biomarkers and insulin resistance in obese men. *Physiology & Behavior* 205, 15–21. doi: 10.1016/j.physbeh.2018.11.033

Krotkiewski, M., Seidell, J. C., and Björntrop, P. (1990). Glucose tolerance and hyperinsulinaemia in obese women: role of adipose tissue distribution, muscle fibre characteristics and androgens. *Journal of Internal Medicine* 228, 385–392. doi: 10.1111/j.1365-2796.1990.tb00250.x

Layne, A. S., Nasrallah, S., South, M. A., Howell, M. E., McCurry, M. P., Ramsey, M. W., et al. (2011). Impaired muscle AMPK activation in the metabolic syndrome may attenuate improved insulin action after exercise training. *J. Clin. Endocrinol. Metab.* 96, 1815–1826. doi: 10.1210/jc.2010-2532

Lee, D. H., Keum, N. N., Hu, F. B., Orav, E. J., Rimm, E. B., Willett, W. C., et al. (2018). Predicted lean body mass, fat mass, and all cause and cause specific mortality in men: prospective US cohort study. *BMJ (Clinical Research Ed.)* 362, k2575. doi: 10.1136/bmj.k2575

Lee, S. J., Bacha, F., Hannon, T., Kuk, J. L., Boesch, C., and Arslanian, S. (2012). Effects of aerobic versus resistance exercise without caloric restriction on abdominal fat, intrahepatic lipid, and insulin sensitivity in obese adolescent boys a randomized, controlled trial. *Diabetes* 61, 2787–2795. doi: 10.2337/db12-0214

Lenasi, H., and Klonizakis, M. (2016). Assessing the evidence: Exploring the effects of exercise on diabetic microcirculation. *Clinical Hemorheology and Microcirculation* 64, 663–678. doi: 10.3233/CH-168022

Leon, B., Jenkins, S., Pepin, K., Chaudhry, H., Smith, K., Zalos, G., et al. (2013). Insulin and extremity muscle mass in overweight and obese women. *International Journal of Obesity* 37, 1560–1564. doi: 10.1038/ijo.2013.45

Lexell, J. (1995). Human aging, muscle mass, and fiber type composition. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences* 50, 11–16. doi: 10.1093/gerona/50a.special_issue.11

Lillioja, S., Young, A. A., Culter, C. L., Ivy, J. L., Abbott, W. G., Zawadzki, J. K., et al. (1987). Skeletal muscle capillary density and fiber type are possible determinants of in vivo insulin resistance in man. *The Journal of Clinical Investigation* 80, 415–424. doi: 10.1172/JCI113088

Lundsgaard, A. M., and Kiens, B. (2014). Gender differences in skeletal muscle substrate metabolism - molecular mechanisms and insulin sensitivity. *Frontiers in Endocrinology* 5:195. doi: 10.3389/fendo.2014.00195

Mancuso, P., and Bouchard, B. (2019). The Impact of Aging on Adipose Function and Adipokine Synthesis. *Frontiers in Endocrinology* 10:137. doi: 10.3389/fendo.2019.00137

Mårin, P., Andersson, B., Krotkiewski, M., and Björntorp, P. (1994). Muscle fiber composition and capillary density in women and men with NIDDM. *Diabetes Care* 17, 382–386. doi: 10.2337/diacare.17.5.382

Marks, B. L., and Rippe, J. M. (1996). The importance of fat free mass maintenance in weight loss programmes. *Sports Medicine* 22, 273–281. doi: 10.2165/00007256-199622050-00001

Mavros, Y., Kay, S., Anderberg, K. A., Baker, M. K., Wang, Y., Zhao, R., et al. (2013). Changes in insulin resistance and HbA1c are related to exercise-mediated changes in body composition in older adults with type 2 diabetes: Interim outcomes from the GREAT2DO trial. *Diabetes Care* 36, 2372–2379. doi: 10.2337/dc12-2196

Miller, J. P., Pratley, R. E., Goldberg, A. P., Gordon, P., Rubin, M., Treuth, M. S., et al. (1994). Strength training increases insulin action in healthy 50- to 65-yr-old men. *Journal of Applied Physiology* 77, 1122–1127. doi: 10.1152/jappl.1994.77.3.1122

Miller, W. J., Sherman, W. M., and Ivy, J. L. (1984). Effect of strength training on glucose tolerance and post-glucose insulin response. *Medicine and Science in Sports and Exercise* 16, 539–543. doi: 10.1249/00005768-198412000-00003

Moro, T., Brightwell, C. R., Phalen, D. E., McKenna, C. F., Lane, S. J., Porter, C., et al. (2019). Low skeletal muscle capillarization limits muscle adaptation to resistance exercise training in older adults. *Experimental Gerontology* 127, 110723. doi: 10.1016/j.exger.2019.110723

Mortensen, S. P., Winding, K. M., Iepsen, U. W., Munch, G. W., Marcussen, N., Hellsten, Y., et al. (2019). The effect of two exercise modalities on skeletal muscle capillary ultrastructure in individuals with type 2 diabetes. *Scandinavian Journal of Medicine and Science in Sports* 29, 360–368. doi: 10.1111/sms.13348

Netreba, A., Popov, D., Bravyy, Y., Lyubaeva, E., Terada, M., Ohira, T., et al. (2013). Responses of knee extensor muscles to leg press training of various types in human. *Ross. Fiziol. Zh. Im. I.M. Sechenova* 99, 406–416.

Nyholm, B., Qu, Z., Kaal, A., Pedersen, S. B., Gravholt, C. H., Andersen, J. L., et al. (1997). Evidence of an increased number of type IIb muscle fibers in insulin-resistant first-degree relatives of patients with NIDDM. *Diabetes* 46, 1822–1828. doi: 10.2337/diab.46.11.1822

Olver, T. D., and Laughlin, M. H. (2016). Endurance, interval sprint, and resistance exercise training: impact on microvascular dysfunction in type 2 diabetes. *American Journal of Physiology-Heart and Circulatory Physiology* 310, H337–H350. doi: 10.1152/ajpheart.00440.2015

Parry, H. A., Roberts, M. D., and Kavazis, A. N. (2020). Human Skeletal Muscle Mitochondrial Adaptations following Resistance Exercise Training. *International Journal of Sports Medicine* 41, 349–359. doi: 10.1055/a-1121-7851

Perreault, K., Lagacé, J. C., Brochu, M., and Dionne, I. J. (2016). Association between fat free mass and glucose homeostasis: Common knowledge revisited. *Ageing Research Reviews* 28, 46–61. doi: 10.1016/j.arr.2016.04.007

Pesta, D. H., Goncalves, R. L. S., Madiraju, A. K., Strasser, B., and Sparks, L. M. (2017). Resistance training to improve type 2 diabetes: Working toward a prescription for the future. *Nutrition and Metabolism* 14, 24. doi: 10.1186/s12986-017-0173-7

Petersen, K. F., Dufour, S., Befroy, D., Garcia, R., and Shulman, G. I. (2004). Impaired Mitochondrial Activity in the Insulin-Resistant Offspring of Patients with Type 2 Diabetes. *New England Journal of Medicine* 350, 664–671. doi: 10.1056/nejmoa031314

Petersen, K. F., and Shulman, G. I. (2002). Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. *The American Journal of Cardiology* 90, 11–18. doi: 10.1016/S0002-9149(02)02554-7

Phu, S., Boersma, D., and Duque, G. (2015). Exercise and Sarcopenia. *Journal of Clinical Densitometry* 18, 488–492. doi: 10.1016/j.jocd.2015.04.011

Poehlman, E. T., Dvorak, R. V., DeNino, W. F., Brochu, M., and Ades, P. A. (2000). Effects of resistance training and endurance training on insulin sensitivity in nonobese, young women: a controlled randomized trial. *J. Clin. Endocrinol. Metab.* 85, 2463–2468. doi: 10.1210/jcem.85.7.6692

Praet, S. F. E., and Van Loon, L. J. C. (2009). Exercise therapy in Type 2 diabetes. *Acta Diabetologica* 46, 263–278. doi: 10.1007/s00592-009-0129-0

Prior, S. J., Blumenthal, J. B., Katzel, L. I., Goldberg, A. P., and Ryan, A. S. (2014). Increased skeletal muscle capillarization after aerobic exercise training and weight loss improves insulin sensitivity in adults with IGT. *Diabetes Care* 37, 1469–1475. doi: 10.2337/dc13-2358

Prior, S. J., Goldberg, A. P., Ortmeyer, H. K., Chin, E. R., Chen, D., Blumenthal, J. B., et al. (2015). Increased skeletal muscle capillarization independently enhances insulin sensitivity in older adults after exercise training and detraining. *Diabetes* 64, 3386–3395. doi: 10.2337/db14-1771

Ravussin, E., and Bogardus, C. (1990). Energy expenditure in the obese: Is there a thrifty gene? *Infusionstherapie* 17, 108–112. doi: 10.1159/000222456

Richey, J. M. (2013). The vascular endothelium, a benign restrictive barrier? no! role of nitric oxide in regulating insulin action. *Diabetes* 62, 4006–4008. doi: 10.2337/db13-1395

Rimbert, V., Boirie, Y., Bedu, M., Hocquette, J. F., Ritz, P., and Morio, B. (2004). Muscle fat oxidative capacity is not impaired by age but by physical inactivity: association with insulin sensitivity. *The FASEB Journal?: Official Publication of the Federation of American Societies for Experimental Biology* 18, 737–739. doi: 10.1096/fj.03-1104fje

Roberts, C. K., Croymans, D. M., Aziz, N., Butch, A. W., and Lee, C. C. (2013). Resistance training increases SHBG in overweight/obese, young men.

Metabolism: Clinical and Experimental 62, 725–733. doi: 10.1016/j.metabol.2012.12.004

Roden, M., and Shulman, G. I. (2019). The integrative biology of type 2 diabetes. *Nature* 576, 51–60. doi: 10.1038/s41586-019-1797-8

Rosenquist, K. J., and Fox, C. S. (2009). "Chapter 36: Mortality Trends in Type 2 Diabetes," in *Diabetes in America*, 1–14. https://www.niddk.nih.gov/about-niddk/strategic-plans-reports/Documents/DiabetesinAmerica3rdEdition/DIA_Ch36.pdf*accessed q

Russell, A. P. (2010). Molecular regulation of skeletal muscle mass. *Clinical and Experimental Pharmacology and Physiology* 37, 378–384. doi: 10.1111/j.1440-1681.2009.05265.x

Russell, R. D., Hu, D., Greenaway, T., Blackwood, S. J., Dwyer, R. M., Sharman, J. E., et al. (2017). Skeletal Muscle Microvascular-Linked Improvements in Glycemic Control From Resistance Training in Individuals With Type 2 Diabetes. *Diabetes Care* 40, 1256–1263. doi: 10.2337/dc16-2750

Schalkwijk, C. G., and Stehouwer, C. D. A. (2005). Vascular complications in diabetes mellitus: The role of endothelial dysfunction. *Clinical Science* 109, 143–159. doi: 10.1042/CS20050025

Shaibi, G. Q., Cruz, M. L., Ball, G. D. C., Weigensberg, M. J., Salem, G. J., Crespo, N. C., et al. (2006). Effects of resistance training on insulin sensitivity in overweight Latino adolescent males. *Medicine and Science in Sports and Exercise* 38, 1208–1215. doi: 10.1249/01.mss.0000227304.88406.0f

Shepherd, S. O., Cocks, M., Tipton, K. D., Witard, O. C., Ranasinghe, A. M., Barker, T. A., et al. (2014). Resistance training increases skeletal muscle oxidative capacity and net intramuscular triglyceride breakdown in type I and II fibres of sedentary males. *Experimental Physiology* 99, 894–908. doi: 10.1113/expphysiol.2014.078014

Shou, J., Chen, P.-J., and Xiao, W.-H. (2020). Mechanism of increased risk of insulin resistance in aging skeletal muscle. *Diabetology & Metabolic Syndrome* 12, 14. doi: 10.1186/s13098-020-0523-x

Sjøberg, K. A., Frøsig, C., Kjøbsted, R., Sylow, L., Kleinert, M., Betik, A. C., et al. (2017). Exercise increases human skeletal muscle insulin sensitivity via coordinated increases in microvascular perfusion and molecular signaling. *Diabetes* 66, 1501–1510. doi: 10.2337/db16-1327

Snijders, T., Nederveen, J. P., Joanisse, S., Leenders, M., Verdijk, L. B., van Loon, L. J. C., et al. (2017). Muscle fibre capillarization is a critical factor in muscle fibre hypertrophy during resistance exercise training in older men. *Journal of Cachexia, Sarcopenia and Muscle* 8, 267–276. doi: 10.1002/jcsm.12137

Sparks, L. M., Johannsen, N. M., Church, T. S., Earnest, C. P., Moonen-Kornips, E., Moro, C., et al. (2013). Nine months of combined training improves Ex vivo skeletal muscle metabolism in individuals with type 2 diabetes. *Journal of Clinical Endocrinology and Metabolism* 98, 1694–1702. doi: 10.1210/jc.2012-3874

Srikanthan, P., and Karlamangla, A. S. (2011). Relative muscle mass is inversely associated with insulin resistance and prediabetes. Findings from the Third National Health and Nutrition Examination Survey. *Journal of Clinical Endocrinology and Metabolism* 96, 2898–2903. doi: 10.1210/jc.2011-0435

St-Jean-Pelletier, F., Pion, C. H., Leduc-Gaudet, J. P., Sgarioto, N., Zovilé, I., Barbat-Artigas, S., et al. (2017). The impact of ageing, physical activity, and pre-frailty on skeletal muscle phenotype, mitochondrial content, and intramyocellular lipids in men. *Journal of Cachexia, Sarcopenia and Muscle* 8, 213–228. doi: 10.1002/jcsm.12139

Stuart, C. A., Lee, M. L., South, M. A., Howell, M. E. A., and Stone, M. H. (2017). Muscle hypertrophy in prediabetic men after 16 wk of resistance training. *Journal of Applied Physiology* 123, 894–901. doi: 10.1152/japplphysiol.00023.2017

Tam, T. T. T., Gross, R., Lukito, W., and Rumawas, J. S. (1999). Chronic energy deficiency and relative abdominal overfatness coexist in free−living elderly individuals in Ho Chi Minh City. *Vietnam. Asia Pacific Journal of Clinical Nutrition* 8, 129–135. doi: 10.1046/j.1440-6047.1999.00082.x

Tesch, P. A., Thorsson, A., and Kaiser, P. (1984). Muscle capillary supply and fiber type characteristics in weight and power lifters. *Journal of Applied Physiology Respiratory Environmental and Exercise Physiology* 56, 35–38. doi: 10.1152/jappl.1984.56.1.35

Tiraby, C., and Langin, D. (2005). PGC-1α a transcriptional coactivator involved in metabolism. *Médecine/Sciences* 21, 49–54. doi: 10.1051/medsci/200521149

Trappe, S. W., Costill, D. L., Fink, W. J., and Pearson, D. R. (1995). Skeletal muscle characteristics among distance runners: A 20-yr follow-up study. *Journal of Applied Physiology* 78, 823–829. doi: 10.1152/jappl.1995.78.3.823

Tsekoura, M., Kastrinis, A., Katsoulaki, M., Billis, E., and Gliatis, J. (2017). Sarcopenia and its impact on quality of life. *Advances in Experimental Medicine and Biology* 987, 213–218. doi: 10.1007/978-3-319-57379-3_19

Tuomilehto, J., Lindström, J., Eriksson, J. G., Valle, T. T., Hämäläinen, H., Ilanne-Parikka, P., et al. (2001). Prevention of Type 2 Diabetes Mellitus by Changes in Lifestyle among Subjects with Impaired Glucose Tolerance. *New England Journal of Medicine* 344, 1343–1350. doi: 10.1056/nejm200105033441801

Umpierre, D., Ribeiro, P. A. B., Kramer, C. K., Leitão, C. B., Zucatti, A. T. N., Azevedo, M. J., et al. (2011). Physical activity advice only or structured exercise training and association with HbA1c levels in type 2 diabetes: A systematic review and meta-analysis. *JAMA - Journal of the American Medical Association* 305, 1790–1799. doi: 10.1001/jama.2011.576

Van Der Heijden, G. J., Wang, Z. J., Chu, Z., Toffolo, G., Manesso, E., Sauer, P. J. J., et al. (2010). Strength exercise improves muscle mass and hepatic insulin sensitivity in obese youth. *Medicine and Science in Sports and Exercise* 42, 1973–1980. doi: 10.1249/MSS.0b013e3181df16d9

Volpi, E., Nazemi, R., and Fujita, S. (2004). Muscle tissue changes with aging. *Current Opinion in Clinical Nutrition and Metabolic Care* 7, 405–410. doi: 10.1097/01.mco.0000134362.76653.b2

Wood, R. J., Gregory, S. M., Sawyer, J., Milch, C. M., Matthews, T. D., and Headley, S. A. E. (2012). Preservation of fat-free mass after two distinct weight loss diets with and without progressive resistance exercise. *Metabolic Syndrome and Related Disorders* 10, 167–174. doi: 10.1089/met.2011.0104

Yang, C.-W., Li, C.-I., Li, T.-C., Liu, C.-S., Lin, C.-H., Lin, W.-Y., et al. (2017). The joint association of insulin sensitivity and physical activity on the skeletal muscle mass and performance in community-dwelling older adults. *Experimental Gerontology* 95, 34–38. doi: 10.1016/j.exger.2017.05.006

Yang, J. (2014). Enhanced skeletal muscle for effective glucose homeostasis. *Progress in Molecular Biology and Translational Science* 121, 133–163. doi: 10.1016/B978-0-12-800101-1.00005-3

Yki Jarvinen, H., and Koivisto, V. A. (1983). Effects of body composition on insulin sensitivity. *Diabetes* 32, 965–969. doi: 10.2337/diab.32.10.965

Zampino, M., Semba, R. D., Adelnia, F., Spencer, R. G., Fishbein, K. W., Schrack, J. A., et al. (2020). Greater Skeletal Muscle Oxidative Capacity Is Associated With Higher Resting Metabolic Rate: Results From the Baltimore Longitudinal Study of Aging. *The Journals of Gerontology: Series A* 75, 2262–2268. doi: 10.1093/gerona/glaa071

Zhang, H., Lin, S., Gao, T., Zhong, F., Cai, J., Sun, Y., et al. (2018). Association between Sarcopenia and Metabolic Syndrome in Middle-Aged and Older Non-Obese Adults: A Systematic Review and Meta-Analysis. *Nutrients* 10, 364. doi: 10.3390/nu10030364

Zou, H., Yin, P., Liu, L., Duan, W., Li, P., Yang, Y., et al. (2020). Association between weight cycling and risk of developing diabetes in adults: A systematic review and meta−analysis. *Journal of Diabetes Investigation* 12, 625–632.

Zou, H., Yin, P., Liu, L., Liu, W., Zhang, Z., Yang, Y., et al. (2019). Body-weight fluctuation was associated with increased risk for cardiovascular disease, all-cause and cardiovascular mortality: a systematic review and meta-analysis. *Frontiers in Endocrinology* 10:728.

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.

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