OPTIMIZING EXERCISE FOR THE PREVENTION AND TREATMENT OF TYPE 2 DIABETES

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PUBLISHED IN: Frontiers in Endocrinology and Frontiers in Physiology

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88945-514-0
DOI 10.3389/978-2-88945-514-0

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OPTIMIZING EXERCISE FOR THE PREVENTION AND TREATMENT OF TYPE 2 DIABETES

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This eBook contains a collection of peer-reviewed original and review articles published in either Frontiers in Endocrinology or Frontiers in Physiology focused on the research topic Optimizing Exercise for the Prevention and Treatment of Type 2 Diabetes.

Citation: Little, J. P., Karstoft, K., Safdar, A., eds. (2018). Optimizing Exercise for the Prevention and Treatment of Type 2 Diabetes. Lausanne: Frontiers Media. doi: 10.3389/978-2-88945-514-0

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frontiers in Endocrinology

EDITORIAL
published: 11 May 2018
doi: 10.3389/fendo.2018.00237

Editorial: Optimizing Exercise for the Prevention and Treatment of Type 2 Diabetes

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Keywords: exercise, diabetes mellitus, type 2, motivation, lifestyle interventions, prediabetes

Editorial on the Research Topic

Optimizing Exercise for the Prevention and Treatment of Type 2 Diabetes

OPEN ACCESS

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

Received: *08 November 2017*
Accepted: *25 April 2018*
Published: *11 May 2018*

Citation:
Karstoft K, Safdar A and Little JP (2018) Editorial: Optimizing Exercise for the Prevention and Treatment of Type 2 Diabetes. Front. Endocrinol. 9:237. doi: 10.3389/fendo.2018.00237

INTRODUCTION

That exercise is beneficial for the prevention and treatment of type 2 diabetes (T2D) is not a novelty, and exercise is indeed regarded as a front-line therapy in T2D (1). In recent years, increasing attention has focused on how to manipulate the exercise stimulus to optimize beneficial responses. As such, factors, including intensity, volume, timing, and potential interactions with diet and medication have each, and in various combinations, been suggested to play pivotal roles in exercise efficacy. Despite this encouraging research, the optimal exercise strategy is far from determined, which is why this Research Topic was introduced.

This Research Topic consists of 10 articles, of which five contain original data and five are review/opinion articles. A broad range of themes are covered, ranging from clinical effects of different types of exercise, to mechanisms underlying exercise-induced improvements in metabolic markers, and expanding to perspectives on why exercise may be important for hard endpoints and how motivation toward physical activity may be regulated.

INTERVAL TRAINING MODALITIES

Interval training, especially high-intensity interval training (HIIT), has in the recent years gained momentum in prevention and treatment of T2D. As a result, HIIT was recently—for the first time—included in the ADA position stand about physical activity/exercise and diabetes (2), as outlined by Colberg, with HIIT now being recommended as an alternative approach to continuous aerobic exercise for some individuals with diabetes. Since some researchers have argued that the inclusion of HIIT in the treatment of metabolic diseases is premature given that only few and small studies exist in relevant populations (3), it is of high interest that several large HIIT studies are included in this Research Topic (Phillips et al.; Francois et al.; Alvarez et al.). This includes the largest published HIIT trial, to our knowledge, in individuals with prediabetes [$N = 189$ (Phillips et al.)]. Overall, these studies suggest that supervised HIIT robustly improves glycemic control and other cardiovascular risk factors in individuals with or at risk for T2D. In contrast, HIIT does not seem to affect basic metabolic rate (Karstoft et al.). All together, these studies report results from $N = 304$ individuals undergoing HIIT, advancing the notion that HIIT is a feasible and effective training strategy, also in participants with metabolic disease.

MECHANISMS

Bearing the above-standing beneficial effects of HIIT in mind, and also acknowledging that HIIT may be superior to moderate-intensity continuous training (4–6), it is of interest to assess which mechanisms that are responsible for the improvements in cardiovascular risk factors seen with HIIT. In this context, several insightful articles are included in the Research Topic. As reviewed by Carson, myokines are proteins that are released by muscles and have auto-, para-, and/or endocrine functions; some of which are known to affect cardiovascular risk factors. Several of the known myokines are induced by contraction, and given that this induction is dependent on exercise intensity (7), it is intriguing to speculate that some of the effects of HIIT are mediated *via* contraction-induced myokines. Also relevant in this context, Eshghi et al. showed that the timing of exercise may be important, since exercise-induced increase in systemic levels of the myokine IL-6 is only seen following the first of two similar exercise bouts performed at one single day. The idea of so-called "non-response" or individualized responses to exercise training is a hot, yet somewhat controversial, topic in the field (8). This was addressed in a preliminary report from Alvarez et al., which suggested that baseline insulin resistance might influence certain cardiometabolic responses to HIIT in women.

In a comprehensive review, Parker et al. reviewed the complex interplay between oxidative stress, antioxidant defense, and physical activity. Whereas both inactivity/obesity on one side and acute exercise on the other side results in increased systemic levels of oxidative stress, the effects on glycemic control and insulin sensitivity are opposing. Parker et al. suggests that differences in intracellular signaling and antioxidant defense may be responsible for these discrepancies. Again, given that the effect of exercise on oxidative stress is dependent on exercise intensity (9), it may be speculated that some of the improvements seen with HIIT are dependent on changes in oxidative stress and antioxidant defense.

NEW INSIGHTS

Whereas the HIIT-induced improvements in cardiovascular risk factors are interesting, it must be acknowledged that little is known about the effects of HIIT (and other types of exercise) on hard endpoints. Given that high postprandial glucose excursions are suggested to be more deleterious than elevated mean blood glucose levels for cardiovascular risk factors (10, 11), and since postprandial exercise is known to effectively reduce glucose excursions (12), Erickson et al. suggest that exercise for T2D subjects should in general be prescribed post-meal and individualized according to the need, with large glucose excursions requiring longer and more intense exercise bouts compared to small glucose excursions.

For benefits of any type of exercise, the need to adhere is fundamental. The review by Ruegsegger and Booth provides exciting new insights into the importance of the mesolimbic system in controlling motivation and physical activity behavior *via* dopaminergic signaling. Understanding these processes is imperative if the general trend in the population, where physical activity levels are decreasing, is to be reversed.

PERSPECTIVES

Papers in this Research Topic highlight that exercise has a role in the prevention and treatment of T2D. Whereas HIIT seems to be effective for improving cardiovascular risk factors, we still need to characterize the mechanisms underlying the improvements seen in order to develop even more effective training programs for individuals with or at risk for T2D. Moreover, whereas efficacy of supervised HIIT is evident, effectiveness of unsupervised "real-life" HIIT is largely unknown and limited to small studies (13, 14). In order for HIIT and other novel types of exercise to be implemented clinically, more work is needed. Interdisciplinary research involving mechanisms like myokines, oxidative stress, and brain reward systems coupled with innovative real-world trials of HIIT and traditional exercise seem an exciting avenue for optimizing exercise for the prevention and treatment of T2D.

AUTHOR CONTRIBUTIONS

KK and JL drafted the manuscript with input from AS. All authors approved the final version.

FUNDING

JL is supported by a Canadian Institutes of Health Research (CIHR) New Investigator Salary Award (MSH-141980) and a Michael Smith Foundation for Health Research (MSFHR) Scholar Award (16890).

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

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 08 September 2017
doi: 10.3389/fendo.2017.00229

A Practical and Time-Efficient High-Intensity Interval Training Program Modifies Cardio-Metabolic Risk Factors in Adults with Risk Factors for Type II Diabetes

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OPEN ACCESS

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

Received: 10 April 2017
Accepted: 22 August 2017
Published: 08 September 2017

Citation:
Phillips BE, Kelly BM, Lilja M, Ponce-González JG, Brogan RJ, Morris DL, Gustafsson T, Kraus WE, Atherton PJ, Vollaard NBJ, Rooyackers O and Timmons JA (2017) 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. 8:229. doi: 10.3389/fendo.2017.00229

Introduction: Regular physical activity (PA) can reduce the risk of developing type 2 diabetes, but adherence to time-orientated (150 min week^{-1} or more) PA guidelines is very poor. A practical and time-efficient PA regime that was equally efficacious at controlling risk factors for cardio-metabolic disease is one solution to this problem. Herein, we evaluate a new time-efficient and genuinely practical high-intensity interval training (HIT) protocol in men and women with pre-existing risk factors for type 2 diabetes.

Materials and methods: One hundred eighty-nine sedentary women ($n = 101$) and men ($n = 88$) with impaired glucose tolerance and/or a body mass index >27 kg m^{-2} [mean (range) age: 36 (18–53) years] participated in this multi-center study. Each completed a fully supervised 6-week HIT protocol at work-loads equivalent to ~100 or ~125% $\dot{V}O_2$ max. Change in $\dot{V}O_2$ max was used to monitor protocol efficacy, while Actiheart™ monitors were used to determine PA during four, weeklong, periods. Mean arterial (blood) pressure (MAP) and fasting insulin resistance [homeostatic model assessment (HOMA)-IR] represent key health biomarker outcomes.

Results: The higher intensity bouts (~125% $\dot{V}O_2$ max) used during a 5-by-1 min HIT protocol resulted in a robust increase in $\dot{V}O_2$ max (136 participants, +10.0%, $p < 0.001$; large size effect). 5-by-1 HIT reduced MAP (~3%; $p < 0.001$) and HOMA-IR (~16%; $p < 0.01$). Physiological responses were similar in men and women while a sizeable proportion of the training-induced changes in $\dot{V}O_2$ max, MAP, and HOMA-IR was retained 3 weeks after cessation of training. The supervised HIT sessions accounted for the entire quantifiable increase in PA, and this equated to 400 metabolic equivalent (MET) min week^{-1}. Meta-analysis indicated that 5-by-1 HIT matched the efficacy and variability of a time-consuming 30-week PA program on $\dot{V}O_2$ max, MAP, and HOMA-IR.

Conclusion: With a total time-commitment of <15 min per session and reliance on a practical ergometer protocol, 5-by-1 HIT offers a new solution to modulate cardio-metabolic risk factors in adults with pre-existing risk factors for type 2 diabetes while approximately meeting the MET min week^{-1} PA guidelines. Long-term randomized controlled studies will be required to quantify the ability for 5-by-1 HIT to reduce the incidence of type 2 diabetes, while strategies are required to harmonize the adaptations to exercise across individuals.

Keywords: health, exercise, high-intensity interval training, variability, $\dot{V}O_2$max, blood pressure, detraining, homeostatic model assessment of insulin resistance

INTRODUCTION

Substantial correlative evidence indicates that exercise capacity and greater self-reported physical activity (PA) (1) both positively relate to health. In fact, aerobic capacity ($\dot{V}O_2$ max) measured in the laboratory appears to be a better predictor of health status and risk of disease than many other risk factors (2). Furthermore, guidance aimed at concurrently improving diet and increasing levels of PA has successfully demonstrated substantial reductions in the incidence or progression-rates of type 2 diabetes after 10 years of follow-up (3–5). It is currently a (reasonable) assumption that the increased levels of PA in these trials (3–5) made a major contribution to the improved metabolic health. Shorter-term exercise training intervention studies (6 weeks–6 months) attempt to quantify the physiological responses to exercise, relying on surrogates or "biomarkers" of health to explore the potential efficacy of very divergent training programs. These studies typically observe gains in aerobic capacity (6) and reductions in blood pressure (7) and insulin resistance (IR) following 6–40 weeks of supervised training (8). The format of each exercise training program (time and exercise intensity) have reflected PA guidelines developed from epidemiological observations, e.g., high-volume continuous submaximal aerobic training carried out on 3–5 days each week (7, 9) with the aim of meeting a time-commitment for voluntary exercise of 150 min week^{-1}.

Studies using lower volume very high-intensity interval training (HIT) and highly specialist cycle ergometers have demonstrated that modulation of risk factors for type 2 diabetes can be achieved by exercising a total of 70–90 min week^{-1} in small groups of individuals (10–14). Nevertheless, while the total time for the "bouts" of exercise can be very low (≤5 min day^{-1}), these formats of HIT require long recovery periods between each bout such that they do not substantially reduce the total time-commitment to a level that might substantially improve exercise participation. Some investigators have raised the possibility of gender-specific benefits, which most likely reflect the large amount of inter-individual variability observed in any exercise training study (15–17) and the small number of subjects studied when evaluating any particular variant of HIT (10–14, 18). The reliance on a wide range of HIT protocols has meant that neither the effect size nor the inter-individual variability has been properly quantified (10–14, 18) and such divergent protocols limit the validity of any meta-analysis approach to address these important questions. Indeed, the design of future large-scale outcome studies of novel exercise paradigms requires reliable estimates of the effect size in target at-risk populations and this study evaluated a more time-efficient protocol that overcomes some of the practical limitations of earlier studies. The initial HIT protocol was based on a 1981 study by Ready et al. (19). While the present project was not a randomized clinical trial, we did embrace the multi-arm multi-stage clinical trial *philosophy* (20), whereby we monitored the HIT protocol efficacy on a rolling basis, by aggregating the $\dot{V}O_2$ maxtraining responses as we went along. This resulted in us discontinuing a 7-by-1 min HIT protocol (~100% $\dot{V}O_2$ max cycling intensity), in favor of a lower volume, higher intensity protocol (5-by-1 min HIT, at ~125% $\dot{V}O_2$ max cycling intensity). We were able to confirm that a practical and time-efficient 5-by-1 HIT protocol not only improved $\dot{V}O_2$ max (on average), but also that this particular time-efficient exercise regime was equally effective in both men and women at modifying cardio-metabolic disease risk factors.

MATERIALS AND METHODS

The experimental design for the 7-by-1 HIT protocol and clinical testing procedures were discussed at a work-shop, in Las Palmas on January 30, 2012, with the following people, in addition to authors, in attendance: Martin Gibala, Jorn Helge, Fleming Dela, Ruth Loos, Laurie Goodyear, Claude Bouchard, Tuomo Rankinen, Jose Calbet, Urho Kujala, Heikki Kainulainen, Steen Larsen, Lauren Koch, and Paul Greenhaff.

Participant Characteristics

For the METAPREDICT HIT trial, we recruited 189 participants (**Figure 1**) across 5 geographical regions: Nottingham (n = 37) and Loughborough (n = 18) in the UK, Stockholm (Sweden, n = 36), Copenhagen (Denmark, n = 48), and Las Palmas de Gran Canaria (Spain, n = 50). All methods relied on across-site standard operating procedures. Participants were recruited *via* advertisements in local media, through publicity on the EU and University websites, and *via* links with radio and TV stations. We also used demographic databases to post information to potential volunteers and put out adverts in local community groups, particularly those involving sedentary adults. Participants were male (n = 88) and female (n = 101), with a mean (range) age of 36 (18–53) years and body mass index (BMI) of 32.0 (26.6–48.0) kg m^{-2}. All participants were classified as sedentary [<600 metabolic equivalents (METs) min week^{-1}] using a modified International Physical Activity Questionnaire (21), and had a fasting blood glucose level consistent with World Health Organization criteria

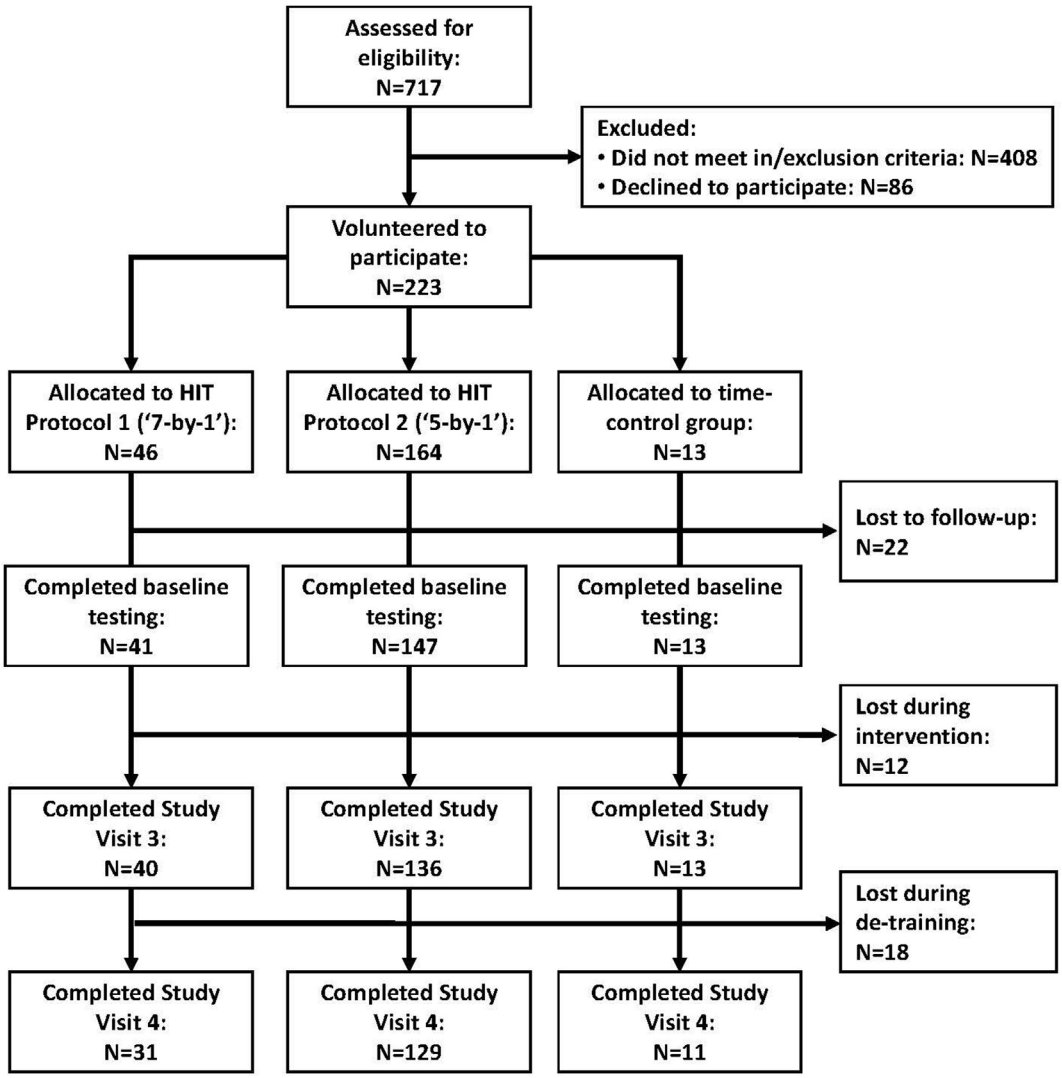

FIGURE 1 | Flow chart of participant contact, screening, and retention through the phases of the study.

for impaired glucose tolerance (IFG; >5.5, <7.0 mmol l^{-1}), and/or a BMI > 27 kg m^{-2}.

All participants were initially screened and excluded if they displayed evidence of active cardiovascular, cerebrovascular, respiratory, gastrointestinal, or renal disease. They were also excluded for history of malignancy, coagulation dysfunction, musculoskeletal or neurological disorders, recent steroid or hormone replacement therapy, or any condition requiring long-term drug prescriptions. All participants gave their written, informed consent to participate. This study was approved by local ethics committees at all sites (the University of Nottingham Medical School Ethics Committee: D8122011 BMS; the Regional Ethical Review Board Stockholm: 2012/753-31/2; the ethics committee of the municipality of Copenhagen and Frederiksberg in Denmark: H-3-2012-024; Comité Ético de Investigación Humana de la ULPGC: CEIH-2012-02; and the Loughborough University Ethics Approvals Human Participants Sub-Committee: 12/EM/0223) and complied with the 2008 Declaration of Helsinki. To ensure accurate results, we were obliged to discontinue training for individuals who (i) failed to attend for more than two consecutive sessions, (ii) missed more than three (~15%) training sessions in total, or (iii) failed to complete their set exercise regime on two occasions or more. This was not the case for any participants.

HIT—Protocol 1 ("7-by-1")

Forty participants [$n = 20$ men/20 women; age: 37 (20–53) years; BMI: 31.0 (27.0–45.5) kg m^{-2}] completed a 7-by-1 HIT protocol (**Table 1**) developed using information from the literature (19, 22). 7-by-1 HIT protocol consisted of three fully supervised cycling sessions per week for 6 weeks. Sessions began with a 2-min warm-up at 50 W followed by seven sets of 1 min cycling at 100% of the work required to elicit $\dot{V}O_2$ max (Corival or Excalibur Sport, Lode, Groningen, the Netherlands) with 1 min recovery between bouts. For 1 h before, during, and for 1 h after each training

TABLE 1 | Participant characteristics.

	Comparison group (n = 13)	7-by-1 HIT (n = 40)	5-by-1 HIT (n = 136)
Gender (men/women)	4/9	20/20	64/72
Age (years)	31 ± 11 (20–51)	37 ± 10 (20–53)	36 ± 9 (18–50)
Height (m)	1.66 ± 0.09 (1.52–1.81)	1.72 ± 0.09 (1.53–1.94)	1.72 ± 0.09 (1.50–2.01)
Body mass (kg)	93.1 ± 18.0 (68.6–130.5)	92.6 ± 17.5 (63.5–138.8)	95.1 ± 15.2 (64.0–136.4)
BMI (kg m^{-2})	33.4 ± 5.0 (27.5–41.4)	31.0 ± 4.2 (27.0–45.5)	32.2 ± 4.1 (26.5–48.1)
IPAQ score	305 ± 150 (118–578)	362 ± 157 (73–594)	313 ± 188 (0–597)
Baseline $\dot{V}O_2$ max (mL kg^{-1} min^{-1})	24.1 ± 5.5 (13.2–32.0)	28.8 ± 7.0 (17.3–46.9)	27.2 ± 5.2 (15.8–44.6)
Systolic blood pressure (mmHg)	120 ± 9 (107–138)	124 ± 12 (106–161)	124 ± 11 (99–168)
Diastolic blood pressure (mmHg)	76 ± 9 (66–92)	78 ± 8 (67–105)	80 ± 10 (59–106)
Mean arterial pressure (mmHg)	91 ± 8 (80–104)	94 ± 9 (80–124)	95 ± 9 (74–127)
Log HOMA-IR	0.34 ± 0.17 (−0.06 to 0.51)	0.27 ± 0.24 (−0.27 to 0.92)	0.30 ± 0.26 (−0.46 to 0.86)

Values shown are mean ± SD and range.

BMI, body mass index; IPAQ, International Physical Activity Questionnaire; $\dot{V}O_2$max, maximal aerobic capacity; HOMA-IR, homeostatic model assessment of insulin resistance; HIT, high-intensity interval training.

Based on the study monitoring process, allocation of the participants to the HIT groups was sequential. The comparison group was utilized to examine test–retest performance over the study duration and not to adjust the HIT intervention responses. Direct physical activity monitoring was utilized to better link the HIT training directly to the changes in health biomarkers.

session, the participants were only allowed to consume water. No adverse events or unintended effects were observed with this intervention. However, based on interim analysis, 7-by-1 HIT was found to result in a relatively modest increase in $\dot{V}O_2$ max (+6.2%) and, thus, was insufficient to assess inter-individual variability in response to training [SD of individual responses (SD_{IR}): 106 mL O_2; 95% CI: −6 to 218 mL (see Data Processing and Statistical Analysis)]. Reliance on a 25-W stepwise $\dot{V}O_2$ max protocol was considered one limitation of work-load setting for 7-by-1.

HIT—Protocol 2 ("5-by-1")

The decision was made to use a higher intensity protocol, while subjects who had started the 7-by-1 HIT protocol completed the protocol and underwent a full clinical assessment (as the protocol may still have had benefits on IR). A further 136 participants completed baseline visits, HIT, and the post-HIT assessment [n = 64 men/72 women; age: 36 (18–50) years; BMI 32.2 (26.6–48.0) kg m^{-2}] for a new higher intensity lower volume (5-by-1) HIT protocol (**Table 1**). The exercise training was fully supervised and consisted of three cycling sessions per week for 6 weeks. All sessions began with a 2-min warm-up at 50 W followed by five sets of 1 min high-intensity cycling work with 90 s recovery between sets with the exception of week 1 where three sets per session were performed in sessions 2 and 3. Work-load was determined in session 1 of week 1, where participants were asked to perform a 2-min warm-up at 50 W followed by 1-min bouts of exercise with 90 s recovery. Exercise started at 85% of the work required to elicit $\dot{V}O_2$ max (Wmax), and increased by 10% (e.g., 95, 105%, etc.) until the participant was unable to complete a full 1-min bout. Intensity for the last bout participants could complete was used thereafter for training, with a 10% increase in intensity after 2 weeks. No adverse events or unintended effects were observed for this intervention.

Non-Exercise Participants

Thirteen participants were allocated at random, within a center, to serve as a non-exercise comparison group [**Table 1**, n = 4 men/9 women; age: 31 (20–51) years; BMI 33.4 (27.5–41.4) kg m^{-2}]. These participants underwent all screening and assessment procedures but did not participate in any training. Their data served to complement the short-term test–retest variability data collected in the intervention groups at the two baseline sessions with "test–retest" data covering the full duration of the study.

Pre-Training Physiological Characterization

Participants were instructed to refrain from exercise for 3 days prior to their visit (baseline session 1) and from alcohol and caffeine for 1 day (fasting from ~09:00 p.m. and reporting to the laboratory 12 h later at ~09:00 a.m.). After 30 min supine rest, blood pressure (BP; Omron M2, Omron Healthcare, Kyoto, Japan) and resting heart rate (RHR) were measured, with mean arterial pressure (MAP) calculated as: 2/3 diastolic blood pressure + 1/3 systolic blood pressure. BP and RHR were determined as the average of three consecutive measurements. A blood sample was taken from a dorsal hand vein for the assessment of IR *via* the homeostatic model assessment (HOMA). Blood was immediately analyzed for glucose concentration (YSI 2300 STAT Plus glucose analyzer, Yellow Springs Inc., OH, USA) and aliquoted in to lithium heparin spray-coated vacutainers (Becton Dickinson, NJ, USA) and centrifuged at 2,000 *g* for 10 min at 4°C to yield plasma. Plasma was stored at −80°C and shipped for centralized analysis of insulin levels by a "high-sensitivity" ELISA (K6219, Dako Sweden AB, Stockholm) according to manufacturer's instruction. HOMA-IR was calculated using the standard equation of [glucose (mmol/l) × insulin (mU/l)]/22.5 (23).

A $\dot{V}O_2$ max test was then conducted using a cycle ergometer (Lode Corival/Excalibur Sport) and a continuous ramp protocol. After a 5-min warm-up at 50 W, the work rate was increased by 1 W every 4 s. Participants were instructed to cycle to volitional exhaustion. For the duration of the test, expired air was analyzed using an inline gas analyzer (e.g., Metamax 3B, Cortex, Leipzig, Germany; Vmax N29, Sensormedics, Anaheim, CA, USA; COSMED, Rome, Italy) with HR continuously monitored.

$\dot{V}O_2$ max was estimated as the highest value obtained in a 15-breath rolling average and a test was deemed valid when the participants achieved two of the following three criteria: (i) volitional exhaustion and/or no longer able to maintain a pedal rate of 50 revolutions per minute despite strong verbal encouragement, (ii) heart rate within 10 beats min^{-1} of age-predicted maximum, and (iii) respiratory exchange ratio (RER) ≥1.10. These criteria were met in all but one test, which was excluded from analysis of $\dot{V}O_2$ max. To assess the reproducibility of this $\dot{V}O_2$ max test, the assessment was repeated 7 days later at baseline session 2 (as well as across 6 weeks in the non-training group). The coefficient of variation (CV) for repeated measurements for $\dot{V}O_2$ max was 4.4%. As group mean $\dot{V}O_2$ max was not different for visits 1 and 2 (2.59 ± 0.60 vs. 2.59 ± 0.63 L min^{-1}, respectively) the mean of the two visits was taken as the subjects' baseline value that reduces the influence of technical and biological variation and so should provide a better estimate of baseline $\dot{V}O_2$ max. At 72–96 h after the last exercise training session, participants underwent a third study day, identical to visit 1.

PA and Post-Training Monitoring

Physical activity was monitored using Actiheart devices (CamNTech, Cambridge, UK), a chest-worn monitor that records heart rate and movement *via* an accelerometer. The device senses the frequency and intensity of torso movements and has been shown to be comparable to doubly labeled water for measuring energy expenditure (24). Activity data were obtained for 7 days prior to study visit 1, prior to study visit 2, during week 3 or 4 of HIT, and prior to study visit 4 (during the detraining period). Participants were instructed to wear the Actiheart device at all times during the monitoring periods (using waterproof Actiheart chest strap or using standard ECG electrodes). Participants using the ECG electrodes were instructed to place one electrode at the site of the fourth intercostal with the second electrode ~10 cm to the left (equivalent to V1 and V4 on a 12-lead ECG). These participants were instructed to wear the monitor at all times with the exception of a very short period each day when they were instructed to thoroughly wash and dry the skin under the electrodes in order to minimize the risk of contact dermatitis or other skin irritation. After completion of the exercise training intervention, participants were asked to return to their habitual PA levels for 3 weeks (confirmed by Actiheart) and then a fourth study day, identical to visit 3, was carried out.

Data Processing and Statistical Analysis

To bench mark these HIT protocols with literature values, a robust post-training group average increase in $\dot{V}O_2$ max had to be evident. Power analysis indicated that >29 participants would be required to detect a 4% difference between pre- and post-training $\dot{V}O_2$ max with a power of 95% and alpha = 0.05, based on a CV of 5.7%. To detect a difference of 4% between men and women, for change in $\dot{V}O_2$ max, with alpha = 0.05 and a power of 95%, >53 participants were required. Thus, the analysis was powered for primary statistical analysis presented in this paper.

Statistical analysis was performed using SPSS statistical software (version 20.0, SPSS Inc., Chicago, IL, USA). Data were either tested for normality using the Shapiro–Wilks test and analyzed with non-parametric tests or log transformed. Differences between pre- and post-training values were evaluated using paired sample *t*-tests [$n = 40$ and $n = 136$ for $\dot{V}O_2$ max, and $n = 36$ and $n = 133$ for HOMA-IR for 7-by-1 and 5-by-1 protocols, respectively (reflecting any missing values)]. Effect size was quantified using Cohen's *d* (25). Gender differences in training response were analyzed using independent sample *t*-tests. Bivariate correlations were assessed using Pearson's correlation coefficient. Repeated measures ANOVAs with *post hoc* Bonferroni tests for multiple comparisons were used to assess retention of training effects following 5-by-1 HIT for those participants who completed study visit 4 (**Figure 1**). All data are presented as mean ± SD unless stated otherwise.

Quantification of inter-individual responses to training, corrected for estimates of random variation (technical/day-to-day biological) was performed according to the procedures proposed by Hopkins (26). SD for individual responses (SD_{IR}) were calculated by taking the square root of the difference between the squares of the SD of the training effect (SD_{exp}) and the SD of either the double baseline measurement (for variables measured twice before training) or the SD of the repeated measures carried out in the comparison group (SD_{con}). In addition, paired sample *t*-tests were performed to determine differences between SD_{exp} and SD_{con}, and Levene's test was performed to determine differences between the SD_{exp} for 5-by-1 HIT and the SD_{exp} for an earlier study that utilized high volume combined aerobic/resistance training [STRRIDE AT/RT study (27)].

Actiheart data were scanned for missing values using a heuristic code in R, and data accepted only when ≥80% of minute-by-minute activity data were available for a 24-h recording period. Furthermore, at least 4 days of valid data had to be available for a participant to be included in the group analysis (leaving $n = 58$ for 5-by-1 HIT). Mean daily energy expenditure (METs) for each of the four measurement periods was calculated, and standard thresholds were used to determine the percentage of time engaged in activity within predetermined intensity zones (sedentary: <1.5 METs; light ≥1.5 < 3 METs; moderate ≥3 < 6 METs; vigorous ≥6 < 10.2 METs; very vigorous ≥10.2 METs). Reliability of the Actiheart data, using this data selection criteria, was excellent ($R^2 = 0.87$ for the repeated baseline measure; CV = 4.8%). The mean values obtained prior to study visits 1 and 2 were used as the baseline values.

RESULTS

Training Responses

Following 6 weeks of 7-by-1 HIT, there were modest improvements in mean $\dot{V}O_2$ max (+6.2%, 95% CI: 3.5–8.9%, $p < 0.001$). This equates to a moderate effect size, i.e., Cohen's $d = 0.71$ (95% CI = 0.25–1.16) for the primary outcome. As expected, Wmax (5.3%; $p < 0.001$) was also increased by 7-by-1 HIT, but no other outcomes were significantly altered. For the control group that undertook two assessments 6 weeks apart, we observed no significant changes between baseline assessment and reassessment 6 weeks later in any parameter.

Following 6 weeks of the more time-efficient 5-by-1 HIT protocol, greater changes were observed for $\dot{V}O_2$ max (+10.0%,

TABLE 2 | Mean physiological changes following 6 weeks of time-efficient high-intensity cycle-training.

	7-by-1 HIT (n = 40)			5-by-1 HIT (n = 136)		
	Baseline	**Post-HIT**	***p*-Value**	**Baseline**	**Post-HIT**	***p*-Value**
BMI (kg m^{-2})	31.0 ± 4.2	30.8 ± 4.2	0.138	32.2 ± 4.1	32.1 ± 4.2	0.98
Body mass (kg)	92.4 ± 17.3	92.0 ± 17.8	0.210	95.1 ± 15.2	95.1 ± 15.4	0.98
$\dot{V}O_2$max (L min^{-1})	2.61 ± 0.60	2.77 ± 0.68	0.00005	2.59 ± 0.62	2.85 ± 0.68	<1E–20
Wmax at $\dot{V}O_2$max (W)	189 ± 50	199 ± 51	0.001	198 ± 48	226 ± 53	<1E–23
SBP (mmHg)	124 ± 12	122 ± 11	0.169	124 ± 11	122 ± 12	0.007
DBP (mmHg)	78 ± 8	77 ± 8	0.281	80 ± 10	77 ± 10	0.0006
MAP (mmHg)	94 ± 9	92 ± 8	0.183	95 ± 9	92 ± 9	0.0001
Fasting glucose (mmol L^{-1})	4.56 ± 0.32	4.57 ± 0.40	0.855	4.63 ± 0.41	4.60 ± 0.45	0.61
Fasting insulin (pmol L^{-1})	10.6 ± 6.4	10.2 ± 6.8	0.494	11.3 ± 6.6	10.5 ± 6.7	0.005
Log HOMA-IR	0.27 ± 0.24	0.23 ± 0.30	0.187	0.30 ± 0.26	0.25 ± 0.27	0.004

Values shown are mean ± SD.

BMI, body mass index; $\dot{V}O_2$max, maximal aerobic capacity; Wmax, maximum power output; SBP, supine systolic blood pressure; DBP, supine diastolic blood pressure; MAP, supine mean arterial pressure; HOMA-IR, homeostatic model assessment of insulin resistance; HIT, high-intensity interval training.

Note that the intensity of each bout during the 7-by-1 protocol was 20–30% lower than during the 5-by-1 protocol, indicating that relying on supramaximal (from the perspective of aerobic capacity) is probably important for gains in aerobic capacity. Changes in a measure of peripheral insulin resistance (HOMA-IR) were more variable with 7-by-1 and not improved with the n = 40 sample size for the selected threshold for statistical significance.

95% CI: 8.4–11.6%; $p < 0.001$) presenting a larger and less variable size effect (Cohen's $d = 1.24$, 95% CI = 0.97–1.50) (**Table 2**). The increase in $\dot{V}O_2$ max with 5-by-1 HIT was also greater than we observed with 7-by-1 HIT ($p < 0.05$) supporting our interim analysis and decision to discontinue that protocol. The *absolute* increase in $\dot{V}O_2$ max with 5-by-1 HIT was significantly higher for men (0.32 ± 0.3 L min^{-1}) compared to women (0.19 ± 0.2 L min^{-1}; $p < 0.001$) as expected, but the relative benefits were not (**Figure 2**). Furthermore, 5-by-1 HIT yielded reductions in MAP (2.8%; $p < 0.001$) and HOMA-IR (16%; $p < 0.01$) (**Table 2**). Similarly, no significant gender differences were apparent for the relative training response for MAP or HOMA-IR (**Figure 2**). Thus, we found no evidence that HIT-induced physiological adaptations were subject to gender-related dimorphism.

Actiheart-derived PA data demonstrated a small increase in PA energy expenditure during the 6-week intervention period of 5-by-1 HIT (mean 24-h activity level: 1.46 ± 0.38 vs. 1.50 ± 0.34 METs) equivalent to an increase of ~400 MET min week^{-1} ($p < 0.05$). This increase was accounted for by increases in the percentage of time spent performing vigorous (0.30 ± 0.47 vs. 0.43 ± 0.43%, $p < 0.001$) and very vigorous activities (0.02 ± 0.09 vs. 0.07 ± 0.16%, $p < 0.001$), i.e., the 18 HIT sessions. No change was observed in the percentage of time spent in sedentary (74.1 ± 16.3 vs. 74.0 ± 14.9%), light (18.0 ± 9.1 vs. 17.8 ± 8.6%), and moderate activity zones (7.6 ± 8.2 vs. 8.2 ± 7.0%). Thus, carrying out 5-by-1 HIT did not alter PA behavior out with the trial.

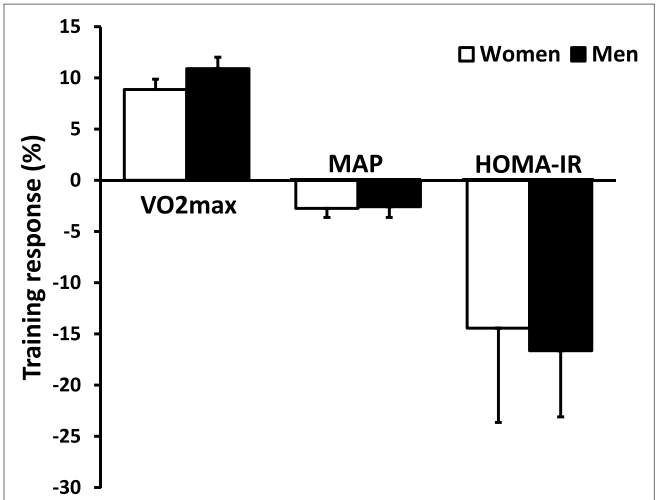

FIGURE 2 | An analysis of the training response to 5-by-1 HIT for $\dot{V}O_2$max, MAP and HOMA-IR in men and women. Inter-participant variability in response to HIT is large for both men and women, but on average both genders respond to a similar extent. Abbreviations: $\dot{V}O_2$max, maximal aerobic capacity; MAP, mean arterial pressure; HOMA-IR, homeostatic model assessment of insulin resistance; HIT, high-intensity interval training.

Comparison of Inter-Individual Variability between HIT and High-Volume Training

Inter-individual variability (**Figure 3**) in training responses reflects the fact that there are genuine low and high responders for major physiological traits, following any type of exercise training program. This variability will be partly due to random contributions from technical and day-to-day biological variation, and partly due to genetic differences between individuals (28). For 5-by-1 HIT, change in $\dot{V}O_2$ max $\left(\Delta\dot{V}O_2\text{ max}\right)$ was not correlated to baseline $\dot{V}O_2$ max ($R^2 = 0.01$, NS) such that low baseline aerobic capacity was not associated with a greater training response nor *vice versa*. By contrast, ΔMAP was negatively correlated to baseline MAP ($R^2 = 0.18$, $p < 0.001$), and Δ log HOMA-IR was negatively correlated to baseline log HOMA-IR ($R^2 = 0.07$, $p < 0.01$). In a population sample that had a range of blood pressure and log HOMA-IR spanning normal to above normal (74 to 127 mmHg and −0.46 to 0.86, respectively), such a correlation is expected as both parameters are regulated toward a physiologically "normal" value. Nevertheless, on an individual basis, this analysis, such as others before it, demonstrates that baseline physiological measures are not, on their own, useful at

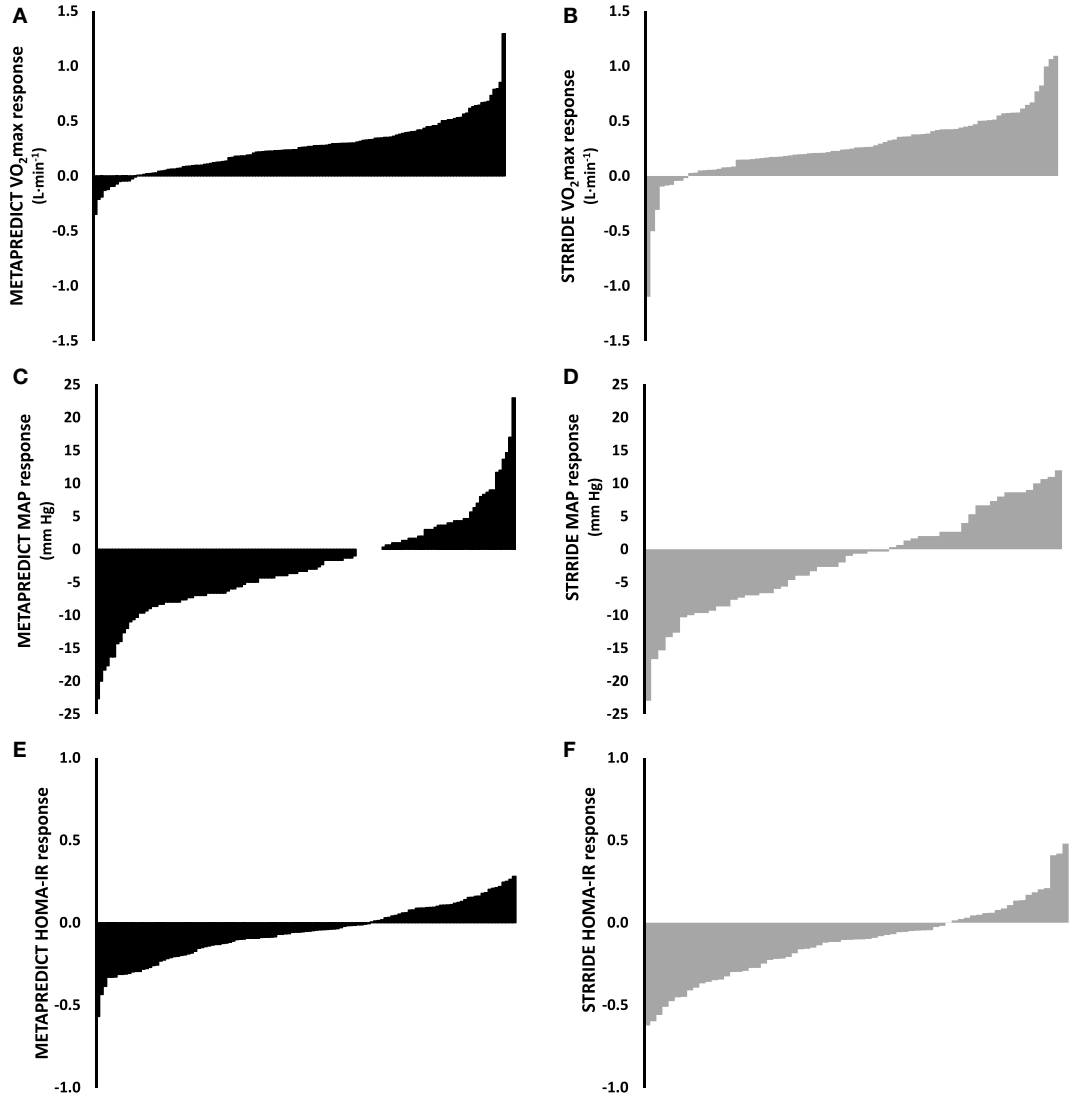

FIGURE 3 | Comparison of the inter-individual variability to exercise training contrasting short-term high-intensity training with longer-term high-volume submaximal training. The training response to 6-weeks 5-by-1 high-intensity interval training [**(A,C,E)**; black bars] and our previously published 8-month STRRIDE AT and AT/RT exercise training study [**(B,D,F)**; gray bars] for $\dot{V}O_2$max , MAP, and HOMA-IR. Training-induced changes in both $\dot{V}O_2$max **(A,B)**, MAP **(C,D)**, and HOMA-IR **(E,F)** vary considerably in both studies and to a similar extent. Abbreviations: AT, aerobic training; RT, resistance training; $\dot{V}O_2$max , maximal aerobic capacity; MAP, mean arterial pressure; HOMA-IR, homeostatic model assessment of insulin resistance.

predicting the health biomarker outcomes of an exercise training regime, indicating that more sophisticated strategies will be required to fulfill such an aim (29).

To contrast the variation observed in response to 5-by-1 HIT with traditional higher volume exercise training (**Figure 3**) (30), we estimated the "added" variation caused by the training intervention (SD_{IR}), over and above the random variation by comparing the variability in repeated measures at baseline [or in a control group (SD_{con})] with the observed variability in response to the training intervention (SD_{exp}). For 5-by-1 HIT, the SD_{con} for $\dot{V}O_2$ max (visit 1 vs. visit 2; 112 ± 94 mL) was lower than SD_{exp} (visit 2 vs. visit 3; 204 ± 150 mL; $p < 0.001$). For 5-by-1 HIT, the SD_{IR} was calculated to be 170 mL (95% CI: 23–311 mL). In standardized units, the magnitude of the effect for the individual responses was large (0.67; 95% CI: 0.11–1.22). For $\dot{V}O_2$ max, the SD_{exp} from our previously published data (30) was not significantly different from 5-by-1 HIT (204 vs. 234 mL O_2). Based on this analysis, 95% of people performing 5-by-1 HIT can be expected to have a "true" response for $\dot{V}O_2$ max between −79 and +587 mL O_2. Similarly, for MAP, SD_{exp} exceeded SD_{con} for 5-by-1 HIT (4.2 vs. 2.6 mmHg, respectively) resulting in an SD_{IR} of 3.3 mmHg (95% CI: 0.3–6.3 mmHg). This is also a large effect in standardized units (−1.32; 95% CI: −2.50 to 0.13) and indicates that for 5-by-1 HIT, 95% of people can be expected to have a response for MAP within −9.0 and +4.0 mmHg, i.e., considerable inter-individual variability in response to HIT. Despite the extreme differences in

the format (volume and intensity) of exercise training between 5-by-1 HIT and STRRIDE AT/RT (30), no significant differences were observed between their respective SD_{exp} for blood pressure (MAP: 4.2 vs. 4.5 mmHg) (**Figure 3**).

The fact that the pattern of variability in response for $\dot{V}O_2$ max, MAP, and HOMA-IR (three key biomarkers for cardio-metabolic health) to 6 weeks of 5-by-1 HIT is not different from that observed in an 6-month high-volume aerobic/resistance training intervention suggests that inter-individual variability in responses to training is not dependent on exercise mode, exercise-session duration, total volume, or the duration of the intervention, but rather depends on genetics, epigenetics, and other biological factors (28). One important practical consideration is the proportions of subjects that demonstrates "real" improvements in each of the main health biomarkers. To address such a question, we counted the frequency of people with 0, 1, 2, or 3 positive changes in $\dot{V}O_2$ max, MAP, and HOMA-IR defined as an improvement over and above technical error for that physiological parameter. As can be observed in **Figure 4**, whether one considers the frequency of observing a numerical improvement (unreliable) or a gain that is greater than the normal technical error for the test, ~50% of subjects improve at least two of the three health biomarkers following 6 months endurance training or 6 weeks of 5-by-1 HIT.

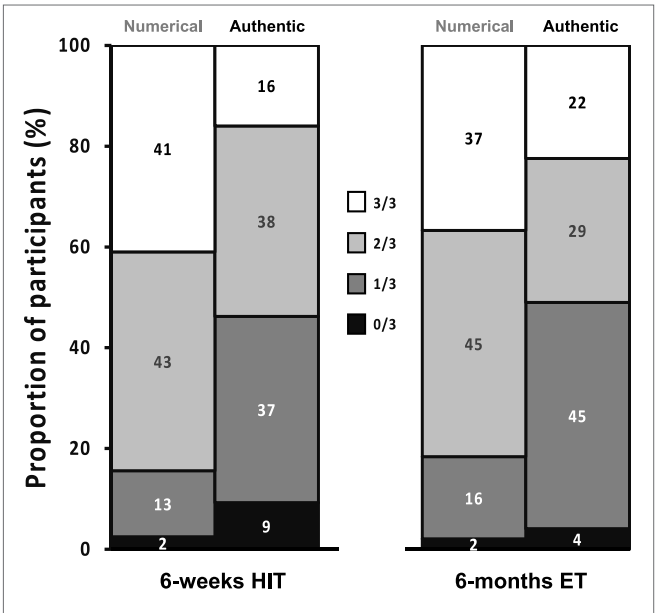

FIGURE 4 | Presentation of the responder frequency for the three main clinical biomarkers considered in this study [high-intensity interval training (HIT)] and comparison with our previously published endurance training (ET) study. Each individual was assessed for improvement in $\dot{V}O_2$max, mean arterial pressure, or HOMA-IR, greater than the laboratory error, and the percentile frequency of 0, 1, 2, or 3 from three improvements was calculated. For sake of comparison, this is plotted side-by-side with the percentile frequency of 0, 1, 2, or 3 gains based on numerical improvements (a criteria that would be considered unreliable by most). Approximately 40% of subjects demonstrate improvement in only one health biomarker, while between 4 and 9% demonstrate no reliable improvement in any.

Physiological Changes during Detraining

As a secondary objective, we evaluated the status of training-induced changes in physiological parameters, from 6 weeks of 5-by-1 HIT, during a 3-week period where subjects returned to their baseline sedentary lifestyle (**Figure 5**). Seven participants (~5%) were lost to follow-up during this period. Actiheart-derived PA measures confirmed that subjects had returned to baseline sedentary behavior (1.48 ± 0.37 METs). $\dot{V}O_2$ max tended toward pre-training levels (32% reversal; $p < 0.001$) after 3 weeks of Actiheart-verified sedentary behavior, yet remained elevated above pre-training values ($p < 0.001$). The reversal of exercise induced changes in MAP following detraining were partial, whereas the HIT-induced changes in HOMA-IR were fully retained during this 3-week period, consistent with some earlier pilot metabolic protein data (31).

$\dot{V}O_2$ max, MAP, and HOMA-IR each displayed negative correlations between the changes following 6 weeks of 5-by-1 HIT and changes following 3 weeks of detraining ($\dot{V}O_2$ max: $R^2 = 0.12$, $p < 0.001$; MAP: $R^2 = 0.30$, $p < 0.001$; HOMA-IR: $R^2 = 0.15$, $p < 0.001$); i.e., high-responding participants tended to lose a greater amount of their training gains compared to low responders, which is logical and further supports that the determinations of training-induced changes were biological in origin. SD_{exp} for $\dot{V}O_2$ max for detraining effects exceeded SD_{con} (178 vs. 112 mL O_2, respectively), resulting in an SD_{IR} of 138 mL O_2 (95% CI: 12–264 mL O_2). Similarly, SD_{exp} for MAP for detraining effects exceeded SD_{con} (4.2 vs. 2.6 mmHg, respectively), resulting in an SD_{IR} of 3.3 mmHg (95% CI: 0.3–6.3 mmHg). This suggests the existence of low and high responders for retention of training effects. However, the amplified effect of technical and day-to-day biological variability on delta-scores compared to absolute scores limits our ability to draw conclusions on whether variability in the responses to training and detraining are strongly linked.

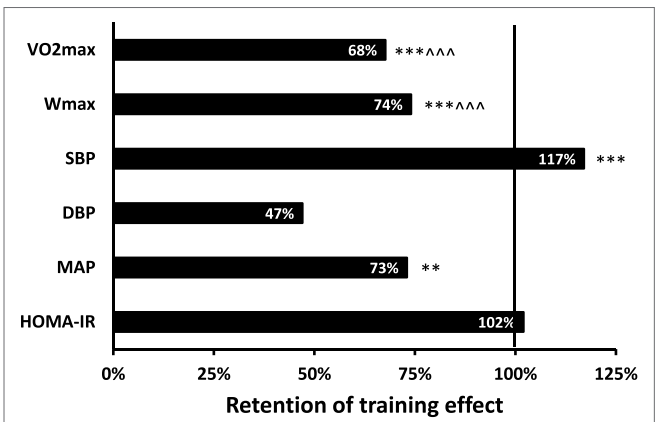

FIGURE 5 | Presentation of the average retention of the training-induced changes observed 3 weeks after cessation of 5-by-1 high-intensity interval training. A value of 100% represents the training effect and a value of 0% indicates that the training effect is lost 3 weeks after training (under sedentary conditions). Significant differences from baseline: $^{**}p < 0.01$, $^{***}p < 0.001$. Significant differences from post-training: $^{\wedge\wedge\wedge}p < 0.001$. Abbreviations: $\dot{V}O_2$max , maximal aerobic capacity; Wmax, maximal power output; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HOMA-IR, homeostatic model assessment of insulin resistance.

DISCUSSION

In this study, we adapted an exercise protocol used by Ready et al. (19) so that it was practical to implement using a standard electrically braked cycle ergometer and involve a total time-commitment of <15 min. We then demonstrated that, on average, 6 weeks of this 5-by-1 protocol was efficacious at reducing blood pressure and peripheral IR, while increasing aerobic capacity and all to an extent identical to high-volume exercise training carried out over 6 months (8). These observations enable us to claim that time-efficient exercise (<45 min week^{-1}) can reduce type 2 diabetes and cardiovascular disease risk factors in overweight men and women. If this exercise behavior was maintained, it should yield long-term health benefits (32) with a fraction of the required time-commitment associated with the recommendations by current public health guidelines (6).

Ready et al. demonstrated over 35 years ago, in Ontario, Canada, that 10 1-min intervals at a work-load equating to ~110% $\dot{V}O_2$ max, interspersed with 1-min recovery intervals, yielded ~8% gains in $\dot{V}O_2$ max. Despite this observation, awareness of the potential utility of HIT has only emerged in recent years. Recently, Little et al. using the same protocol as Ready et al., found improved glycemic control in a small group of people with type 2 diabetes (22) and we now demonstrate that, in a large group of subjects at-risk for developing type 2 diabetes, HIT can improve HOMA-IR with a more time-efficient version of this protocol. 5-by-1 HIT, relying on 50% fewer sprints than Ready et al. (19), but at a greater intensity (~125 vs. ~110% $\dot{V}O_2$ max), produced an equally robust increase in $\dot{V}O_2$ max (~10%) and reductions in HOMA-IR (~16%). We also noted that 6 weeks of 5-by-1 HIT has a mean effect for $\dot{V}O_2$ max comparable with 6 months of traditional high-volume time-consuming exercise training, indicating that time-efficient HIT can match the efficacy of traditional exercise training paradigms for the present health biomarkers, as proposed from earlier pilot studies (33).

This study is the first to attribute improvements in health biomarkers to the HIT sessions *per se*, as we show that performing HIT did not result in alterations in PA out with the supervised training sessions. The lack of "extra-curricular" changes in PA is consistent with observations made during studies involving long-term high-volume exercise training (34). The estimation of energy expenditure using Actiheart monitors enabled us to present the HIT intervention protocol in units consistent with public health orientated measures of PA. The Actiheart device appears sufficiently sensitive to pick up high-intensity exercise performed during the HIT sessions, providing reliable free-living data on both total PA levels and time spent performing activities of different intensities. We found that subjects performing 5-by-1 HIT had an increase in energy expenditure of ~400 MET min week^{-1}, consistent with the lower end of the current US Department of Health "time orientated" recommendations for PA (500–1,000 MET min week^{-1}). Thus, we were able to demonstrate that it is possible to reach these MET targets in a highly time-efficient manner. There were, however, technical limitations of the Actiheart monitoring, namely the devices produced acceptable data for less than half of our participants (reflecting obvious and periodic loss of signal). The participants received clear instructions on how to correctly wear the activity monitor during free-living conditions, and we do not know what caused the loss of signal and further research is needed to make continuous PA monitoring more reliable.

Importantly, we found that response variability in response to 6 weeks of 5-by-1 HIT exceeds technical and day-to-day biological variability for aerobic capacity and blood pressure and that this variability was similar that observed following 6 months of high-volume exercise training (9, 35). We observed, for the present three sessions per week training program, a rate of non-responders for $\dot{V}O_2$ max (~15–20%) comparable to many other high volume training programs, involving thousands of volunteers typically training 4–5 days week^{-1} (15, 36–39). Recently, it has been claimed that non-responders for $\dot{V}O_2$ max "do not exist" (40). This conclusion was based on "under-training", then re-training four groups of 10 subjects with differing frequencies of training per week. The study used a spuriously and low value for the $\dot{V}O_2$ max testing variation, i.e., the Wmax error, and failed to consider that this "error" applies to both the pre-test and post-test values, seriously undermining the validity of the study. In addition, they could not replicate in *phase one* of their "study," the known non-response rate for $\dot{V}O_2$ max seen in much larger studies using their 4–5 days week^{-1} training protocol (15, 36–39), suggesting some form of recruitment bias. Careful consideration of their data, claims, and an appropriate cutoff value for measurement variance indicates that the conclusions reached (40) are misleading. Thus, large and robust studies have found that physiological responses are heterogeneous to every type of exercise training program. Indeed, we present a meta-analysis of the genuine response frequencies for our three clinically relevant health biomarkers, $\dot{V}O_2$ max, BP, and HOMA-IR (**Figure 4**), demonstrating that at least 50% of the population can expect to be a non-responder for one of these biomarkers. This is somewhat in agreement with the efficacy noted in the long-term diabetes prevention studies (3–5), where type 2 diabetes risk is reduce but not eliminated.

We can, therefore, conclude that the present 5-by-1 HIT protocol is consistent with other exercise programs, and that it is *on average* sufficient to yield improvements in cardiovascular and metabolic parameters in both men and women. Weston et al. (41) recently conducted a meta-analysis and concluded that improvements in the $\dot{V}O_2$ max of sedentary males (10.0%; 90% CI: 4.9–15.1%) was greater than for sedentary females (7.3%; 2.5–12.1%). We would argue that an accurate estimation of the size effect of HIT using meta-analysis methodology and numerous very disparate small studies is not robust due to large variations in protocol design. While the large confidence intervals presented by Weston et al. were indicative of a high level of uncertainty in their analysis, this study relied on a large cohort of men and women undertaking an identical training program and measurement protocol, and found gains in $\dot{V}O_2$ max were in fact comparable in men and women. The same conclusion can be reached regarding blood pressure and fasting IR.

Various HIT-like protocols have been utilized in patient groups to promote rehabilitation and control risk factors for disease (42–44). In fact, many HIT-type protocols have been utilized safely in cardiac patients for many years (45). In this

study, we did not observe any adverse clinical events in a group of sedentary participants with risk factors for cardiovascular and metabolic disease. However, we do not have the required size or duration of follow-up to make recommendations on safety (or disease prevention), as such an analysis will require thousands of participants (as serious acute clinical events are rare during exercise training). Nevertheless, given that the 5-by-1 protocol yields a PA MET "score" comparable to current PA targets, is equally effective at improving aerobic capacity and reducing IR, it would seem reasonable to conclude that it can emerge as an effective alternative to high-volume time-consuming aerobic exercise training. This is particularly true as the majority of the adult population do not meet the lower-intensity time-orientated targets and, thus, do not gain some of the benefits of an active lifestyle. Thus 5-by-1 HIT could substantially reduce the incidence or progression-rates of type 2 diabetes similar to previous long-term lifestyle interventions (3–5). Notably, the improvement in HOMA-IR following 6 weeks HIT is comparable in magnitude to 2 years of calorie restriction (46) supporting the idea that increased levels of PA *via* HIT could directly contribute to the prevention of type 2 diabetes more rapidly that other types of intervention.

ETHICS STATEMENT

This study was approved by local ethics committees at all sites [University of Nottingham (D8122011 BMS), Karolinska Institutet (2012/753-31/2), the University of Copenhagen (H-3-2012-024), the University of Las Palmas de Gran Canaria (CEIH-2012-02), and Loughborough University (12/EM/0223)] and complied with the 2008 Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

The META-PREDICT application was written in 2010 by JT, and awarded in 2011 with written contributions from PA and OR. BP, NV, PA, TG, BK, WK, JT, and OR contributed to the design of the study. All authors contributed to data acquisition (BP, BK, ML, JP-G, TG, and OR), data analysis (BP, BK, OR, RB, DM, JT, and NV), or interpretation of data (BP, BK, NV, OR, RB, PA, WK, and JT). JT, BP, WK, RB, OR, and NV drafted the manuscript for publication, while all authors contributed to critically reviewing the manuscript for intellectual content. All authors gave final written approval of the manuscript for publication and agreed to be accountable for the accuracy and integrity of the data.

ACKNOWLEDGMENTS

We would like to acknowledge the important technical support at the clinical sites, including from Aisling Burns, Tariq Taylor, Michael Melin, Joao Viana, Jose Losa-Reyna, David Morales-Alamo, Rafael Torres-Peralta, Stine Dam Søndergaard, Julie Hagstrøm Danielsen, Cathrine Munk Scheuer, Tine Dohlmann, Morten Hindsø, Jose Navarro de Tuero, Cecilia Dorado, Jaime de La Calle Herrero, Ismael Perez-Suarez, and Arima Santana.

FUNDING

The META-PREDICT study was supported by the European Union Seventh Framework Programme (HEALTH-F2-2012-277936). STRRIDE AT and AT/RT were supported by NIH/NHLBI (R01-HL-057354). The raw data assimilation, data-basing, and data analysis were supported through additional funds, from JT.

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Conflict of Interest Statement: JT, TG, OR, BP, and NV are shareholders in XRGenomics LTD. The authors have no further interests to declare and the present article does not represent any protected information.

frontiers in Physiology

ORIGINAL RESEARCH
published: 25 July 2017
doi: 10.3389/fphys.2017.00528

Combined Interval Training and Post-exercise Nutrition in Type 2 Diabetes: A Randomized Control Trial

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Background: High-intensity interval training (HIIT) can improve several aspects of cardiometabolic health. Previous studies have suggested that adaptations to exercise training can be augmented with post-exercise milk or protein consumption, but whether this nutritional strategy can impact the cardiometabolic adaptations to HIIT in type 2 diabetes is unknown.

Objective: To determine if the addition of a post-exercise milk or protein beverage to a high-intensity interval training (HIIT) intervention improves cardiometabolic health in individuals with type 2 diabetes.

Design: In a proof-of-concept, double-blind clinical trial 53 adults with uncomplicated type 2 diabetes were randomized to one of three nutritional beverages (500 mL skim-milk, macronutrient control, or flavored water placebo) consumed after exercise (3 days/week) during a 12 week low-volume HIIT intervention. HIIT involved 10 X 1-min high-intensity intervals separated by 1-min low-intensity recovery periods. Two sessions per week were cardio-based (at ∼90% of heart rate max) and one session involved resistance-based exercises (at RPE of 5–6; CR-10 scale) in the same interval pattern. Continuous glucose monitoring (CGM), glycosylated hemoglobin (HbA_{1c}), body composition (dual-energy X-ray absorptiometry), cardiorespiratory fitness ($\dot{V}O_{2peak}$), blood pressure, and endothelial function (%FMD) were measured before and after the intervention.

Results: There were significant main effects of time (all $p < 0.05$) but no difference between groups (Interaction: all $p > 0.71$) for CGM 24-h mean glucose (−0.5 ± 1.1 mmol/L), HbA_{1c} (−0.2 ± 0.4%), percent body fat (−0.8 ± 1.6%), and lean mass (+1.1 ± 2.8 kg). Similarly, $\dot{V}O_{2peak}$ (+2.5 ± 1.6 mL/kg/min) and %FMD (+1.4 ± 1.9%) were increased, and mean arterial blood pressure reduced (−6 ± 7 mmHg), after 12 weeks of HIIT (all $p < 0.01$) with no difference between beverage groups (Interaction: all $p > 0.11$).

Conclusion: High-intensity interval training is a potent stimulus for improving several important metabolic and cardiovascular risk factors in type 2 diabetes. The benefits of HIIT are not augmented by the addition of post-exercise protein.

Keywords: dairy, exercise, lifestyle, body composition, glycemic control, endothelial function, blood pressure

OPEN ACCESS

Edited by:
Billy Sperlich, University of Würzburg, Germany

Reviewed by:
Christian Brinkmann, German Sport University Cologne, Germany
Karsten Müssig, German Diabetes Center (LG), Germany

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

Received: *17 May 2017*
Accepted: *07 July 2017*
Published: *25 July 2017*

Citation:
Francois ME, Durrer C, Pistawka KJ, Halperin FA, Chang C and Little JP (2017) Combined Interval Training and Post-exercise Nutrition in Type 2 Diabetes: A Randomized Control Trial. Front. Physiol. 8:528. doi: 10.3389/fphys.2017.00528

INTRODUCTION

Worldwide more than 257 million people have type 2 diabetes, a figure projected to reach 395 million by 2030 (Shaw et al., 2010). Of those, 71% have hypertension and 40% have three or more coexisting chronic conditions, with cardiovascular disease the leading cause of mortality (Centers for Disease Control and Prevention, 2014). Accordingly, interventions that improve both glycemic control and reduce cardiovascular risk factors are central to reducing the burden of type 2 diabetes (Inzucchi et al., 2012). Lifestyle interventions, including exercise and nutrition are at the forefront for the prevention of diabetes complications (Inzucchi et al., 2012). Intensive glucose lowering with multiple pharmacological treatments leads to reduced microvascular complications (UK Prospective Diabetes Study Group, 1998), but the effect on macrovascular complications is unclear.

Large controlled trials and numerous experimental studies reveal the widespread benefits of exercise for people with type 2 diabetes (Marwick et al., 2009; Lin et al., 2015). The Look AHEAD (Action for Health in Diabetes) trial showed that moderate continuous exercise and a caloric restrictive diet leads to sustained reductions in cardiometabolic risk factors, diabetes complications, and health costs (Wing et al., 2013). However, the number of cardiovascular events between the intervention and control groups was not different. The addition of vigorous exercise may be required to elicit substantial changes in cardiovascular function (Baldi et al., 2016), as it appears that vigorous, but not low-moderate exercise, reduces cardiovascular disease (Tanasescu et al., 2002; Lee et al., 2003). Studies using higher exercise intensities, such as interval and resistance exercise, show strong effects on cardiometabolic outcomes (Wisløff et al., 2007; Weston et al., 2014).

Cardiorespiratory fitness is an independent predictor of all-cause mortality and cardiovascular events (Kodama et al., 2009). A recent meta-analysis revealed that the increase in fitness after interval training is ∼2-fold greater than continuous training (Weston et al., 2014). In the longest trial to date comparing interval and continuous exercise in diabetes, Karstoft et al. (2013) randomized participants to 4 months interval walking ($n = 12$), energy and time-matched continuous walking ($n = 12$; 60-min, 5 days/week), or non-exercise control ($n = 8$). Greater improvements in fitness, body fat, and glycemic control were observed after interval compared to continuous walking and control (Karstoft et al., 2013). These findings clearly support the benefit of interval exercise, however the volume of exercise (300 min/week) is far greater than usually attained by the general population, many of whom cite lack of time as a considerable exercise barrier (Korkiakangas et al., 2009). Emerging evidence from small short-term trials show that low-volume high-intensity interval training (HIIT) rapidly improves glycemic control in type 2 diabetes (Little et al., 2011; Madsen et al., 2015). Low-volume HIIT involves alternating brief periods of vigorous exercise with periods of recovery, typically taking ∼20 min per session and performed three times per week (Little et al., 2011). Further research is needed to confirm changes in cardiometabolic health outcomes after several months of low-volume HIIT in studies with larger sample sizes.

Sarcopenic obesity disproportionately affects people with type 2 diabetes (Park et al., 2009). Diminished lean muscle leads to poor physical functioning, glycemic control and cardiovascular health (Anton et al., 2013). The anabolic effects of exercise (Robinson et al., 2017) and high-quality protein (Reitelseder et al., 2011) are important for counteracting the age-associated decline in muscle, and when combined, provide synergistic effects on muscle protein synthesis (Esmarck et al., 2001; Hartman et al., 2007). In particular, it appears that consuming milk-protein after exercise promotes significant lean mass accretion and fat loss (Hartman et al., 2007; Josse et al., 2010). HIIT was recently shown to promote increased protein synthesis in the skeletal muscle of older adults, an effect linked to improved insulin sensitivity and mitochondrial function (Robinson et al., 2017). Combining HIIT with post-exercise protein supplementation therefore holds potential for maximizing skeletal muscle adaptations in order to improve cardiometabolic health outcomes, particularly in older adults.

The purpose of this study was to determine whether post-exercise milk augments the cardiometabolic benefits of low-volume HIIT in individuals with type 2 diabetes. The primary outcome of glycemic control was assessed across 3 days before and after the intervention using continuous glucose monitoring (CGM). Secondary outcomes of body composition, HbA_{1c}, fasting blood parameters, fitness, blood pressure, and endothelial function were also examined to determine how low-volume HIIT impacted key cardiometabolic health parameters.

RESEARCH DESIGN AND METHODS

Study Design

This double-blind, randomized clinical trial was conducted at The University of British Columbia Okanagan between January 2015 and December 2016 (clinicaltrials.gov #NCT02251301). The Clinical Research Ethics Board (CREB #H14-01636) approved the study and participants provided written informed consent. Randomization was by a third-party using variable permuted block sizes with computer-generated random numbers and sealed envelopes. A researcher not involved in data analysis prepared the beverages so participants and study personnel were blinded to the beverage condition.

Participants

Men and women between 40 and 75 years with physician-diagnosed type 2 diabetes (>6 months) were recruited from the Kelowna Diabetes Program via mail-out advertisements and sign-up sheets. Exogenous insulin users, diagnosed cardiovascular disease and diabetes complications, or contraindications to exercise (Thompson et al., 2013)

Abbreviations: HIIT, High-intensity interval training; FMD, Flow mediated dilation; $\dot{V}O_{2peak}$, Cardiorespiratory fitness; CGM, Continuous glucose monitoring; MAGE, mean amplitude of glycemic excursions; QoL, Quality of Life; HR_{max}, Peak heart rate; RPE, Rating of perceived exertion; VAT, Visceral Adipose Tissue.

were excluded. After telephone/email interviews interested participants attended a screening visit, which included a medical history questionnaire, physical activity readiness questionnaire-plus (PARQ+), and informed consent. Eligible participants then completed a 12-lead stress test using a modified Bruce protocol and a cardiologist provided clearance for vigorous exercise.

Intervention

Experimental Protocol Overview

Fifty-three participants were randomized to one of three beverages; (i) low-fat milk, (ii) macronutrient control, or (iii) placebo, consumed after exercise (details below). Baseline and post-intervention outcomes were assessed over 5 days before and after the intervention (48–72 h after the last training session). Fasted blood and body composition measures were obtained on day 1 and CGM was performed across days 2–4 while participants followed a standardized diet. Blood pressure, endothelial function and fitness were assessed on day 5. Body weight, waist circumference, blood pressure, and endothelial function were also assessed at 6 weeks (Mid).

Exercise Training

All groups performed supervised low-volume HIIT 3d/week for 12 weeks. To be consistent with exercise recommendations by the American Diabetes Association and the American College of Sports Medicine (Colberg et al., 2016) both resistance and cardio-based exercises were included in the HIIT program. The first and last sessions per week were cardio-based (cycle ergometer, treadmill, or elliptical) involving 1-min bursts of exercise at 85–90% of the participants' maximum heart rate (HR_{max}; obtained during baseline $\dot{V}O_{2peak}$ test) with 1 min of easy recovery in between. The middle session each week involved whole-body resistance exercises (using resistance bands or multi-gym). Similar to cardio-based HIIT, each resistance exercise was performed for 1 min (as many repetitions as possible) at an intensity eliciting an RPE of 5 "hard" on the CR-10 scale (Borg, 1962) followed by 1 min of recovery. A 3-min warm-up and cool-down was performed with all sessions. The number of 1-min intervals in each session progressed from four in week one to ten in week six of training. Thereafter, 10 X 1-min intervals eliciting ∼90% of HR_{max} (cardio-based) or RPE ∼5 (resistance-based) were completed in each session. Previous short-term training studies in individuals with, and at risk for, diabetes, have shown this low-volume HIIT protocol is effective for improving cardiometabolic health (Little et al., 2011; Francois et al., 2016). A heart rate monitor was worn to closely prescribe intensity, and capillary blood glucose and blood pressure measures were obtained before and after each exercise session.

Post-exercise Nutrition Supplementation

After each session participants consumed 500 mL of either: (i) low-fat milk; (ii) milk protein macronutrient-matched control; or (iii) placebo (water), within 1 h. The beverages were designed to look and taste similar and distributed in opaque containers. To accomplish this, one-teaspoon of cocoa powder and ¼ teaspoon of stevia (Stevia In The Raw®, Cumberland Packing Corp; containing ∼28 mg stevia) were added to each beverage. Low-fat milk was prepared from skim-milk powder (MedallionMilk Co., Canada) providing 187 calories, 19 g protein, 26 g carbohydrate, and <1 g of fat. Macronutrient-matched control (milk protein concentrate; Vitalus Nutrition Inc., Canada plus lactose; NOW® Foods, IL, US) provided 186 calories, 21 g protein, 24 g carbohydrate, and <1 g of fat; i.e., providing the same macronutrient and protein composition as milk but without the micronutrients and other bioactive factors. The placebo beverage provided <10 calories from the cocoa powder.

Outcomes

Continuous Glucose Monitoring (CGM)

A continuous glucose monitor (iPro 2, Medtronic Inc.) was used to continuously measure blood glucose across 3 days before and after the intervention. CGM provides valuable insight (that a one-off fasting blood or HbA_{1c} sample cannot) into glycemic variability and the magnitude of postprandial excursions across several days under free-living conditions (Klonoff, 2005). The CGM continuously samples interstitial fluid from the abdomen, measuring glucose concentration every 5-min using the glucose oxidase reaction (Rossetti et al., 2010). Participants took capillary glucose samples (4X/d), which were used to retrospectively calculate retrospective blood glucose concentration via an algorithm within the online software program (CareLink Pro, Medtronic; Rossetti et al., 2010). All food, drink, and medication were recorded (including time eaten, amount, brand) for pre-testing, and then replicated exactly for post-intervention.

The primary outcome was 24-h average glucose (from 00:00 to 23:55), calculated as the mean of the 3 CGM days. Standard deviation of 24-h blood glucose and mean amplitude of glycemic excursions (MAGE; Molnar et al., 1970) were calculated from the same 24-h periods to assess glycemic variability.

Body Composition

Waist circumference (WHO Expert Consultation, 2008), height and weight (Seca 700, Hamburg, Deutschland) were measured to the nearest 0.1 cm and 0.1 kg, respectively. Percent fat, visceral adipose tissue (VAT) and lean body mass (LBM) were measured by dual-energy X-ray absorptiometry (Hologic Discovery DXA, MA, USA). All measures were performed and analyzed by the same researcher, with calibrations and quality control testing performed daily.

Cardiorespiratory Fitness ($\dot{V}O_{2peak}$)

$\dot{V}O_{2peak}$ was assessed using a maximal incremental ramp test on a cycle ergometer (Lode Excalibur, Netherlands) with continuous sampling of expired gases (Parvomedics TrueOne2400, USA). Beginning at 30 W, the test increased by 1 W every 4 s (15 W/min) until volitional exhaustion or contraindication (Fletcher et al., 2013). $\dot{V}O_{2peak}$ and RER were calculated from the highest 30-s average, while HR_{max} was recorded as the highest value obtained during the test.

Biochemical Analyses

Fasting blood samples were collected by venipuncture into EDTA containing tubes, centrifuged for 15 min (1,550 g at 4°C) and

the plasma stored at −80°C for subsequent batch analyses. Medications were withheld the morning of the fasting blood sample. Fasting glucose was measured by the hexokinase method, high-sensitivity C-reactive protein by latex particle enhanced immunoturbidimetric assay and triglycerides by the enzymatic glycerol kinase and glycerol phosphate oxidase method. Pre and post-intervention samples were analyzed concurrently in duplicate (average coefficient of variation 6.8%) on a clinical chemistry analyzer (Chemwell 2910, Awareness Technologies) using assays from Pointe Scientific (MI, USA). HbA_{1c} was analyzed from a separate EDTA tube by a medical laboratory that routinely performs this analysis according to the National Glycohemoglobin Standardization Program (NGSP).

Blood Pressure and Endothelial Function

All measures were assessed 4 h postprandial, after abstaining from alcohol and caffeine for 12 h and, within participants, at the same time of day with meal and medication standardized. After 20 min of rest in a supine position, blood pressure was measured manually using the auscultatory method, at least twice to the nearest 2 mmHg.

Flow-mediated dilation

Brachial artery flow-mediated dilation (FMD) is an important prognostic indicator of endothelial function and incident cardiovascular disease (Yeboah et al., 2007). The ability of the vessel to dilate (%FMD) is measured in response to a physiological (shear stress) stimulus (Thijssen et al., 2011). In the current study, brachial artery FMD was assessed according to current guidelines (Thijssen et al., 2011). Briefly, simultaneous measures of diameter and blood velocity were obtained with high-resolution ultrasound (Terason 3200), 2 cm from the antecubital fossa. Data were collected over a 1-min baseline, for the last 30 s of a 5 min period of forearm ischemia (pneumatic cuff inflated 60 mmHg above systolic blood pressure) and for 3 min thereafter.

Brachial artery dilatory capacity

The peak blood flow and diameter response to ischemic handgrip exercise provides an index of resistance artery size or remodeling and the maximal dilatory capacity (Naylor et al., 2005). This is important since changes in artery function (%FMD) with exercise training are thought to occur rapidly (i.e., first few weeks) after which are superseded by changes in structure, potentially concealing further changes in function (Tinken et al., 2010). After 15 min of rest, following the FMD procedure, baseline diameter and blood velocity were recorded for 1 min. This was followed by 5 min of forearm ischemia (as above), including 3 min of isotonic handgrip exercise (1 contraction every 2 s using a dynamometer) between 1-min periods of ischemia alone (Naylor et al., 2005). Again recording resumed 30 s before cuff deflation and continued for 3 min thereafter.

Absolute FMD (peak diameter – baseline diameter), %FMD (peak – baseline diameter/baseline diameter), and time to peak diameter were measured using custom designed edge-detection and wall-tracking software, which minimizes user bias (Woodman et al., 2001). This protocol is routinely performed in our lab using the methods outlined in Francois et al. (2016); coefficients of variation for diameter and %FMD are 2.1 and 7.3%, respectively.

TABLE 1 | Baseline characteristics of participants.

	Milk ($n = 18$)	Macronutrient control ($n = 16$)	Placebo ($n = 19$)
Sex	11 F	12 F	11 F
Age (y)	62 ± 8	56 ± 9	55 ± 9
BMI (kg/m^2)	36 ± 7	35 ± 6	33 ± 6
Years of diagnosis	6 ± 6	7 ± 7	5 ± 6
MEDICATIONS			
Lifestyle only	5	5	3
Metformin	10	11	13
Sulfonylureas	6	1	3
SGLT2 inhibitors	1	2	3
DPP4 inhibitors	1	2	3
GLP1 analogs	1	2	0
Lipid lowering	9	7	7
Antihypertensive	7	6	8
BASELINE PHYSICAL ACTIVITY			
LTPA score	17 ± 15	14 ± 10	21 ± 17
MVPA (min/day)	14 ± 15	13 ± 13	30 ± 19
Dairy intake (servings/day)	2.3 ± 2.4	2.7 ± 2.1	2.1 ± 1.6

F, Females; LTPA, Leisure-Time Physical Activity; MVPA, Moderate-Vigorous Physical Activity.

Quality of Life (QoL)

Participants completed the Medical Outcomes Study Short Form 36 (SF-36) questionnaire before and after the intervention (McHorney et al., 1994). The SF-36 is a self-report QoL questionnaire; the scores are used to provide two norm-based T scores, physical component summary (PCS) and mental component summary (MCS).

Diet and Exercise Standardization

Participants maintained their usual diet, lifestyle, and medication habits throughout the testing and training sessions, verified by physical activity and diet records. Baseline dairy consumption was assessed using a food frequency questionnaire, and dietary intake before and during the study was assessed using 3-day diet records analyzed using FoodWorks16 (The Nutrition Company, NJ, USA). Baseline activity was examined using both accelerometry (Actigraph GT3x, FL, USA) over a 7-day period to assess minutes of moderate-vigorous physical activity (MVPA, Freedson et al., 1998 cut-points) and a Godin leisure-time exercise questionnaire (Godin and Shephard, 1997; **Table 1**).

Statistical Analyses

Sample Size

Using means and standard deviations from previously published data on the change in CGM assessed hyperglycemia in type 2 diabetes after HIIT (Little et al., 2011), power calculations determined that $n = 17$ per group would be sufficient to detect

a 30% reduction in glucose (Cohen $d = 0.7$) with a power of 80% and alpha of 0.05.

Statistics

Analyses were performed on all participants that completed the intervention. Characteristics of the intervention groups are shown in **Table 1**. Linear mixed models using SPSS 22.0 (SPSS, Chicago, Illinois) examined changes in trial outcomes (pre-post or pre-mid-post) between groups. Significant interactions were probed with pre-planned contrasts comparing the change within each group, whereas isolated significant main effects of time were examined by pairwise comparisons with groups collapsed using Least Significant Difference (LSD) test (Hopkins et al., 2009). Results are reported as means and standard deviations with 95% confidence limits. Magnitude based inferences were used to identify clinically meaningful changes in major outcomes using techniques described by Batterham and Hopkins (2006). The threshold for clinically beneficial changes in 24-h glucose and HbA_{1c} were reductions of 0.5 mmol/l and 0.7%, respectively, based on the reduced risk for diabetes complications (Mazzone, 2010). For cardiorespiratory fitness an increase of 1 metabolic equivalent (MET) was used for a 15% risk reduction in cardiovascular disease (Kodama et al., 2009). For %FMD +1% was used, based on the 13% risk reduction in cardiovascular events (Inaba et al., 2010). In line with previous studies, a 2 mmHg reduction in MAP was considered to be the smallest clinical threshold change for BP (Cook et al., 1995). The clinically meaningful difference in QOL was determined as a change >3 points (Warkentin et al., 2014).

RESULTS

Participant Compliance and Adverse Events

Figure 1 shows the CONSORT flow diagram of study progression. Fifty-three participants were eligible after screening; four required additional 24-h blood pressure monitoring ($n = 2$) and stress echo ($n = 2$) cardiologist clearance following the 12-lead ECG stress test. Baseline characteristics of randomized participants are shown in **Table 1**. The majority (51/53) were of European descent, while two were Southeast Asian (2/53).

Of the 53 participants randomized, 51 successfully completed 36 sessions of HIIT in 12 ± 1 wk. One participant suffered a non-fatal myocardial infarction in week eight (after 23 HIIT sessions) and one dropped out for personal reasons. There were no reports of hypoglycemia after exercise or at home throughout the intervention. Exercise sessions were rescheduled on 10 occasions ($n = 6$ due to sickness and $n = 4$ due to systolic blood pressure >144 mmHg prior to exercise). No musculoskeletal injuries were reported as a result of the training. $\dot{V}O_{2peak}$ testing was truncated in three participants because systolic pressure exceeded 250 mmHg during the test (Fletcher et al., 2013). For CGM analyses three participants were excluded due to sensor failure ($n = 1$) and medication changes ($n = 2$; required reduced medication). All other analyses are reported for $n = 51$ unless otherwise stated. Overall the exercise intensity achieved was 88 ± 7% of HR_{max} during cardio-based intervals, and an average RPE of 5 ± 1 and 4 ± 1, for cardio- and resistance-based intervals, respectively.

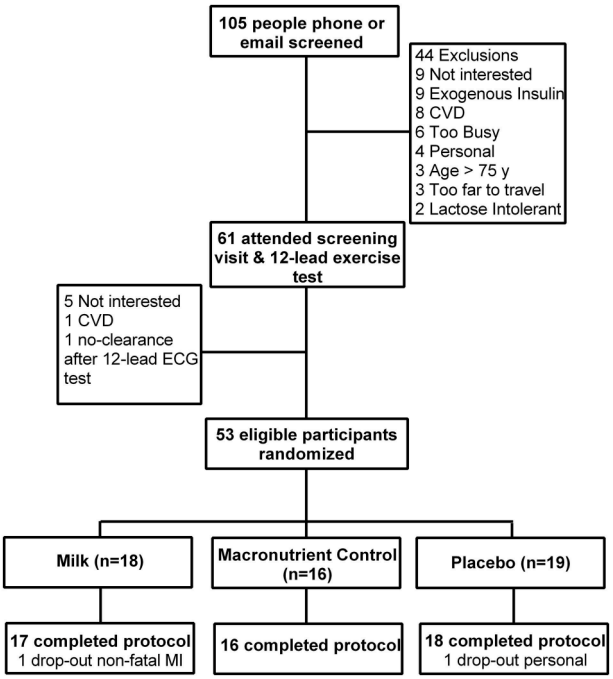

FIGURE 1 | Consolidated Standards of Reporting Trials (CONSORT) flow diagram.

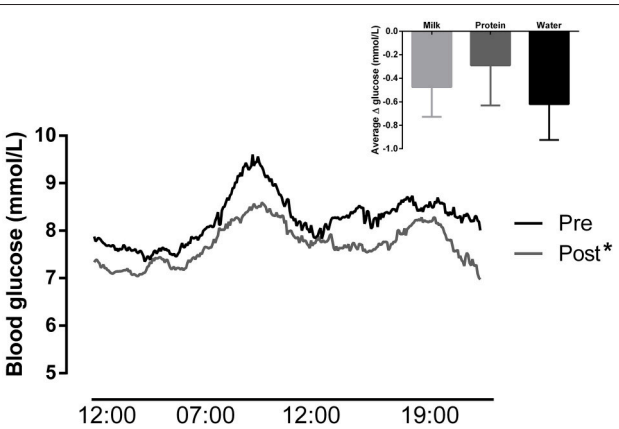

FIGURE 2 | Continuous blood glucose across 24-h ($n = 48$) before and after the intervention (groups collapsed, *main effect of time: $p = 0.01$). Inset: Change in blood glucose after the intervention in the milk, protein, and water groups.

Glycemic Control

There was a significant reduction in mean 24-h glucose following 12 weeks of HIIT (by −0.5 ± 1.1 mmol/L, **Figure 2**) with no difference between groups (**Table 2**). The probability that the

TABLE 2 | Body composition, cardiorespiratory fitness, blood pressure, flow-mediated dilation, triglycerides, C-reactive protein, and glycemic control measures before and after 12 weeks of HIIT and nutritional beverage.

	Milk ($n = 18$)		Macronutrient control ($n = 16$)		Placebo ($n = 19$)		*P*-value	
	Pre	Post	Pre	Post	Pre	Post	Interaction	Time
BODY COMPOSITION								
Mass (kg)	97.7 ± 19.3	96.8 ± 20.5	95.9 ± 17.3	94.5 ± 17.3	89.5 ± 21.1	89.1 ± 20.9	0.46	0.03*
VAT (g)	1057 ± 335	1033 ± 316	1007 ± 260	981 ± 212	815 ± 337	802 ± 285	0.75	0.25
CARDIORESPIRATORY FITNESS								
$\dot{V}O_{2peak}$ (L/min)	1.7 ± 0.4	2.0 ± 0.7	1.8 ± 0.5	2.0 ± 0.6	1.9 ± 0.5	2.1 ± 0.5	0.53	<0.01*
BLOODS								
HbA_{1c} (%; mmol/mol)	7.1 ± 0.8	6.9 ± 0.7	6.9 ± 0.8	7.0 ± 0.7	6.9 ± 0.8	6.6 ± 0.9	0.92	<0.01*
	54 ± 9	52 ± 8	54 ± 8	53 ± 8	51 ± 8	49 ± 9		
Fasting glucose (mmol/L)	8.6 ± 2.3	8.3 ± 1.7	9.2 ± 1.9	9.5 ± 2.3	8.9 ± 2.7	8.5 ± 2.1	0.35	0.53
Triglycerides (mg/dL)	149 ± 82	152 ± 70	161 ± 62	139 ± 65	152 ± 93	142 ± 80	0.36	0.17
C-reactive protein (mg/dL)	7.1 ± 10.3	4.4 ± 5.3	4.7 ± 4.3	4.9 ± 4.7	3.7 ± 4.1	3.1 ± 3.6	0.33	0.21
CGM GLUCOSE CONCENTRATION								
24-h mean (mmol/L)	8.4 ± 1.4	7.7 ± 1.2	8.1 ± 1.4	7.8 ± 1.7	8.4 ± 2.1	7.8 ± 1.5	0.74	0.01*
SD (mmol/L)	1.6 ± 1.0	1.3 ± 0.5	1.6 ± 0.6	1.1 ± 0.4	1.7 ± 0.8	1.5 ± 0.7	0.51	0.01*
MAGE (mmol/L)	4.3 ± 3.5	3.1 ± 1.3	4.1 ± 2.0	2.8 ± 1.3	4.1 ± 2.2	3.7 ± 1.6	0.60	0.02*
BLOOD PRESSURE								
Systolic (mmHg)	130 ± 10	119 ± 7	132 ± 13	129 ± 9	128 ± 13	117 ± 11	0.03#	<0.01*
Diastolic (mmHg)	79 ± 6	75 ± 5	83 ± 11	79 ± 6	81 ± 7	75 ± 7	0.20	<0.01*
FLOW-MEDIATED DILATION								
Absolute FMD (mm)	0.020 ± 0.01	0.027 ± 0.01	0.018 ± 0.01	0.024 ± 0.01	0.019 ± 0.01	0.023 ± 0.01	0.61	<0.01*
Baseline diameter (mm)	0.41 ± 0.10	0.41 ± 0.09	0.41 ± 0.08	0.41 ± 0.07	0.41 ± 0.07	0.42 ± 0.07	0.77	0.71
Time to peak (s)	64 ± 26	57 ± 25	60 ± 30	46 ± 23	56 ± 21	50 ± 21	0.75	0.05*
Total energy intake (Kcal/day)	2053 ± 881	2039 ± 898	1810 ± 525	2017 ± 706	1912 ± 629	1888 ± 710	0.35	0.25

HbA_{1c}, Glycosylated Hemoglobin; BMI, Body Mass Index; VAT, Visceral Adipose Tissue; MAP, Mean Arterial Pressure; FMD, Flow Mediated Dilation; TE, Total Energy.
**Time effect $p < 0.05$.*
*#Interaction group*time $p < 0.05$.*

change in glucose was clinically beneficial was 54% (95% CI: −0.8, 0.1 mmol/L). Glycemic variability assessed by both *SD* (by −0.33 ± 0.78 mmol/L) and MAGE (by −0.98 ± 2.27 mmol/L) was significantly reduced, with no differences between groups (**Table 2**). HbA_{1c} was significantly reduced after 12 weeks of HIIT (by −0.22 ± 0.39%, **Figure 3**) with no differences between groups (**Table 2**). The probability that the change in HbA_{1c} was clinically beneficial was 0% (95% CI: −0.33, 0.16%), with the change being most likely trivial. Fasting glucose was not significantly different after HIIT in all groups (**Table 2**).

Body Composition

Body mass was significantly lower after 12 weeks of HIIT (by −0.9 ± 3.9 kg, **Table 2**), with no difference between groups. There was a significant reduction in waist circumference after 12 weeks of HIIT (by −2.9 ± 3.5 cm, main effect of time: $p < 0.01$) with no difference between groups (Interaction: $p = 0.21$, **Figure 4**). Percent body fat was significantly reduced (by −0.76 ± 1.63%, main effect of time: $p = 0.02$) and lean body mass significantly increased (by +1.07 ± 2.76 kg, main effect of time: $p = 0.01$) after 12 weeks of HIIT, with no difference between groups (Interactions: all $p > 0.83$, **Figure 3**).

Cardiorespiratory Fitness ($\dot{V}O_{2peak}$) and Blood Pressure

$\dot{V}O_{2peak}$ significantly increased 9.8% after 12 weeks of HIIT (main effect of time: $p < 0.01$, **Figure 3**) with no difference between groups (Interaction: $p = 0.55$). The probability that the change in fitness was clinically beneficial was 5% (95% CI: 1.8, 3.1 mL/kg/min), with the change being 95% very likely trivial.

Mean arterial blood pressure was significantly reduced after 12 weeks of HIIT (by −5.7 ± 7.0 mmHg, main effect of time: $p < 0.01$) with no difference between groups (Interaction: $p = 0.11$, **Figure 4**). The probability that the change in MAP pre-post intervention was clinically beneficial was 99% (95% CI: −9, −2 mmHg).

Flow-Mediated Dilation

%FMD significantly increased after 12 weeks of HIIT (by +1.4 ± 1.9%, main effect of time: $p < 0.01$), with no difference between groups (Interaction: $p = 0.72$, **Figure 4**). The probability that the change in %FMD was clinically beneficial was 94% likely (95% CI: 0.86, 1.94%). Absolute FMD also increased after HIIT (**Table 2**), with no difference between groups. Time to peak dilation was significantly lower (by 9.1 ± 31.1 s, **Table 2**) after 12 weeks of HIIT, with no difference between groups. Peak dilator capacity

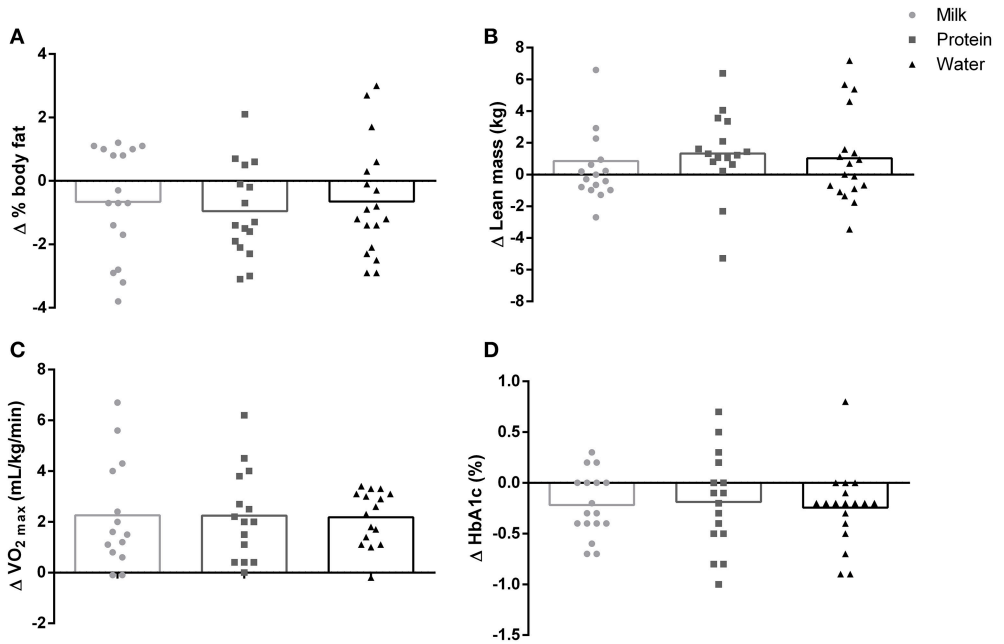

FIGURE 3 | Change from pre intervention for **(A)** % body fat, **(B)** lean body mass, **(C)** cardiorespiratory fitness ($\dot{V}O_{2peak}$) and **(D)** glycosylated hemoglobin (HbA_{1c}) in the milk, protein, and water groups (all main effect of time $p < 0.05$, no group interaction $p > 0.05$).

did not change across the intervention; Pre: 9.6 ± 5.2%, Mid: 8.1 ± 4.2%, Post: 10.4 ± 3.6% (main effect of time: $p = 0.36$).

Quality of Life

PCS scores significantly increased after 12 weeks of HIIT ($n = 49$, by 8.1 ± 12.1, main effect of time: $p < 0.01$) with no difference between groups (Interaction: $p = 0.11$). The probability that the change in PCS pre-post intervention was clinically beneficial was 99% likely (95% CI: 4.4, 11.8). The change in MCS post-intervention was different between groups ($n = 49$, Interaction: $p = 0.02$); *post hoc* testing revealed significant improvements in the protein group (+12.1 ± 9.69, $p < 0.01$) but not skim-milk (−1.1 ± 13.5, $p = 0.79$) or placebo (+5.6 ± 10.7, $p = 0.06$).

Dietary Intake Records

Analysis of the 3-day diet records collected before and during the last week of the intervention showed no difference in the total daily energy intake between groups and/or across time (**Table 2**). Macronutrient composition of the diet was not different between groups ($p = 0.32$), or across time: for % carbohydrate (Pre: 48.0 ± 12.5% vs. Post: 48.4 ± 13.0% of total energy, $p = 0.47$), % protein (Pre: 20.4 ± 4.9% vs. Post: 19.9 ± 4.9% of total energy, $p = 0.15$) and % fat (Pre: 30.3 ± 12.5% vs. Post: 30.7 ± 13.3% of total energy, $p = 0.49$).

DISCUSSION

This study comprehensively examined the cardiometabolic benefits of HIIT in individuals with type 2 diabetes. We show for the first time that 12 weeks of low-volume HIIT, with or without post-exercise milk or protein, improves glycemic control, blood pressure, cardiorespiratory fitness, body composition, and endothelial function. Low-volume HIIT therefore appears to be a feasible and efficacious lifestyle intervention, involving minimal time and resource, to improve health in type 2 diabetes. Reducing the interval length and total exercise time has previously been shown to increase enjoyment and compliance (Martinez et al., 2015). To this end, we experienced very low dropout rates and high compliance to low-volume HIIT. In addition, we show that 12 weeks of HIIT improves quality of life, similar to previous studies in hypertensive (Molmen-Hansen et al., 2012) and heart failure (Wisløff et al., 2007) patients.

Exercise interventions generally result in modest weight loss, however exercise promotes lean mass accretion; which has important implications for whole-body metabolism, glucose disposal, and quality of life (Anton et al., 2013). Indeed, in the current study HIIT significantly increased lean mass and reduced body fat. Although weight loss was not a goal of the intervention, participants lost, on average, ∼0.9 kg of body mass, which was a statistically significant change yet small in magnitude (∼1%). Generally studies report significant benefits of weight loss in the magnitude of 5–7% (Wadden et al., 2012) but it is possible that improvements in some cardiometabollic outcomes were related to the small amount of weight loss seen. Consuming high-quality protein after exercise is known to further potentiate muscle protein synthesis (Esmarck et al., 2001; Hartman et al., 2007). Despite this, comparable changes in body composition and cardiometabolic health were seen with post-exercise milk, milk-protein, or water. In agreement, Parr et al. (2016) found changes in body composition after a combined resistance training

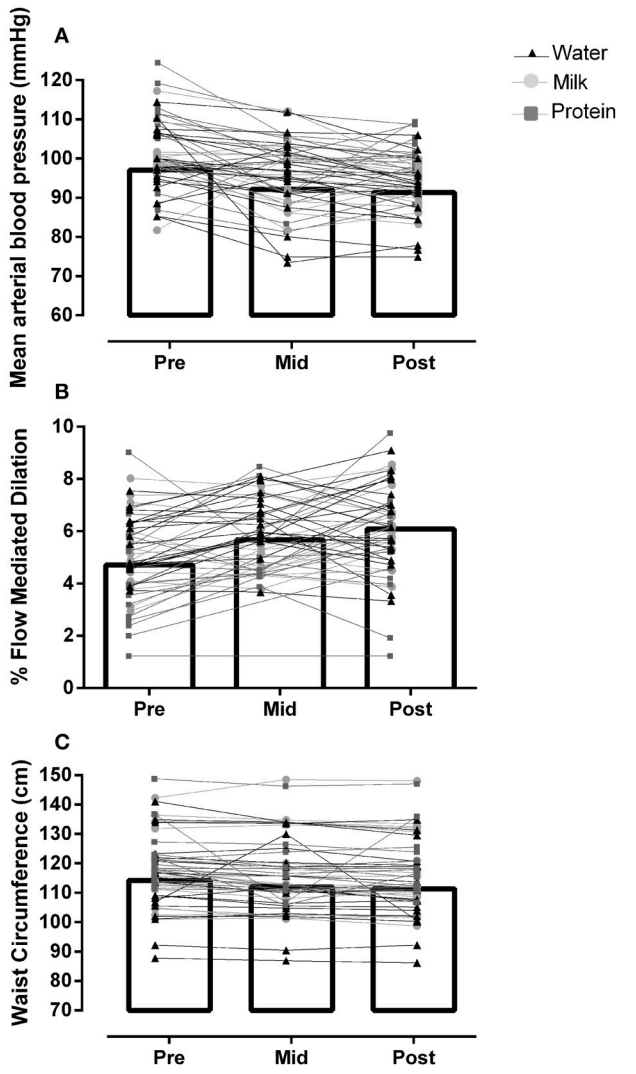

FIGURE 4 | Data for **(A)** mean arterial blood pressure (MAP), **(B)** percentage flow-mediated dilation (%FMD), and **(C)** waist circumference, before (Pre), after 6 weeks (Mid), and 12 weeks (Post) mean for all participants (bar graph, all main effect of time $p < 0.05$) and individual data (line and symbols per beverage group, no group interaction $p > 0.05$).

and diet intervention were independent of the amount and type of protein (high/low dairy). Epidemiological data shows an inverse relationship between low-fat dairy consumption and the risk of type 2 diabetes (Aune et al., 2013) and the addition of four servings of low fat dairy per day has been shown to improve insulin resistance (Rideout et al., 2013). Therefore, additional milk/protein supplementation (e.g., on non-exercise days) may have been needed to elucidate effects of nutritional supplementation. Indeed, some previous studies showing benefits on lean mass have provided milk/protein after exercise 5 days per week (Hartman et al., 2007; Josse et al., 2010). However, ∼20 g of post-exercise protein (similar to the current study) has been shown to maximize muscle protein synthesis (Churchward-Venne et al., 2016). To this end, a non-exercising control group may be required to detect effects of post-exercise protein added to a potent training intervention such as, HIIT. However, we feel a non-exercise control group in type 2 diabetes is unethical since numerous studies have shown worsening of glycemic control and cardiovascular risk factors in control group participants (Church et al., 2010; Karstoft et al., 2013).

Current research suggests that HIIT is more effective than continuous training for improving insulin resistance (Jelleyman et al., 2015). A recent meta-analysis revealed that absolute changes in HbA_{1c} are 0.5 and 0.25% greater with HIIT than control and continuous exercise, respectively (Jelleyman et al., 2015). The small, yet significant change in HbA_{1c} in the current study is in line with previous HIIT interventions (Madsen et al., 2015; Cassidy et al., 2016) yet robust changes in 24-h glucose were observed (**Figure 2**). Interestingly, the changes in 24-h glucose are similar to Karstoft et al. (2013) after 4 months of high-volume HIIT (300 min/week). This is an important finding given the perceived time barrier to exercise participation in type 2 diabetes (Korkiakangas et al., 2009). The use of CGM is a strength as it allows for additional insight into the changes in postprandial hyperglycemia and overall glycemic variability (Klonoff, 2005). Mean 24-h glucose and glycemic variability were reduced by 7 and 23%, respectively, after HIIT, regardless of post-exercise nutritional supplementation. Glycemic variability may be a stronger predictor than HbA_{1c} for diabetes complications (Praet et al., 2006). Previous research also shows that HIIT has the potential to improve beta cell function as Madsen et al. (2015) demonstrated an increase in the oral disposition index and HOMA-%β after 8 weeks. The mechanisms underlying the improvements in glycemic control could not be ascertained from the present study design but likely involve a combination of improvements in peripheral insulin sensitivity, beta cell function, and hepatic insulin resistance (Karstoft et al., 2014; Madsen et al., 2015; Cassidy et al., 2016). Collectively, these findings show the potential of HIIT to improve several underlying aspects of glycemic dysfunction in type 2 diabetes.

The added benefits of vigorous exercise for cardiovascular health are well known (Marwick et al., 2009; Baldi et al., 2016) and many studies have demonstrated superior cardiovascular effects of HIIT compared to continuous training (Wisløff et al., 2007; Marwick et al., 2009; Weston et al., 2014). Extending on this work, we observed an ∼10% increase in cardiorespiratory fitness, a 6 mmHg reduction in MAP and ∼1.4% improvement in FMD following 12 weeks of HIIT in type 2 diabetes. In itself, cardiorespiratory fitness is a strong predictor for cardiovascular mortality with each MET increase associated with a 10–20% improvement in survival (Kodama et al., 2009). Although only a 0.7 MET increase was observed, this is in line with previous low-volume HIIT studies (Madsen et al., 2015) and participants are likely to have gained significant health benefits given their low baseline fitness (<6 MET). A meta-analysis showed that the greatest mortality benefits occur for even small increases in fitness for those progressing from the least fit category (Kodama et al., 2009). Furthermore, the low-volume nature of the HIIT protocol involved only 45–78 min of exercise per week with one session being resistance training. The

combination of resistance and cardio exercise may be superior to either type alone for improving health in type 2 diabetes (Church et al., 2010). Indeed, in hypertensive patients blood pressure is reduced more with combination training than cardio alone (Lamberti et al., 2016); the 5–6 mmHg reduction is in line with the current study. Our findings suggest that HIIT performed as combined aerobic and resistance exercise clearly promotes beneficial cardiovascular adaptations in type 2 diabetes patients.

In conclusion, we show that low-volume HIIT, with or without post-exercise milk or protein supplementation, improves metabolic and cardiovascular risk factors in individuals with type 2 diabetes. The combination of resistance and aerobic-based HIIT increases lean mass, reduces fat mass, and improves endothelial function. This study, the largest and longest low-volume HIIT study in type 2 diabetes to date, provides further evidence that HIIT is a feasible and efficacious exercise intervention to improve glycemic control, cardiovascular fitness, and body composition.

AUTHOR CONTRIBUTIONS

MF, JL, and CD designed the study. MF, CD, KP, FH, and CC conducted the research. MF, JL, KP, and FH analyzed the data. MF and JL wrote the initial draft of the manuscript. All authors edited the manuscript and approved the final draft.

FUNDING

This study was supported by a research grant awarded to JL by the Dairy Farmers of Canada.

ACKNOWLEDGMENTS

We would like to thank all our participants for their commitment to this training study, and to our undergraduate students from UBC Okanagan Courtney, Trevor, Marcel, and Michael for their help with training. In addition thanks to Jacqueline and Jordelle from Coach Cardiology.

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

frontiers in Endocrinology

CLINICAL TRIAL
published: 13 June 2017
doi: 10.3389/fendo.2017.00132

Resting Metabolic Rate Does Not Change in Response to Different Types of Training in Subjects with Type 2 Diabetes

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Background and objectives: Ambiguous results have been reported regarding the effects of training on resting metabolic rate (RMR), and the importance of training type and intensity is unclear. Moreover, studies in subjects with type 2 diabetes (T2D) are sparse. In this study, we evaluated the effects of interval and continuous training on RMR in subjects with T2D. Furthermore, we explored the determinants for training-induced alterations in RMR.

Methods: Data from two studies, both including T2D subjects, were encompassed in this manuscript. Study 1 was a randomized, crossover study where subjects (n = 14) completed three, 2-week interventions [control, continuous walking training (CWT), interval-walking training (IWT)] separated by washout periods. Training included 10 supervised treadmill sessions, 60 min/session. CWT was performed at moderate walking speed [aiming for 73% of walking peak oxygen uptake (VO_2peak)], while IWT was performed as alternating 3-min repetitions at slow (54% VO_2peak) and fast (89% VO_2peak) walking speed. Study 2 was a single-arm training intervention study where subjects (n = 23) were prescribed 12 weeks of free-living IWT (at least 3 sessions/week, 30 min/session). Before and after interventions, RMR, physical fitness, body composition, and glycemic control parameters were assessed.

Results: No overall intervention-induced changes in RMR were seen across the studies, but considerable inter-individual differences in RMR changes were seen in Study 2. At baseline, total body mass (TBM), fat-free mass (FFM), and fat mass were all associated with RMR. Changes in RMR were associated with changes in TBM and fat mass, and subjects who decreased body mass and fat mass also decreased their RMR. No associations were seen between changes in physical fitness, glycemic control, or FFM and changes in RMR.

Conclusion: Neither short-term continuous or interval-type training, nor longer term interval training affects RMR in subjects with T2D when no overall changes in body composition are seen. If training occurs concomitant with a reduction in fat mass, however, RMR is decreased.

Clinical Trials Registration (www.ClinicalTrials.gov): NCT02320526 and NCT02089477.

Keywords: resting metabolic rate, exercise interventions, exercise training, body composition, physical fitness, glycemic control, diabetes type 2

OPEN ACCESS

Edited by:
Jan Polák, Charles University in Prague, Czechia

Reviewed by:
Jørgen Jensen, Norwegian School of Sport Sciences, Norway
Ondřej Šeda, Charles University in Prague, Czechia

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

Received: *30 March 2017*
Accepted: *30 May 2017*
Published: *13 June 2017*

Citation:
Karstoft K, Brinkløv CF, Thorsen IK, Nielsen JS and Ried-Larsen M (2017) Resting Metabolic Rate Does Not Change in Response to Different Types of Training in Subjects with Type 2 Diabetes. Front. Endocrinol. 8:132. doi: 10.3389/fendo.2017.00132

INTRODUCTION

Most subjects with type 2 diabetes (T2D) are overweight or obese, and overweight/obesity is considered to be a central component of the pathogenesis and pathology of T2D (1, 2). Indeed, weight loss is associated with improvements in glycemic control and other cardiovascular risk factors, and weight loss is recommended for all overweight/obese subjects with T2D (3). Classically spoken, body weight is dependent on the balance between energy intake and energy consumption, and a decrease in energy intake and/or an increase in energy consumption will lead to a weight loss. Energy consumption is dependent on several factors, with resting metabolic rate (RMR) being responsible for 60–70% of the total energy consumption in subjects who are not very active (4). As such, an increase in RMR will increase the likelihood of a weight loss, and interventions that may increase RMR would be attractive in subjects with T2D.

Exercise increases energy expenditure during and after the exercise (5). The increased energy expenditure in the hours following an exercise session is known as excess post-exercise oxygen consumption (EPOC), and this is dependent on both exercise duration and exercise intensity (6). Moreover, training interventions may indirectly increase RMR since fat-free mass (FFM), which is known to be the predominant determinant of RMR (4), is often maintained or increased with training. Since subjects with similar FFM may differ substantially in RMR (7), FFM is, however, not the only determinant of RMR, and although data are conflicting (8), it has been suggested that training may directly influence RMR. As such, it has been found that endurance-trained subjects have higher RMR than sedentary matched controls (9–11) and that training interventions may increase RMR (10, 12). Conversely, other studies have found that training interventions do not affect RMR (13, 14). Whereas these discrepancies between studies may be dependent on different factors, it has been suggested that VO_2max is an important determinant for changes in RMR (9, 15), and so the ability of a training intervention to increase VO_2max may be essential.

Only a few studies examining the effect of training interventions on RMR in diabetic subjects have been performed, and, as for healthy subjects, findings are conflicting. Araiza et al. found that a training intervention increased RMR (16), whereas Mourier et al. and Jennings et al. found no effect of training interventions on RMR (17, 18). In subjects with T2D, RMR is typically higher compared to matched normal glucose tolerant subjects, something which is considered to be due to the compromised glycemic control (19, 20). Whereas Araiza et al. found no improvements in glycemic control with their training intervention, both Mourier and Jennings et al. did see training-induced improvements in glycemic control. Thus, it might be speculated whether training-induced improvements in RMR were blunted or even completely offset by the training-induced improvements in glycemic control in the two latter studies.

Exercise intensity is an important determinant for training-induced changes in body composition (21), and we have previously found that 17 weeks of interval-walking training (IWT) results in a substantial weight loss (on average 4 kg) in opposition to time duration and energy-expenditure matched continuous walking training (CWT) (22). Whereas part of this differential weight loss between CWT and IWT may be explained *via* differential EPOC (23), the main reason for the discrepancy remains unclear. There are some indications that training with higher intensity may increase RMR more than training with lower intensity, but it is unclear if this is due to differential effects on VO_2max and other potential determinants for RMR, or if there is a direct effect of higher training intensity on RMR (24). As such, we aimed to examine the direct effects (independent of changes in body composition and VO_2max) of short-term (2 weeks) IWT/CWT and the effects of longer term (12 weeks) IWT on RMR in subjects with T2D. Moreover, we aimed to assess the associations between potential determinants for RMR (VO_2max, body composition, glycemic control) and RMR, both at baseline and in relation to the changes induced with 12 weeks of IWT.

MATERIALS AND METHODS

This manuscript builds on data from two different studies, both including subjects with T2D (25). Exclusion criteria were pregnancy, smoking, contraindication to increased levels of physical activity (26), insulin dependence, and evidence of thyroid, liver, lung, heart, or kidney disease. All subjects underwent a screening consisting of a medical interview and examination, an oral glucose tolerance test (OGTT), a walking VO_2peak test with indirect calorimetry (Cosmed K4B2, Rome, Italy) and a familiarization VO_2max test performed on a treadmill (Katana Sport, Lode, Groningen, the Netherlands) with indirect calorimetry (Cosmed Quark, Rome, Italy) as previously described (22, 27). Written and informed consent was obtained from all research participants before any investigations were performed and the studies were approved by the Ethical Committee of the Capital Region of Denmark (H-6-2014-043 and H-1-2013-116) and registered at www.ClinicalTrials.gov (NCT02320526) and (NCT02089477).

Study Designs and Interventions

Study 1 was a randomized, crossover trial where subjects were included in three different interventions, each lasting 2 weeks. The interventions were CWT (ten 60-min walking sessions performed with a continuous speed, aiming for oxygen uptake rates at 73% of VO_2peak); IWT [ten 60-min walking sessions performed with cycles of alternating 3-min slow (54% of VO_2peak) and 3-min fast (89% of VO_2peak) walking]; control (CON) (no walking), and interventions were performed in randomized order. All walking sessions were performed at a treadmill (Katana Sport) and controlled with indirect calorimetry at the first and sixth session (in order to determinate the walking speed that corresponded to the correct oxygen uptake rates). Between interventions, washout periods (8 weeks after CWT/IWT, 4 weeks after CON), where subjects returned to their habitual activity level, were applied to ensure that any intervention-induced effects disappeared before initiation of the next intervention. Other data from this study have previously been published (28).

Study 2 was a single-arm intervention study, where subjects were prescribed free-living IWT for 12 weeks. Subjects were told

to complete at least three weekly training sessions, each lasting at least 30 min and with repeated cycles of 3-min fast and 3-min slow walking. Training was controlled by a smartphone application (InterWalk®), and data from training sessions were uploaded to a central server (29). Other data from this study have previously been published (27).

Investigations

Before (pre) and after (post) interventions, subjects underwent one experimental day (meaning that subjects in Study 1 completed 6 experimental days in total). Subjects met fasting (~12 h for all except water) in the laboratory, by means of passive transport (car, bus, etc.). After confirming that no subjective feeling of acute disease and no fever was present, subjects voided. Subjects were then weighted, had an antecubital vein catheter inserted, and were placed in a bed in a temperature controlled (20°C) and calm room. After an acclimatization period of at least 30 min, the RMR measurements commenced: a standardized head tilt (15°) was applied to the bed and a ventilated hood (Cosmed, rounded canopy) was placed over the subject's head and connected to an indirect calorimetric system (Cosmed Quark) *via* a canopy blower (Cosmed). Carbon dioxide concentrations in the system were kept below 1% to avoid excess breathing (30). Subjects were instructed to breathe normally and not to fall asleep. RMR measurements were performed for 20 min.

Following the RMR measurements, fasting blood samples (lithium-heparin and EDTA tubes) were obtained and subjects included in Study 1 underwent supine resting whereas subjects in Study 2 underwent a 2 h standard OGTT (75 g anhydrate glucose dissolved in water to a total volume of 300 ml) with bedside blood glucose measurements (ABL 8 series, Radiometer, Herlev, Denmark) obtained every 30 min. Finally, following resting/OGTT procedures, all subjects were given a light meal and underwent a dual-energy X-ray absorptiometry scan (Lunar Prodigy Advance; GE Healthcare, Madison, WI, USA) and a VO_2max test comparable to the one performed at the screening day.

TABLE 1 | Pre- and (for Study 2) post-intervention characteristics.

	Study 1	Study 2 pre	Study 2 post
n	14	23	
Sex (M/F)	11/3	7/16	
Age (years)	65.3 ± 1.7	64.8 ± 1.5	
Time since diagnosis (years)	8.6 ± 1.3	6.0 ± 0.9	
Glucose-lowering medication (n)			
Metformin	14	19	
Sulfonylureas	3	2	
GLP-1 analogs/DPP-4 inhibitors	3	7	
SGLT2 inhibitors	0	1	
RMR			
Absolute (ml O_2/min)	1,736 ± 85	1,659 ± 51	1,646 ± 69
Relative to body mass (ml O_2/min/kg TBM)	18.1 ± 0.6	21.4 ± 0.8*	21.0 ± 0.7
Relative to FFM (ml O_2/min/kg FFM)	28.8 ± 1.1	34.4 ± 0.8*	34.0 ± 1.1
Physical fitness (VO_2max)			
Absolute (ml O_2/min)	2,438 ± 147	1,961 ± 90	2,065 ± 92‡
Relative to body mass (ml O_2/min/kg TBM)	25.3 ± 1.1	25.1 ± 1.0	26.3 ± 0.8‡
Relative to FFM (ml O_2/min/kg FFM)	41.3 ± 3.7	40.0 ± 1.1	42.1 ± 0.8(‡)
Body composition			
BMI (kg/m^2)	31.6 ± 1.1	28.8 ± 1.3	28.7 ± 1.2
TBM (kg)	98.3 ± 4.7	79.7 ± 3.5*	79.3 ± 3.4
FFM (kg)	61.5 ± 3.2	48.8 ± 1.8*	48.7 ± 1.8
Fat mass (kg)	36.8 ± 2.1	30.6 ± 2.5	30.2 ± 2.5
Fat percentage (%)	38.0 ± 1.5	38.8 ± 2.0	38.5 ± 2.1
Glycemic control			
Fasting glucose (mmol/l)	7.7 ± 0.5	6.9 ± 0.4	7.1 ± 0.4
Fasting insulin (pmol/l)	119 ± 35	72 ± 10	83 ± 10
Two-hour OGTT glucose (mmol/l)	13.1 ± 1.3	14.4 ± 0.7	14.1 ± 0.7
Mean OGTT glucose	13.0 ± 0.7	13.4 ± 0.6	13.2 ± 0.6
HbA1c (mmol/mol)	47.7 ± 2.4	50.1 ± 2.5	50.5 ± 2.3
Thyroid hormones			
TSH (×10^{-3} IU/L)	1.8 ± 0.3	1.6 ± 0.2	1.7 ± 0.2
Triiodothyronine (nmol/l)	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1
Thyroxine (nmol/l)	96.7 ± 6.1	87.3 ± 2.7	89.5 ± 2.9

Data are numbers (n) or mean ± SEM.

GLP-1, glucagon-like peptide-1; DPP-4, dipeptidyl peptidase-4; SGLT2, sodium-glucose cotransporter-2; RMR, resting metabolic rate; VO_2max, maximal oxygen consumption rate; TBM, total body mass; FFM, fat-free mass; BMI, body mass index; OGTT, oral glucose tolerance test; HbA1c, hemoglobin A1c; TSH, thyroid-stimulating hormone.

*Statistical differences are indicated by *($p < 0.05$, Study 1 vs. Study 2 pre, Student's unpaired t-test) and ‡($p < 0.05$, Study 2 pre vs. study 2 post, Student's paired t-test).*

(‡)indicates $p < 0.10$.

Post-intervention investigations were in Study 1 initiated 39–43 h after the last exercise bout (in CWT/IWT interventions), and in Study 2 at least 48 h after the last exercise bout.

Analyses and Calculations

Fasting blood samples were centrifuged (2,000 g, 15 min, 4°C). Lithium-heparin plasma was analyzed for thyroid hormones (thyroid-stimulating hormone, triiodothyronine, and thyroxine) and insulin *via* Electrochemiluminescence immunoassay (Cobas 8000, Roche Diagnostics, IN, USA). EDTA plasma was analyzed for HbA1c *via* absorption photometry (Tosoh G7; Tosoh, San Francisco, CA, USA).

Mean oxygen uptake and carbohydrate excretion rates were calculated from the indirect calorimetric measurements. RMR was calculated according to the equations by Weir (31).

Statistics

First, intervention-induced effects on RMR were compared using two-way (time × intervention) repeated-measures (RM) ANOVA (Study 1) and Student's paired *t*-test (Study 2).

Next, simple linear regression analyses between potential determinants of RMR (VO_2max, body composition, and glycemic control variables) and RMR were performed on baseline data (both studies) and on post–pre intervention (delta) values (Study 2). To avoid regression toward the mean, all delta values were controlled for baseline values, and this did not change the results of the regression analyses.

Finally, due to large between-subject heterogeneity in RMR responses in Study 2, subjects were stratified into three groups according to the intervention-induced effect on RMR as (1) decreased RMR (≥ 10% decrease); (2) unchanged RMR; (3) increased RMR (≥10% increase). The specific cutoff levels were chosen to ensure that subjects categorized in group 1 and 3 with certainty had intervention-induced alterations in RMR and that the differences measured were not just due to imprecision of the measurements or biological day-to-day variation (30, 32). Stratified analyses were performed as one-way ANOVA of baseline values (to assess baseline differences between strata), as one-way RM ANOVA of delta values between strata (to assess differential changes in potential determinants of RMR between strata), and as two-way (time × stratification) RM ANOVA's (to assess differential changes in potential determinants of RMR within each strata).

Data are reported as mean ± SEM or delta values with confidence intervals (CI). All analyses were performed using Prism v6.03 (Graphpad Software, CA, USA) and statistical significance was accepted when $p < 0.05$.

RESULTS

Baseline data are given in **Table 1**. $N = 14$ subjects were included in Study 1 with all subjects being included in the analyses. $N = 32$ subjects were included in Study 2, but only 23 subjects underwent RMR measurements. As such, $N = 37$ subjects were overall included in the current analyses. No subjects changed glucose-lowering medication during the study period. In Study 1, glucose-lowering medication was continued unchanged during the entire study, whereas, in Study 2, glucose-lowering medication was paused from 2 days before each experimental day and until the end of the experimental day.

Training Data

In Study 1, training adherence (amount of training performed relative to prescribed) was 99% in both CWT and IWT. As previously published (28), mean oxygen consumption and heart rates were comparable between CWT and IWT, whereas fast and slow

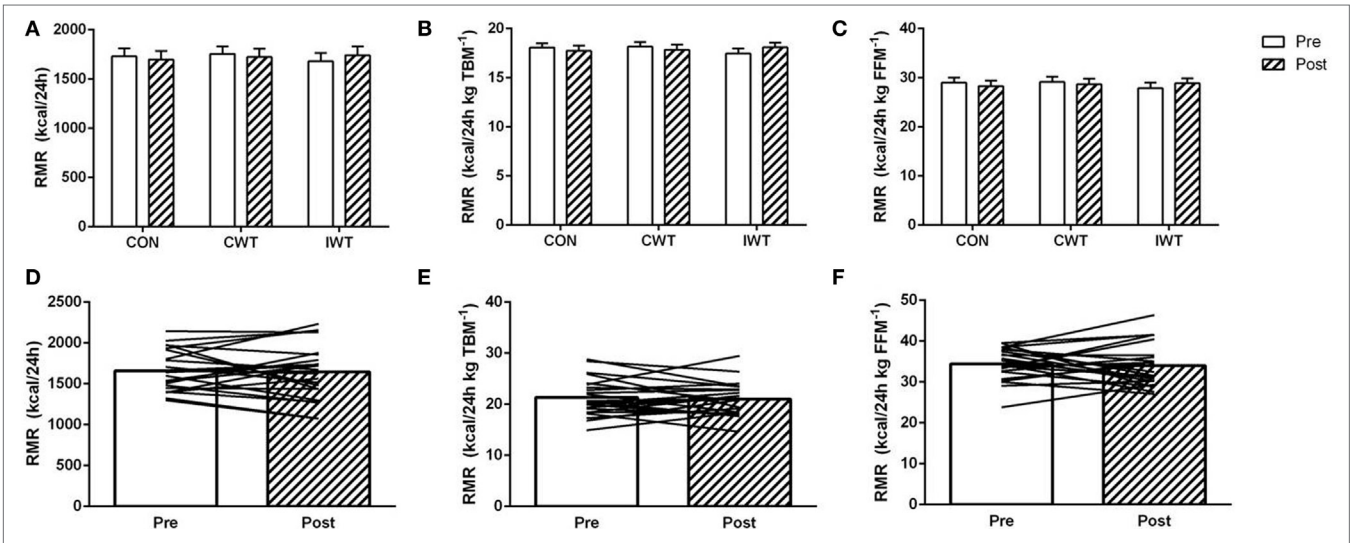

FIGURE 1 | The effect of training interventions on resting metabolic rate (RMR). Subjects with type 2 diabetes underwent three 2-week interventions **(A–C)**; no training [control (CON)], continuous walking training (CWT), interval-walking training [IWT or 12 weeks of IWT training **(D–F)**]. RMR was measured before and after interventions and is reported as total RMR **(A,D)**, RMR relative to total body mass [TBM **(B,E)**] and RMR relative to fat-free mass [FFM **(C,F)**]. Data are shown as mean ± SEM **(A–C)** and mean + individual data **(D–F)**. Statistical analyses [two-way repeated-measures ANOVA in Panels **(A–C)**] and Student's paired *t*-test **(D–F)** did not result in any significant changes within interventions ($p > 0.05$ for all comparisons).

IWT intervals were performed with higher and lower oxygen consumption and heart rates, respectively, compared to CWT.

In Study 2, the mean uploaded IWT time was 68 ± 9 min/week, corresponding to 75% of the minimal volumes of prescribed training. There were, however, substantial between-subject differences in uploaded IWT time, with six individuals uploading less than 30% of the minimal volumes of prescribed training. If excluding these six, apparently non-adherent, subjects from the analyses, mean uploaded IWT time was 85 ± 7 min/week, corresponding to 94% of the minimal amounts of prescribed training. It was not possible to assess training intensity from the uploaded data.

Intervention-Induced Effects on RMR

In Study 1, no effect of any intervention was found on RMR [delta CON = −33 (95% CI: −122 to 57) kcal/24 h, delta CWT = −32 (95% CI: −122 to 58) kcal/24 h, delta IWT = 62 (95% CI: −28 to 152) kcal/24 h, $p > 0.05$ for all comparisons] (**Figure 1**). Likewise, in Study 2, no overall intervention-induced change in RMR was found [delta IWT = −13 (95% CI: −125 to 98) kcal/24 h, $p > 0.05$], and exclusion of the subjects who were apparently non-adherent to the training ($n = 6$), did not change this. Moreover, no association was seen between uploaded IWT time and changes in RMR ($r^2 = 0.05$, $p = 0.34$) However, intervention-induced changes in RMR varied considerably between subjects (**Figures 1D–F**). As such, $n = 7$ subjects decreased RMR (≥10%), $n = 9$ subjects did not change RMR and $n = 7$ subjects increased RMR (≥10%) with the intervention. Normalization of RMR to total body mass (TBM) or FFM did not alter the above-standing results.

Potential Determinants of RMR

In Study 1, no intervention-induced effects on physical fitness or body composition was seen with any of the interventions ($p > 0.05$ for all comparisons, data not shown) (**Table 1**). Conversely, and as previously described (28), measures of glycemic control (mean

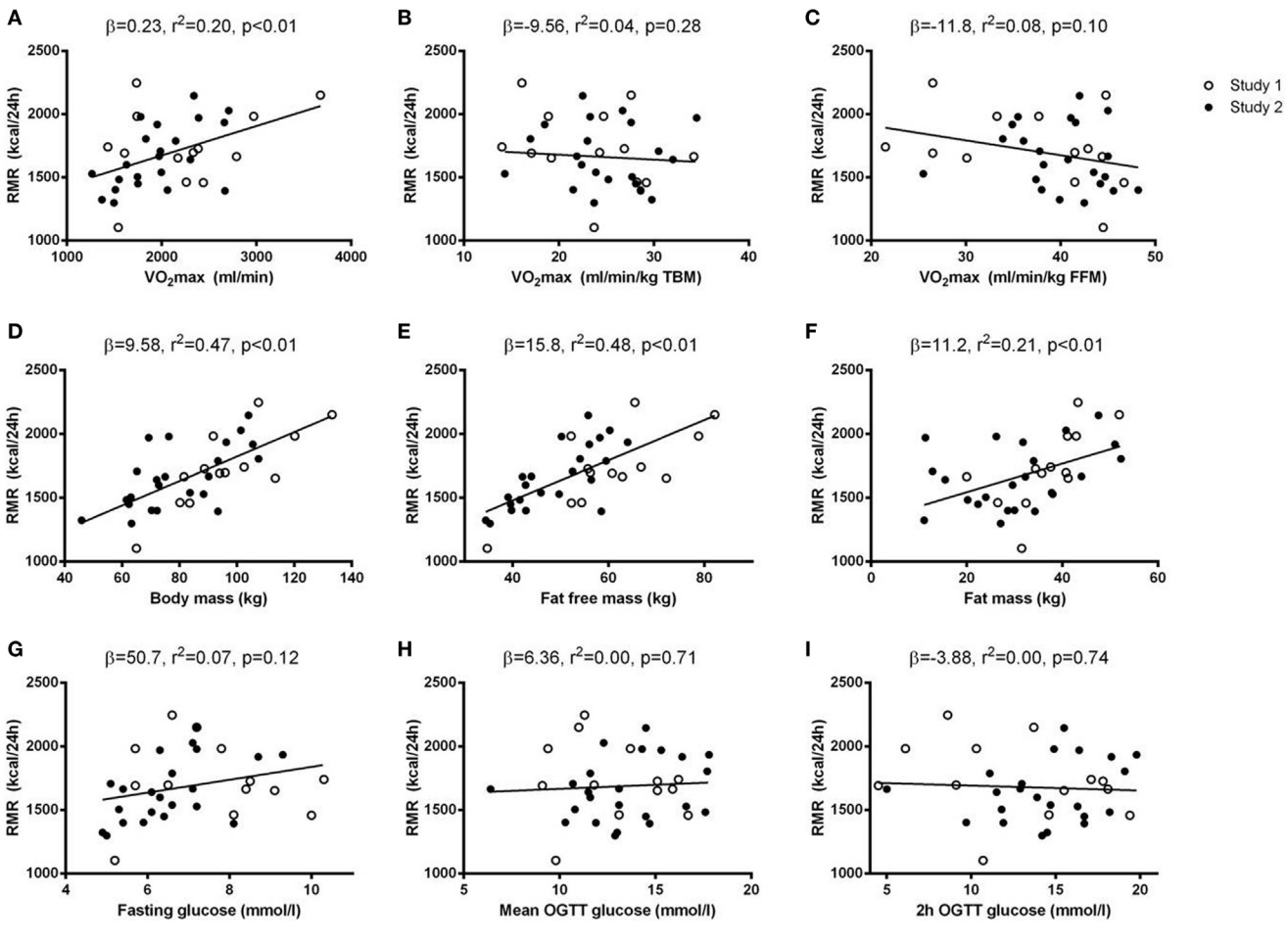

FIGURE 2 | Baseline associations between resting metabolic rate (RMR) and potential determinants of RMR. Simple regression analyses were performed between baseline values of potential determinants of RMR (*x*-axis) and baseline RMR (*y*-axis). The potential determinants were VO_2max [absolute, relative to total body mass (TBM), and relative to fat-free mass (FFM) **(A–C)**], body composition [body mass, FFM, and fat mass **(D–F)**] and glycemic control [fasting glucose, mean oral glucose tolerance test (OGTT) glucose, and 2 h OGTT glucose **(G–I)**]. Data from both Study 1 (open circles) and Study 2 (black circles) were included in the regression analyses and results (β-coefficients, r^2-, and *p*-values) are given in each panel.

and maximum 24 h glucose levels) were improved with IWT, with no effects of CON or CWT.

In Study 2, physical fitness improved with the intervention [delta VO_2max = 104 (CI: 11–197) ml/min, $p < 0.05$], whereas neither body compositional nor glycemic control variables improved with the intervention (**Table 1**, $p > 0.05$ for all comparisons).

Associations between Potential RMR-Determinants and RMR

Baseline levels of VO_2max were positively correlated with RMR (**Figures 2** and **3**). When normalizing VO_2max to body mass or FFM, however, the association disappeared. No significant associations between delta values in VO_2max and RMR were seen.

Body compositional variables (TBM, FFM, and fat mass), were all positively correlated with RMR at baseline. When analyzing delta values, the associations between TBM/fat mass and RMR were maintained, whereas no association between FFM and RMR was seen. The association between changes in fat mass and RMR was maintained when a sequential correction for changes in the other potential RMR-determinants was performed.

No associations between glycemic control variables (fasting glucose, mean OGTT glucose, 2 h OGTT glucose, HbA1c) and RMR were seen, neither at baseline nor when analyzing delta values.

Changes in Potential RMR-Determinants in Stratified Analyses

No baseline differences in RMR or any potential determinants of RMR (measures of VO_2max, body compositional variables,

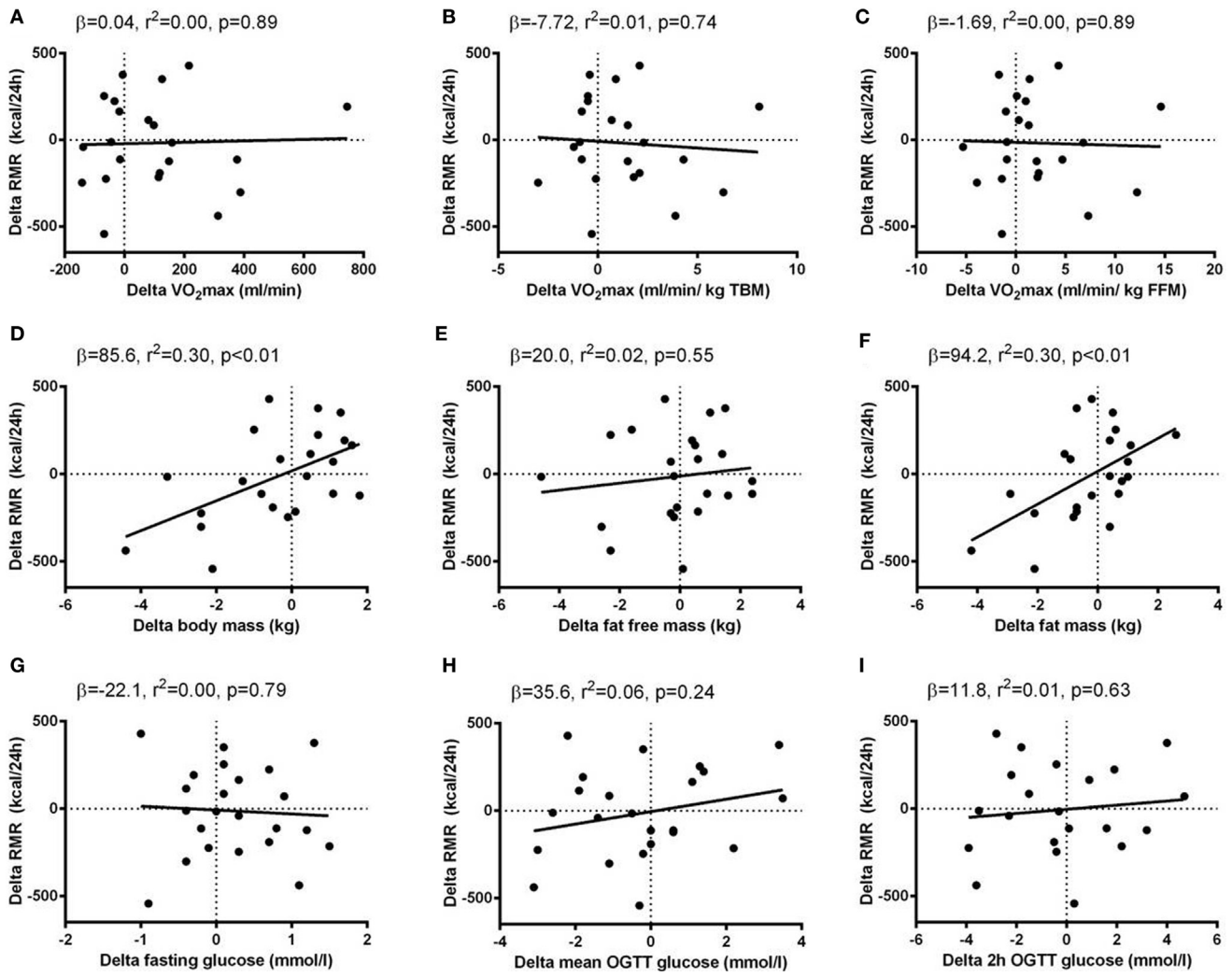

FIGURE 3 | Associations between delta (post minus pre intervention) values of resting metabolic rate (RMR) and delta values of potential determinants of RMR. Simple regression analyses were performed between delta values of potential determinants of RMR (*x*-axis) and delta values of RMR (*y*-axis). The potential determinants were VO_2max [absolute, relative to total body mass (TBM) and relative to fat-free mass (FFM) **(A–C)**], body composition [body mass, FFM and fat mass **(D–F)**] and glycemic control [fasting glucose, mean oral glucose tolerance test (OGTT) glucose, and 2 h OGTT glucose **(G–I)**]. Data from Study 2 were included in the regression analyses and results (β-coefficients, r^2-, and *p*-values) are given in each panel.

glycemic control variables) were seen between strata ($p > 0.05$ for all comparisons) (**Table 2**; **Figure 4**).

For measures of VO_2max and glycemic control, no intervention-induced changes within strata were seen, nor were there any intervention-induced differences between strata.

An intervention-induced reduction in body mass was seen in subjects who also decreased RMR, and a between-strata difference in body mass was seen between subjects who decreased and subjects who increased RMR ($p < 0.05$ for both).

Whereas the results for fat mass mirrored those seen for TBM, no differences within or between strata was seen for FFM.

Hormone Levels

In Study 1, no intervention-induced effects on fasting insulin or thyroid hormones were seen with any of the interventions (data not shown, $p > 0.05$ for all comparisons).

In Study 2, fasting insulin and thyroid hormones did not change with the intervention, and likewise, no differences were seen in the stratified analyses (data not shown, $p > 0.05$ for all comparisons).

DISCUSSION

The most important finding of this study is that neither short-term continuous or interval-based training nor longer term interval-based training altered RMR in subjects with T2D as long as the training did not alter body composition. Body composition, both FFM and fat mass, were important determinants for RMR at baseline, but, interestingly, only training-induced changes in fat mass and not in FFM were associated with training-induced changes in RMR. This was supported by the stratified analyses, were subjects with a training-induced loss of fat mass had an accompanying decrease in RMR.

The lack of training-induced changes in RMR is in line with most previous studies. Both in healthy subjects (13, 14) and in subjects with T2D (17, 18), it is most commonly reported that RMR does not change with a training intervention. However, some studies have found increased RMR after a training intervention (10, 12, 16). Whereas parts of the explanation for the conflicting findings may be due to different training modalities (33), and differential changes in body composition, it is also possible that the post-intervention RMR measurement has been performed too early after the last exercise bout in some studies, implying that EPOC has been included in the measurement (6). Whereas we did not see any significant changes in RMR in any of the two studies in the primary analyses, a paired *t*-test indicated a tendency for increased RMR with IWT in Study 1 ($p = 0.06$). Since EPOC is increased with IWT compared to both CON and CWT (23), and since our RMR measurements were performed ~40 h after the last exercise bout, it is possible that the tendency for increased RMR seen with IWT in Study 1 in fact was prolonged EPOC (8).

In contrast to previous observations (19, 20), we did not find any indication that glycemic control affected RMR. Increased RMR has mainly been reported in subjects with dysregulated diabetes (5) and the subjects included in our studies had a fairly good glycemic control both at baseline and after the

TABLE 2 | Stratified analyses in Study 2.

	Decreased RMR		Unchanged RMR		Increased RMR	
	Pre	Post	Pre	Post	Pre	Post
RMR						
Total (kcal/24 h)†#	1,616 ± 102	1,308 ± 69*	1,751 ± 85	1,735 ± 95	1,586 ± 78	1,871 ± 92*
Relative to body mass (kcal/24 h/kg TBM)#	36.4 ± 0.9	30.0 ± 0.6*	34.9 ± 1.2	34.4 ± 1.7	31.8 ± 1.7	37.6 ± 2.1*
Relative to FFM (kcal/24 h/kg FFM)#	23.7 ± 1.4	19.7 ± 1.2*	21.6 ± 1.0	21.4 ± 1.0	18.7 ± 1.1	21.9 ± 1.4*
Physical fitness (VO_2max)						
Absolute (ml O_2/min)‡	1,681 ± 88	1,721 ± 154	2,141 ± 135	2,225 ± 151	2,034 ± 190	2,171 ± 146
Relative to body mass (ml O_2/min/kg TBM)‡	24.8 ± 1.6	25.3 ± 1.0	28.2 ± 2.2	29.2 ± 2.3	24.0 ± 2.2	25.2 ± 1.4
Relative to FFM (ml O_2/min/kg FFM)(‡)	38.0 ± 1.0	40.5 ± 2.3	43.3 ± 2.2	43.9 ± 2.3	40.4 ± 3.0	43.1 ± 1.2
Body composition						
Body mass (kg)#	70.1 ± 7.0	68.4 ± 6.4*	82.0 ± 5.1	81.9 ± 4.8	86.4 ± 5.7	87.0 ± 5.6
FFM (kg)	44.6 ± 3.2	43.9 ± 3.1	50.6 ± 3.0	51.1 ± 3.0	50.6 ± 3.3	50.5 ± 3.1
Fat mass (kg)#	25.4 ± 5.0	24.0 ± 4.6*	30.9 ± 4.2	30.7 ± 4.2	35.2 ± 3.4	35.9 ± 3.4
Fat percentage (%)	36.0 ± 3.9	35.2 ± 4.0	38.2 ± 3.8	37.9 ± 3.9	42.2 ± 2.1	42.7 ± 2.0
Glycemic control						
Fasting glucose (mmol/l)	6.2 ± 0.5	6.5 ± 0.6	6.5 ± 0.2	6.7 ± 0.2	8.0 ± 1.2	8.2 ± 1.0
2 h OGTT glucose (mmol/l)(†)	15.5 ± 0.9	14.5 ± 1.4	12.4 ± 1.4	12.6 ± 1.0	15.8 ± 1.2	15.7 ± 1.1
Mean OGTT glucose (mmol/l)(†)	13.8 ± 0.9	13.0 ± 1.3	12.2 ± 0.9	11.9 ± 0.6	14.7 ± 1.1	15.1 ± 0.7
HbA1c (mmol/mol)	49.9 ± 4.6	48.6 ± 3.4	47.1 ± 1.2	48.6 ± 1.6	53.9 ± 6.2	54.7 ± 6.4

Subjects with type 2 diabetes underwent a 12-week interval-walking training intervention, with measurements of resting metabolic rate (RMR), physical fitness, body composition, and glycemic control before (Pre) and after (Post) the intervention. Subjects were stratified according to the intervention-induced change in RMR as decreased RMR (≥10%), unchanged RMR or increased RMR (≥10%), and the intervention-induced changes in potential determinants of RMR were analyzed using a two-way repeated-measures ANOVA for within-strata changes. Data are presented as mean ± SEM.

*Statistical differences are indicated by ‡(main effect of time), †(main effect of stratification), #(time × stratification interaction), *(within group, pre vs. post, $p < 0.05$). Parenthesis indicates $p < 0.10$.*

TBM, total body mass; FFM, fat-free mass; OGTT, oral glucose tolerance test; HbA1c, hemoglobin A1c.

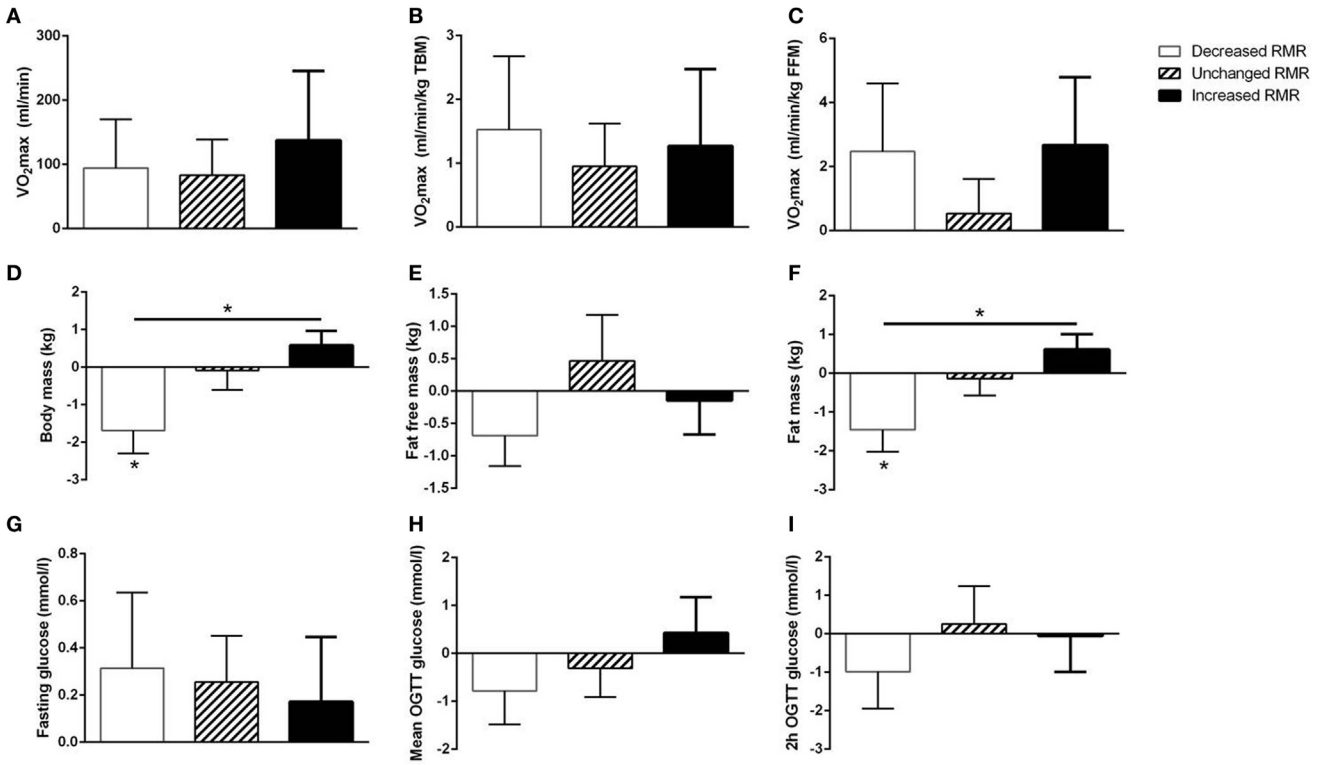

FIGURE 4 | Stratified analyses of potential determinants for resting metabolic rate (RMR). Subjects included in Study 2 were stratified according to their change in RMR as increased RMR (≥10%, $n = 7$), unchanged RMR ($n = 9$), or decreased RMR (≥10%, $n = 7$). Delta (post minus pre intervention) values ± SEM of potential determinants for RMR {VO_2max [absolute, relative to total body mass (TBM), and relative to fat free mass (FFM); panel **(A–C)**], body composition [body mass, fat free mass, and fat mass; panel **(D–F)**], and glycemic control [fasting glucose, mean OGTT glucose, and 2 h OGTT glucose; panel **(G–I)**]} are shown for the different strata. Within-strata changes in potential determinants of RMR were analyzed by two-way (strata × time) repeated-measures (RM) ANOVA (significant changes indicated by an asterisk above the bar) and between-strata differences were analyzed by one-way RM ANOVA of the delta values (significant changes indicated by a connecting line and an asterisk).

interventions; potentially too good to affect RMR. Also, the previously reported positive correlation between VO_2max and RMR (9, 15), was not replicated in our data when VO_2max relative to body weight or FFM was used, neither for baseline values nor for intervention-induced changes. Since an association between changes in VO_2max and glycemic control has previously been described in subjects with T2D (34), it would hypothetically be possible that subjects who increased VO_2max the most also improved glycemic control the most, and that the combined and opposing effect of these determinants resulted in no changes in RMR. However, since no associations were seen between changes in VO_2max/glycemic control and changes in RMR and since no associations were found between changes in VO_2max and changes in glycemic control (data not shown), we find this unlikely.

The strong baseline associations we found between FFM and fat mass on one side and RMR on the other side have previously been reported (35). Interestingly, when comparing intervention-induced changes in Study 2, the association between FFM and RMR disappeared, whereas the association between fat mass and RMR persisted. Moreover, subjects who decreased RMR with the training intervention also lost fat mass. Whereas FFM is considered to be the primary determinant for RMR and training-induced changes in RMR are most often explained by changes in FFM (4, 8), it has also been reported that a training-induced loss of fat mass may "overrule" the effect of an increase in FFM on RMR since this combination has been shown to decrease RMR (36). The mechanisms underlying these results cannot readily be derived from our data. It is generally believed, however, that the body responds to a weight loss with a homeostatic energy sparring, which is mainly seen as decreased RMR dependent on reductions in hormones like insulin and triiodothyronine (37) and reduced activity of the sympathetic nervous system (38). This has mainly been shown for a weight loss arising from dietary energy restriction (39–41), but may also be seen when at least parts of the weight loss is mediated *via* increased physical activity (42, 43). Whereas we did not see any changes in insulin or thyroid hormones with any of the training interventions, we did not measure sympathetic nervous system activity. Since changes in sympathetic nervous system activity are closer associated with changes in fat mass than with changes in FFM (44), it is plausible that the subjects who lost fat mass in Study 2 had a reduction in RMR due to a decreased activity of the sympathetic nervous system.

While Study 1 was fully supervised efficacy trial with high training adherence, Study 2 was a free-living effectiveness trial

without supervision. Whereas the training adherence in Study 2, in terms of volume, was fairly good compared to other free-living training studies (45, 46), the training adherence, in terms of intensity, was not possible to assess. Since no overall changes in glycemic control and body composition were seen in Study 2, it must be speculated how good the training adherence was. Subjects improved their VO_2max, however, indicating that some effect of the training was seen. Still, when compared to our previous study, where 17 weeks of IWT resulted in considerable weight loss and improvements in glycemic control and a much greater increase in VO_2max (22), it is clear that subjects included in the current Study 2 had suboptimal training responses. The prescribed volume of IWT (at least three times a week with at least 30 min duration pr. session) in the current Study 2 was approximately 1/3 of the previously prescribed training volume (22), indicating that at least training volume is important for the metabolic improvements seen. As such, and given that higher training volume and intensity (24) and greater improvements in VO_2max (9, 15) may be central for increases in RMR, it may be speculated whether a more intense training intervention would have resulted in different RMR results.

Given that the analyses in this paper were secondary to other analyses (27, 28) and that no power calculations were performed for RMR outcomes, it may be speculated whether the negative findings were a result of a lack of power. This is further important since a large heterogeneity was seen for changes in RMR. As such, despite the overall analyses indicated that RMR was unaffected by the training interventions, no final conclusions can be drawn from this paper.

In summary, neither short-term continuous or interval-type training, nor longer term interval training affects RMR in subjects with T2D when no overall changes in body composition are seen. Whereas both FFM and fat mass are important determinants of RMR at baseline, only training-induced changes in fat mass and not in FFM seem to be important for training-induced changes in RMR.

AUTHOR CONTRIBUTIONS

KK designed the studies, analyzed and interpreted the data, and wrote the manuscript. MR-L contributed to the study design. KK, CB, IT, and JN researched the data. All authors reviewed and revised the manuscript, approved the final version, and agreed to be accountable for the content of the work.

ACKNOWLEDGMENTS

We thank all participants for their participation. Laura Staun Valentiner (CopenRehab, Department of Public Health, Section of Social Medicine, University of Copenhagen) is acknowledged for technical assistance.

FUNDING

The Centre for Physical Activity Research (CFAS) is supported by a grant from TrygFonden. During the study period, the Centre of Inflammation and Metabolism (CIM) was supported by a grant from the Danish National Research Foundation (DNRF55). The study was further supported by grants from Diabetesforeningen, Augustinusfonden, Fonden til Laegevidenskabens Fremme, and Krista og Viggo Petersens Fond. CIM/CFAS is a member of DD2—the Danish Center for Strategic Research in Type 2 Diabetes (the Danish Council for Strategic Research, grant no. 09-067009 and 09-075724). MR-L was funded by a post doc grant from the Danish Diabetes Academy (through the Novo Nordic Foundation).

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

The reviewer, OS, and handling editor declared their shared affiliation, and the handling editor states that the process nevertheless met the standards of a fair and objective review.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 11 July 2017
doi: 10.3389/fendo.2017.00154

Glycemic and Metabolic Effects of Two Long Bouts of Moderate-Intensity Exercise in Men with Normal Glucose Tolerance or Type 2 Diabetes

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

Received: 24 February 2017
Accepted: 20 June 2017
Published: 11 July 2017

Citation:
Eshghi SR, Fletcher K, Myette-Côté É, Durrer C, Gabr RQ, Little JP, Senior P, Steinback C, Davenport MH, Bell GJ, Brocks DR and Boulé NG (2017) Glycemic and Metabolic Effects of Two Long Bouts of Moderate-Intensity Exercise in Men with Normal Glucose Tolerance or Type 2 Diabetes. Front. Endocrinol. 8:154. doi: 10.3389/fendo.2017.00154

Background: The glycemic and insulinemic responses following 30–60 min of exercise have been extensively studied, and a dose–response has been proposed between exercise duration, or volume, and improvements in glucose tolerance or insulin sensitivity. However, few studies have examined the effects of longer bouts of exercise in type 2 diabetes (T2D). Longer bouts may have a greater potential to affect glucagon, interleukin-6 (IL-6) and incretin hormones [i.e., glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP)].

Aim: To examine the effect of two bouts of long-duration, moderate-intensity exercise on incretins, glucagon, and IL-6 responses before and after exercise, as well as in response to an oral glucose tolerance test (OGTT) conducted the following day.

Methods: Twelve men, six with and six without T2D, participated in two separate conditions (i.e., exercise vs. rest) according to a randomized crossover design. On day 1, participants either rested or performed two 90 min bouts of treadmill exercise (separated by 3.5 h) at 80% of their ventilatory threshold. All participants received standardized meals on day 1. On day 2 of each condition, glucose and hormonal responses were measured during a 4-h OGTT.

Results: On day 1, exercise increased IL-6 at the end of the first bout of exercise (exercise by time interaction $p = 0.03$) and GIP overall (main effect of exercise $p = 0.004$). Glucose was reduced to a greater extent in T2D following exercise (exercise by T2D interaction $p = 0.03$). On day 2, GIP and active GLP-1 were increased in the fasting state ($p = 0.05$ and $p = 0.03$, respectively), while plasma insulin and glucagon concentrations were reduced during the OGTT ($p = 0.01$ and $p = 0.02$, respectively) in the exercise compared to the rest condition for both healthy controls and T2D. Postprandial glucose was elevated in T2D compared to healthy control ($p < 0.05$) but was not affected by exercise.

Conclusion: Long-duration, moderate-intensity aerobic exercise can increase IL-6. On the day following exercise, fasting incretins remained increased but postprandial insulin and glucagon were decreased without affecting postprandial glucose. This long duration of exercise may not be appropriate for some people, and further research should investigate why next day glucose tolerance was unchanged.

Keywords: aerobic exercise, glucose tolerance, glucagon, insulin, glucagon-like peptide-1, glucose-dependent insulinotropic peptide

INTRODUCTION

Exercise recommendations for the prevention and treatment of type 2 diabetes (T2D) emphasize exercise prescriptions designed to target insulin sensitivity or body composition (1, 2). These outcomes have been extensively studied, and it is generally recognized that typical exercise-induced changes in body composition are modest and that changes in insulin sensitivity are short lived (1, 2). Evidence to support, adapt, and fine-tune these recommendations are rapidly accumulating. The most recent (November 2016) position statement of the American Diabetes Association on Physical Activity/Exercise and Diabetes (2) currently recommends:

- To enhance insulin action: daily exercise or at least not allowing more than 2 days to elapse between exercise sessions.
- For optimal glycemic and health outcomes: adults with T2D should ideally perform both aerobic and resistance exercise training.
- To prevent or delay the onset of T2D in populations at high risk and with prediabetes: structured lifestyle interventions that include at least 150 min/week of physical activity and dietary changes resulting in weight loss of 5–7%.

While exercise interventions based on this paradigm clearly contribute to meaningful reductions in the incidence of diabetes (3, 4) or hyperglycemia (5, 6), an unintended consequence of this success may have been a substantially smaller emphasis on the effects of exercise on other pathophysiologic disturbances present in T2D. For example, Defronzo (7) proposed an "ominous octet" of potential pathophysiologic targets that also includes an increased glucagon secretion and a decreased incretin effect. The effects of exercise on many of these other outcomes are largely unknown in people with T2D.

Insight regarding how exercise could potentially affect glucagon or incretins in T2D may be obtained from studies in other populations. For example, repeated long bouts (e.g., two bouts of 90 min) of moderate-intensity exercise performed on the same day have been shown to lead to reductions in glucagon and other counter-regulatory hormones, as well as reductions in sympathetic nerve activity, which persist until at least the next day in people with type 1 diabetes (T1D) (8) and in healthy participants (9). This has been studied as part of the concept known as hypoglycemia-associated autonomic failure or HAAF (10). Reduced glucagon responses may be problematic in T1D who can experience hypoglycemia in response to exercise or excess insulin and has been studied more extensively. However, T2D and impaired glucose tolerance are characterized by impaired postprandial suppression of glucagon and could potentially benefit from non-pharmacological reductions in glucagon (11–13).

Incretin hormones, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), are secreted from the gastrointestinal tract into the portal circulation in response to nutrients. In a nutrient-dependent manner, incretins have been shown to contribute to lowering blood glucose by increasing insulin secretion, decreasing glucagon secretion, and decreasing the rate of gastric emptying [as reviewed by Drucker (14)]. On the other hand, GIP can increase glucagon secretion when glucose is low (15). The GLP-1 receptor has been found in cardiac muscle, smooth muscle of the vasculature, and perhaps skeletal muscle (16, 17). These findings, combined with the established heart rate (HR) increasing effect of GLP-1 (18), suggest that incretins could play a role in cardiometabolic responses to exercise.

Ellingsgaard et al. (19) have shown that increased interleukin-6 (IL-6) during exercise could stimulate secretion of GLP-1 from intestinal L cells and also pancreatic alpha cells. As reviewed by Pedersen (20), it is likely that increased circulating IL-6 during exercise is secreted directly by skeletal muscle and is proportional to the amount of glycogen depletion. *In vivo* human studies in healthy, obese, or T2D have often not observed an effect of exercise on incretin concentrations (21, 22), whereas a study in healthy runners observed increased GLP-1 following a marathon (23). It is unclear if this difference among human studies is due to differences in exercise duration or volume.

The effects of exercise clinical relevant outcomes such as glycated hemoglobin have been extensively studied (6, 24). The objective of this study was to examine the effect of two bouts of long-duration, moderate-intensity exercise on biomarkers, such as plasma glucagon, IL-6, GLP-1, and GIP. It was hypothesized that, compared to rest, two bouts of long-duration (i.e., 90 min) moderate-intensity exercise would increase plasma IL-6 and incretin hormone concentrations in T2D and in healthy participants. On the day after the exercise or rest conditions, participants returned to the laboratory for a 4-h oral glucose tolerance test (OGTT) and it was hypothesized that two bouts of long-duration moderate-intensity exercise would reduce the following day glucagon concentrations immediately before (fasted state) and during the OGTT. These objectives were examined using large amounts of exercise as a proof of concept with the understanding that this large amount of exercise (i.e., 3 h in a single day) is unlikely for most people and unsafe for some.

MATERIALS AND METHODS

Research Design

The experimental design involved two conditions that each required visits to the laboratory on two consecutive days (i.e., a total of four visits). On day 1 of each condition, participants were assigned to either exercise or control (i.e., rest) according to a randomized crossover design (**Figure 1**). On day 2 of each condition, participants return to the lab following an overnight fast for a 4-h OGTT. The 2-day exercise and control conditions were separated by at least 2 weeks.

Participants

Twelve men, six without diabetes and six with physician diagnosed T2D, were recruited for this study. Men were selected since they have higher glucagon concentrations in response to various stimuli (e.g., exercise or hypoglycemia) (25) and previous exercise studies of this nature have shown larger reductions in counter-regulatory responses in men (22). To minimize heterogeneity in T2D and the risk of hypoglycemia with prolonged exercise, participants were required to be treated with lifestyle intervention(s) and metformin only. In order to be eligible, all participants also had to be non-smokers and not taking any beta-blockers. Furthermore, participants were excluded if they had cardiovascular or orthopedic limitations to exercise, or felt they would be unable to walk for 90 min without interruption. Many of the participants with T2D were recruited from our previous exercise studies (26, 27) and were purposefully identified due to their above average level of fitness as potential volunteers due to the long bouts of walking required in the present study. Comparable cohorts of men without diabetes had not previously been studied in our lab, therefore, recruited a convenience sample of healthy counterparts with similar body mass indices.

This study was carried out in accordance with the recommendations of the Tri-Council Policy Statement on "Ethical Conduct for Research Involving Humans" with written informed consent from all subjects. The protocol was approved by the University of Alberta Health Research Ethics Board.

Baseline Assessment

Participants attended a baseline visit to measure glycated hemoglobin (A1c; DCA Vantage™ A1C Analyzer, Siemens Medical Solutions, Malvern, PA, USA), resting metabolic rate (RMR), and perform a graded submaximal exercise test with indirect calorimetry (TrueMax metabolic measurement system, Parvo Medics, Salt Lake City, UT, USA). HR was measured using a Polar heart rate monitor (Polar Electro, Finland). The submaximal exercise test was performed according to a modified Balke–Ware treadmill protocol where each participant walked at a self-selected speed, determined as comfortable but brisk, while the grade was increased by 1% each minute. The test was ended shortly after participants reached their individual ventilatory threshold (VT) using the V-slope criteria (28) as determined by a trained exercise physiologist.

Once eligibility was confirmed and baseline assessments were performed, a 1-month exercise habituation phase was completed by every participant that included three sessions of exercise per week at 80% of their VT. The duration began with 30 min and gradually progressed until participants could walk for 90 min continuously.

Experimental Protocol

On day 1 of each condition, participants arrived at lab at 08h00 after a minimum 10-h overnight fast. They were asked to avoid vigorous exercise the day before each testing condition. An intravenous catheter was inserted into an antecubital vein and was kept patent with sterile saline. The exercise condition contained

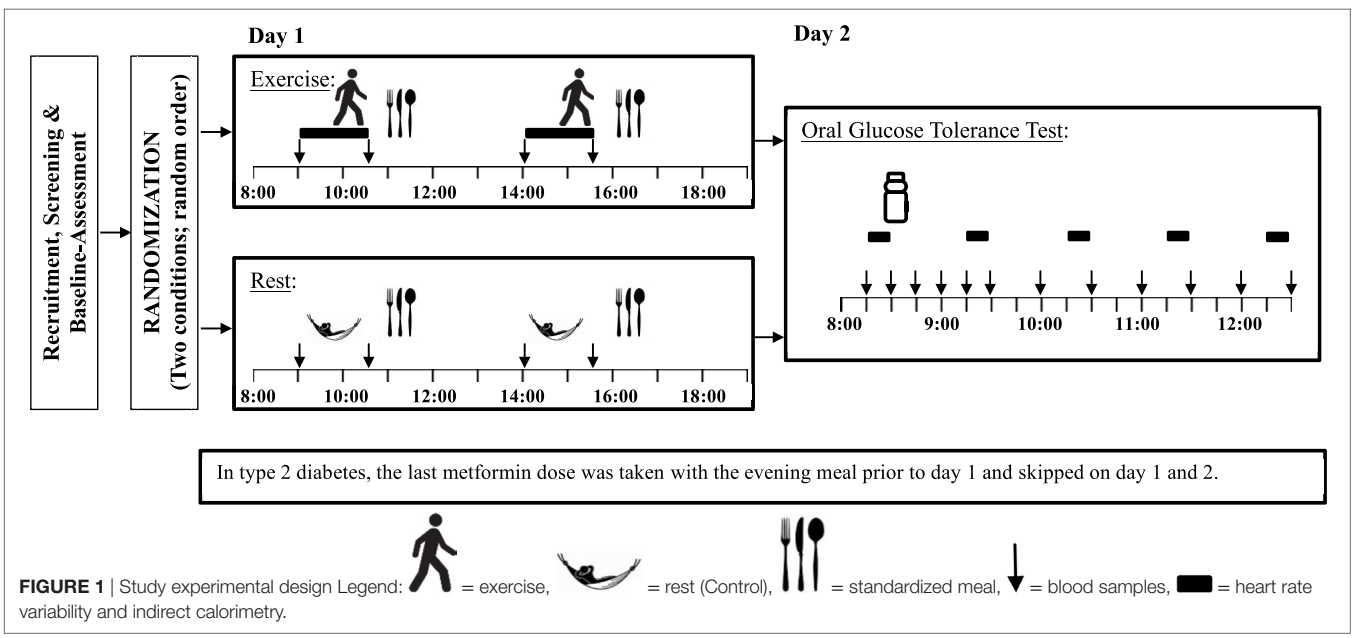

FIGURE 1 | Study experimental design Legend: variability and indirect calorimetry.

two 90-min bouts of treadmill exercise at intensity of 80% of the previously determined VT, as described in previous studies (9, 29). The first exercise bout began at 09h00 in the fasted state and the second at 14h00. Blood samples were taken immediately before and after each bout of exercise. Indirect calorimetry and HR measurements were collected during the first and last 10 min of each 90-min bout of exercise. During the non-exercise condition, participants remained sedentary but the above measures (except for HR) were collected at the same times as during the corresponding exercise condition.

The energy intake required to maintain energy balance during non-exercise condition was estimated based on participants' previously measured RMR multiplied by a physical activity level (PAL) of 1.4 (Note: this PAL is typically used to characterize a sedentary lifestyle (30)). As in previous studies of this nature (31, 32), energy intake was kept the same on the exercise and non-exercise conditions. Energy intake was divided in two equal standardized meals (59% carbohydrate, 22% fat, 19% protein) provided 30 min after each exercise bout (see **Figure 1**). As such, the first exercise bout was performed in the fasting state and the second bout started 150 min after the first meal.

On day 2, participants returned to the lab after a minimum 10-h fast. Two fasting blood samples were taken; one 15 min before and the other immediately before the beginning of the OGTT containing 75 g of glucose (Trutol, Thermo Fisher Scientific, Canada). Ten blood samples were collected at specific time points following consumption of the glucose beverage (i.e., 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min). Oxygen consumption and carbon dioxide production were collected for 10 min before the OGTT and for the last 10 min of each of the next 4-h periods (see **Figure 1**) using the same metabolic measurement system. Respiratory exchange ratio (RER) was determined as ratio between carbon dioxide production and oxygen consumption, while energy expenditure was calculated assuming non-protein energy equivalents. A metabolic equivalent (MET) was calculated as 1 kcal/kg/h (33). Participants were asked sit continuously throughout the test, with the exception of a bathroom break if required. Appetite rating and HR were collected during the same intervals. Appetite ratings were measured by a 150 mm visual analog scale and included questions on hunger, fullness, prospective food consumption, and desire to eat something sweet, salty, or fatty (34). HR was measured using a standard three-lead ECG over the same intervals as for the indirect calorimetry. Heart rate variability (HRV) is a tool that can be used to investigate the sympathetic and parasympathetic function of the autonomic nervous system. Autonomic nervous system activity can be affected by both hypo- and hyperglycemia. HRV indices included the root mean squared of the successive differences between R–R intervals (rMSSD), the SD of the R–R intervals (SDRR), and the ratio of low frequency spectral power to high frequency spectral power. For both day 1 and day 2, the first and last minutes of the 10-min indirect calorimetry and HR periods were excluded to allow for more stable data.

Participants with T2D refrained from taking their metformin dose on all four testing days. The last metformin dose was consumed more than 12 h prior to first blood sample which was taken on day 1 of each testing condition.

Blood Samples

Each blood sample was first collected into a 10-mL EDTA vacutainer tube. Subsequently, 2.0 mL was transferred into a tube with 20 μL of a dipeptidyl peptidase (DPP-4) inhibitor (Millipore, MA, USA), 2.0 mL was transferred into a tube with 6.7 μL aprotinin (Millipore, MA, USA), and 0.25 mL whole blood was transferred into 1.0 mL ice-cold 8% perchloric acid. Aprotinin was added to inhibit proteases known to interfere with the determination of glucagon. The DPP-4 inhibitor was added to prevent degradation of active GLP-1. Perchloric acid was added to deproteinize the samples. The EDTA tubes were centrifuged at 1,500 × *g* for 10 min at 4°C. The tubes containing perchloric acid and aprotinin were centrifuged at 2,000 × *g* for 15 min at 4°C. Following centrifugation, the samples were immediately moved to a −80°C freezer until assays were completed.

Non-esterified fatty acids (NEFAs) were analyzed using commercially available kits (Wako Diagnostics, CA, USA), while plasma glucose and lactate were determined enzymatically using spectrophotometric assays. Total GIP, glucagon and insulin were measured using a Multi-Spot® Assay System with a Sector® Imager 2400 (Meso Scale Discovery®, MD, USA). Active GLP-1 was measured separately (Meso Scale Discovery®, MD, USA). Hematocrit was measured only on day 1 for both exercise and non-exercise conditions. Plasma IL-6 was also measured from day 1 plasma samples using a high-sensitivity ELISA (Quantikine HS human IL-6, R&D Systems Ltd., Abingdon, UK). Plasma metformin concentrations were assessed by high performance liquid chromatography in all plasma samples from day 1 as well as fasting samples from day 2. The concentration of phosphate solution used in the mobile phase was 20 mmol/L. The metformin assay was validated to a lower limit of quantitation of 8 ng/mL metformin based on 0.1 mL of human plasma (35). All assays were run in duplicate and the average of the two was reported.

Statistical Analysis

The primary analyses were conducted using a three-way mixed factorial design ANOVA with *Diabetes* as a between group factor (i.e., T2D vs. healthy control), as well as *Exercise* (i.e., exercise vs. rest conditions) and *Time* (i.e., consecutive blood samples) as repeated measures factors. The number of levels for the time factor differed depending on the time period examined (e.g., day 1 had four consecutive blood samples). For day 2, The *Diabetes* by *Exercise* by *Time* ANOVA showed a significant effect of *Time* for all of the blood sample results (all $p < 0.01$). Therefore, it was deemed more informative to separate the fasting from the post glucose beverage results. For the 10 blood samples taken at different intervals postprandially, we considered both the area under the curve (AUC) and incremental AUC (iAUC). The AUC was calculated by the trapezoid method. The iAUC was calculated by subtracting the average of the two fasting values from the AUC. For the variables that were measured at 1-h intervals postprandially (e.g., calorimetry, HRV, and appetite) the four postprandial values were averaged. Age was a known confounder for HRV

and was significantly associated with our HRV outcomes; we, therefore, considered age as a covariate for the statistical analyses on HRV. For each ANOVA, we examined interaction effects and main effects, but did not conduct the many possible *post hoc* comparisons due to lack of statistical power. Sphericity was tested using Mauchly's test of sphericity. In the events where Mauchly's sphericity test was significant the Greenhouse–Geisser correction was used.

Baseline characteristics were compared between groups using independent *t*-tests. Secondary analyses also examined the bivariate correlations among variables (e.g., IL-6 vs. GLP-1). Statistical tests were two-tailed, and *p*-values ≤ 0.05 were considered significant. Statistical analyses were performed with SPSS 21 (SPSS, Inc., Chicago, IL, USA).

RESULTS

Participants

All 12 participants (6 T2D and 6 healthy) completed the study. Baseline characteristics are presented in **Table 1**. T2D and healthy participants had an average age of 60.5 ± 8.5 and 42.5 ± 10.5 years ($p < 0.01$) and an average body mass index (BMI) of 24.8 ± 4.3 and 26.7 ± 3.2 kg/m^2 ($p = 0.39$), respectively.

TABLE 1 | Baseline characteristics.

	T2D	Healthy	*p*-Value
n	6	6	–
Age (years)	60.5 ± 8.5	42.5 ± 10.5	0.009
BMI (kg/m^2)	24.8 ± 4.3	26.7 ± 3.2	0.39
Body weight (kg)	75.5 ± 16.2	81.6 ± 10.2	0.45
Duration of T2D (years)	3.9 ± 2.3	–	–
A1c (%)	6.4 ± 0.3	5.6 ± 0.1	<0.001
VO_2@VT (mL/kg/min)	28.5 ± 5.6	37.2 ± 8.4	0.06
SBP (mmHg)	125 ± 14	131 ± 11	0.44
DBP (mmHg)	75 ± 10	71 ± 5	0.45

T2D, type 2 diabetes; BMI, body mass index; A1c, glycated hemoglobin; NA, not applicable; NS, not significant; VT, ventilatory threshold; SBP, systolic blood pressure; DBP, diastolic blood pressure; RMR, resting metabolic rate.
Data presented as mean ± SD.

All T2D participants had a well-controlled glycemia as suggested by their A1c (6.4 ± 0.3%). They were treated with 500 to 1,500 mg of metformin per day and the average duration of diabetes diagnosis was 3.9 ± 2.3 years.

Day 1

Energy Expenditure and HR

All participants completed both 90-min exercise bouts without requiring adjustments to the exercise intensity. Indirect calorimetry and HR results from the exercise bout and corresponding rest conditions are presented in **Table 2**. As expected, energy expenditure and RER were significantly increased with exercise (main effect of *Exercise* $p < 0.001$). Energy expenditure corresponded to approximately one MET on the rest day and seven METs during exercise with no significant difference between T2D and healthy participants (see **Table 2** for details). In addition to an increased RER with exercise, a significant *Exercise* by *Diabetes* interaction ($p = 0.036$) and an *Exercise* by *Time* interaction ($p < 0.001$) were observed for RER. These interactions were the result of RER being lower on the rest day in the healthy participants and after lunch in the exercise condition but greater after lunch in the rest condition. During the exercise bouts, HR averaged 121 ± 3 beats per minute during the first 10 min of each bout of exercise, or 72 ± 2% of age predicted maximum HR, and drifted upwards throughout exercise (main effect of time $p = 0.001$).

Plasma Samples

Results of blood sample analyses from day 1 are summarized in **Table 3** and **Figure 2**. There was a significant *Time* by *Diabetes* interaction ($p = 0.03$) suggesting that glucose changed to a greater extent in T2D over time. In addition, there was a significant main effect of *Exercise* leading to lower overall glucose concentrations in the exercise condition ($p = 0.02$).

There was a significant *Exercise* by *Time* interaction for IL-6 ($p = 0.03$). Visual Inspection of the graph in **Figure 2** suggests that exercise increased IL-6 compared to rest when performed in the fasting state but not when performed after lunch when IL-6 was increased overall compared to fasting. The IL-6 responses

TABLE 2 | Indirect calorimetry and HR at the beginning and at the end of two bouts of exercise or control (i.e., rest) on day 1.

		Healthy				T2D				*p*-Value
		9h00–9h10	10h20–10h30	2h00–2h10	3h20–3h30	9h00–9h10	10h20–10h30	2h00–2h10	3h20–3h30	
RER (VCO_2/VO_2)	Ex	0.87 ± 0.04	0.84 ± 0.02	0.90 ± 0.02	0.85 ± 0.03	0.89 ± 0.03	0.82 ± 0.03	0.89 ± 0.03	0.84 ± 0.04	Ex < 0.001
	Rest	0.76 ± 0.03	0.74 ± 0.05	0.83 ± 0.03	0.80 ± 0.03	0.79 ± 0.04	0.79 ± 0.05	0.86 ± 0.05	0.84 ± 0.06	Time < 0.001 Ex × T2D = 0.036 Ex × Time < 0.001
EE (METs)	Ex	7.25 ± 1.81	7.59 ± 1.65	7.29 ± 1.25	7.08 ± 1.14	6.78 ± 1.06	6.86 ± 1.01	6.56 ± 0.98	6.49 ± 1.19	Ex < 0.001
	Rest	0.93 ± 0.15	0.91 ± 0.11	1.12 ± 0.14	1.01 ± 0.10	0.86 ± 0.18	0.94 ± 0.11	1.08 ± 0.11	1.01 ± 0.16	
HR (bpm)	Ex	120 ± 13	135 ± 23	139 ± 23	145 ± 25	121 ± 6	139 ± 9	129 ± 9	141 ± 11	Time = 0.001
	Rest	NA	NA	NA	NA	NA	NA	NA	NA	

Outcomes were measured during the first 10 min and last 10 min of each bout of exercise (or rest).
T2D, type 2 diabetes; Ex, exercise; RER, respiratory exchange ratio; EE, energy expenditure; METs, metabolic equivalent (kcal/kg/h); HR, heart rate; bpm, beats per minute; NA, not available.
Data presented as mean ± SD. ANOVA examined main effect of exercise, diabetes, time, and their interactions. Only significant p-values are shown.

TABLE 3 | Concentrations of energy substrates and hormones before and after two 90-min moderate-intensity exercise bouts or rest on day 1.

		Healthy				T2D				*p*-value
		9h00 (pre-first bout)	10h30 (post-first bout)	2h00 (pre-second bout)	3h30 (post-second bout)	9h00 (pre-first bout)	10h30 (post-first bout)	2h00 (pre-second bout)	3h30 (post-second bout)	
Glucose (mmol/L)	Ex	4.7 ± 0.2	4.3 ± 0.3	4.8 ± 0.3	3.8 ± 0.2	6.1 ± 0.5	5.2 ± 0.2	8.8 ± 1.1	4.3 ± 0.3	Ex = 0.02
										Time, T2D < 0.001
	Rest	4.5 ± 0.3	4.7 ± 0.2	5.1 ± 0.8	4.5 ± 0.5	6.2 ± 0.6	6.2 ± 0.5	9.3 ± 1.1	6.9 ± 1.0	Time × T2D = 0.03
Lactate	Ex	0.9 ± 0.2	1.1 ± 0.2	1.0 ± 0.2	1.0 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.2	Ex × Time = 0.05
(mmol/L)	Rest	1.0 ± 0.2	0.7 ± 0.0	1.1 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	
NEFA (mmol/L)	Ex	0.4 ± 0.1	1.2 ± 0.3	0.2 ± 0	1.1 ± 0.2	0.5 ± 0.1	1.4 ± 0.2	0.3 ± 0.1	1.1 ± 0.2	Ex, Time < 0.001
										Time × T2D = 0.02
	Rest	0.5 ± 0.1	0.5 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.6 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	Ex × Time < 0.001
Insulin	Ex	39.1 ± 8.9	20.8 ± 6.2	308.6 ± 81.7	23.9 ± 10.5	30.6 ± 9.5	36.3 ± 11.2	168.8 ± 48.5	37.7 ± 7.61	Time < 0.001
(pmol/L)	Rest	37.2 ± 7.6	32.5 ± 8.9	319.9 ± 103	58.9 ± 21.2	31.3 ± 8.8	24.6 ± 8.3	392.9 ± 159.7	20.6 ± 42	
Glucagon	Ex	54.7 ± 6.5	81 ± 14.7	79.1 ± 5.5	114 ± 16.1	71.3 ± 6.7	78.9 ± 6.3	97.3 ± 19	103.6 ± 9.9	Ex, Time < 0.001
(ng/L)	Rest	50.2 ± 6.9	49.8 ± 7.7	77.7 ± 6.2	62.8 ± 6.6	58.9 ± 5.9	47.3 ± 9.3	86.4 ± 13.8	69.2 ± 4.6	Ex × Time = 0.02

Outcomes were measured immediately before and immediately after of each bout of exercise (or rest). T2D, type 2 diabetes; Ex, exercise; NEFA, non-esterified fatty acids. Results presented as mean ± SEM. ANOVA examined main effect of exercise, diabetes, time, and their interactions. Only significant p-values are shown.

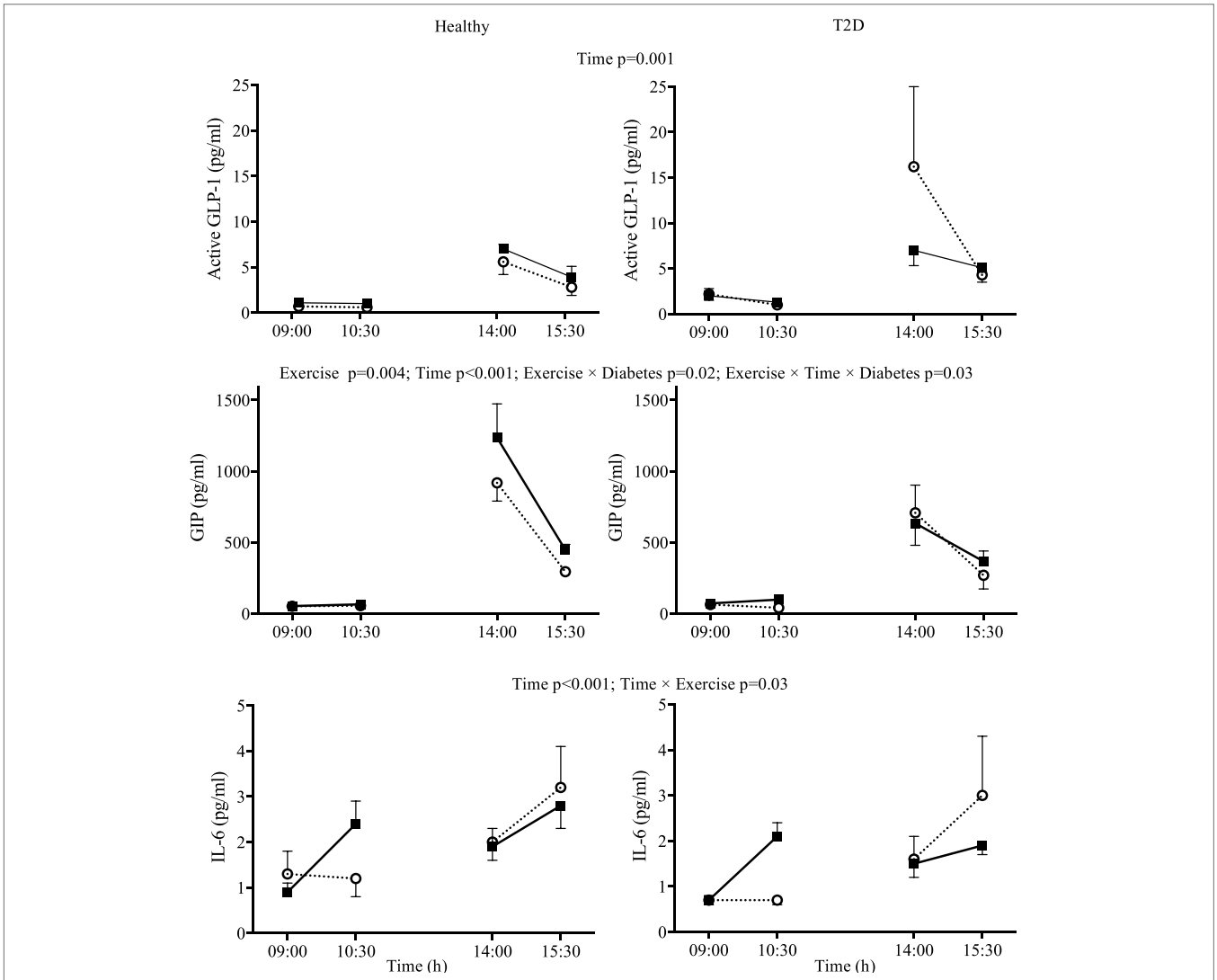

FIGURE 2 | Day 1 plasma concentrations for interleukin-6 (IL-6), active glucagon-like peptide-1 (GLP-1), and glucose-dependent insulinotropic peptide (GIP) in response to two 90-min bouts of exercise (■) vs. rest (○) in healthy participants (left panels) and in type 2 diabetes (T2D) (right panels). Data shown as mean ± SEM.

were similar for participants with and without T2D (**Figure 2**). GIP followed a similar pattern during the first exercise bout compared to rest, but increased to a greater extent in healthy participants following lunch, leading to an *Exercise* by *Time* by *Diabetes* interaction ($p = 0.03$). Overall plasma metformin concentrations decreased from 402 ± 120 to 191 ± 52 ng/ml (main effect of *Time* $p = 0.03$) throughout day 1 and were not affected by exercise.

Day 2

Energy Expenditure, HR, and Appetite

There were main effects of *Time* on energy expenditure and RER during the OGTT (both $p < 0.001$). There were no effects of *Exercise* or *T2D* on energy expenditure in the fasting state or postprandially. RER was greater in participants with diabetes but lower after exercise throughout day 2 (main effect of *T2D* and *Exercise,* both $p \leq 0.01$ see **Table 4**). There was no statistically significant effect of exercise or diabetes and HRV indices during the OGTT. Overall, ratings for prospective food consumption were higher during the OGTT from the exercise condition compared to the rest condition ($p = 0.03$). However, postprandial fullness decreases following exercise in T2D only (Diabetes by Exercise interaction $p = 0.04$ for fasting; $p = 0.056$ for the mean postprandial values). Participants with T2D had a lower desire to eat something sweet in the fasting ($p = 0.01$) and postprandial state ($p = 0.03$), but a Diabetes by Exercise interaction ($p = 0.045$) indicated that exercise tended to increase the desire to eat something sweet in T2D while decreasing this rating in healthy participants during the OGTT.

Plasma Samples

There were significant main effects of *Time* on all energy substrates and hormones on day 2. Therefore, analyses were conducted separately for the fasting and postprandial values. There was a main effect of *Diabetes* on fasting, AUC, and iAUC glucose (all $p < 0.05$) but no main effect of *Exercise* on the AUC and iAUC. However, there was a main effect of *Exercise* on fasting glucose ($p = 0.05$), with a 0.5 and 0.1 mmol/L decrease fasting glucose in the morning following exercise in the T2D and healthy control group, respectively (note: the *Diabetes* by *Exercise* interaction was not significant, $p = 0.35$), see **Figure 3**.

Exercise increased fasting glucagon in the healthy control group but not in T2D (*Exercise* by *Diabetes* interaction, $p = 0.02$), whereas the postprandial iAUC for glucagon was reduced by exercise (main effect of E*xercise*, $p = 0.01$). Fasting insulin was not affected by exercise but iAUC and AUC insulin were reduced (main effect of *Exercise*, $p = 0.08$, $p = 0.01$ and $p = 0.001$, respectively). In terms of the insulin:glucagon ratio, both the iAUC and AUC were reduced following exercise (main effect of *Exercise* $p = 0.04$ and $p = 0.004$, respectively), see **Figure 3**.

Fasting active GLP-1 and GIP concentrations showed a small increase with exercise (main effect of *Exercise*, both $p < 0.05$). Exercise on the previous day did not affect postprandial incretin hormones during the OGTT, see **Figure 3** and **Table 5**.

Upon arrival on day 2, fasting plasma metformin concentrations were very low and similar between exercise and rest (i.e., control) conditions (76 ± 17 to 83 ± 18 ng/ml, respectively, main effect of *Time,* $p = 0.03$).

Bivariate Correlations

There was no significant bivariate correlation between changes in IL-6, insulin or glucagon and changes in active GLP-1 or GIP, either when examining the participants with and without T2D together or separately on day 1. On day 2, there was an inverse association ($r = -0.60$, $p = 0.038$) between the exercise-induced changes in lactate and HRV as assessed by RMSSD. No associations were found between incretins and glucagon or insulin.

TABLE 4 | Indirect calorimetry and heart rate variability during fasting and following an oral glucose tolerance test on day 2.

		Fasting			Mean postprandial			ΔPostprandial		
		Healthy	**T2D**	***p***	**Healthy**	**T2D**	***p***	**Healthy**	**T2D**	***p***
RER (VCO_2/VO_2)	Ex	0.77 ± 0.04	0.73 ± 0.03	T2D < 0.01	0.82 ± 0.04	0.76 ± 0.02	T2D = 0.01	0.04 ± 0.03	0.03 ± 0.01	
	Rest	0.80 ± 0.03	0.76 ± 0.02		0.84 ± 0.03	0.80 ± 0.02	Ex < 0.01	0.05 ± 0.02	0.04 ± 0.02	
EE (METs)	Ex	0.85 ± 0.12	0.90 ± 0.11		0.91 ± 0.08	0.95 ± 0.12		0.05 ± 0.10	0.05 ± 0.05	
	Rest	0.85 ± 0.13	0.88 ± 0.08		0.91 ± 0.07	0.92 ± 0.11		0.06 ± 0.08	0.03 ± 0.07	
HR (bpm)	Ex	60 ± 5	59 ± 15		64 ± 4	61 ± 13		4 ± 2	2 ± 2	
	Rest	56 ± 5	61 ± 15		59 ± 4	62 ± 13		3 ± 1	1 ± 4	
RMSSD	Ex	51 ± 23	34 ± 15		40 ± 10	28 ± 11		−11 ± 12	−6 ± 10	
	Rest	60 ± 20	27 ± 14		49 ± 16	26 ± 11		−10 ± 5	−1 ± 9	
SDRR	Ex	80 ± 38	46 ± 17		66 ± 19	54 ± 26		−14 ± 23	8 ± 18	
	Rest	74 ± 25	46 ± 20		70 ± 20	48 ± 27		−1 ± 19	2 ± 13	
LF/HF	Ex	1.52 ± 1.14	0.91 ± 9.34		1.61 ± 0.72	1.32 ± 0.94		−0.04 ± 1.12	0.41 ± 1.17	
	Rest	0.77 ± 0.27	1.80 ± 1.03		1.27 ± 0.84	1.46 ± 1.28		0.53 ± 0.66	−0.34 ± 1.53	

T2D, type 2 diabetes; Ex, exercise; RER, respiratory exchange ratio; EE, energy expenditure; METs, metabolic equivalent or kilocalories divided by kilograms of body mass and hours (kcal/kg/h); HR, heart rate; bpm, beats per minute; SDRR, SD of the R–R intervals; rMSSD, root mean squared of the successive differences between R–R intervals; LF/HF, the ratio of low frequency spectral power to high frequency spectral power; mean postprandial, average from 10 min at the end of each of the four 1-h postprandial periods; ΔPostprandial, mean postprandial minus fasting. Data presented as mean ± SD. ANOVA examined main effect of exercise, diabetes, time, and their interactions. Only significant p-values are shown.

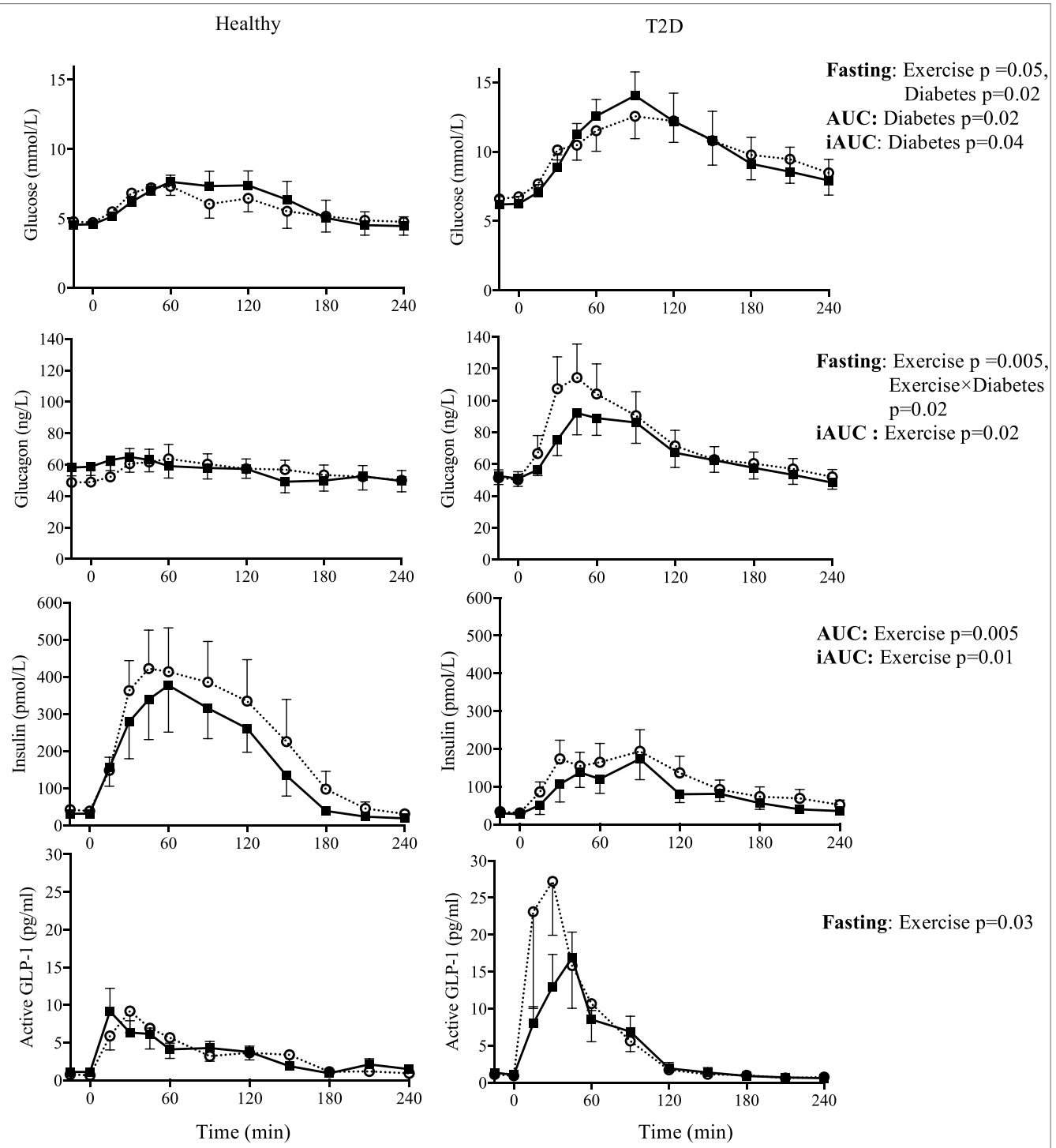

FIGURE 3 | Day 2 fasting plasma concentrations (−15 and 0 min) and responses to an oral glucose tolerance test (area under the curve = AUC; incremental AUC = iAUC) for glucose, glucagon, insulin, and active glucagon-like peptide-1 (GLP-1), the day after two 90-min bouts of exercise (■) vs. rest (○) in healthy participants (left panels) and in type 2 diabetes (T2D) (right panels). Results from 2 × 2 ANOVA showing main effects of exercise vs. rest, diabetes vs. control, and their interaction. Data shown as mean ± SEM.

DISCUSSION

To our knowledge, no other study in T2D has examined the glycemic, hormonal, and metabolic responses to exercise of such a high volume in a single day (i.e., 3 h walking). Although other studies have suggested a dose–response relationship between exercise duration and improvements in glucose tolerance or insulin sensitivity (36–38), we did not

TABLE 5 | Concentrations of energy substrate and hormones during fasting and following an oral glucose tolerance test on day 2.

		Fasting			iAUC			AUC		
		Healthy	T2D	*p*	Healthy	T2D	*p*	Healthy	T2D	*p*
Glucose	Ex	4.6 ± 0.1	6.2 ± 0.5	Ex = 0.05	372 ± 171	1,006 ± 264	T2D < 0.05	1,469 ± 190	2,499 ± 345	T2D < 0.05
(mmol/L)	Rest	4.7 ± 0.2	6.7 ± 0.6	T2D < 0.05	269 ± 138	902 ± 193		1,408 ± 185	2,504 ± 304	
Lactate	Ex	0.8 ± 0.1	0.8 ± 0	T2D < 0.05	38.8 ± 5.3	51.9 ± 11.5		221.4 ± 15.0	241.3 ± 9.8	Ex = 0.05
(mmol/L)	Rest	0.7 ± 0.1	1.0 ± 0.1	Ex × T2D < 0.05	49.6 ± 7.4	26.6 ± 12.2		222.9 ± 10.0	264.8 ± 15.3	
NEFA	Ex	0.7 ± 0.1	0.6 ± 0.1		−73.4 ± 23.2	−55.7 ± 8.9		90.3 ± 14.2	76.5 ± 13.1	
(mEq/L)	Rest	0.6 ± 0.1	0.5 ± 0.1		−75.4 ± 12	−48.1 ± 6.5		66.7 ± 5.2	72.0 ± 17.0	
Insulin	Ex	31.9 ± 7.6	28.7 ± 8.7		36,182 ± 9,974	13,992 ± 3,841	Ex = 0.01	43,836 ± 11,595	20,881 ± 5,203	Ex < 0.01
(pmol/L)	Rest	40.7 ± 9.2	32.6 ± 8.7		47,004 ± 14,486	20,144 ± 5,078		56,777 ± 16,265	27,958 ± 6,422	
Glucose-dependent insulinotropic peptide (pg/mL)		59.9 ± 7.2	68.7 ± 10.7	Ex = 0.05	43,630 ± 6,230	34,329 ± 7,530		58,016 ± 5,804	50,827 ± 7,208	
		55.1 ± 6.4	57.2 ± 7.6		39,297 ± 6,526	36,856 ± 4,640		52,515 ± 5,716	50,594 ± 4,441	

T2D, type 2 diabetes; Ex, exercise; AUC, area under the curve; iAUC, incremental area under the curve; T2D, type 2 diabetes; NEFA, non-esterified fatty acids. For all outcomes, there was a sigificant main effect of time. Data presented as mean ± SEM. ANOVA examined main effect of exercise, diabetes, and their interactions. Only significant p-values are shown.

observe any improvements in glucose tolerance following 3 h of exercise.

Unlike other studies in T2D or obesity which utilized shorter bouts of exercise (21, 22, 39), we observed elevated incretin hormones, particularly GIP, immediately after exercise (**Figure 2**). This increase persisted to the following day in the fasted state but not during the OGTT. It is unclear if these increases are practically meaningful as the increases were small in absolute terms and occurred at times when incretins were low. The increase was nonetheless consistent as we were able to detect these differences with a small sample size. In the participants with T2D who had relatively well-controlled glycemia, postprandial plasma incretin concentrations were not lower in T2D compared to healthy controls. While earlier studies suggested lower incretins in T2D, recent meta-analyses suggest that this is not always the case for both GIP (40) and GLP-1 (41). Another possibility to explain the strong incretin response (particularly for GLP-1) during the OGTT in our participants with T2D was that they were prescribed metformin, an oral hypoglycemic medication that has been shown to increase incretins (21). However, the last metformin dose had been consumed at least 36 h before the OGTT and metformin concentrations had been reduced less than 5% of the concentrations we observed in the hours following a morning dose of metformin (21). However, it is not known if the effect of long term metformin treatment could have persisted beyond 36 h.

According to Ellingsgaard et al. (19) an increased GLP-1 following exercise may be due to increased IL-6. Importantly, the increase in plasma IL-6 during exercise can be directly attributed to secretion from skeletal muscle and IL-6 is thought to be secreted in proportion to glycogen depletion [as reviewed by Pedersen (20)]. We observed that plasma IL-6 only increased compared to rest during the first exercise bout, which was performed in the fasting state and not during the second bout performed after lunch. The design of the present study does not allow us to conclude if the absence of an effect of exercise on plasma IL-6 after lunch was due to the meal itself or to a reduced effect when sequential exercise bouts are performed. However, a recent study found that consuming a carbohydrate beverage during 120 min of cycling abolished leg IL-6 release even though muscle glycogen was reduced to a similar extent compared to fasting exercise (42). IL-6 was also increased by lunch itself, which is consistent with previous studies (43, 44). Therefore, it appears that exercise-induced IL-6 secretion requires, or at least is more pronounced, with fasting exercise protocols.

A notable finding in the present study was that, in accordance with the hypothesis, two long bouts of exercise enhanced the postprandial suppression of glucagon (i.e., reduced iAUC). The postprandial suppression of glucagon is thought to be impaired in people with T2D (7). While both insulin and glucagon were lowered by exercise during the OGTT, insulin was reduced to a greater extent as reflected as a decrease in both the iAUC and AUC for the insulin:glucagon ratio. Insulin acts to suppress glucagon secretion; therefore, the observation of a lower glucagon in the presence of lower insulin is noteworthy since previous studies using hyperinsulinemic clamp protocols reported a reduced glucagon following exercise when insulin was maintained in the exercise and rest conditions (8, 9, 29). The mechanism by which this form of exercise suppresses postprandial glucagon concentration in T2D cannot be elucidated from this study and is indeed a topic of continued interest and debate (7, 45). Despite postprandial glucagon being reduced in our participants with T2D, postprandial hyperglycemia was not improved. While this may be disappointing from a clinical perspective, the similar concentrations of plasma glucose in both conditions may be considered fortuitous to examine changes in glucoregulatory hormones without needing to clamp glucose at a fixed concentration.

From a theoretical perspective, the absence of a glucose lowering effect of exercise during an OGTT performed on the following day in T2D was unexpected. It is generally believed that the glucose lowering effect of exercise is proportional to the duration or volume of exercise (37, 38). Although our study had a small sample size, the absence of the expected glucose lowering effect of exercise is unlikely to be due low statistical power as the post OGTT glucose AUC was slightly higher (1%)

in the exercise condition. The reasons for the unchanged glucose were unclear, although postprandial insulin was reduced suggesting improved insulin sensitivity. Other studies have documented an absence of improvement in OGTT following longer bouts of exercise. For example, Tremblay et al. observed an increase in glucose AUC during an OGTT performed 16 h after 90 min of cycling at 67% of VO_{2max} (46). They attributed this increased to an increased adipose tissue lipolysis, increased NEFA, and a decreased glucose oxidation (46). This explanation is consistent with our observation of a decreased RER (i.e., indicating a decreased carbohydrate oxidation) and a tendency for increased NEFA ($p = 0.09$) during the OGTT from the exercise condition.

A primary limitation of the present study was the small sample size. As a result, our study was underpowered to detect potentially meaningful effects of exercise or diabetes. Our randomized crossover design helped to reduce the impact of this limitation on statistical power when comparing the exercise and rest conditions. However, the between participant comparison of T2D ($n = 6$) to healthy control ($n = 6$) was particularly underpowered for some outcomes.

The validity of conclusions regarding comparisons between T2D and healthy controls was further impaired by these small subgroups which were not matched for possible confounders (e.g., age, BMI, and fitness). BMI and exercise-induced energy expenditure ended up being relatively similar between the healthy and T2D participants; however, the healthy control group was younger and likely had different body composition (e.g., more fat free mass). Age was not associated with most outcomes in our study with the notable exception of HRV. HRV was lower in our T2D participants but these differences were no longer significance after adjusting for age. Although not statistically significant, there were trends to suggest an increase in indices of HRV following exercise in T2D but not in healthy participants. In addition to detecting differences in glucose, our study was able to detect other expected differences between healthy participants and T2D [e.g., RER (47) and glucagon (11)]. Nonetheless, the primary contributions to be retained from this article should be in regards to the exercise vs. control comparison.

The participants with T2D that were recruited for our study were likely more fit, more physically active, and leaner than many people with T2D. Such participants were selected to increase the likelihood of completing the exercise protocol and reduce the risk of injury. However, this selection also introduces potential bias. The phenotypic differences in our participants could influence many of the hormonal and metabolic responses to exercise. For example, participants with lower fitness or greater adiposity may have a different inflammatory profile or different inflammatory response to exercise (48, 49). This could potentially reduce generalizability of our result.

Another limitation of the study was the reliance on plasma concentrations of hormones taken from peripheral blood samples. These concentrations from the systemic circulation often do not reflect the exposure of other organs to these hormones (e.g., pancreas or liver). In addition, our multiplex hormone assay used has limitations in regards to specificity. For example, the glucagon assay has been shown to have some cross-reactivity with glicentin (or to a lesser extent oxyntomodulin) (50).

In conclusion, exercise can affect a variety of pathological features that can contribute to hyperglycemia. Potential benefits include decreasing postprandial hyperglucagonemia and increasing incretin concentrations. The exercise protocol (i.e., two 90 min bouts of exercise) used in this study is likely not feasible for most people. Larger samples sizes and closer matching of participant characteristics would be required to more carefully address differences between participants with normal glucose tolerance and those with T2D. Future studies should seek to better understand if similar results can be obtained with shorter exercise protocols, as well as the persistency of the observed changes.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Tri-Council Policy Statement on "Ethical Conduct for Research Involving Humans" with written informed consent from all subjects. The protocol was approved by the University of Alberta Health Research Ethics Board.

AUTHOR CONTRIBUTIONS

NB, SRE, CS, MD, GP, PS, JL, and DB contributed to the conception of the study and obtained funding for this project. SRE, NB, and ÉM-C collected the data. SRE, NB, ÉM-C, KF, RG, and CD analyzed the data. SRE and NB drafted the manuscript, and all authors critically reviewed the manuscript to provide important intellectual content.

FUNDING

This study was funded by an Alberta Diabetes Institute Pilot Project Award. SRE and ÉM-C were supported by Alberta Diabetes Institute Graduate Scholarship and KF was supported by an Alberta Diabetes Institute Summer Studentship. JL was supported by a CIHR New Investigator Salary Award (MSH-141980).

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

frontiers in Physiology

ORIGINAL RESEARCH
published: 06 July 2017
doi: 10.3389/fphys.2017.00479

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

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

Received: 23 November 2016
Accepted: 22 June 2017
Published: 06 July 2017

Citation:
Álvarez C, Ramírez-Campillo R, Ramírez-Vélez R and Izquierdo M (2017) 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. 8:479. doi: 10.3389/fphys.2017.00479

Background: Exercise training improves performance and biochemical parameters on average, but wide interindividual variability exists, with individuals classified as responders (R) or non-responders (NRs), especially between populations with higher or lower levels of insulin resistance. This study assessed the effects of high-intensity interval training (HIIT) and the prevalence of NRs in adult women with higher and lower levels of insulin resistance.

Methods: Forty adult women were assigned to a HIIT program, and after training were analyzed in two groups; a group with higher insulin resistance (H-IR, 40 ± 6 years; BMI: 29.5 ± 3.7 kg/m^2; $n = 20$) and a group with lower insulin resistance (L-IR, 35 ± 9 years; 27.8 ± 2.8 kg/m^2; $n = 20$). Anthropometric, cardiovascular, metabolic, and performance variables were measured at baseline and after 10 weeks of training.

Results: There were significant training-induced changes [delta percent (Δ%)] in fasting glucose, fasting insulin, and homeostasis model assessment of insulin resistance (HOMA-IR) scores in the H-IR group (−8.8, −26.5, −32.1%, $p < 0.0001$), whereas no significant changes were observed in the L-IR. Both groups showed significant pre-post changes in other anthropometric variables [waist circumference (−5.2, $p < 0.010$, and −3.8%, $p = 0.046$) and tricipital (−13.3, $p < 0.010$, and −13.6%, $p < 0.0001$), supra-iliac (−19.4, $p < 0.0001$, and −13.6%, $p < 0.0001$), and abdominal (−18.2, $p < 0.0001$, and −15.6%, $p < 0.010$) skinfold measurements]. Systolic blood pressure decreased significantly only in the L-IR group (−3.2%, $p < 0.010$). Both groups showed significant increases in $1RM_{LE}$ (+12.9, $p < 0.010$, and +14.7%, $p = 0.045$). There were significant differences in the prevalence of NRs between the H-IR and L-IR groups for fasting glucose (25 vs. 95%, $p < 0.0001$) and fasting insulin ($p = 0.025$) but not for HOMA-IR (25 vs. 45%, $p = 0.185$).

Conclusion: Independent of the "magnitude" of the cardiometabolic disease (i.e., higher vs. lower insulin resistance), no differences were observed in the NRs prevalence with regard to improved HOMA-IR or to anthropometric, cardiovascular, and muscle performance co-variables after 10 weeks of HIIT in sedentary adult women. This research demonstrates the protective effect of HIIT against cardiometabolic disease progression in a sedentary population.

Keywords: high-intensity interval training, non-responders, insulin resistance, women

INTRODUCTION

Exercise training is a strategy for the prevention and treatment of several inactivity-related metabolic diseases, such as insulin resistance (Álvarez et al., 2014) and type 2 diabetes mellitus (T2DM) (Alvarez et al., 2016). Similarly, exercise-based interventions, including resistance training (RT), together with pharmacological, and dietary interventions, represent the cornerstones of T2DM management (ADA, 2011). In addition to the beneficial effects on glycemic control (Umpierre et al., 2013) and other risk factors of T2DM (Chudyk and Petrella, 2011; Figueira et al., 2014), physical exercise is effective in improving muscle strength (Dunstan et al., 2002), cardiovascular function (Cano-Montoya et al., 2016), and functional capacity (Cadore and Izquierdo, 2015). In this regard, combining RT and endurance training is an effective intervention to promote overall physical fitness in T2DM patients (Balducci et al., 2012). More recently, high-intensity interval training (HIIT, i.e., repeated short bursts of high intensity activity with rest breaks in between each bout of exercise) is a time-efficient exercise modality that has emerged as an alternative to continuous traditional endurance exercise training to improve cardiometabolic health (Gibala et al., 2012).

However, despite the frequent reports of "average" exercise-related changes, there is wide interindividual variability in the results of exercise training (Astorino and Schubert, 2014). Under the same stimulus, some subjects, termed responders (R), achieve benefits after training, while others, termed non-responders (NRs), show an unchanged or worsened response (Bouchard et al., 2012; Bonafiglia et al., 2016; Álvarez et al., 2017). In the literature, this phenomenon has been characterized using several terms, such as low/high responders (Davidsen et al., 2011), non-responders/responders (Sisson et al., 2009), or as an adverse response (Bouchard et al., 2012); in these studies, similar but slightly different methodological criteria have been applied for identifying R and NRs. Genetic (Stephens et al., 2015) and environmental factors (Bouchard and Rankinen, 2001) have been suggested to be responsible for this variability, although not all of the potential environmental factors (e.g., different health status, magnitude of disease, or different mode of exercise training) have been explored.

Furthermore, the prevalence of these unchanged or worsened responses, known as NRs prevalence (i.e., percentage of subjects who do not improve/show a worsened response with regard to a variable), has been reported predominantly after endurance training (Sisson et al., 2009; Bouchard et al., 2012) and RT (Moker et al., 2014; Churchward-Venne et al., 2015). There have been no studies reporting the NRs prevalence associated with risk factors for T2DM after HIIT, which has been shown to improve anthropometric, cardiovascular, metabolic, and performance variables in different cohorts (Astorino and Schubert, 2014; Alvarez et al., 2016). For example, in one study of insulin resistance adult women, there were reductions of −12 to −14% in fasting glucose, −27 to −37% in fasting insulin and ∼40% in homeostasis model assessment of insulin resistance (HOMA-IR) scores after 8 weeks of HIIT (Álvarez et al., 2014). In another study of T2DM subjects, there was a decrease of ∼14% in fasting glucose, with additional decreases of ∼4 mmHg in blood pressure, ∼2% in body mass, ∼4% in waist circumference, and ∼19% in subcutaneous fat after 16 weeks of HIIT (Alvarez et al., 2016). Notably, another study showed that only 2 weeks of HIIT decreased the average 24 h fasting glucose level by approximately −13% (Little et al., 2011). Finally, a study of subjects with poor glucose control showed an improvement (−12%) in the area under the curve for the oral glucose tolerance test (OGTT) results and a 4.2 kg decrease in fat mass after 10 weeks of HIIT (Mancilla et al., 2014).

Latin America has experienced an epidemiological transition characterized by an increasing burden of cardiometabolic disease due to physical inactivity and shifts in diet and lifestyle patterns (Rivera et al., 2014). Evidence in Chilean adults suggests similar associations between low physical activity levels and cardiometabolic risk factors and between health status and overweight/obesity (Vio et al., 2008). Thus, the aim of the present study was to assess the effects 10 weeks of HIIT and the NRs prevalence (as indicated by glucose control variables) in groups with higher and lower levels of insulin resistance. A second aim was to assess other anthropometric, cardiovascular, and performance variables. We hypothesized that independent of the magnitude of the metabolic disease [i.e., higher (HOMA-IR > 5.0) or lower (HOMA-IR < 3.0) levels of insulin resistance], there would be no differences in the NRs prevalence for changes in glucose control parameters after HIIT between women with higher

Abbreviations: T2DM, type 2 diabetes mellitus; R, responders; NRs, non-responders: HIIT, high-intensity interval training; H-IR: higher insulin resistance group; L-IR, lower insulin resistance group; BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; 1RM, one repetition maximum strength test; $1RM_{LE}$, one repetition maximum test of leg extension; $1RM_{UR}$, one repetition maximum test of upper row; HbA1c, glycated hemoglobin, OGTT, oral glucose tolerance test; VO_2peak, maximum peak of oxygen uptake, VO_2max, maximum oxygen uptake.

and lower levels of insulin resistance using the HOMA-IR criteria.

MATERIALS AND METHODS

Participants

The first stage of the study was to recruit, using a short telephone survey, adult patients who were previously identified at their last clinical exam as at risk for T2DM and who had dropped out from their regular appointments at the healthcare center. In this first stage, 168 sedentary adult women (aged ≥18 years) with no background of regular exercise training volunteered to be screened.

Screening and Preliminary Testing

First, subjects were screened for insulin resistance based on a HOMA-IR > 2.6 using both fasting glucose and fasting insulin, and after intervention the subjects were separated into two groups and analyzed based on differences in the "magnitude" of insulin resistance (i.e., a group with a higher level of insulin resistance: HOMA-IR > 5.0, and a group with a lower level insulin resistance: HOMA-IR < 3.0). In the first screening before intervention, 65 individuals classified as "higher insulin-resistance" subjects ($n = 65$) were excluded for multiple reasons (16 due to age > 40 years; 2 due to being recently physically active; 5 due to a diagnosis of hypertension; 5 due to a diagnosis of hypothyroidism; 3 due to musculoskeletal injury; 21 due to no history of T2DM; 6 due to stationary asthma/respiratory disease; and 7 due to having a rural address). Similarly, 25 subjects identified as "lower insulin-resistance" subjects ($n = 25$) were also excluded for similar reasons (6 due to age ≥40 years; 12 due to being physically active; 2 due to a diagnosis of hypothyroidism; and 5 due to having a rural address). Finally, 78 screened subjects with a higher level of insulin resistance ($n = 78$) were assigned to 10 weeks of a HIIT program and were analyzed after intervention in two different groups: a group with a higher level of insulin resistance (H-IR, $n = 20$) and a group with a lower level of insulin resistance (L-IR, $n = 20$). None of the subjects were taking oral hypoglycemic medications to improve metabolic control of glucose because they all had been recently diagnosed with insulin resistance by our research team. Subjects with < 70% attendance at training sessions were excluded from all statistical analyses after the intervention; after excluding those subjects, the characteristics of the analyzed subject groups were as follows: H-IR group, age 40 ± 6 years, $n = 20$; L-IR group, age 35 ± 9 years, $n = 20$ (see flow chart in **Figure 1**). The treatment allocation is described in the flow chart in **Figure 1**.

All participants were informed about the experimental procedures and about possible risks and benefits associated with participation in the study. Written informed consent was obtained before any of the assessments were performed. The study was conducted in accordance with the Declaration of Helsinki and was approved by the institutional review board for the use of human subjects of the local Ethics Committee of the University of los Lagos (Comité de Revisión Científica y Ética Institucional del Departamento de Ciencias de la Actividad Física de la Universidad de Los Lagos). Characteristics of the study participants are provided in **Tables 1**, **2**.

Eligibility criteria included the following: (a) diagnosed with insulin resistance based on the HOMA-IR metabolic marker method and using a cut-off point of HOMA-IR ≥2.6 in a Chilean population (Garmendia et al., 2009), (b) physical inactivity (≤150 min/week of low-moderate physical activity or <75 min/week of vigorous physical activity; O'Donovan et al., 2010), (c) no familial (parents/siblings) history of T2DM, (d) living only in urban areas, and (e) with care provided under the public Chilean healthcare system (i.e., not a private healthcare system). Exclusion criteria included participants with the following characteristics: (a) potential medical or musculoskeletal problems, (b) osteoarthritis, (c) history of ischemic disease, (d) arrhythmia, (e) asthma, (f) chronic obstructive pulmonary disease, and (g) utilization of drugs that modulate metabolic or respiratory control.

Classification of Responder (R) and Non-responders (NRs)

Using previous criteria applied in exercise-based interventions (Bonafiglia et al., 2016), the interindividual variability in the response to exercise training of the subjects was used to categorize them as responders (R) or non-responders (NRs) using the typical error measurement (TE). Thus, the TE was calculated for all independents variables 3 weeks before the pre-test measurements as described previously (Álvarez et al., 2017) using the following equation:

$$TE = SD_{diff}/\sqrt{2} \quad (1)$$

where SD_{diff} is the variance (standard deviation) of the difference in scores observed between the two repeats of each test. A non-responders participant for HOMA-IR assessments, as well as for all other included co-variables, was defined as an individual who failed to demonstrate a decrease or increase (in favor of beneficial changes) that was greater than two times the TE from zero. A change greater than two times the TE means that there is a high probability (i.e., 12 to 1 odds) that this response is a true physiological adaptation beyond what might be expected to result from technical and/or biological variability (Hopkins, 2000).

Procedures

Anthropometric and Cardiovascular Assessments

Anthropometric and blood pressure assessments were carried out during the first week of the allocation stage. Body mass was assessed using a digital scale with an accuracy of 0.1 kg (Omron HBF-INTTM, Omron Healthcare Inc., Lake Forest, IL, USA). Height was assessed with a professional stadiometer (Health o Meter™ Professional, Sunbeam Products Inc., Chicago, IL, USA) with an accuracy of 0.1 cm, and body mass index (BMI) was calculated according to the following formula: kg/m^2. Waist circumference was assessed with an inextensible measuring tape with and accuracy of 0.1 cm (HoechstmassTM, Sulzbach, Germany). Three skinfold measurements of subcutaneous adipose tissue (i.e., tricipital,

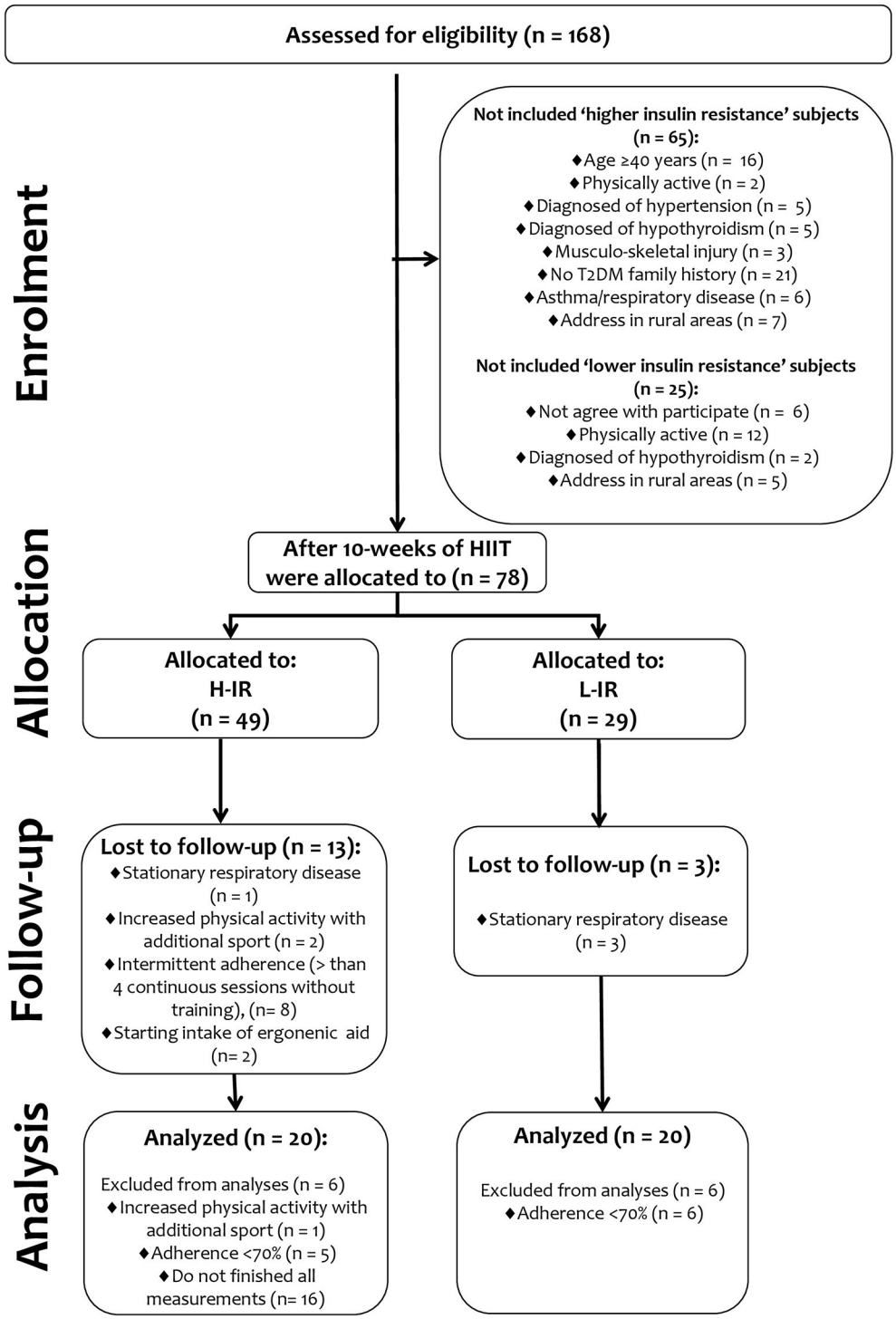

FIGURE 1 | Study design.

supra-iliac, and abdominal skinfolds) were assessed using a Langue™ skinfold caliper (Beta Technology Inc., Santa Cruz, California, USA) according to standard protocols (Marfell-Jones et al., 2006).

Systolic and diastolic blood pressure were assessed using an automatic monitor (Omron HEM 7114™, Omron Healthcare Inc., Lake Forest, IL, USA) in triplicate (2-min interval between measurements) after 15 min of rest and with the subjects in

a seated position following standard classification procedures (Chobanian et al., 2003).

Plasma Metabolic Markers

The metabolic measurements were carried out in the second week. Subjects arrived at the laboratory of the Riñihue clinic between 8 and 10 in the morning after a 10 h overnight fast. Blood samples (approximately 3.5 mL) were collected in tubes with specific anticoagulant gels for fasting glucose and fasting insulin measurements at baseline and at the 10 week follow-up. Samples were placed on ice and centrifuged at 4,000 rpm (1,700 × g) for 5 min at 4°C. Plasma samples were immediately transferred to pre-chilled microtubes and stored at −20°C for later analysis.

Plasma glucose was analyzed via enzymatic methods using standard kits (Wiener Lab Inc., Rosario, Argentina) on an automatic analyzer (Metrolab 2300 Plus™, Metrolab Biomed Inc., Buenos Aires, Argentina). Fasting insulin was measured via RIA (DPC, Los Angeles, CA, USA). The HOMA-IR index was calculated using the Matthews equation (Matthews et al., 1985): HOMA-IR = [Fasting glucose (mg/dL) × Fasting insulin (μU/dL)]/405). The same blood sampling and preparation procedure was performed at the end of the 10 week follow-up 48 h after the last exercise session in the morning to avoid possible acute effects of exercise.

Familiarization with the Exercise Training Program

In weeks 3 and 4, in three sessions, the subjects in both the H-IR and L-IR groups underwent a familiarization period for the HIIT protocol, as well as for the $1RM_{LE}$- and $1RM_{UR}$-tests. In the first and second sessions, the subjects were educated about the cycling machines and the free weights, as well as the exercise machine used for the strength test. In the following four sessions, the subjects underwent HIIT.

One-Repetition Maximum Test

In week 5, after a familiarization process with the test and before the intervention, both groups performed one-repetition maximum strength tests to establish $1RM_{LE}$- and $1RM_{UR}$-values as previously described (Izquierdo et al., 2004). The $1RM_{LE}$-test involved an exercise machine (OXFORD™, model EE4002, Santiago, Chile), and in the $1RM_{UR}$-test, free weights with bars were used. In brief, for the $1RM_{LE}$-test, the subjects began by lifting a load of weights on the machine with both legs. For the $1RM_{UR}$-test, the subjects adopted a body flexion angle of 90°, grabbed a bar with weights and plates, and with both arms extended, lifted the bar to approximately knee height. The highest load from three attempts per exercise was reported.

Experimental Protocol

The HIIT program was started in the sixth week and was performed three times per week, for a total of 30 sessions, using exercise bikes (OXFORD™, model BE2601, OXFORD Inc., Santiago, Chile). Each participant performed a range of 8–12 cycling intervals during the intervention period. The time of each cycling work interval was 60 s, with 120 s of passive rest (sitiing on the bicycle without movement) between work intervals. This rest period was progressively decreased (2 min weeks 1–2, 1.45

TABLE 1 | Anthropometric characteristic before and after 10-weeks of high-intensity interval training in a higher (H-IR), and lower insulin resistance adult women group (L-IR).

	H-IR baseline	H-IR 10-weeks	L-IR baseline	L-IR 10-weeks	*p*-Values†		*p*-Values†		*P*-values‡	*P*-values§
					H-IR pre-post	Δ% (ES)	L-IR pre-post	Δ% (ES)	H-IR baseline	H-IR 10-weeks
n =	20		20							
Age (years)	40 ± 6		35 ± 9							
Height (cm)	155 ± 0.04		158 ± 0.05							
Body mass (kg)	71.4 ± 9.4	69.1 ± 9.1	70.0 ± 7.3	67.8 ± 7.7	0.089	−3.2 (−0.23#)	0.061	−3.1 (−0.29#)	0.603	0.397
Body mass index (kg/m^2)	29.5 ± 3.7	28.6 ± 3.5	27.8 ± 2.8	27.0 ± 3.0	0.067	−3.0 (−0.24#)	0.189	−2.8 (−0.31#)	0.112	0.365
Waist circumference (cm)	98.8 ± 8.2	93.6 ± 8.0	99.7 ± 7.1	95.9 ± 6.7	**<0.010**	−5.2 (−0.61&)	**0.046**	−3.8 (−0.52#)	0.719	0.167
Tricipital skinfold (mm)	24.7 ± 7.2	21.4 ± 6.9	24.1 ± 6.3	20.8 ± 6.1	**<0.010**	−13.3 (−0.48#)	**<0.0001**	−13.6 (−0.57#)	0.772	0.399
Supra-iliac skinfold (mm)	31.4 ± 6.4	25.3 ± 7.3	33.7 ± 7.6	29.1 ± 8.1	**<0.0001**	−19.4 (−0.83&)	**<0.0001**	−13.6 (−0.55#)	0.309	0.112
Abdominal skinfold (mm)	40.5 ± 12.1	33.1 ± 9.4	33.2 ± 7.4	28.0 ± 6.0	**<0.0001**	−18.2 (−0.65&)	**<0.010**	−15.6 (−0.85&)	**0.028**	0.126

*Data are means and ± SD; Delta changes (Δ%) = (10-weeks * 100/baseline). ES, effect size. Bold values denotes significant differences in each respective comparison at (P ≤ 0.05).*

† Analyzed by Repeated Measures group × time.

‡ Analyzed by ANOVA one-way.

§ Analyzed by Bonferroni post-hoc.

 # Indicates "small" standardized ES at level p ≤ 0.05.

& Indicates "moderate" standardized ES at level p ≤ 0.05.

TABLE 2 | Cardiovascular, metabolic, and performance characteristics of the subjects before and after 10-weeks of high-intensity interval training in a group of adult women with a higher level of insulin resistance: HOMA-IR > 5.0 (H-IR), and a group with a lower level insulin resistance: HOMA-IR < 3.0 (L-IR).

	H-IR baseline	H-IR 10-weeks	L-IR baseline	L-IR 10-weeks	*p*-Values†		*p*-Values†		*p*-Values‡	*P*-values§
					H-IR pre-post	Δ% (ES)	L-IR pre-post	Δ% (ES)	H-IR vs. L-IR baseline	H-IR vs. L-IR post
n =	20		20							
CARDIOVASCULAR										
Systolic blood pressure (mmHg)	127 ± 4	124 ± 3	125 ± 4	121 ± 4	0.059	−2.3 (−1.09[&])	**<0.010**	−3.2 (−0.95[&])	0.065	0.134
Diastolic blood pressure (mmHg)	74 ± 6	73 ± 6	72 ± 4	71 ± 4	0.076	−1.3 (−0.29[#])	0.070	−1.3 (−0.16[#])	0.083	0.377
METABOLIC										
Fasting glucose (mg/dL)	113 ± 7	103 ± 6	93 ± 4	91 ± 5	**<0.0001**	−8.8 (−1.65[¶])	0.179	−2.1 (−0.50[#])	**<0.0001**	**<0.0001**
Fasting insulin (μU/dL)	20.0 ± 4.7	14.7 ± 6.6	12.4 ± 2.7	10.8 ± 2.8	**<0.0001**	−26.5 (−1.06[&])	0.145	−12.9 (−0.57[#])	**<0.0001**	**<0.0001**
HOMA-IR	5.6 ± 1.6	3.8 ± 2.0	2.9 ± 0.7	2.4 ± 0.7	**<0.0001**	−32.1 (−1.23[¶])	0.165	−17.2 (−0.62[&])	**<0.0001**	**<0.0001**
PERFORMANCE										
$1RM_{LE}$ (kg)	31 ± 3	35 ± 5	34 ± 4	39 ± 4	**<0.010**	+12.9 (0.96[&])	**0.045**	+14.7 (1.25[¶])	0.068	0.211
$1RM_{UR}$ (kg)	23 ± 3	25 ± 2	22 ± 2	24 ± 3	0.078	+8.6 (0.62[&])	0.067	+9.0 (0.44[#])	0.193	0.267

*Data are means and ± SD; Delta changes (Δ%) = [10-weeks * 100/beseline]; HOMA-IR, homeostasis model assessment of insulin resistance; $1RM_{LE}$, one-maximum repetition of leg extension; $1RM_{UR}$, one-maximum repetition of upper row.*

Bold values denotes significant ($p < 0.05$) differences in all the respectives comparisons.

† *Analyzed by Repeated Measures group × time.*

‡ *Analyzed by ANOVA one-way/or ANCOVA.*

§ *Analyzed by Bonferroni post-hoc.*

[#] *Indicates "small" standardized ES at level $p \leq 0.05$.*

[&] *Indicates "moderate" standardized ES at level $p \leq 0.05$.*

[¶] *Indicates "large" standardized ES at level $p \leq 0.05$.*

min weeks 3–5, 1.30 min weeks 6–8, and 1.15 min weeks 9–10), reaching a time of 1.15 min in the tenth week. Cycle revolutions were maintained at a range of 50–70 rpm and a speed between 20 and 40 km/h during each work interval. Subjects were required to cycle at levels between 8 and 10 points on a modified 0–10 Borg scale during the work interval (Ciolac et al., 2015). This subjective intensity corresponds to a range of 70–100% of the maximum heart rate according to the Karvonen formula (Karvonen and Vuorimaa, 1988). All subjects had good exercise tolerance, and none of the participants reported an injury. Exercise compliance was 82.0 ± 3% in the H-IR group and 79.3 ± 4% in the L-IR group.

Statistical Analysis

Data are presented as the mean ± standard deviation (SD). Normality and homoscedasticity assumptions for all data were assessed using the Shapiro-Wilk-test and Levene's-test, respectively. The Wilcoxon-test was used for non-parametric data. One-way ANOVA was performed to test differences between groups at baseline. An ANCOVA was conducted to analyze variables that were significantly different at baseline. A repeated-measures ANOVA (group × time) was used to determine differences in all dependent variables between pre- and post-tests using each group × time. A chi-square test (X^2) was used to determine differences between categorical variables for R and NRs by group (H-IR × L-IR). After the intervention, the typical error (TE) were calculated for the pre-post changes for each dependent variable. The subjects were categorized as a R or NRs according to the previously described criteria of a change greater than two times the TE (Bonafiglia et al., 2016). The Bonferroni *post-hoc* test was applied to establish differences among groups. Additionally, Cohen's-test was used to detect effect sizes (ESs), with threshold values at 0.20, 0.60, 1.2, and 2.0 for small, moderate, large, and very large effects, respectively (Hopkins et al., 2009). ES-values are presented as the mean with 95% confidence limits. Odds ratios (ORs) were used to assess differences in dichotomous NRs variables between groups. All statistical analyses were performed with SPSS statistical software version 18 (SPSS™ Inc., Chicago, Illinois, USA). The alpha level was fixed at $p \leq 0.05$ for determining statistical significance in all cases.

RESULTS

Anthropometric Measurements

At baseline, there were significant ($p \leq 0.05$) differences between groups for abdominal skinfold thickness (**Table 1**). There were significant ($p \leq 0.05$) pre-post changes [presented as delta percent (Δ%)] in waist circumference (−5.2, −3.8%) and in tricipital (−13.3, −13.6%), supra-iliac (−19.4, −13.6%), and abdominal skinfold thicknesses (−18.2, −15.6%) in both the H-IR and L-IR groups (**Table 1**).

Cardiovascular Measurements

At baseline, there were no significant ($p > 0.05$) differences between the groups for diastolic or systolic blood pressure (**Table 1**). After intervention, the L-IR group showed significant pre-post changes in systolic blood pressure (−2.3%) (**Table 2**), whereas there were no significant changes in diastolic blood pressure in any group (**Table 2**).

Metabolic Measurements

At baseline, there were significant ($p \leq 0.05$) differences between the groups for fasting glucose, fasting insulin, and HOMA-IR scores (**Table 2**). After intervention, there were no pre-post changes in fasting glucose, fasting insulin, or HOMA-IR scores in L-IR group (**Table 2**). There were significant ($p \leq 0.05$) pre-post changes [presented as delta percent (Δ%)] in fasting glucose (−8.8%), fasting insulin (−26.5%), and HOMA-IR scores (−32.1%) in the H-IR group (**Table 2**). The ES-values were high for fasting glucose (−1.65; 95%CI −2.07, −1.22) and HOMA-IR scores (−1.23; 95%CI −1.60, −0.85) in the H-IR group (**Table 2**).

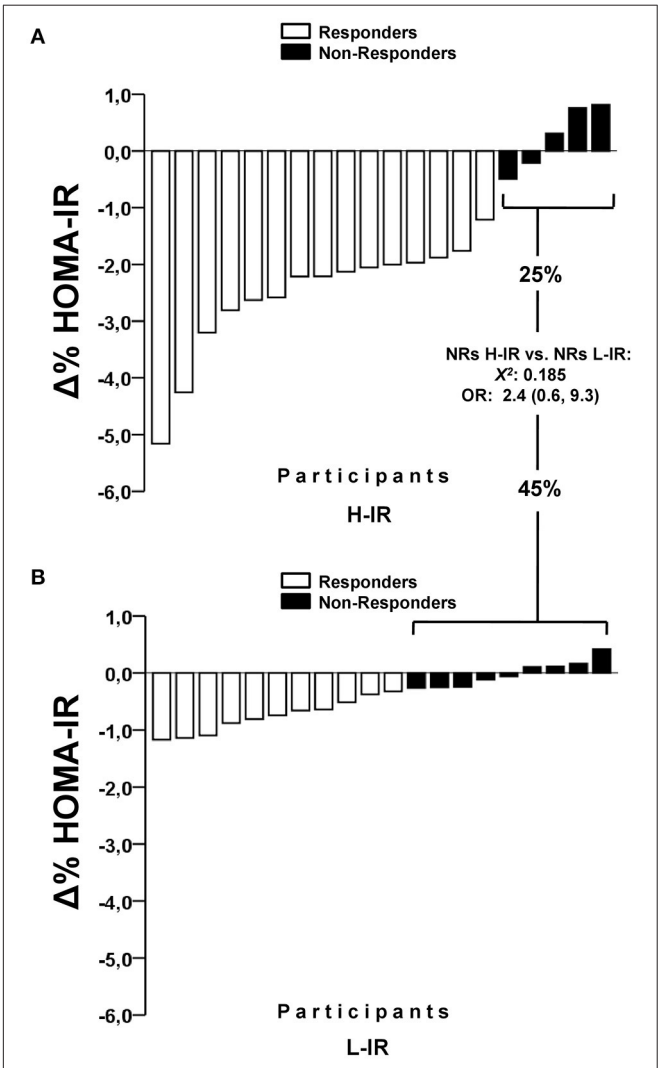

FIGURE 2 | Differences in the prevalence of non-responders to decrease HOMA-IR after high-intensity interval training in a higher insulin resistance (H-IR, $n = 20$), and lower insulin resistance group (L-IR, $n = 20$) of adult women. OR, odds ratios for suffering a non-response.

Muscle Performance Measurements

At baseline, there were no significant ($p \leq 0.05$) differences between groups for $1RM_{LE}$ and $1RM_{UR}$ (**Table 2**). After intervention, there were significant ($p \leq 0.05$) pre-post changes in $1RM_{LE}$ in the H-IR (+12.9) and L-IR (+14.7%) groups (**Table 2**), whereas $1RM_{UR}$ remained unchanged in both groups. The ES-value was high for $1RM_{LE}$ (1.25; 95%CI 1.04, 1.45) in the L-IR group (**Table 2**).

Differences in NRs Prevalence between the H-IR vs. L-IR Groups with Respect to Glucose Control Variables

There were significant differences between the H-IR vs. L-IR groups in NRs prevalence with regard to improved fasting glucose (25.0 vs. 95.0%, $p < 0.0001$) and fasting insulin (25.0 vs. 60.0%, $p = 0.025$). There were no significant differences between the H-IR vs. L-IR groups in NRs prevalence with regard to a decrease in HOMA-IR scores (25.0 vs. 45.0%, $p = 0.185$; **Figure 2**).

Differences in the NRs Prevalence between the H-IR vs. L-IR Groups with Respect to Other Anthropometric, Cardiovascular, and Performance Variables

There were no significant differences between the H-IR vs. L-IR groups in NRs prevalence with regard to improvements in anthropometric parameters (i.e., body mass, BMI, waist circumference, and tricipital, supra-iliac, and abdominal skinfolds), muscle performance (i.e., $1RM_{LE}$ and $1RM_{UR}$), or cardiovascular parameters (i.e., systolic/diastolic blood pressure; **Table 3**).

The OR analysis for NRs prevalence detected a high risk of being a NRs (>2-fold) associated with improved waist circumference (OR: 2.1, 95%CI 0.1, 3.2), diastolic blood pressure (OR: 2.1, 95%CI 1.5, 2.9), fasting glucose (OR: 4.0, 95%CI 2.2, 14.4), and $1RM_{UR}$ (OR: 2.1, 95%CI 0.5, 9.0; **Table 3**).

DISCUSSION

The present study was designed to assess the effects 10 weeks of HIIT and NRs prevalence (as indicated by glucose control parameters) in adult women with higher and lower levels of insulin resistance to test if the "magnitude" of a metabolic disease play a role in increasing or decreasing the NRs prevalence. The major findings of this study indicate that (i) HIIT promotes significantly more benefits in training-induced changes in fasting glucose, fasting insulin and HOMA-IR scores in adult women with higher insulin resistance; (ii) the NRs prevalence was significantly different between the H-IR vs. L-IR groups with regard to improve fasting glucose and fasting insulin but not for HOMA-IR scores; and (iii) both the H-IR and L-IR groups experienced similar positive training-induced changes and similar NRs prevalence with regard to anthropometric (body mass, BMI), cardiovascular (systolic/diastolic blood pressure), and muscle strength performance ($1RM_{LE}$, $1RM_{UR}$) measures.

Several environmental factors related to NRs prevalence have been reported after training interventions. For example, a recent report assessed the effects of RT at different frequencies (3 and 2 days/week) to tests the effect of frequency in older NRs subjects for 12 and 24 weeks. Major differences between both training regimens were found for body mass, which decreased by ∼4.5% at 12 weeks and 23% at 24 weeks. Interestingly, other results included increases in type I (+34.5 vs. +29.4%) and type II muscle fibers (+22.7 vs. +21.1%), as well as increasing leg strength in extension exercises (+0.9 vs. +1.17%) at 12 and 24 weeks, with relatively similar results obtained independent of the training frequency. These results indicated that the frequency of training was not necessarily related to the NRs prevalence for some variables (Churchward-Venne et al., 2015).

There is limited evidence about interindividual variability in exercise training with regard to the NRs prevalence in subjects with low glucose control, and there are several methodological differences in studies comparing the NRs prevalences observed in previous studies (Boulé et al., 2005; Gremeaux et al., 2012; Yates et al., 2013; Moker et al., 2014; Winett et al., 2014; Higgins et al., 2015). For example, for glucose control variables, several authors have observed that after 3 months of strength training (2 days/week, 2 strength exercises at maximal effort), the NRs prevalence for improvements in an OGTT in pre-diabetic patients was 44%. In the present study, we found a NRs prevalence of 15 and 25% in the H-IR and L-IR groups, respectively, for decreased fasting glucose, with no significant difference between the groups (to see **Table 3**; Winett et al., 2014). Regarding HOMA-IR, the HERITAGE study (Boulé et al., 2005) showed that after 20 weeks of endurance training [30–50 min/session, 55–75% maximum oxygen uptake (VO_2max) for 20 weeks], 42% of subjects were NRs for a decrease in HOMA-IR scores. We found similar results regarding a decrease in HOMA-IR scores, with a NRs prevalence of 15 and 20% for the H-IR and L-IR groups, respectively (**Figure 2**). Therefore, considering our 10 weeks of HIIT-based exercise vs. the 20 weeks of endurance exercise in the abovementioned study (Boulé et al., 2005), increasing the environmental "volume" factor of exercise may not necessarily be related to a decrease in the NRs prevalence for improved glucose control variables such as HOMA-IR scores. In a different study (Yates et al., 2013), NRs prevalence of 3% was reported for decreased fasting glucose after 12 months of exercise-based intervention. Similarly, when T2DM subjects were tested after 9 months of endurance training, RT or concurrent training in another study (Stephens et al., 2015), 21% of subjects were NRs for decreased glycated hemoglobin, as well as other body composition and protein markers.

Understanding the NRs prevalence after exercise modes such as HIIT and including populations with higher and lower risks of T2DM, such as those with higher and lower levels of insulin resistance, can be useful for designing more efficient exercise interventions: in this case, populations with higher levels of insulin resistance, such as the H-IR group, defined based on fasting glucose and HOMA-IR scores, are less likely to be NRs after 10 weeks of HIIT. This altered baseline, which we termed previously as "higher insulin resistance," may be in some way related to potential factors for predicting responses

TABLE 3 | Prevalence of non-responders (NRs) on anthropometric, cardiovascular, metabolic, and performance parameters after 10-weeks high-intensity interval training in a group of adult women with a higher level of insulin resistance: HOMA-IR > 5.0 (H-IR), and a group with a lower level insulin of resistance: HOMA-IR < 3.0 (L-IR).

	Response	H-IR	L-IR	OR (95% CI)	*p*-Values NRs H-IR vs. NRs L-IR
n =		20	20		
ANTHROPOMETRIC					
Body mass (%/*n*=)	NRs	20.0 (4)	10.0 (2)	0.4 (0.7, 2.7)	0.376
	R	80.0 (16)	90 (18)		
Body mass index (%/*n*=)	NRs	25.0 (5)	10.0 (2)	0.3 (0.5, 1.9)	0.212
	R	75.0 (15)	90.0 (18)		
Waist circumference (%/*n*=)	NRs	5.0 (1)	10.0 (2)	2.1* (0.1, 3.2)	0.548
	R	95.0 (19)	90.0 (18)		
Tricipital skinfold (%/*n*=)	NRs	5.0 (1)	5.0 (1)	1.0 (0.5, 0.9)	0.987
	R	95.0 (19)	95.0 (19)		
Supra-iliac skinfold (%/*n*=)	NRs	30.0 (6)	30.0 (6)	1.0 (0.2, 3.8)	0.944
	R	70.0 (14)	70.0 (14)		
Abdominal skinfold (%/*n*=)	NRs	10.0 (2)	5.0 (1)	0.4 (0.3, 5.6)	0.543
	R	90.0 (18)	95.0 (19)		
CARDIOVASCULAR					
Systolic blood pressure (%/n=)	NRs	55.0 (11)	70.0 (14)	1.9 (0.5, 7.0)	0.327
	R	45.0 (9)	30.0 (6)		
Diastolic blood pressure (%/*n*=)	NRs	90.0 (18)	100 (20)	2.1* (1.5, 2.9	0.147
	R	10.0 (2)	0 (0)		
METABOLIC					
Fasting glucose (%/*n*=)	NRs	25.0 (5)	95.0 (19)	4.0* (6.2, 14.4)	**<0.0001**
	R	75.0 (15)	5.0 (1)		
Fasting insulin (%/*n*=)	NRs	25.0 (5)	60.0 (12)	4.5 (1.1, 4.3)	**0.025**
	R	75.0 (15)	40.0 (8)		
PERFORMANCE					
$1RM_{LE}$ (%/*n*=)	NRs	10.0 (2)	0 (0)	0.4 (0.3, 0.6)	0.147
	R	90.0 (18)	100 (20)		
$1RM_{UR}$ (%/*n*=)	NRs	20.0 (4)	35.0 (7)	2.1* (0.5, 9.0)	0.288
	R	80.0 (16)	65.0 (13)		

Data are percentage, %/n = number of cases. $1RM_{LE}$, one-maximum repetition of leg extension; $1RM_{UR}$, one-maximum repetition of upper row. Bold values denotes significant ($p < 0.05$) differences between NRs from H-IR vs. NRs from the L-IR group at level ($p < 0.05$).

**Denotes a high risk (>2-fold) for suffering a non-response.*

in future long-term studies. Collectively, and in combination with previous reports (Hecksteden et al., 2013b), these findings indicate that the "magnitude" of changes in response to an acute exercise session can be a potential factor for predicting responses to chronic exercise-based interventions. In this study, the magnitude of changes in plasma variables after volitional exercise was very similar to results from another study where subjects showed decreased fasting insulin after chronic exercise training [walking/running at 60% peak oxygen consumption (VO_2peak) for 4 weeks].

Another study (Moker et al., 2014) exploring another co-variable, blood pressure, showed that after 5 months of endurance training (65–80% VO_2peak, walking/jogging), RT (8–12 repetitions per set, 8 exercises, 70–85% of their one-maximum repetition value), or concurrent training, approximately ∼60% of subjects were NRs for a decrease in systolic and diastolic blood pressure. In our study, we found a NRs prevalence of 20 and 15% for decreased systolic blood pressure in the H-IR and L-IR groups, respectively, as well as a more pronounced NRs prevalence of 30 and 45% for decreased diastolic blood pressure in the H-IR and L-IR groups, respectively (**Table 3**). Because none of the intervention groups were diagnosed with hypertension, we hypothesized that genetic together with environmental factors, such as time of intervention, mode of training, and non-hypertensive baseline profiles, may be responsible for these results. However, these results were more positive after 10 weeks of HIIT than the 60% NRs prevalence observed in the aforementioned study following 5 months of intervention. Thus, the volume of training does not appear to play a role in NRs prevalence for decreases in systolic or diastolic blood pressure. Evidence has shown the benefit in terms of decreased systolic blood pressure after HIIT interventions (Ciolac, 2012); however, in this non-hypertensive cohort, we did not observe significant training-induced changes in systolic or

diastolic blood pressure (**Table 2**). In other studies, there was an ∼60% NRs prevalence for decreased systolic or diastolic blood pressure after 6 weeks (Higgins et al., 2015) or 6 months (Moker et al., 2014) of HIIT. Interestingly, a study that explored the magnitude of the changes in blood pressure after an acute exercise session reported that this response can be used as a predictive factor for decreases in blood pressure after long-term exercise training (Hecksteden et al., 2013a).

In this study, we found significant training-induced changes in $1RM_{LE}$-test results in the H-IR (+12.9%) and L-IR (+14.7%) groups (**Table 2**). Similarly, we found a 10% NRs prevalence for an increase in $1RM_{LE}$ results in the H-IR group and no NRs (0%) in the L-IR group (**Table 3**). However, in previous studies, RT (10–15 repetitions, four sets of leg extension, 60–80% of the one-maximum repetition value) resulted in a minimum NRs prevalence of ∼1% for an increase in $1RM_{LE}$ after 12 and 24 weeks of RT (Churchward-Venne et al., 2015). Additionally, despite the fact that our HIIT mode of training is very different methodologically than what was reported in previous studies, the HIIT protocol was able to increase the strength of the lower limbs. These findings are consistent with a previous HIIT-based study (90 s, 6 bouts, 6 weeks), in which HIIT improved several parameters related to power cycling in the lower limbs in adult men (Ziemann et al., 2011).

We observed different ranges of NRs prevalence for other anthropometric (5–30%), blood pressure (55–100%), metabolic (25–95%), and performance (0–35%) variables. These results are consistent with literature reports for blood pressure (59–60%) (Moker et al., 2014), metabolic (7–44%) (Boulé et al., 2005; Yates et al., 2013; Winett et al., 2014; Osler et al., 2015; Stephens et al., 2015), and performance (1%) variables (Churchward-Venne et al., 2015). Finally, our study has some important limitations. Our sample size was limited, but it is similar to the sample sizes used in other exercise training studies (∼10–20 subjects). Additionally, we lacked a true no-exercise control group, and we did not control the physical activity patterns and diet of subjects after training, although subjects were reminded each week to maintain their baseline patterns of activity and food consumption. The strengths of this study were that we included both the effects of HIIT and NRs prevalences for changes in anthropometric, cardiovascular, and metabolic risk factors and in performance variables. We also included a statistical estimate of the ES for each variable studied.

CONCLUSION

In summary, independent of the "magnitude" of the cardiometabolic disease (i.e., higher vs. lower insulin resistance), no differences were observed in the NRs prevalence with regards to improved HOMA-IR scores, other anthropometric, cardiovascular, and muscle performance variables after 10 weeks of HIIT in sedentary adult women. This research demonstrates the protective effect of HIIT against cardiometabolic disease progression in a sedentary population.

AUTHOR CONTRIBUTIONS

CA conceived and designed the project. CA and RR-C reviewed the literature studies and conducted data extraction. CA conducted data analyses. CA, RR-C, and MI were responsible for data interpretation. CA, RR-C, and MI drafted the manuscript, and RR-V, and MI revised it critically for intellectual contributions. CA and RR-C coordinate the study development. All authors reviewed and edited the manuscript. All authors read and approved the final manuscript.

FUNDING

This work was supported by the health promotion program of the Public Health Service of Los Ríos Government (SSVV). MI was funded by research grants from the Spanish Net on Aging and frailty (RETICEF) (ISCIII, FEDER) and Centro de Investigación Biomédica en Red (CIBER) en Fragilidad y Envejecimiento Saludable (CIBERFES; CB16/10/00315) del Instituto de Salud Carlos III (FEDER).

ACKNOWLEDGMENTS

We are thankful with Mr. Farid (physician), Miss Paulina Carrasco (nursing), and Mrs. Elizabeth Mariangel (nursing), as well as to Mrs Sandra Castro, and Marcial Leal (technician). Finally, we are thankful with Miss DorkaNorambuena, director of the Family Healthcare Center Tomás Rojas Vergara for their regular interest to include the exercise training at level of public health.

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

The reviewer AS and handling Editor declared their shared affiliation, and the handling Editor states that the process nevertheless met the standards of a fair and objective review.

frontiers in Endocrinology

OPINION
published: 20 February 2017
doi: 10.3389/fendo.2017.00033

Key Points from the Updated Guidelines on Exercise and Diabetes

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Keywords: physical activity, exercise, diabetes, guidelines, American Diabetes Association

INTRODUCTION

No doubt remains that the adoption and maintenance of physical activity is important for overall health and blood glucose management in individuals with diabetes and prediabetes. Recently, the American Diabetes Association (ADA) published updated recommendations and precautions about physical activity and exercise in people with type 1 diabetes, type 2 diabetes, and gestational diabetes (1). Given the importance of these topics, it is worth discussing the key changes and updates included in this ADA position statement (PS).

Pre-Exercise Health Screening and Evaluation

This PS reiterates that "pre-exercise medical clearance is not necessary for asymptomatic, sedentary individuals who wish to begin low- or moderate-intensity physical activity not exceeding the demands of brisk walking or everyday living" (1). This stance directly opposes a recent recommendation from the American College of Sports Medicine (ACSM) (2) that requires anyone with a metabolic disease (in this case, diabetes) who desires to begin exercising at any level—even doing light activities—to obtain medical clearance from a health-care provider first. The authors of the ADA PS did not agree with this restriction and took the same stance as the prior ADA PS on type 2 diabetes and exercise (3), which I believe is a much better recommendation. Making adults obtain any type of medical clearance prior to starting walking, for example, is an unnecessary barrier that will not necessarily make exercising any safer for them. However, ADA agrees with ACSM that adults with diabetes who plan to exercise at higher intensities than currently undertaken or who would be considered at high risk for cardiovascular disease (e.g., have elevated blood cholesterol, smoke, have a strong family history, etc.) or other health complications from doing such activities are recommended to obtain a pre-training examination from a health-care provider who may or may not recommend exercise stress testing (3).

RECOMMENDED PHYSICAL ACTIVITY/EXERCISE

All physical movement has the potential to improve physical and mental health (4–6). Since blood glucose management varies with a number of factors, it is critical for recommendations to be tailored for activity type and health complications to be effective (3, 7). In the PS, physical activity is defined as any movement that increases energy use, and exercise is a subset of physical activity that is more planned or structured (1), which is an important distinction.

Aerobic Exercise Training

As previously recommended, most adults with type 1 or type 2 diabetes should undertake at least 150 min or more of moderate- to vigorous-intensity activity weekly; it is also recommended that these activities occur on at least 3 or more days during the week and that individuals should not allow more than 2 days to elapse between activity sessions to maintain higher levels of insulin sensitivity

OPEN ACCESS

Edited by:
Jonathan Peter Little, University of British Columbia, Canada

Reviewed by:
Normand Boule, University of Alberta, Canada
Jamie F. Burr, University of Guelph, Canada

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

Received: *14 January 2017*
Accepted: *07 February 2017*
Published: *20 February 2017*

Citation:
Colberg SR (2017) Key Points from the Updated Guidelines on Exercise and Diabetes. Front. Endocrinol. 8:33. doi: 10.3389/fendo.2017.00033

(**Table 1**). However, it is now recognized in this PS that younger or more physically fit individuals may receive similar cardiovascular and fitness benefits from undertaking vigorous-intensity or high-intensity interval training (HIIT), assuming it adds up to a minimum of 75 min/week (1, 8, 9).

Also included in the PS this time for the first time is HIIT, which is a type of training that includes short bursts (seconds to minutes) of very intense activity with recovery periods interspersed that may involve a lower intensity activity or rest. Such training has been demonstrated to result in greater insulin sensitivity and better overall blood glucose levels, at least in adults with type 2 diabetes (9, 10). Adults with type 1 diabetes can engage in HIIT and manage blood glucose with appropriate regimen changes (8, 11), which may include more insulin during and following and activity and reduced dosing overnight, along with food intake to prevent overnight hypoglycemia. Since its safety and efficacy remain unclear for some adults (12, 13), individuals who undertake such training should be clinically stable, already exercising regularly in activities that are moderate in intensity or harder, and possibly supervised when HIIT is started (14). This type of training is definitely not right for everyone.

Resistance Exercise Training

The PS recommends 2–3 sessions/week of resistance exercise on non-consecutive days using a variety of strength training modalities (1), which is also unchanged from prior recommendations and from guidelines for all adults. Although heavier resistance training improves glycemic control and strength more than lighter weights or home-based activities (15), all resistance training has the potential to result in greater strength, which can translate into improved balance and ability to live independently and undertake activities of daily living.

The main PS update is related to discussing the glycemic impact of resistance exercise in adults with type 1 diabetes (1), which remains unclear (8). It may lower the risk of developing exercise-induced hypoglycemia in type 1 diabetes (16). When both aerobic and resistance exercise are undertaken during a solitary activity session, it has been shown that doing the bout of resistance work first may actually help maintain glycemic balance more so than when aerobic exercise occurs before resistance training (17). Varying the order of the activities based on blood glucose levels may minimize the risk of hypoglycemia.

Flexibility and Balance Exercises

One major change of this PS is a greater focus on the inclusion of flexibility exercise to improve range of motion around joints in individuals of all ages (18) and balance activities to improve gait and prevent falls in older adults (19). Both flexibility exercises and balance training are recommended to be done minimally 2–3 times/week, especially by older adults (1). Including both is vitally important to living well since limited joint mobility is common in older adults and long-standing diabetes due to advanced glycation end products formed by normal aging and hyperglycemia (20). Stretching increases range of motion around joints and flexibility (18), and balance training can reduce falls risk by improving balance and gait (19).

TABLE 1 | Exercise training recommendations.

	Aerobic	Resistance	Flexibility and balance
Type of exercise	Prolonged, rhythmic activities using large muscle groups (e.g., walking, cycling, and swimming) May be done continuously or as high-intensity interval training	Resistance machines, free weights, resistance bands, and/or body weight as resistance exercises	Stretching: static, dynamic, and other stretching, yoga Balance (for older adults): practice standing on one leg, exercises using balance equipment, lower-body and core resistance exercises, tai chi
Intensity	Moderate to vigorous (subjectively experienced as "moderate" to "very hard")	Moderate (e.g., 15 repetitions of an exercise that can be repeated no more than 15 times) to vigorous (e.g., 6–8 repetitions of an exercise that can be repeated no more than 6–8 times)	Stretch to the point of tightness or slight discomfort Balance exercises light to moderate intensity
Duration	At least 150 min/week at moderate to vigorous intensity for most adults with diabetes. For adults able to run steadily at 6 mph/9.7 kmph for 25 min, 75 min/week of vigorous activity may provide similar cardioprotective and metabolic benefits	At least 8–10 exercises with completion of 1–3 sets of 10–15 repetitions to near fatigue per set on every exercise early in training	Hold static or do dynamic stretch for 10–30 s; 2–4 repetitions of each exercise Balance training can be any duration
Frequency	3–7 days/week, with no more than 2 consecutive days without exercise	A minimum of 2 non-consecutive days/week, but preferably 3	Flexibility: ≥2–3 days/week Balance: ≥2–3 days/week
Progression	A greater emphasis should be placed on vigorous-intensity aerobic exercise if fitness is a primary goal of exercise and not contraindicated by complications; both high-intensity interval and continuous exercise training are appropriate activities for most individuals with diabetes	Beginning training intensity should be moderate, involving 10–15 repetitions per set, with increases in weight or resistance undertaken with a lower number of repetitions (8–10) only after the target number of repetitions per set can consistently be exceeded; increase in resistance can be followed by a greater number of sets and lastly by increased training frequency	Continue to work on flexibility and balance training, increasing duration and/or frequency to progress over time

Lower-body and core strengthening exercises may be considered part of balance training. Yoga may promote improvement in glycemic control, lipid levels, and body composition in adults with type 2 diabetes (21). Tai chi training may improve glycemic control and balance neuropathic symptoms and some dimensions of quality of life in adults with diabetes and neuropathy (22).

Daily Movement

Engaging in more unstructured daily activity, such as errands, household tasks, dog walking, and gardening, increases daily energy expenditure and assists with weight loss and maintenance (23–25). Increasing daily movement appears to acutely lower postprandial hyperglycemia and possibly improve blood glucose management, especially when undertaken after meals (26–34). It is recommended as part of a whole-day approach and as a starting place for anyone who is currently sedentary and either unwilling or unable to start engaging in more structured activities. For many deconditioned and older individuals with diabetes, increasing daily movement may be an appropriate place to start with physical activity rather than with more structured activities.

Reduced Sedentary Time and Interrupted Sitting

As demonstrated in adults with type 2 diabetes, encouraging them to interrupt prolonged periods of sitting with 15 min of walking after meals (26) and either light walking or simple bodyweight resistance activities undertaken for 3 min after every 30 min of inactivity (29) improves overall glycemic control. The PS recommends that all adults attempt to lower the total amount of time that they spend each day in sedentary activities and break up prolonged bouts of sitting with some type of light activity for a few minutes at least every 30 min to improve their glycemic management; both should be added to daily structured exercise and unstructured movement rather than being a replacement for them. Research in this area, however, is still in its infancy—especially in populations with diabetes—and more studies are needed to better define the best types and timing of activity, not only for managing blood glucose levels but also for preventing type 2 diabetes and reversing prediabetes in the first place.

PHYSICAL ACTIVITY AND TYPE 2 DIABETES

The impact of exercise on insulin action is transient and, accordingly, activities should be undertaken daily or no less frequently than every other day. It is important to continue to recommend that exercise be undertaken regularly since in many cases, acute effects of aerobic exercise may not last even 24 h. At least one study has shown that if the same volume of exercise is done—either as 30 min of moderate exercise daily or 1 h at the same intensity every other day—the glycemic effects over the ensuing 48-h period are similar (35). Exercise does not necessarily need to be prolonged to result in enhanced insulin sensitivity, but if shorter in duration, engaging in harder workouts or high-intensity intervals will increase its impact (36, 37). However, daily moderate or high-intensity aerobic or resistance exercise is likely optimal (38–40). Aerobic training may improve overall glycemic control more than resistance training, but both reduce cardiovascular risk markers similarly (41), and a single bout of either may have a similar acute effect in any case (42). To achieve better glycemic management, engaging in combined aerobic and resistance training appears to be superior to undertaking either type of training on its own (43, 44). In fact, the PS states, "Adults should ideally perform both aerobic and resistance exercise training for optimal glycemic and health outcomes" (1), which I firmly believe to be an excellent recommendation.

It is also important for type 2 diabetic youth (children and adolescents) to be more physically active. Their goal should be to meet the activity goals recommended for all youth, which consists of 60 min/day or more of moderate- or vigorous-intensity aerobic activity, with vigorous, muscle-strengthening, and bone-strengthening activities at least 3 days/week (1). Few studies have been done to examine the impact of exercise training and interventions in youth with type 2 diabetes, and those are inconclusive, although it can be assumed that the health and glycemic benefits they would gain are similar to those experienced by adults with type 2 diabetes (45).

PHYSICAL ACTIVITY AND TYPE 1 DIABETES

This PS is the first in many decades to address the complexities of managing blood glucose with exercise in adults and children with type 1 diabetes (46). Both aerobic and resistance training are recommended for these adults (47–49), and youth with type 1 diabetes should follow general recommendations for children and adolescents (47). Blood glucose responses are impacted by the type, timing, intensity, and duration of exercise, as well as by many other factors. Different activities will likely require individualized adjustments to carbohydrate and food intake and insulin dosing during and after exercise.

Aerobic exercise after meals usually decreases blood glucose levels (50), especially during prolonged activity (34, 51, 52). Doing activity during fasting conditions, however, results in more stable glycemia, with less of a decline or even a small increase in overall levels (53). Engaging in very intense activities either maintains or raises blood glucose (16, 54), depending on duration, which is an important point to keep in mind.

Variable glycemic responses to physical activity (46) make uniform recommendations nearly impossible. In general, individuals will need to increase their carbohydrate intake and/or reduce circulating insulin levels when engaging in longer duration aerobic activities, along with frequently monitoring blood glucose. These additional recommendations are stated in the PS (1) but come from other studies: for low- to moderate-intensity aerobic activities lasting 30–60 min during fasting or basal insulin conditions, ~10–15 g of carbohydrate may suffice to prevent hypoglycemia (55). For activities done after bolus insulin, 30–60 g of carbohydrate per hour may be needed (56, 57), or insulin can be reduced 25–75% to reduce or eliminate the need for carbohydrate intake (58). Basal rate reductions for exercise may reduce hypoglycemia (59).

Continuous glucose monitors (CGM) are more widely available nowadays and have increased in accuracy; for many individuals, wearing such a device may decrease the fear of developing exercise-induced hypoglycemia. They are able to provide blood glucose trends, which can potentially assist the user in preventing hypoglycemia or treating it sooner (60–63). Some issues with CGM use during activity remain, however, as stated in the PS (1): inadequate accuracy (64), sensor filament breakage (62, 63), inability to calibrate (61) time lags between the change in blood glucose and its detection by CGM (65), and variations in sensor performance (66–68). CGM devices are currently being paired with insulin pumps into closed-loop systems run by algorithms. These technological issues with CGM use during exercise are continuing to make regular participation in physical activity a huge hurdle to creating an effective system.

PHYSICAL ACTIVITY AND PREGNANCY WITH DIABETES

The PS recommends, "Females with pre-existing diabetes of any type should be advised to engage in regular physical activity prior to and during pregnancy" (1). It also reiterates prior recommendations from other organizations that state that "pregnant females with or at risk for gestational diabetes should engage in 20–30 min of moderate-intensity exercise on most or all days of the week" (69–71). Undertaking any type of training (aerobic or resistance) has the ability to improve insulin sensitivity and overall blood glucose management (72). Ideally, physical activity should start prior to pregnancy to reduce gestational diabetes risk (73) but can be initiated safely during pregnancy (69). Regular physical activity is important for other positive pregnancy outcomes as well and should be recommended to all females of childbearing age, both prior to and during pregnancy.

MINIMIZING EXERCISE-RELATED ADVERSE EVENTS

In the PS (1), it is reiterated that, "Exercise-induced hypoglycemia is common in type 1 diabetes, and to a lesser extent, people with type 2 diabetes using insulin or insulin secretagogues." Some medications (other than insulin) may increase exercise risk, and doses may need to be adjusted (74, 75). Given that fear of hypoglycemia related to exercise is a proven barrier to exercise participation (76), any strategies that will assist in minimizing its occurrence have the potential to increase adherence to exercise training. Other acute strategies to prevent hypoglycemia involve including short sprints, performing resistance exercise before aerobic exercise in the same session, and activity timing (77–82), which are primarily based on the ability of a greater release of counterregulatory hormones during intense activities to maintain blood glucose levels more effectively. Exercise-induced nocturnal hypoglycemia is a major concern (83). Hypoglycemic events occur typically within 6–15 h postexercise (84), although risk can extend out to 48 h (85). Risk of nocturnal hypoglycemia following physical activity may be mitigated with lower basal insulin doses overnight, bedtime snacks, and/or use of CGM, and these strategies should be recommended to assist in preventing delayed-onset lows.

Very intense exercise like sprinting (79), brief but intense aerobic exercise (86), and heavy powerlifting (87, 88) may promote hyperglycemia, especially with elevated starting blood glucose levels (86). A number of strategies can mitigate exercise-induced hyperglycemia, though. For example, it may be modulated with insulin administration, interspersing moderate aerobic activity between intense bouts, and a low-intensity cooldown (89, 90). Another stance taken in the PS (1) is "Overconsumption of carbohydrates before or during exercise, along with aggressive insulin reduction, can promote hyperglycemia during any exercise (58). Exercising with hyperglycemia and elevated blood ketones is not recommended."

Aging combined with diabetes may result in worse blood glucose control; moreover, peripheral neuropathy may be present and skin blood flow and sweating impaired (91–93), which increases the risk of heat-related illness. Chronic hyperglycemia also causes dehydration. These are all fairly new findings. For these reasons, the PS (1) recommends, "Older adults with diabetes or anyone with autonomic neuropathy, cardiovascular complications, or pulmonary disease should avoid exercising outdoors on very hot and/or humid days to prevent heat-related illnesses."

In addition, these statements from the PS are aimed at avoidance of other exercise-related adverse responses (1), which is critical for continued participation: "Active individuals with type 1 diabetes are not at increased risk of tendon injury (94), but this may not apply to sedentary or older individuals with diabetes. Diabetes may lead to exercise-related overuse injuries due to changes in joint structures related to glycemic excursions (95), so exercise training should progress appropriately to avoid excessive aggravation to joint surfaces and structures, particularly when taking statin medications for lipid control (96)."

MANAGING HEALTH COMPLICATIONS

Finally, many individuals with diabetes carry the burden of having associated health concerns, many of which can impact their ability to exercise safely and effectively. None of these are new ideas, but here is a summary of recommended actions as stated in the PS (1): macrovascular and microvascular diabetes-related complications can develop and worsen with inadequate blood glucose management (97, 98). Physical activity with vascular diseases can be undertaken safely, but with appropriate precautions. Being active with peripheral neuropathy necessitates proper foot care to prevent, detect, and treat problems early to avoid ulceration and amputation. Autonomic neuropathy may complicate being active; certain precautions are warranted to prevent problems during activity, such as avoiding rapid directional changes (if orthostatic hypotension is present) and preventing dehydration and overheating during exercise with adequate fluid intake. Vigorous aerobic or resistance exercise, jumping, jarring, and head-down activities, and breath-holding should be avoided in anyone with severe non-proliferative and unstable proliferative diabetic retinopathy. Exercise with diabetic kidney disease can be undertaken safely, even during dialysis sessions. Regular

stretching and appropriate progression of activities should be done to manage joint changes and diabetes-related orthopedic limitations.

CONCLUSION

This PS really does not contain any controversial recommendations, other than ADA disagreeing (strongly) with the requirement that ACSM put forth that all individuals with a metabolic condition who are currently sedentary must seek medical clearance prior to getting up off the couch. It is good to be reminded as well that although everyone can benefit from being physically active, specific recommendations and precautions will vary by the type of diabetes, age, activity done, and presence of complications, and exercise prescriptions should be tailored to meet the specific needs of each individual. Overall, this ADA PS provides a comprehensive and current guide to assist individuals of all ages with any type of diabetes with engaging in recommended amounts of regular physical activity safely and effectively and is a much-needed publication. For even more specifics about type 1 diabetes and exercise participation, however, readers are referred to a very recent consensus statement (99) sponsored by the Juvenile Diabetes Research Foundation, which is far more comprehensive for this group of exercisers than this ADA PS ever intended to or realistically could be since it covered all types of diabetes, not just type 1.

AUTHOR CONTRIBUTIONS

SC wrote and edited this opinion piece with no input from any other authors.

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

frontiers in Endocrinology

HYPOTHESIS AND THEORY
published: 19 September 2017
doi: 10.3389/fendo.2017.00228

Exercise after You Eat: Hitting the Postprandial Glucose Target

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We discuss a novel hypothesis: the effect size of postmeal exercise for attenuating postprandial glucose will be a function of the exercise bout vs. the size of the postprandial glucose response, specifically peak and duration of the postprandial glucose excursion.

Keywords: postmeal exercise, postprandial glucose, type 2 diabetes, continuous glucose monitoring, glycemic control

INTRODUCTION

Hyperglycemia is a hallmark feature of type 2 diabetes. Sustained high glucose concentrations play a central role in the development of diabetes-related complications (1). Importantly, restoration of glycemic control reduces cardiovascular disease (2). Thus, the primary goal of type 2 diabetes treatment is to achieve and maintain glycemic control. While various therapeutic options are available, glycemic control remains challenging. For example, the long-term performance of hypoglycemic agents is unsatisfactory (2–4).

Traditional markers for glycemic control include fasting glucose, hemoglobin A_{1C} (HbA_{1C}), and postprandial glucose. The gold standard for assessing glycemic control is HbA_{1C}, which represents a 3-month average of glucose exposure. Postprandial glucose is gaining more recognition as a key glycemic target for therapeutic intervention, as multiple lines of evidence support its use as a clinical marker. Epidemiological studies have shown that postprandial glucose is a better cardiovascular disease predictor than HbA_{1C} (5–7), as well as fasting glucose (8). In addition, interventional studies have shown that reducing postprandial glucose improves glycemic control (9) and leads to reductions in cardiovascular disease risk in people with type 2 diabetes (10).

Due to the growing body of evidence supporting the link between postprandial glucose and cardiovascular disease, the International Diabetes Federation (IDF) published guidelines for postmeal glucose management. Specifically, the target glucose value 1–2 h after meal ingestion is 160 mg/dL (9.0 mmol/L) (11). Recommendations for treatment of postprandial glucose include pharmacologic and non-pharmacological strategies (11). Interestingly, exercise was not described as a treatment option in the IDF recommendation.

Exercise has been shown to be important for both prevention and treatment of type 2 diabetes, and the American Diabetes Association and American College of Sports Medicine have developed exercise guidelines for people with type 2 diabetes (12). However, these guidelines are not specific to postprandial glucose, and they do not mention exercise timing in relation to meal ingestion. The lack of attention to exercise timing in treatment guidelines highlights a need for more research on postmeal exercise and its effects on diabetes-related outcomes.

Postprandial exercise has been shown to be safe (13) and effective in people with type 2 diabetes (**Figure 1**) (14). Exercise acutely increases glucose uptake in skeletal muscle. This occurs through an insulin-independent process (15, 16) and, therefore, is applicable to type 2 diabetes. Muscle contraction serves as a signal for GLUT-4 receptor translocation on the skeletal muscle plasma membrane (17). GLUT-4 receptors are responsible for transporting glucose from systemic circulation and into skeletal muscle. This effect occurs after just a single bout of exercise, meaning

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Edited by:
Kristian Karstoft, University of Copenhagen, Denmark

Reviewed by:
Barry Braun, Colorado State University, United States
Carmine Grieco, Colorado Mesa University, United States

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

Received: *19 June 2017*
Accepted: *22 August 2017*
Published: *19 September 2017*

Citation:
Erickson ML, Jenkins NT and McCully KK (2017) Exercise after You Eat: Hitting the Postprandial Glucose Target. Front. Endocrinol. 8:228. doi: 10.3389/fendo.2017.00228

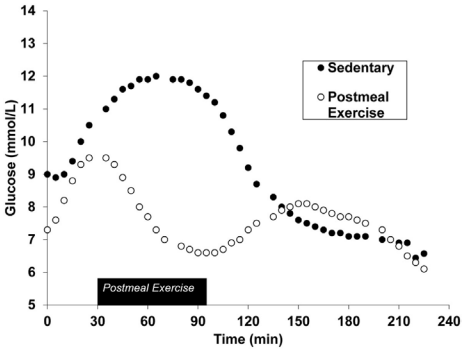

FIGURE 1 | Continuous glucose monitoring data during the postprandial phase of both sedentary and postmeal exercise conditions after a standardized meal in the same individual. Figure has been adapted from previously published works (20). American Physiological Society, permissions for reuse not required due to original authorship.

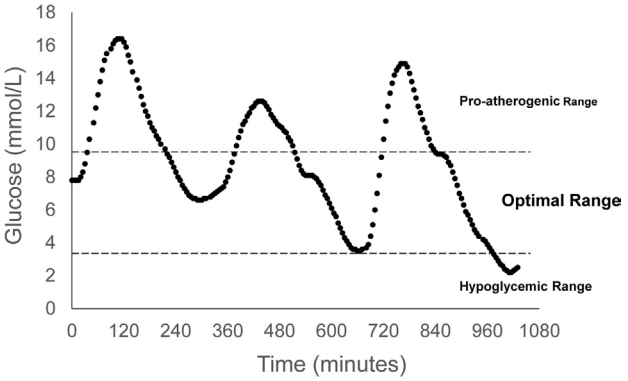

FIGURE 2 | Indicates optimal range of postprandial glucose control. The upper glucose bound is set by the International Diabetes Federation Guidelines, while the lower glucose bound is defined by hypoglycemic risk. Continuous glucose monitoring data are representative of a 24-h glucose profile of an individual with type 2 diabetes. Summary data have been published previously (23).

the glucose-lowering effects can be realized immediately (17). Furthermore, the acute nature of this response indicates that long-term training adaptations are not necessary for beneficial effects on blood glucose to occur. The optimal timing for postmeal exercise has been suggested to be 30 min after the start of a meal (18). This is because peak postmeal glucose values typically occur within 90 min (19), and initiating exercise during this time window will blunt peak glucose excursions protecting the endothelial wall from pro-atherogenic glucose concentrations.

EFFECTIVE EXERCISE CONSIDERS GLUCOSE LEVELS

A key characteristic of type 2 diabetes is an exaggerated glucose response to a meal, and studies using continuous glucose monitoring (CGM) have shown that glucose excursions in people with type 2 diabetes are well above those of non-diabetic controls, even when treated with hypoglycemic agents (21, 22). An optimal postprandial glucose treatment will produce a glucose response that mimics normal glucose tolerance. Measurable parameters of the postprandial glucose response that can be used for interventional guidance include glucose peak and duration of elevation. Those with normal glucose tolerance do not exceed 140 mg/dL and glucose levels return to preprandial levels after 2 h. Given these parameters, postmeal exercise can be strategically applied to lower peak glucose as well as time of elevation, thus resembling normal glycemic control. Effective exercise for type 2 diabetes requires balance, in that, clinically meaningful glucose reductions should be pursued, while minimizing risk for hypoglycemia (**Figure 2**).

We propose that the effectiveness of exercise will be dependent on the postprandial glucose response, including the glucose peak and duration of elevation. Larger amplitude, longer duration glucose excursions will require more intervention than smaller, shorter glucose excursions to produce a normoglycemic pattern. Therefore, the measurable effect size of an exercise bout will be dependent on the glycemic excursion itself, in that, a higher

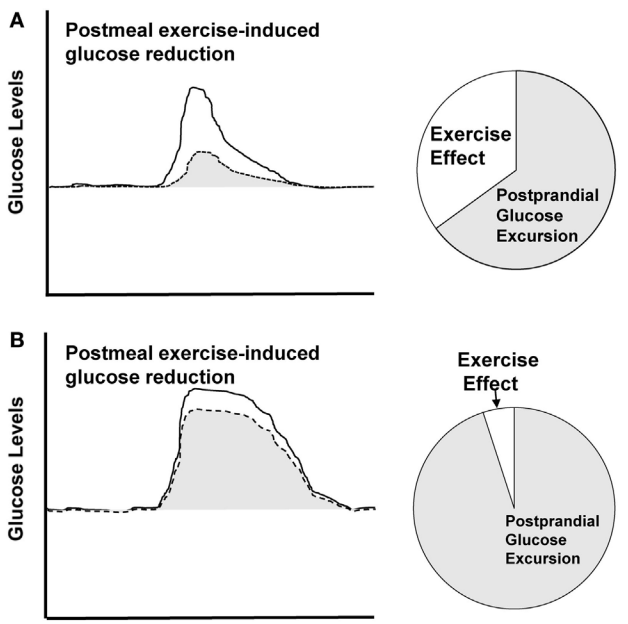

FIGURE 3 | Theoretical depiction. Solid line represents sedentary condition and dashed line represents postmeal exercise condition. **(A)** Displays larger effect size for postmeal exercise-induced glucose reduction in a smaller, shorter excursion. **(B)** Displays smaller effect size for a higher, longer glucose excursion.

and longer excursion will experience less reduction from the same exercise bout compared to a smaller and shorter excursion (**Figure 3**). This concept is supported by quantitative comparisons of our previous work. In two distinct studies, we used CGM to assess the effects of postmeal exercise on postprandial glucose excursions in those treated with metformin (20), as well as those treated with more advanced T2D requiring metformin plus additional hypoglycemic agents (23).

We observed different effect sizes for postmeal exercise-induced reductions in 2-h glucose peak, including a large effect

size (0.81) in individuals treated with only metformin (20) vs. a moderate effect size (0.56) in those treated with additional hypoglycemic agents (23). We propose that these differential results can be explained by two key differences among studies, including (1) differences in the applied exercise bout, as well as (2) differences in the amplitude of the postprandial glucose peak. The larger effect size was observed in the study that applied the larger exercise stimulus, i.e., exercise that was longer in duration (50 vs. 30 min) and modestly higher in intensity (60% VO_2 max vs. 50% VO_2 max). In addition, the amplitude of the 2-h glucose peak measured in the control condition was lower in the metformin study participants compared to that of the metformin plus add-on hypoglycemic agent study participants (12.0 vs. 14.5 mmol/L, respectively), indicative of an easier glucose "target" for reduction. This comparison suggests that a larger exercise stimulus (longer in duration, higher in intensity) applied to the smaller glucose peak resulted in a more effective strategy for glucose attenuation. One interpretation of this comparison is that the effectiveness of a postmeal exercise bout is a function of the amount of exercise vs. the size of the glucose peak.

To further explore this concept, a second comparison of the two studies was completed. The aim of this analysis was to use CGM data to quantify the amount of time exercising vs. the amount of time spent in postprandial hyperglycemia. This can be thought of as the percentage of time in which the glucose excursion was being intervened upon by exercise. This analysis revealed differences among our two studies. The percentage of time that was "treated" by exercise was 34% in the metformin study (20), vs. only 16% in the add-on therapy (23). These findings are consistent with the measured effect sizes, in that the larger effect size corresponded with the larger percentage of treated time (ES: 0.81; 34% of time was "treated" with exercise) and the smaller effect size corresponded with the small percentage of treated time (ES: 0.56; 16% of time was "treated" with exercise). These findings further support the concept that the effectiveness of exercise will be a function of the exercise bout vs. the size of the postprandial glucose response.

Significant glucose reductions have been reported in people with type 2 diabetes using a large variety of exercise strategies. This includes continuous (24, 25) and interval protocols (20, 23). In addition, various durations (20–60 min) and intensities have been shown to be effective (26, 27). Furthermore, multiple modes of exercise have been used including walking and cycling (24, 25). More recently, high-intensity interval training has been shown to be a promising approach for improving health outcomes in the people with type 2 diabetes (28–30). Taken together, these studies show there are numerous strategies to prescribe exercise. It is currently not clear if one variable is more important than another for postprandial glucose control. However, in the case of postmeal exercise approaches, it seems evident that maximizing the glucose-lowering power of an exercise bout will require taking the size of the glucose excursion itself, into account.

COMPLEMENTARY EFFECTS OF DRUGS AND EXERCISE

Hypoglycemic agents are a mainstay in type 2 diabetes treatment. Thus, the combined effects of hypoglycemic agents and exercise should remain a high priority for future investigations. Metformin is the first-line therapy (31) for the prevention and treatment of type 2 diabetes. During disease progression, a variety of hypoglycemic agents can be used for glycemic control. Currently, there are nine available FDA-approved classes of oral hypoglycemic agents (32) and several injectable agents. Some drug classes specifically target and reduce postprandial glucose, including α-glucosidase inhibitors, DPP-4 inhibitors, glinides, GLP-1 derivatives, short-acting sulfonylureas, and insulin regimens (31). All of these medications are recommended to be taken along with regular exercise.

The target tissues and mechanisms of action widely vary among drug classes. Subsequently, these drug classes have differing effects on the 24-h glycemic profile. Some drugs effectively lower fasting glucose, while others target postprandial glucose. Postmeal exercise may be an effective complement to these agents. In fact, the combination of postmeal exercise and hypoglycemic agents has been shown to produce further glucose-lowering effects compared to drug treatment alone (20, 23). Additional experimental studies are needed to determine the interactive effects of postmeal exercise among various drug classes. This will involve appropriately timing medication and exercise in order to avoid hypoglycemia.

Insulin and insulin-analog regimens have been specifically designed to reduce postprandial glucose. Incorporation of postmeal exercise alongside insulin therapy may also have beneficial health effects. If postmeal exercise is effective enough, it could potentially lead to a reduction in insulin dose. A study in participants with type 1 diabetes found that prolonged walking (~40–50 km) led to profound reductions in insulin administration (26%) compared to a sedentary day (33). Future studies should investigate the effectiveness of more feasible exercise strategies, including postmeal exercise, as a complementary treatment to insulin.

For safety reasons, optimal diabetes treatments should have a low probability for eliciting hypoglycemia. The counter-regulatory response is a natural physiological process that defends against hypoglycemia, and this can occur if glucose falls too low during exercise. The counter-regulatory release of hormones into the circulation, including glucagon, catecholamines (epinephrine and norepinephrine), cortisol, and growth hormone is triggered when glucose drops below 3.8 mmol/L (34). This effect has been demonstrated experimentally in people with type 2 diabetes (25, 35) and should remain a consideration with prescribing exercise alongside hypoglycemic agents.

GLUCOSE-GUIDED APPROACH

Our current hypothesis that the effectiveness of an exercise bout will be dependent on the size of the glucose excursion itself. Therefore, optimal exercise approaches will require knowledge of glucose values in real time. One commonly used approach is self-monitoring capillary glucose with finger sticks and glucometers. When timed correctly, this method can be used to assess acute fluctuations in glucose after meals. In addition, CGM will likely play an important role. CGM technology uses a small probe within the subcutaneous tissue that samples and measures

glucose concentrations in the interstitial fluid. Future studies should determine the most effective and feasible approach for glucose-guided exercise prescriptions, which may involve a hybrid approach of glucometers and CGM.

CONCLUSION

Improving the treatment of type 2 diabetes is a major health care need. Taming postprandial glucose excursions can be accomplished by exercising after meals. The effectiveness of an exercise bout for lowering glucose will be dependent upon the size (peak and duration) of the postprandial glucose excursion. Larger excursions necessitate more aggressive intervention, while smaller excursions are easier targets for attenuation. Glucose monitoring techniques, such as glucometers and CGM technology, may have an important role in quantifying the effectiveness of exercise bouts.

AUTHOR CONTRIBUTIONS

ME, NJ, and KM developed ideas. ME drafted manuscript. NJ and KM edited and approved manuscript.

ACKNOWLEDGMENTS

The dissertation entitled "Effects of postmeal exercise and hypoglycemic agents on postprandial glucose excursions" informed concepts presented here (36).

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

frontiers in Endocrinology

MINI REVIEW
published: 23 May 2017
doi: 10.3389/fendo.2017.00109

Running from Disease: Molecular Mechanisms Associating Dopamine and Leptin Signaling in the Brain with Physical Inactivity, Obesity, and Type 2 Diabetes

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Physical inactivity is a primary contributor to diseases such as obesity, cardiovascular disease, and type 2 diabetes. Accelerometry data suggest that a majority of US adults fail to perform substantial levels of physical activity needed to improve health. Thus, understanding the molecular factors that stimulate physical activity, and physical inactivity, is imperative for the development of strategies to reduce sedentary behavior and in turn prevent chronic disease. Despite many of the well-known health benefits of physical activity being described, little is known about genetic and biological factors that may influence this complex behavior. The mesolimbic dopamine system regulates motivating and rewarding behavior as well as motor movement. Here, we present data supporting the hypothesis that obesity may mechanistically lower voluntary physical activity levels *via* dopamine dysregulation. In doing so, we review data that suggest mesolimbic dopamine activity is a strong contributor to voluntary physical activity behavior. We also summarize findings suggesting that obesity leads to central dopaminergic dysfunction, which in turn contributes to reductions in physical activity that often accompany obesity. Additionally, we highlight examples in which central leptin activity influences physical activity levels in a dopamine-dependent manner. Future elucidation of these mechanisms will help support strategies to increase physical activity levels in obese patients and prevent diseases caused by physical inactivity.

Keywords: physical activity, physical inactivity, motivation, dopamine, obesity, leptin

OPEN ACCESS

Edited by:
Jonathan Peter Little, University of British Columbia, Canada

Reviewed by:
Catherine M. Kotz, University of Minnesota, United States
Lindsay Spielman, University of British Columbia Okanagan, Canada

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

Received: *15 March 2017*
Accepted: *05 May 2017*
Published: *23 May 2017*

Citation:
Ruegsegger GN and Booth FW (2017) Running from Disease: Molecular Mechanisms Associating Dopamine and Leptin Signaling in the Brain with Physical Inactivity, Obesity, and Type 2 Diabetes. Front. Endocrinol. 8:109. doi: 10.3389/fendo.2017.00109

INTRODUCTION

Physical inactivity presents a major public health problem. Predictions by Lee et al. (1) estimated that physical inactivity accounts for between 6 and 10% of type 2 diabetes (T2D) and coronary heart disease prevalence, with this percentage further elevated for specific diseases (30% for ischemic heart disease) (2). Moreover, the World Health Organization declared physical inactivity as the fourth leading risk factor for death worldwide, responsible for ~6% of the deaths worldwide in 2008 (1, 2). Accelerometry measurements by Troiano et al. (3) reported that less than 5% of adults met the US guidelines for physical activity, while questionnaire data collected globally in 2009 suggested that 31%

of the world's population did not attain minimum recommended levels of physical activity (4). Given the deleterious effects of physical inactivity, understanding molecular mechanisms that influence physical activity adherence is needed. Here, we summarize current knowledge suggesting the mesolimbic dopamine system regulates physical activity, obesity-induced impairments in dopamine signaling may cause physical inactivity, and central leptin resistance in obesity and T2D may alter physical activity in a dopamine-dependent manner. Specifically, our discussion focuses on motivated and self-rewarding (i.e., voluntary wheel running), rather than spontaneous (i.e., cage activity, tremors, etc.), forms of physical activity.

GENETIC CONTROL OF PHYSICAL ACTIVITY

In 1953, Mayer, a leader who helped clarify the natures of hunger and of obesity, demonstrated that physical activity behavior has a biological basis (5). Mayer noted that obese, hyperglycemic mice were far less active than non-obese littermates. However, when the obese mice were bred against mice with a so-called "waltzing gene" physical activity increased to sufficiently prevent the development of obesity. Since Mayer's original speculation of an uncharacterized "waltzing gene," studies in animals and humans have estimated the genetic component for physical inactivity to be between 20 and 80% (6–12). Analysis of 772 same-sex twin pairs concluded that 31% of the variance in daily sedentary time was explained by heritable factors (13). Of these heritable factors, associations between dopamine and motivated physical activity are well established, as discussed below. However, other neuromodulators such as endocannabinoids (14, 15), opioids (16), and brain-derived neurotrophic factor (17) also influence physical activity behavior. Furthermore, interactions between these neuromodulatory systems imply that biological networks control voluntary physical activity (18). Evolutionary perspectives also argue that while selection did not operate to cope with the detrimental effects of long-term physical inactivity, humans adapted to avoid unnecessary exertion due to limited energy supply (19). Additionally, gene–environment interactions influence physical activity. Rowland (20) proposed that through components related to energy balance control an "activity-stat" may regulate the propensity for physical activity. Furthermore, obesity was speculated to be a critical negative influencer of the "activity-stat" (21). Collectively, these findings suggest that physical activity levels have strong genetic control.

DOPAMINERGIC CONTROL OF PHYSICAL ACTIVITY

Although detailed mechanisms describing the neurobiology of wheel running are incomplete, substantial evidence suggests that the mesolimbic dopamine pathway, specifically the ventral striatum and nucleus accumbens (NAc), plays an important role in determining voluntary running behavior (22–24). A detailed review of the mesolimbic dopamine system is beyond the scope of this review; however, a brief overview is provided next [please see Ref. (25, 26) for more detailed review]. In the mesolimbic dopamine system, dopaminergic neurons originating in the ventral tegmental area (VTA) project to various limbic nuclei, including the NAc, and changes in dopamine transmission play central roles in modulating information flow through the limbic system (27–30). These nuclei, through interconnections *via* dopaminergic neurons, have implications in reward, motivation, learning, and motor movement (31). Importantly, the NAc acts as a "filter" and/or "amplifier" of information passing between various limbic, cortical, and motor areas of the brain, suggesting the NAc is instrumental in orchestrating behavioral processes related to motivation (25). Several reports have demonstrated that other mesolimbic structures, such as the VTA and prefrontal cortex, contribute to reward derived from physical activity, potentially through their interactions with the NAc (32–34).

Disruption of dopaminergic transmission and/or dopamine receptor expression in the NAc and ventral striatum can strongly influence voluntary physical activity. The depletion of NAc dopamine by 6-hydroxydopamine decreased wheel running ~40% (35). Knab et al. (22) suggested that differences in dopamine 1-like (D1-like) receptors and tyrosine hydroxylase (*Th*) mRNA, the rate-limiting enzyme in dopamine synthesis, in the NAc influence different running distances between mouse strains.

Selective breeding studies have provided ample insight into voluntary physical activity regulation. Mice bred by Garland et al for high voluntary running distance displayed dysfunctional dopaminergic profiles in the NAc (36, 37) and increased dopamine receptor 2 (*Drd2*) and dopamine receptor 4 (*Drd4*) mRNA ~20% in the hippocampus (38), compared to control mice. Furthermore, agonism (24) and antagonism (37) of D1-like receptors in the NAc paradoxically both decreased wheel running in high-running mice to a greater extent than in control mice. Similar findings from our group using rat lines selectively bred for high (HVR) and low (LVR) wheel-running suggested rats predisposed to run high nightly distances may quickly develop a rewarding response to exercise due to optimal D1-like receptor signaling in the NAc (39). Collectively, these data suggest the following: (1) dopamine signaling is optimally primed to achieve reward associated with running in high-running rats, (2) dopamine is at least partially required for wheel-running behavior, and (3) animals run to achieve the rewarding effects of dopamine but do not want to run when dopamine signaling is artificially activated. Dopamine receptors 1 (*Drd1*), *Drd2*, and dopamine receptor 5 (*Drd5*) mRNA were also inherently 50 to 85% higher in the NAc of HVR compared to LVR (16). Similarly, inherent ~1.3-fold increases in NAc *Drd1* mRNA and ~1.8-fold greater dopaminergic activity were speculated to mediate increased wheel running in rats selectively bred for high, compared to low, aerobic capacity, suggesting that aerobic capacity may influence physical activity levels through alterations in mesolimbic dopamine activity (40, 41). Furthermore, the loss of dopamine receptors or reduced dopamine release in the brain was associated with age-related declines in physical activity across many species (42) and was hypothesized to influence age-related physical activity reductions in humans (43). Single nucleotide polymorphism (SNP) analysis suggested that the *DRD2* gene associated with physical activity levels in women

(44) and that individuals with the CC homozygous variant in rs1800955 of the *DRD4* gene were more prone to sport-specific sensation seeking (45). Similarly, Wilkinson et al. (46) found associations between SNPs in two dopamine pathway genes, angiotensin I converting enzyme (*ACE*) and synaptosomal-associated protein 25 (*SNAP25*), and decreased likelihood for physical activity in youth.

However, whether alterations in the dopamine system are the result or driver of differences in voluntary physical activity is unknown. For example, previous reports show that voluntary wheel running is rewarding, and over time, able to alter behavior and affect the neuroplasticity of the mesolimbic reward pathway (34). Furthermore, endurance exercise training increased central dopamine concentrations up to 1.5-fold (47). Thus, physical activity, itself, could function in a feed-forward mechanism to further elevate physical activity.

OBESITY AND DOPAMINERGIC DYSREGULATION

In the past three decades, obesity prevalence in the US has risen from below 20 to 36.5% (48). Additionally, physical inactivity levels and excessive food intake have increased over a similar period, directly contributing to increases in obesity and T2D (1) (**Figure 1**). Increases in unadjusted food intake from ~1980 to 1994 were associated with initial rapid increases in obesity, but not T2D, prevalence. Furthermore, beginning in ~1998 to 2000, physical activity levels rapidly dropped and sedentary time rapidly increased. This decrease in physical activity and increase in physical inactivity corresponded with increases in both obesity and T2D prevalence, despite food intake staying relatively constant during the same period. In our opinion, more recent increases in obesity are thus better associated with physical inactivity increases as caloric intakes were unchanged. Importantly, while declining physical activity levels contribute to obesity development, obesity contributed to reductions in physical activity in humans, even after controlling for baseline differences in physical activity (49). This interaction may promote the development of self-perpetuating vicious cycles whereby physical inactivity and obesity promote each other's development (50).

The effects of obesity on the mesolimbic dopamine system are well studied, and hypotheses suggesting "reward dysfunction" in obesity have developed given findings that obesity is associated with alterations in striatal dopamine signaling (55).

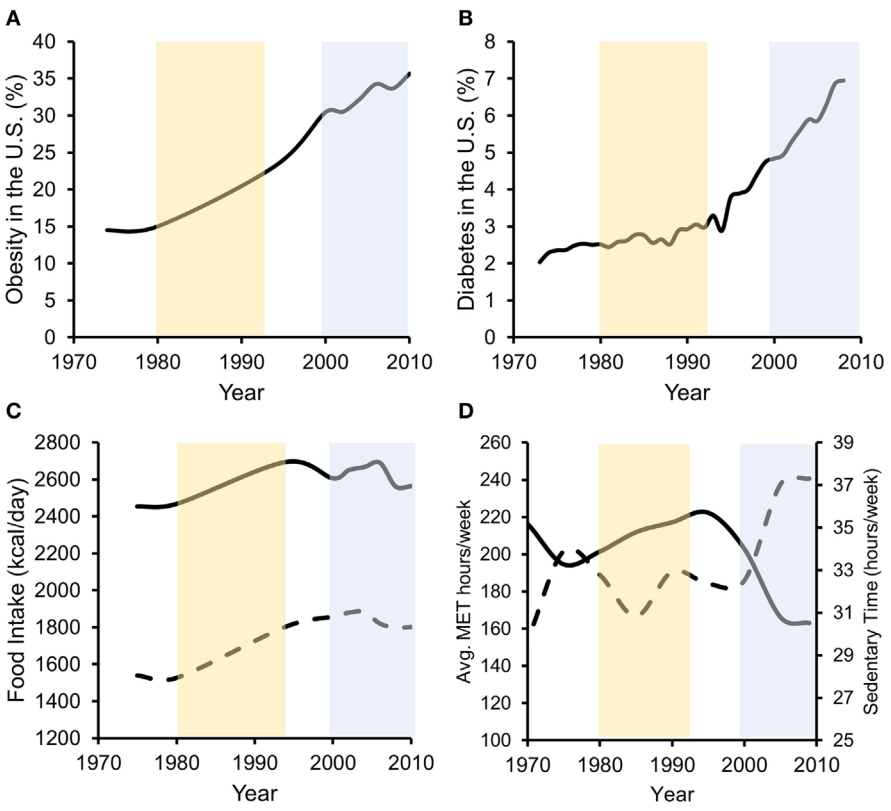

FIGURE 1 | Data suggest that both increases in energy intake and reductions in energy expenditure associate with increased obesity prevalence, while in later years, decreased energy expenditure more strongly associates with T2D prevalence. Percentage of US adults with obesity **(A)** or diagnosed with type 2 diabetes **(B)** over the past ~40 years. **(C)** Unadjusted food intake for male (solid line) and female (dashed line) adults in the US during the same time frame. **(D)** Physical activity (solid line/left axis) [average metabolic equivalent (MET) hours per week] and physical inactivity (dashed line/right axis) (hours per week of sedentary time) performed by US adults. Obesity data redrawn from Ref. (48, 51), diabetes data from the CDC (52), food intake data from Ref. (53), and physical activity data from Ref. (54).

For example, reduced dopamine function, particularly DRD2 signaling, is associated with obesity development in rodents (56–59) and humans (60–62). However, these studies associated hyperphagia with obesity development and did not assess physical activity. Similarly, using positron emission tomography (PET) Guo et al. (63) observed a negative relationship between D2-like receptor binding in the ventral striatum and body mass index (BMI), suggesting that BMI could influence rewarding and effort-based actions. Similar measurements associating D1-like receptor neuron activity with obesity in humans are lacking, although several animal studies found that *Drd1* mRNA is reduced up to ninefold in the NAc of obese rats (64, 65). High-fat diet consumption for 12 weeks decreased tonic dopamine and *Drd1* and *Drd2* mRNA expression ~50% in the NAc of mice (66). Interestingly, following a 4-week recovery from high-fat diet, NAc *Drd1* and *Drd2* mRNA expressions were normalized in female, but not male, mice (66). Similarly, PET studies in humans show that DRD2 binding is not recovered (67) or partially recovered (68) following Roux-en-Y gastric bypass surgery. Collectively, these data suggest that reductions in dopamine function accompanying obesity could persist following weight loss. This notion is consistent with findings that physical inactivity levels remained high in obese humans months after weight loss (69–71), raising the question whether "physical activity resistance" exists temporarily/permanently after weight loss.

Interestingly, animal studies also suggest that high-fat diet exposure, rather than weight gain, may be more predictive of changes in striatal dopamine signaling. Isocaloric high-fat diet feeding in rats resulted in ~40% lower DRD2 in the NAc (72). Furthermore, chronic *ad libitum* high-fat diet reduced dopamine turnover 3.5-fold in the NAc of rats, although similar reductions were observed following isocaloric high-fat diet (73). Additionally, animal studies suggest that longer-term high-fat diet exposure can suppress dopamine synthesis, release, or turnover, ultimately reducing motivated behaviors not limited to motivation for food, such as physical activity (74). Despite considerable variability in experimental outcomes, we conclude that decreased dopamine signaling, particularly decreased D2-type function, could be particularly relevant to obesity.

OBESITY AND PHYSICAL INACTIVITY

Obesity is strongly associated with physical inactivity (75, 76). While sparsely studied, several studies suggest that diet-induced dopaminergic alterations accompanying obesity may promote physical inactivity. Friend et al. (77) noted that diet-induced obesity in mice reduced D2-type receptor binding in the striatum that associated with decreased voluntary physical activity. Furthermore, in the same study the deletion of the *Drd2* gene, specifically in inhibitory medium spiny neurons (iMSNs), decreased wheel revolutions compared to littermate controls, although these mice were surprisingly not more vulnerable to diet-induced weight gain (77). Finally, the restoration of iMSN signaling reversed deficits in wheel running (77). Collectively, these data support the notion that D2-type receptor dysregulation contributes to obesity-induced physical inactivity, but that physical inactivity may be a consequence, rather than effector, of obesity.

Similarly, comparisons between mice bred for excessive exercise or obesity revealed that NAc dopamine content was increased in high running compared to obese and control mice, while *Drd1*, *Drd2*, and adenylate cyclase 5 (*Adcy5*) mRNAs were downregulated 92, 80, and 91%, respectively, in obese compared to control mice (78). Nonetheless, the authors hypothesized that modifications in the dopaminergic system may contribute to the differences in voluntary exercise between the high-running and obese mice (78). Analysis of obesity-resistant, compared to obesity-prone, rats also suggested that reduced physical activity levels in obesity-prone rats may stem from decreased action of hypothalamic orexin on dopamine neurons in the striatum and substantia nigra (79, 80). Finally, lower striatal dopaminergic activity may have contributed to low wheel running activity in rats with low aerobic capacity, who also had greater body weight and metabolic disease risk (40).

A recent study found that decreased DRD2 signaling in the striatum influences obesity development *via* reductions in physical activity rather than increases in food intake. Using *Drd2* knockdown mice, Beeler et al. (81) observed that when presented with voluntary exercise in an enriched environment, *Drd2* knockdown mice were dramatically less active than wild-type mice. Importantly, in the same study reduced voluntary exercise by *Drd2* knockdown mice promoted an obese phenotype despite no differences in food intake (81). These intriguing observations not only suggest a direct link between reduced dopamine function and decreased physical activity, but that the decreases DRD2 signaling can contribute to obesity *via* reduced energy expenditure rather than the initiation of compulsive overeating. Furthermore, obesity-induced reduction in DRD2 signaling could initiate the following feedback mechanism to further amplify obesity and physical inactivity: obesity → ↓ DRD2 signaling → ↑ physical inactivity → ↑ obesity → futile cycle. On the contrary, separate experiments show that dietary restriction increased wheel running (82) and dopamine overflow and receptor expression in the NAc (83, 84), suggesting that obesity and dietary restriction may have opposing effects on dopamine signaling and, in turn, voluntary physical activity. However, future research is needed to dissect causal and consequential relationships between obesity, dopamine, and physical inactivity.

CENTRAL LEPTIN ACTION AND PHYSICAL ACTIVITY

Relationships between leptin and physical activity are well established. Central leptin resistance is a hallmark of obesity (85, 86), and leptin resistance in the VTA following diet-induced obesity has been noted previously (87). Normal leptin signaling in VTA dopaminergic neurons is well characterized, with a general consensus being that leptin receptor (LEPR) signaling inhibits dopamine activity (88–90). Correspondingly, associations between select *DRD2* and *LEPR* allelic gene variations have been associated with the development of severe obesity (91).

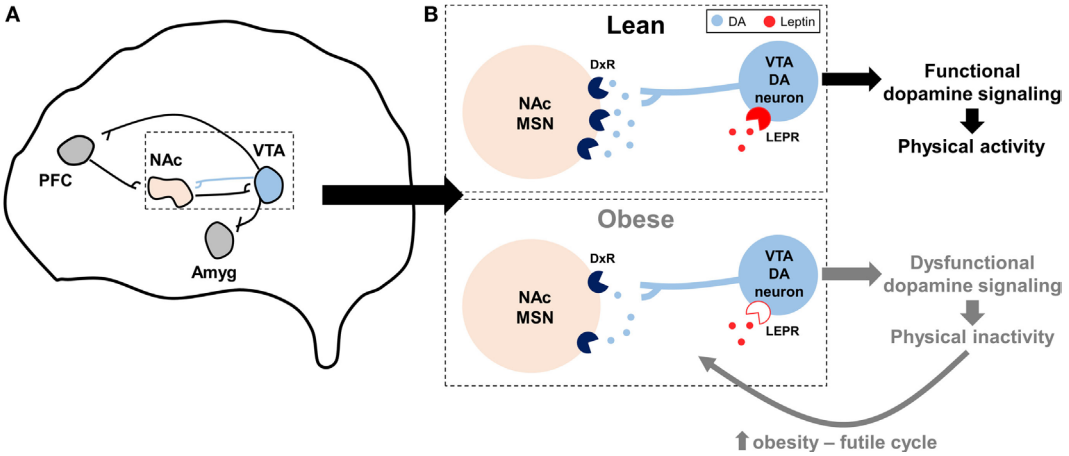

FIGURE 2 | **Hypothesized model by which impaired dopaminergic signaling promotes physical inactivity in obesity**. **(A)** Summary of the reward circuitry in the brain; originally established by Robison and Nestler (95). The blue projection illustrates dopaminergic projections from the ventral tegmental area (VTA) that release dopamine (DA) onto post-synaptic neurons in the nucleus accumbens (NAc). **(B)** Expanded, but simplified, illustration of this dopaminergic VTA to NAc projection as it is hypothesized to relate to physical inactivity in lean and obese individuals. In obesity, dopamine receptor (DxR), particularly dopamine receptor 2, expression is decreased in NAc medium spiny neurons (MSNs). Similarly, mechanisms controlling DA production and release are reduced with obesity, leading to less DA in the synapse. Central leptin resistance in obesity [denoted by open leptin receptor (LEPR) symbol] may influence LEPR signaling in VTA DA neurons, in turn further diminishing downstream DA function. Collectively, these obesity-induced impairments in dopaminergic signaling may lead to exacerbated levels of physical inactivity, which may in turn lead to a futile cycle of increased obesity, dopaminergic dysregulation, and physical inactivity. Other abbreviations: Amyg, amygdala; PFC, prefrontal cortex.

Leptin suppressed the rewarding effects of wheel running in mice *via* activation of signal transducer and activator of transcription-3 (STAT3) signaling in VTA dopamine neurons, an effect which likely influenced dopamine overflow and function in the NAc and suggested that leptin may influence the motivational and rewarding effects of wheel running (92). Additional studies show that dopamine overflow in the NAc is reduced by leptin deficiency (88) and diet-induced obesity (57). In mice bred by Garland et al for high voluntary wheel running, which display dysfunctional dopaminergic profiles in the NAc as described above (36, 37), intraperitoneal leptin injection increased running by 17%, while control mice were unaffected (93). Paradoxically, in the same study high-fat feeding increased wheel running 20% in high-running mice, an effect speculated to be mediated by leptin (93). Intracerebroventricular injection of a recombinant adeno-associated virus (rAAV) overexpressing a mutant of leptin, which produces a protein that acts as a LEPR antagonist, decreased wheel running 25 and 40% in rats fed either a standard chow or high-fat diet, respectively, while rAAV overexpression of functional leptin increased wheel running ~2-fold Matheny et al. (94). However, changes in voluntary physical activity in the Matheny et al. study could be secondary to changes in adiposity following rAAV injection. Collectively, a hypothesis describing the interaction between obesity, dopamine, leptin, and physical inactivity is presented in **Figure 2**.

Further suggesting that leptin may impact the motivational and rewarding effects of running are observations that high serum leptin levels inversely correlated with low marathon run times after BMI adjustment (96), and with running performance (time and speed) in mice bred for high voluntary running (97). Leptin deficiency has also been shown to influence physical activity humans, whereas acute leptin increased locomotor activity in leptin-deficient patients during the fed state (98, 99). Similarly, leptin-deficient *ob/ob* mice increased wheel running 3.5-fold during the fed state following acute subcutaneous leptin injection, while no effect was observed in wild-type mice (100). Collectively, these studies highlight the important role of leptin as an effector of voluntary physical activity, potentially through alternations in dopamine signaling.

CONCLUSION

Physical inactivity and obesity have reached pandemic levels (101). The abovementioned studies strongly suggest that dopaminergic function influences physical inactivity levels. Similarly, obesity-induced suppression of dopamine signaling may contribute to the high prevalence of physical inactivity observed in obese people. Additional understanding of mechanisms by which dopaminergic dysfunction contributes to obesity, physical inactivity, or their interactions may reveal novel approaches for increasing physically activity in obese populations.

AUTHOR CONTRIBUTIONS

GR and FB conceived the idea, wrote, and edited this manuscript.

ACKNOWLEDGMENTS

The authors disclose no conflicts of interest. No funding sources were acquired for this project.

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

frontiers in Endocrinology

REVIEW
published: 05 May 2017
doi: 10.3389/fendo.2017.00087

Exercise and Glycemic Control: Focus on Redox Homeostasis and Redox-Sensitive Protein Signaling

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Physical inactivity, excess energy consumption, and obesity are associated with elevated systemic oxidative stress and the sustained activation of redox-sensitive stress-activated protein kinase (SAPK) and mitogen-activated protein kinase signaling pathways. Sustained SAPK activation leads to aberrant insulin signaling, impaired glycemic control, and the development and progression of cardiometabolic disease. Paradoxically, acute exercise transiently increases oxidative stress and SAPK signaling, yet postexercise glycemic control and skeletal muscle function are enhanced. Furthermore, regular exercise leads to the upregulation of antioxidant defense, which likely assists in the mitigation of chronic oxidative stress-associated disease. In this review, we explore the complex spatiotemporal interplay between exercise, oxidative stress, and glycemic control, and highlight exercise-induced reactive oxygen species and redox-sensitive protein signaling as important regulators of glucose homeostasis.

Keywords: exercise, insulin signaling, stress kinase, glycemic control, oxidative stress, redox

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

Received: *31 January 2017*
Accepted: *04 April 2017*
Published: *05 May 2017*

Citation:
Parker L, Shaw CS, Stepto NK and Levinger I (2017) Exercise and Glycemic Control: Focus on Redox Homeostasis and Redox-Sensitive Protein Signaling. Front. Endocrinol. 8:87. doi: 10.3389/fendo.2017.00087

INTRODUCTION

Physical inactivity and excess adipose tissue are associated with the development of insulin resistance and type 2 diabetes mellitus (T2DM), which has reached epidemic proportions (1). Regular exercise can assist in the prevention and management of metabolic disease (2). Even a single session of exercise can improve glycemic control for up to 48 h postexercise (3–5). Improved glycemic control following acute and regular exercise occurs in part through improved insulin action and substrate metabolism in skeletal muscle (6, 7) by mechanisms that remain largely unknown. One potential mechanism may involve reactive oxygen species (ROS) and their paradoxical dual role in the pathophysiology of glucose homeostasis (8, 9). Considering that acute and chronic exercise training lead to alterations in oxidation–reduction (redox) homeostasis (10, 11), it is not surprising that redox biology has been proposed as a possible modulator of glycemic control and skeletal muscle adaptation to exercise (12–14). This review explores current evidence supporting exercise-induced ROS and skeletal muscle redox-sensitive protein signaling as important regulators of glucose homeostasis.

EXERCISE AND GLYCEMIC CONTROL

Insulin-Stimulated Glucose Uptake

Glucose homeostasis is vital for organism survival and involves the complex interaction between intestinal glucose absorption, liver gluconeogenesis and glycogenolysis, and tissue glucose uptake (15). During conditions of elevated substrate availability, for example, a glucose load from a meal,

elevated blood glucose is sensed by pancreatic β-cells resulting in the secretion of insulin to maintain glucose homeostasis (15). Under normal physiological conditions, insulin binds to the extracellular α-subunit of the insulin receptor promoting autophosphorylation of the transmembrane β-subunit on tyrosine residues 1158, 1162, and 1163 (16). Scaffolding proteins including Shc adapter protein isoforms, signal-regulatory protein family members, Gab-1, Cbl, adapter protein with a PH and SH2 domain, and insulin receptor substrates (IRS) are bound, and tyrosine residues phosphorylated to promote subsequent binding to phosphatidylinositol-3 kinase (PI3K) (17, 18). Activation of PI3K generates phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) that docks to and subsequently induces membrane translocation of the serine/threonine kinase Akt. PIP_3 activation of phosphoinositide-dependent kinase-1 (PDK1) and the Rictor/mTOR complex 2 lead to dual phosphorylation of Akt on serine 473 and threonine residue 308 promoting subsequent activation of Akt kinase (19, 20). Increased Akt activity elicits phosphorylation of Akt substrate of 160 kDa (AS160; also known as TBC1D4) and TBC1D1 (21), promoting GTP loading and activation of Rab proteins releasing glucose transporter 4 (GLUT4) vesicles from intracellular compartments and promoting GLUT4 plasma membrane docking to facilitate glucose uptake (22–24).

Akt phosphorylation not only promotes GLUT4 translocation but also facilitates glycogen synthesis *via* inhibitory phosphorylation of glycogen synthase kinase 3 (GSK3) on Ser23 (GSK3α) and Ser9 (GSK3β) (25–27). PIP_3 and PDK1 also activate atypical protein kinase C (PKC) isoforms ζ and λ, which are reported to facilitate GLUT4 vesicle trafficking and glucose uptake (28, 29). A summary of the canonical insulin signaling pathway is presented in **Figure 1**.

Glucose Uptake during Exercise

Glucose uptake during exercise occurs in an exercise intensity- and exercise duration-dependent manner, which depends largely on a combination of increased glucose delivery, glucose transport, and glucose metabolism (7). Increased trafficking of GLUT4 to the plasma membrane during exercise occurs largely through mechanisms independent of insulin (7). These include the cellular detection of changes in Ca^{2+} concentration (30, 31), changes in the energy status (ATP) of the cell (32–35), remodeling of the actin cytoskeleton *via* GTPase Rac1 (36), and fiber type-specific mediation of nitric oxide (NO) synthase (37). The primary protein signaling pathways include contraction-induced activation of calcium (Ca^{2+})/calmodulin-dependent kinase, atypical PKC, calcineurin, 5′ adenosine monophosphate-activated protein kinase (AMPK), Akt, and mitogen-activated protein kinases (12, 38). Exercise-induced AMPK, and to a lesser extent Ca^{2+} signaling pathways (30, 31), elicits GLUT4 translocation and subsequent glucose uptake through phosphorylation and inactivation of the convergent glucose uptake signaling proteins AS160 and TBC1D1 (21, 24, 39–42) (**Figure 2**).

Postexercise Enhancement of Insulin Sensitivity

Glucose uptake during exercise is maintained in populations who are insulin resistant and/or have been diagnosed with type 2 diabetes (43). In contrast, basal and postexercise insulin-stimulated glucose uptake appears to be impaired and contribute to the development of chronic disease (8, 44, 45). Regular exercise in both healthy and clinical populations improves indices of glycemic control including glycated hemoglobin (HbA1c) and insulin sensitivity in a "dose"-dependent manner (duration and intensity) (2, 46). It is generally conceded that training-induced improvements in glycemic control lead to improved insulin action in part through the upregulation of key skeletal muscle glucose homeostasis regulatory proteins such as Akt1/2, AS160, AMPK, hexokinase 2, and importantly GLUT4 (6, 7). Improved insulin action may also occur through exercise-induced mitochondrial biogenesis and improved mitochondrial function in addition to the upregulation of antioxidant defenses that lead to improved redox homeostasis (6, 13).

In contrast to regular exercise, the transient enhancement of insulin sensitivity in the hours after acute exercise appear to occur independent of modifications to the insulin receptor, IRS1/2, PI3K, Akt, and/or GSK3 α/β proteins (3, 14, 47, 48). It has been reported that AS160 and TBC1D1, which converge downstream of insulin- and contraction-mediated glucose uptake signaling pathways, are associated with the postexercise enhancement of insulin sensitivity (14, 42, 49–53). Although decades of research have contributed to a greater understanding of exercise and glycemic control, the specific exercise-induced signaling mechanisms leading to the acute and long-term adaptations that favor enhanced glycemic control are less clear (3, 7). One potential mechanism may be through exercise-induced ROS and their capacity to act as second messengers for skeletal muscle cell signaling (13, 14, 54, 55).

REDOX HOMEOSTASIS

Biological organisms are constantly undergoing oxidation–reduction (redox) reactions to maintain a redox environment that is optimal for cellular signaling (56). Under certain circumstances, excess ROS production can lead to oxidative damage and/or modification of lipids, proteins, RNA, and DNA, leading to a redox state that is often referred to as oxidative stress (57). ROS production in a biological system occurs through numerous sources including radiation, environmental pollutants, chemotherapeutics, psychological stress (58), normal and abnormal cellular substrate metabolism (9, 59), and mechanical and physiological stress induced through exercise (9, 11). ROS considered to be of biological importance, which includes hydroxyl radical (OH), superoxide anion $\left(O_2^-\right)$, NO, peroxyl radical, peroxynitrite, hypochlorous acid, hydrogen peroxide (H_2O_2), singlet oxygen, and ozone (57, 60). It should be noted that reactive nitrogen species and reactive sulfur species also constitute separate radical groups with independent biological functions (61, 62); however, their discussion lies beyond the scope of this review.

Reactive oxygen species are capable of direct and/or indirect oxidative modification to proteins (63). Sustained oxidation of proteins can result in disruptions in the normal functioning of the proteome including protein inactivation (64), modification of the protein side chains, fragmentation of peptide bonds (65), and structural unfolding and conformational changes (66).

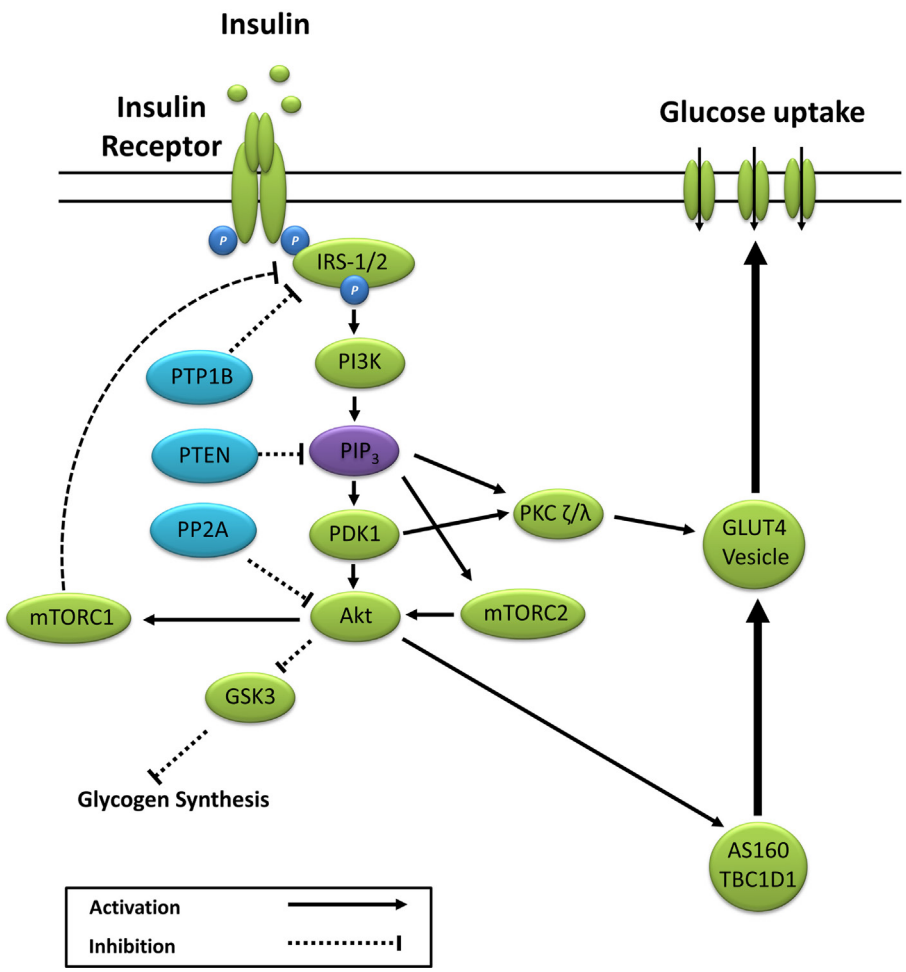

FIGURE 1 | **Primary signaling pathways involved in insulin-stimulated glucose uptake**. Akt, protein kinase B; AS160, Akt substrate of 160 kDa; GLUT4, glucose transporter 4; GSK3, glycogen synthase kinase 3; IRS-1/2, insulin receptor substrates 1 and 2; mTORC1/2, mechanistic target of rapamycin complex 1/2; PDK1, phosphoinositide-dependent kinase-1; PI3K, phosphatidylinositol-3 kinase; PIP_3, phosphatidylinositol (3,4,5)-trisphosphate; PKC, protein kinase C; PP2A, protein phosphatase 2; PTEN, phosphatase and tensin homolog; PTP1B, protein tyrosine phosphatase 1B.

Likewise, ROS are implicated in oxidative damage to DNA, a process that ultimately results in strand breakage, DNA–protein crosslinks and base alterations, and defective DNA transcription and translation leading to the synthesis of less protein and/or defective protein (67–69). In addition to DNA, both messenger and ribosomal RNA are vulnerable to oxidative damage, which can lead to the disturbance of translational process and impairment of protein synthesis (69). ROS-induced damage to mRNA occurs primarily through the formation of highly reactive free radicals such as the OH (70) and appears to be selective and independent of the abundance of the mRNA species (69). Although RNA is highly susceptible to oxidative damage, considerably more so than DNA, protein, and lipids (69), to the authors knowledge, research has yet to investigate the effect of exercise-induced ROS production on RNA damage and the subsequent effects on protein synthesis and exercise adaption.

Lipids, especially polyunsaturated fatty acids, are susceptible to oxidative degradation, a process referred to as lipid peroxidation, which can result in a chain reaction leading to subsequent formation of peroxyl radicals and hydroperoxides (71). In addition to the direct cellular damage caused by ROS-induced lipid peroxidation, secondary products from lipid peroxidation such as malondialdehyde, propanal, hexanal, and the highly toxic 4-hydroxynonenal (4-HNE) can elicit signaling events that contribute to the development of cardiometabolic disease (72–75).

Disturbances in redox homeostasis can lead to perturbed redox signaling and aberrant cellular functioning (56). Therefore, organisms have evolved to encompass a complex and interconnected antioxidant defense system, which helps maintain redox homeostasis through the reduction of ROS and/or ROS intermediates, subsequent termination of ROS-mediated chain reactions, and/or through ROS-induced damage repair mechanisms

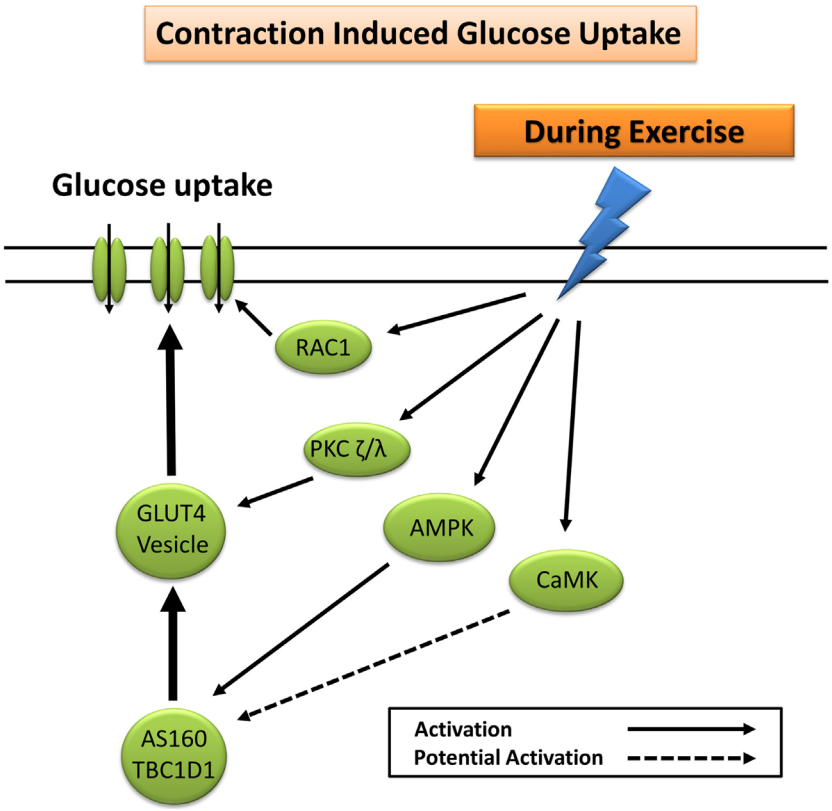

FIGURE 2 | Primary signaling pathways involved in contraction-induced glucose uptake. AMPK, 5′ adenosine monophosphate-activated protein kinase; AS160, Akt substrate of 160 kDa; CaMK, Ca^{2+}/calmodulin-dependent protein kinase; GLUT4, glucose transporter 4; PKC, protein kinase C; RAC1, ras-related C3 botulinum toxin substrate 1.

(60, 76). These defenses include a number of redox-buffering enzymes, proteins, and scavengers, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)/reductase, thioredoxin, peroxiredoxin, inducible nitric oxide synthase (iNOS), gamma-glutamylcysteine synthetase, redox effector factor 1, nuclear factor erythroid 2-related factor 2, antioxidant response element, Kelch-like ECH-associated protein 1, uric acid, lipoic acid, bilirubin, coenzyme Q10, vitamin C, vitamin E, and carotenoids (57, 60, 77–82).

OXIDATIVE STRESS AND METABOLIC HEALTH

Chronically elevated systemic oxidative stress is associated with over 100 pathological conditions including accelerated aging, cardiovascular disorders, insulin resistance, and T2DM (9, 57, 83). Considerable research has reported attenuated antioxidant defense and elevated basal oxidative stress in populations with chronic disease, often correlating with classical cardiometabolic risk factors such as increased circulating high-sensitivity C-reactive protein, greater waist-to-hip ratio, total cholesterol, triglycerides, and fasting blood glucose (84–90). As such, the measurement of basal systemic oxidative stress has been proposed as a marker for predicting the onset of a disease, assessing the progression of a disease, and evaluating the effect of pharmacological (e.g., antioxidant supplementation) and non-pharmacological (e.g., diet and exercise) therapies targeting oxidative stress-associated disease (81, 87, 91).

EXERCISE-INDUCED OXIDATIVE STRESS

Acute exercise elicits a transient state of elevated ROS, which depending on the type of exercise, duration and intensity, and antioxidant capacity of the individual, can result in oxidative stress (11, 87, 92). In contrast to chronic oxidative stress, the transient increase in ROS and oxidative stress elicited by most types of exercise (i.e., non-extreme muscle damaging exercise) are reported to be beneficial and a necessary requirement for optimal cellular functioning and adaptation to physiological stress (79).

Mechanisms for Exercise-Induced Oxidative Stress

The mechanisms of intracellular and extracellular ROS generation in skeletal muscle during exercise are reviewed in detail elsewhere (93–95). In brief, the primary mechanisms are suggested to include NADPH oxidase (96, 97), xanthine oxidase (98), NO synthase (99), and arachidonic acid release from cell

membranes by phospholipase A2 (100), whereas mitochondrial electron leak is suggested to contribute only marginally during muscular contraction (101) (**Figure 3**). Other mechanisms that may contribute to elevated skeletal muscle and/or plasma oxidative stress include the oxidation of catecholamine (102), lactate accumulation (103, 104), elevated core body temperature (105), hemoglobin and myoglobin-mediated autooxidation (106–108), and postexercise inflammatory and phagocytic responses including ischemic reperfusion, cytokine secretion, and respiratory burst (109–111).

Although plasma oxidative stress is commonly measured as an indicator of exercise-induced oxidative stress, the exact sources of systemic oxidative stress following skeletal muscle contraction are not well understood. Nevertheless, due to the large proportion of body mass that is constituted by skeletal muscle, it is proposed that skeletal muscle fibers, vascular cells, endothelial cells, and/or blood cells residing within skeletal tissue are the main contributors of both the exercise-induced local and systemic oxidative stress (95). *Ex vivo* skeletal muscle contraction studies have established the potential for skeletal muscle to elicit systemic oxidative stress (95, 112, 113). The specific cell types that contribute to skeletal muscle ROS production likely include vascular smooth muscle cells, endothelial cells, fibroblasts, erythrocytes, and white blood cells, with skeletal muscle fibers suggested to play the biggest role in the generation of extracellular ROS during and after exercise (95, 114, 115). Other tissues such as the heart, liver, and lungs may also contribute to the systemic increase in oxidative stress following acute exercise, but likely to a lesser degree (95).

Exercise-Induced Oxidative Stress and Metabolic Health

To date, the literature is equivocal in regards to the effect of acute exercise on biomarkers of oxidative stress and antioxidant activity (11). Inconsistencies in the literature likely result from variations in dietary intake, training status, exercise intensity (5, 11, 92, 116, 117), exercise duration (11, 118, 119), exercise mode (11, 119), tissues sampled (119), sampling time points (119, 120), as well as the variety and volatility of the biochemical assays used (121). Nevertheless, the general consensus is that acute exercise elicits a transient increase in systemic and localized oxidative stress and antioxidant defense, which, depending on the intensity and mode of exercise, can be detected for up to 4 days after exercise (11, 116, 122).

Excessive ROS production and/or oxidative stress induced through severe or extreme exercise regimes (e.g., ultraendurance events) in humans is associated with cellular disturbances promoting muscular fatigue (94, 123), aberrant upregulation of endogenous antioxidant defenses (124, 125), and impaired cognitive function (126). Similarly, impaired exercise tolerance and physiological responses have been documented in murine animal models (127). For example, Aoi et al. (128) reported that muscle damaging exercise in mice induced through downhill running increased skeletal muscle oxidative stress [thiobarbituric acid reactive substances (TBARS)] and resulted in 4-HNE-mediated impairment of the canonical insulin protein signaling pathway and decreased insulin-stimulated glucose uptake 24 h after exercise. Thus, under certain conditions, exercise-induced oxidative stress has the potential to elicit a deleterious redox

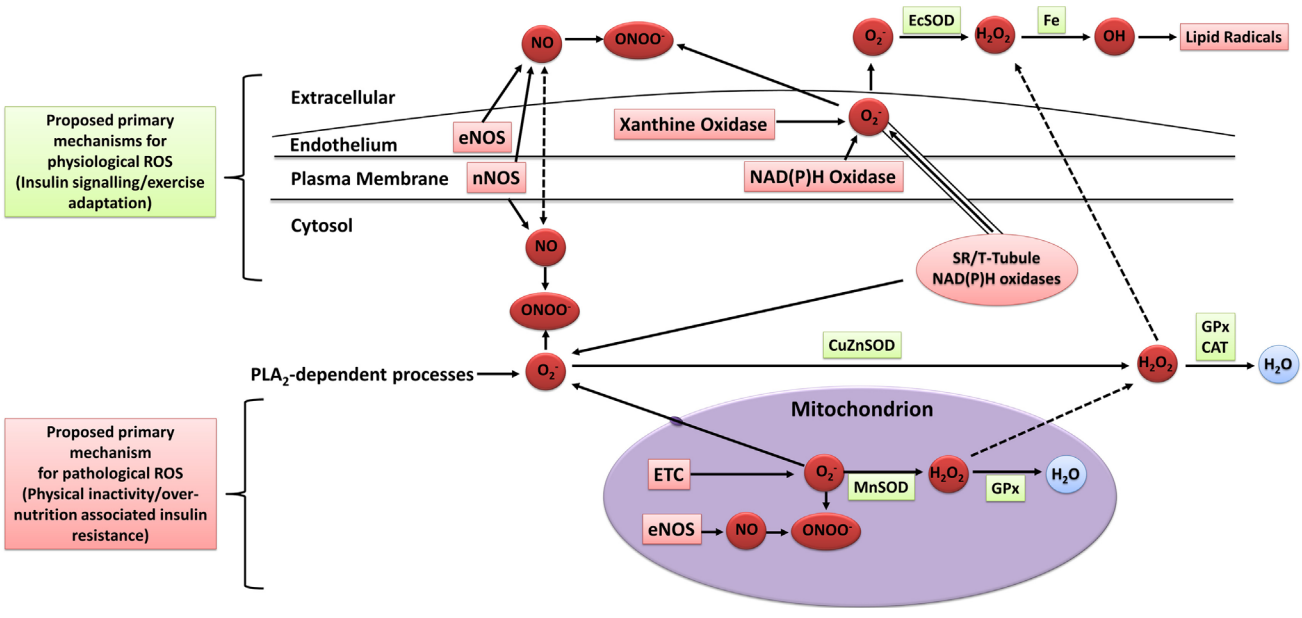

FIGURE 3 | Sources of ROS in skeletal muscle. ETC, electron transport chain; eNOS, endothelial nitric oxide synthase; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; $ONOO^-$, peroxynitrite; OH, hydroxyl radical; O_2^-, superoxide; H_2O_2, hydrogen peroxide; H_2O, water; EcSOD, extracellular superoxide dismutase; MnSOD, manganese superoxide dismutase; CuZuSOD, copper–zinc superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase; PLA_2, phospholipase A2; Fe, iron; ROS, reactive oxygen species. Adapted from the study by Powers and Jackson (93) with permission.

environment conducive to impaired exercise capacity and health (**Figure 4**).

The pathological effects of exercise-induced oxidative stress likely stem from secondary muscle damage leading to phagocytic infiltration into skeletal muscle (129) and subsequent generation of ROS (130, 131). In support, Nikolaidis et al. (122) reported that muscle damaging exercise (75 lengthening knee flexions) significantly increased serum oxidative stress (TBARS, oxidized GSH, and protein carbonyls) and serum antioxidant defense (CAT activity, uric acid, bilirubin, and total antioxidant capacity), which lasted for up to 4 days after exercise. When a second identical bout of exercise was performed 3 weeks later, indices of muscle damage were lower, including improved isometric torque, which coincided with attenuation of the postexercise systemic redox response (122). Thus, the acute exercise-induced oxidative stress impairment of exercise performance, recovery, and metabolic health appears to occur independently from the transient and immediate increase in oxidative stress measured during and after exercise and is likely attenuated with subsequent exercise-induced oxidative stress insults (e.g., exercise training).

The majority of literature supports the idea that transient ROS production and/or oxidative stress elicited through regular exercise regimes (e.g., accustomed and/or non-extreme muscle damaging exercise) is beneficial and a necessary requirement for optimal physiological functioning and adaptation to physiological stress (79). Samjoo et al. (132) reported that 12 weeks of endurance training (2–3 sessions per week of 30–60 min cycling at 50–70% VO_{2peak}) in obese and sedentary men decreased basal skeletal muscle and urinary markers of oxidative stress (4-HNE, protein carbonyls, and 8-isoprostane), increased basal skeletal muscle MnSOD protein abundance, and improved indices of glycemic control. Thus, repetitive sessions of exercise-induced ROS (i.e., exercise training) can improve metabolic health through the upregulation of endogenous antioxidant defense and attenuation of basal chronic oxidative stress. Further support for the beneficial effect of exercise-induced ROS can be found in human and animal studies that have reported antioxidant compounds to impair exercise capacity (133, 134), adaptive gene expression and protein synthesis (133, 135–138), upregulation of antioxidant defense (10, 13, 133, 136, 139, 140), cardiovascular health (141, 142), skeletal muscle inflammatory response and repair capabilities (134, 139), and insulin sensitivity (13, 55, 143, 144). Not all studies have reported the blunting of the aforementioned exercise-mediated adaptations (145–149), with some reports indicating enhanced exercise-induced adaptation with antioxidant supplementation (150, 151). An overview of the diverse role of oxidative stress in metabolic health is presented in **Figure 4**.

STRESS-ACTIVATED PROTEIN KINASE (SAPK) AND MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) SIGNALING

Stress-activated protein kinase and MAPK signaling pathways include, but are not limited to, p38 MAPK (p38 MAPK), c-Jun *N*-terminal kinases (JNK), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and PKC (152, 153). For the purpose of this review, both MAPK and SAPK are collectively referred to as SAPK.

Stress-activated protein kinase signaling pathways are associated with cellular proliferation, differentiation, survival, and cell

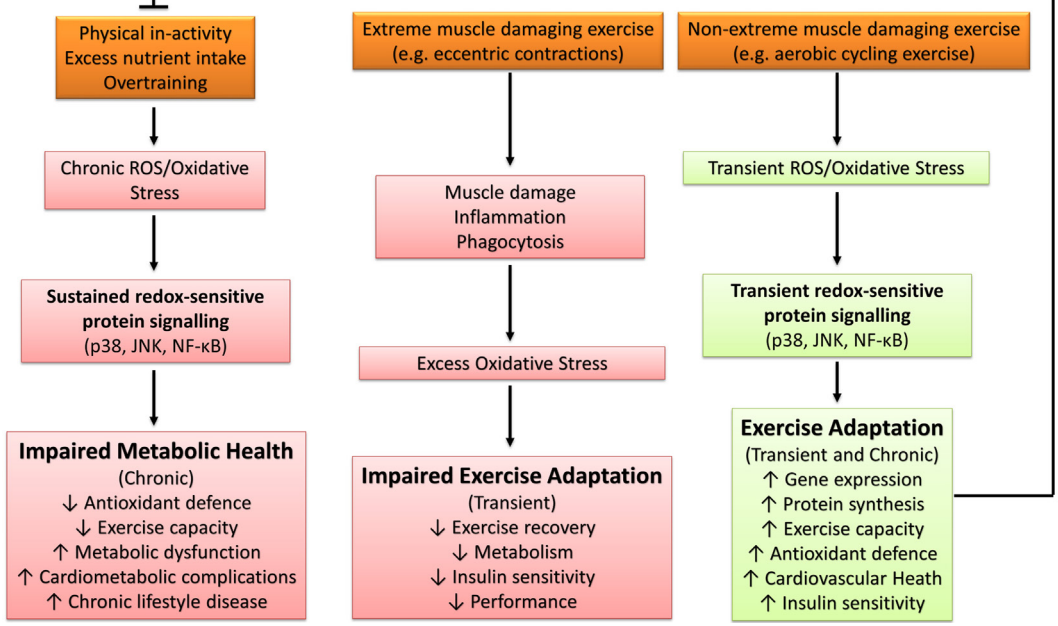

FIGURE 4 | The influence of oxidative stress in health and disease. p38, p38 mitogen-activated protein kinases; JNK, c-Jun *N*-terminal kinases; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells.

death. Uncontrolled or sustained activation of SAPK signaling pathways are associated with the development and progression of cancer, neurodegenerative, and cardiometabolic disease (8, 57, 154). In contrast, controlled and/or transient SAPK activation is required for normal physiological functioning and reported to mediate many of the adaptations and health benefits received from regular exercise (12, 152).

Stress-activated protein kinase pathways are activated through numerous stimuli involving hormones, growth factors, cytokines, agents acting through G protein-coupled receptors, transforming growth factors, pathogens and danger-associated molecular patterns, and physical and chemical stresses (153, 155, 156). Relevant to the current review, however, is the inherent capacity of ROS to both directly and indirectly activate SAPK signaling pathways in skeletal muscle (157–161).

ROS-Induced SAPK Signaling

The direct oxidation of proteins on cysteine residues by ROS act as biological "switches" turning on the catalytic properties of numerous proteins and enzymes (162). Cysteine thiol oxidation produces sulfenic acids, which form irreversible oxidation products or, in many cases, react to form reversible disulfide and sulfenamide bonds. These bonds can later be reduced *via* enzymes or compounds such as thioredoxin and glutathione, acting as an "off switch" and inhibiting protein function and enzymatic activity (163, 164). ROS-induced SAPK signaling can occur through reversible oxidative modification processes that involve MAPK kinase kinases (MAP3K/MAP2Ks) (165) and oxidative inactivation of thioredoxin (166, 167) and MAPK phosphatases (168–171). In addition, SAPK activation can occur through ROS-induced inactivation of glutathione S-transferases (172), tyrosine phosphorylation of protein kinase D (173), tyrosine, and serine phosphorylation of upstream targets such as the nuclear factor of kappa light polypeptide gene enhancer in β-cells inhibitor alpha (174) and the interaction with growth factor and cytokine receptors (163, 175). Crosstalk also exists between SAPK signaling pathways, with activation of one pathway (e.g., JNK and p38 MAPK) often interacting with and activating other pathways (e.g., NF-κB) (176). Irrespective of the mechanisms, considerable research has reported increased SAPK signaling under conditions of elevated ROS production (135, 157–160).

Exercise-Induced SAPK Signaling

The mechanical and physiological stresses elicited by acute exercise are potent stimuli for the transient activation of SAPK signaling in human skeletal muscle in part through increased ROS production (12). Exercise-induced SAPK signaling activate important skeletal muscle transcription factors and coactivators, which include peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), activating transcription factor 2, myocyte-enhancing factor 2, c-jun, c-fos, p53, and Elk-1 (12, 135, 177–185). Exercise-induced SAPK signaling is also associated with increased gene expression and the upregulation of antioxidant defenses such as SOD, iNOS, gamma-glutamylcysteine synthetase, GPx, and CAT (12, 135, 137, 161, 185–187).

Evidence supporting a role for exercise-induced ROS and SAPK signaling in exercise adaptation is primarily derived from research manipulating the redox environment to attenuate or enhance the exercise-induced ROS and protein signaling response. Henriquez-Olguin et al. (161) reported that inhibition of the ROS-producing enzyme complex NADPH oxidase 2 in rats attenuates the exercise-induced skeletal muscle phosphorylation of p38 MAPK and NF-κB p65 and gene expression of MnSOD, GPx, citrate synthase (CS), and mitochondrial transcription factor A (mtTFA). Similar findings have also been published using ROS inhibitors (e.g., antioxidant supplementation) in animals (10, 135, 188). Strobel et al. (189) reported that increased exercise-induced oxidative stress *via* skeletal muscle glutathione depletion in rats resulted in greater PGC-1α gene expression. In humans, antioxidant supplementation attenuates exercise-induced activation of p38 MAPK, NF-κB p65 and JNK protein signaling, and gene expression of SOD isoforms in skeletal muscle (10, 134, 137). Chronic inhibition of exercise-induced oxidative stress also impairs the training-induced upregulation of PGC-1α, nuclear respiratory factor (NRF)-1, and mtTFA in rats (135).

It is important to note that not all studies have reported an association between increased redox-sensitive protein kinase signaling and exercise adaptation. Wadley et al. (190) reported similar PGC-1α, NRF-2, and SOD gene expression after exercise in rats with allopurinol treatment, a xanthine oxidase inhibitor, despite decreased p38 MAPK phosphorylation and mtTFA gene expression. In addition, chronic allopurinol treatment was reported to have no effect on the training-induced upregulation of PGC-1α, mtTFA, cytochrome c, CS, and β-hydroxyacyl-CoA dehydrogenase (190). In humans, Morrison et al. (140) reported vitamin C and E supplementation to have little effect on exercise-induced gene expression of PGC-1α, mtTFA, and PGC-related coactivator, or training-induced improvements in VO_{2peak}, CS activity, and expression of cytochrome oxidase subunit 4. However, SOD activity and protein abundance of SOD and mtTFA were attenuated by vitamin C and E supplementation (140). A summary of key findings from research investigating redox manipulation, exercise, and SAPK signaling are summarized in **Table 1**.

The discrepancy in findings are unclear, but likely include interstudy variations in the method and/or compounds used to modulate exercise-induced ROS, variations in the dose and treatment/supplementation time, and the often non-specific and/or ineffective action of antioxidant supplementation/treatment as a model for ROS inhibition (81, 191–195). Nevertheless, evidence provided so far supports a likely association between redox-sensitive SAPK signaling and skeletal muscle adaptation, specifically with that of mitochondrial biogenesis and endogenous antioxidant upregulation, which both participate in the regulation of glycemic control (6, 9).

POSITIVE AND NEGATIVE REGULATION OF GLYCEMIC CONTROL BY ROS

Physical Inactivity, Excess Nutrient Intake, and Oxidative Stress

Chronic physical inactivity and overnutrition are associated with elevated systemic oxidative stress and the development of

TABLE 1 | Summary of key findings from research investigating the effect of redox state manipulation on acute exercise-induced protein signaling and molecular markers of skeletal muscle adaptation.

Reference	Participants	Exercise	Redox manipulation	Time point	SAPK signaling[a]	Markers of skeletal muscle adaptation[a]
Gomez-Cabrera et al. (10)	25 adults Trained Healthy	Marathon	Allopurinol (n = 14) Placebo (n = 11)	Postex.	Placebo only: ↑ lymphocyte NF-κB p50 activity	
Gomez-Cabrera et al. (188)	15 male Wistar rats	Exhaustive treadmill exercise	Rest (n = 5) Exercise (n = 5) Exercise + allopurinol (n = 5)	Postex.	Placebo only: ↑ p-p38 MAPK, NF-κB activity	Placebo only: ↑ MnSOD, iNOS, and eNOS mRNA
Henriquez-Olguin et al. (161)	20 male BalbC mice	Swimming exercise	Apocynin (n = 10) Vehicle (n = 10)	Postex.	Apocynin: attenuated p-p38 MAPK and p-NF-κB p65	Apocynin: attenuated MnSOD, GPX, CS, and mtTFA mRNA
Kang et al. (135)	18 female Sprague-Dawley rats	Exhaustive treadmill exercise	Allopurinol (n = 9) Vehicle (n = 9)	Postex.	Allopurinol: attenuated p–p38 MAPK, p-IκBα, NF-κB DNA binding	Allopurinol: attenuated PGC-1α, p-CREB, NRF-1, mtTFA content
Michailidis et al. (134)	10 young males Active Healthy	300 unilateral eccentric leg repetitions	Crossover: *N*–acetylcysteine and placebo	2 h postex.	Both: ↑ p-p38 MAPK NC p-NF-κB p65	Both: ↑ p-Akt^{Ser473}, p–p70S6K^{Thr389} and p-rpS6. NC MyoD. Muscle function impaired (mean torque)
				2 days postex.	NAC: greater p-p38 MAPK Attenuated p-NF-κB p65	Both: ↑ p-Akt^{Ser473}. NC MyoD. NAC: attenuated mTOR^{Ser2448}, p-p70S6K^{Thr389} and p-rpS6. Muscle function impaired.
				8 days postex.	Both: NC p-NF-κB p65 NAC: Attenuated p-p38 MAPK	NAC: attenuated p-Akt^{Ser473}, mTOR^{Ser2448}, p-p70S6K^{Thr389}, p-rpS6, and MyoD Placebo only: muscle function completely recovered
Petersen et al. (137)	8 young males Trained Healthy	45 min at 71% VO_{2peak} followed by 92% VO_{2peak} to fatigue	Crossover: *N*–acetylcysteine and Saline infusion	Postex. (45 min at 71% VO_{2peak})	Both: ↑ p-p38 MAPK, ↓ IκBα. NC p-NF-κB p65. NAC: attenuated p-JNK	Both: ↑ PGC-1 α mRNA. NAC: attenuated MnSOD mRNA
				Postex. (fatigue)	NAC: attenuated p-JNK, ↓ p-NF-κB p65.	Both: NC PGC-1 α mRNA and MnSOD mRNA
Strobel et al. (189)	Male Wistar rats	Exhaustive treadmill exercise	DEM and controls	Postex.	Both ↑ p-p38 MAPK	Not measured
				4 h postex.	Not measured	Both: NC NRF-2 DEM: greater ↑ PGC-1α mRNA. Attenuated GPx mRNA
Trewin et al. (55)	7 young adults Active Healthy	55 min at 65% VO_{2peak} followed by 5 min at 85% VO_{2peak}	Crossover: *N*–acetylcysteine and Saline infusion	Postex.	NAC: ↓ p-p38 MAPK	Both: ↑ p-p70S6K^{Thr389} and p-rpS6
Wadley et al. (190)	Male Sprague-Dawley rats	Treadmill exercise	Allopurinol or placebo	Postex.	Allopurinol: attenuated p–p38 MAPK	Not measured
				4 h postex.	Not measured	Both: ↑ mtTFA, NRF-2, PGC-1α, GLUT4, MnSOD, and EcSOD mRNA Allopurinol: attenuated mtTFA mRNA

[a]*Molecular response in skeletal muscle unless otherwise noted.*

NC, no change compared to baseline or control; ↑, significant increase compared to baseline or control; ↓, significant decrease compared to baseline or control; iNOS, inducible nitric oxide synthase; eNOS, endothelial nitric oxide synthase; CS, citrate synthase; mtTFA, mitochondrial transcription factor A; NRF-1/2, nuclear respiratory factor-1 and 2; MyoD, myogenic determination factor; PGC-1 α, peroxisome proliferator-activated receptor gamma coactivator 1 α; young, participants 18–40 years old; middle aged, 40–65 years old; older, >65 years old; active, recreationally active; GPx, glutathione peroxidase; MnSOD, manganese superoxide dismutase; EcSOD, extracellular superoxide dismutase; MAPK, mitogen-activated protein kinase; DEM, diethyl maleate; SAPK, stress-activated protein kinase; IκBα, nuclear factor of kappa light polypeptide gene enhancer in β-cells inhibitor alpha.

lifestyle disease in part through mitochondrial dysfunction (9). Metabolism of carbohydrate and lipids initiates the transfer of electrons from reducing equivalents (i.e., NADH, $FADH_2$) into the mitochondrial electron transport system (ETS) (9). In the absence of energy demand, for example, physical inactivity, increased energy supply results in increased electron flow through the ETS and pumping of protons outside the mitochondrial membrane (9). When the membrane potential exceeds mitochondrial uncoupling capacity, electrons leak through complexes I and III reacting with O_2 to form the free radical O_2^-, where it is catalyzed by MnSOD to H_2O_2 (196–199). Providing there is sufficient antioxidant activity, H_2O_2 is further reduced to H_2O by antioxidants such as GSH and/or CAT (200). In pathological conditions in which antioxidant defense is insufficient, H_2O_2 can accumulate in the mitochondrial matrix and intermembrane space or diffuse outside the permeable mitochondrial outer membrane (201). Excess ROS production results in oxidative stress and the signaling events leading to insulin resistance and chronic metabolic disease (59). This proposed mechanism for physical inactivity and excess nutrient intake-induced chronic disease is supported by reports that mitochondrial-specific antioxidants, which attenuate mitochondrial ROS production, reverse high-fat diet-induced insulin resistance in rodents (198).

Elevated basal and/or postprandial hyperglycemia elicited through excess nutrient intake, physical inactivity, and insulin resistance also increases oxidative stress through the formation of advanced glycation end products (AGEs) (202). Activation of the AGE receptor stimulates ROS production through NADPH oxidase (203), opening of the mitochondrial permeability transition pore (204), and through suppression of enzymatic antioxidant defenses (205–207). Therefore, hyperglycemia has the potential to elicit a potentially deleterious redox environment conducive to insulin resistance.

Numerous studies have reported increased biomarkers of systemic oxidative stress in humans for up to 4 h after the ingestion of pure carbohydrate (208, 209), fat, and protein meals (210); mixed macronutrient meals high in fat (211–214) and high in carbohydrate (215); and high-fat liquid meals (216, 217). Larger meals and meals higher in lipid content elicit greater postprandial oxidative stress (218, 219). This has led to many studies researching the effects of high-fat meal ingestion on postprandial oxidative stress (211–214, 220); however, meals adhering to national recommended dietary guidelines also induce systemic postprandial oxidative stress (5).

A single bout of low to moderate-intensity exercise in healthy males can attenuate the postprandial oxidative stress response to a meal ingested 1–2 h before exercise (5, 216) and 24 h after exercise (215), in part through improved glucose and triglyceride processing and clearance and increased antioxidant activity (214). Acute high-intensity exercise may also attenuate postprandial oxidative stress (212, 213); however findings are inconsistent and likely depend on whether exercise is performed before or after meal consumption (5, 214).

The divergent effects of postexercise oxidative stress (physiological) and postprandial oxidative stress (pathological) on metabolic health may stem from the mechanisms of ROS production (59, 79). The pathological effects of oxidative stress are reported to primarily occur through mitochondrial dysfunction and excess mitochondrial ROS production (9), whereas exercise-induced ROS production are reported to primarily occur through alternative mechanisms such as NADPH oxidase and xanthine oxidase (95). Furthermore, the effects of ROS on glycemic control appear to occur on a spatiotemporal paradigm that involve the concentration of ROS (221), the exposure time of ROS (160), the type of ROS, organs and organelles involved (79), the subcellular localization of redox-sensitive protein signaling (160), and the type of exercise and postexercise recovery timepoint (14, 128, 222).

Negative Regulation of Insulin Signaling by ROS

Sustained activation of redox-sensitive SAPK signaling pathways leads to impaired insulin signaling *via* increased phosphorylation of IRS-1 and IRS-2 on multiple serine and threonine residues, see the study by Copps and White (223) for a detailed review. Sustained IRS-1/2 serine phosphorylation impairs PI3K activity and downstream insulin signaling through attenuated tyrosine phosphorylation and IRS proteasomal degradation and subcellular relocalization (27, 160, 224–232) (**Figure 5**). The prevention of IRS-1 degradation through the inhibition of ROS and SAPK signaling restores insulin signaling and insulin-stimulated glucose uptake (8, 75, 181, 198, 233). Paradoxically, IRS serine phosphorylation may also be necessary for normal insulin signal transduction and glucose uptake (234). However, reports are contradictory (229, 231, 235) and depend largely on the length and degree of phosphorylation on specific serine residues (236). Previous studies have also reported that hyperinsulinemia initiates a negative feedback loop that inhibits insulin signaling and glucose uptake in part through SAPK-induced IRS serine phosphorylation (229, 231, 237–239).

Positive Regulation of Insulin Signaling by ROS

The insulin receptor belongs to a subclass of the protein tyrosine kinase family. Positive regulation of the insulin signaling cascade is mediated in part through the oxidative inactivation of protein tyrosine phosphatases (PTP), which include protein tyrosine phosphatase 1B, phosphatase and tensin homolog, and protein phosphatase 2 (**Figure 5**). Insulin-induced inactivation of PTPs prevents the dephosphorylation of the insulin receptor (240), IRS-1 (241), and Akt proteins (242) and prevents the enzymatic degradation of PIP_3 (243). The PTP superfamily signature motif contains an invariantly low-p*Ka* catalytic cysteine residue making it highly susceptible to reversible oxidation by ROS (244). ROS inactivation of PTP activity is associated with numerous cellular processes, including the regulation of cell proliferation, differentiation, survival, metabolism, and motility (244). Under basal conditions, antioxidant defenses such as CAT and peroxiredoxin create a reduced intracellular redox environment prioritizing PTP activity. Increased PTP activity suppresses kinase activity and maintains a dephosphorylated state of the IR, IRS-1, and inhibition of the PI3K/Akt signaling pathway

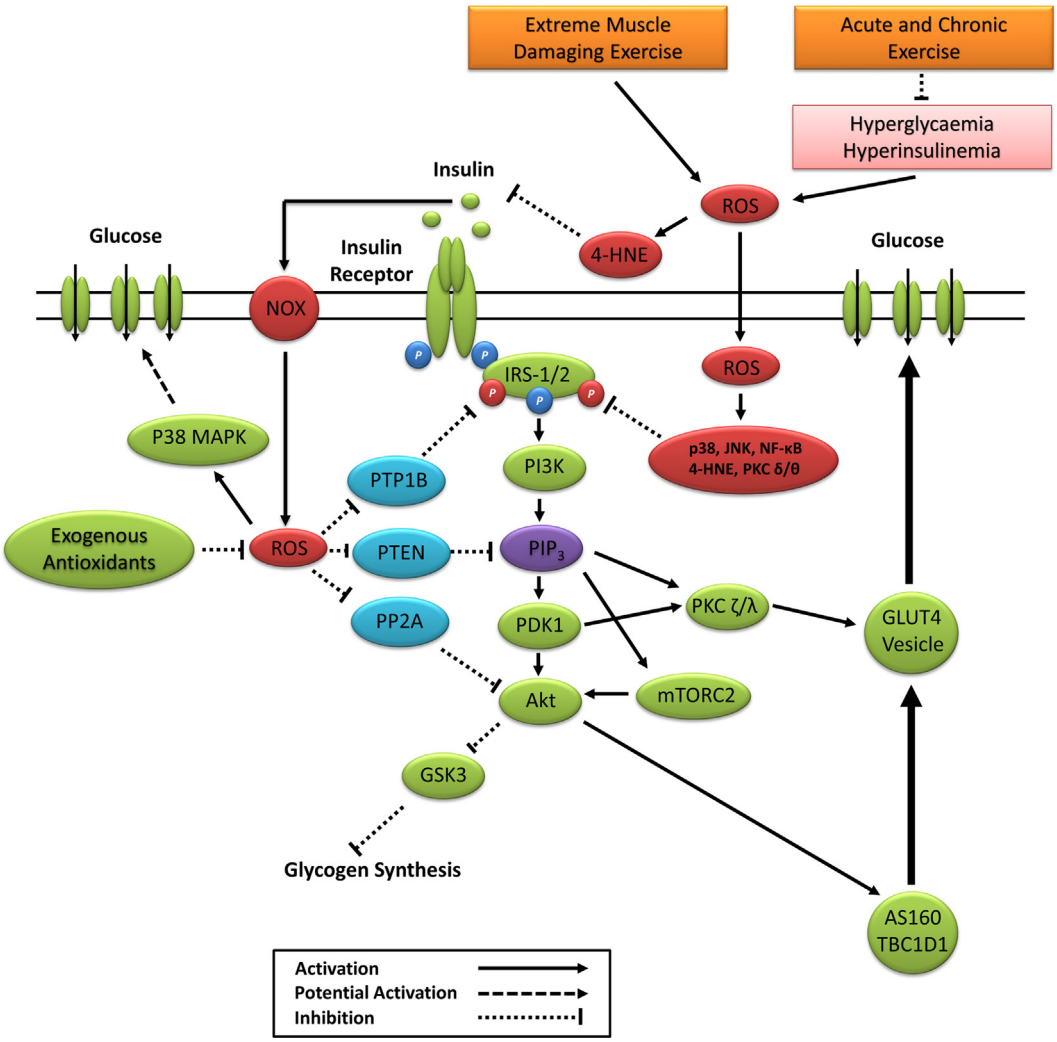

FIGURE 5 | Primary ROS signaling pathways involved in positive and negative regulation of insulin signaling. 4-HNE, 4-hydroxynonenal; Akt, protein kinase B; AS160, Akt substrate of 160 kDa; GSK3, glycogen synthase kinase 3; IRS-1/2, insulin receptor substrates 1 and 2; JNK, c-Jun *N*-terminal kinases; mTORC2, mechanistic target of rapamycin complex 2; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NOX, nicotinamide adenine dinucleotide phosphate oxidase; P38 MAPK, p38 mitogen-activated protein kinases; PDK1, phosphoinositide-dependent kinase-1; PI3K, phosphatidylinositol-3 kinase; PIP_3, phosphatidylinositol (3,4,5)-trisphosphate; PKC, protein kinase C; PP2A, protein phosphatase 2; PTEN, phosphatase and tensin homolog; PTP1B, protein tyrosine phosphatase 1B; ROS, reactive oxygen species.

(243, 245). The binding of insulin to the insulin receptor signals a burst of endogenous superoxide production, which is reduced to H_2O_2 creating a local oxidative redox environment (246–248). This oxidative redox environment favors the oxidation of catalytic cysteine to sulphenic acid, suppressing PTP activity and enhancing kinase activity and propagation of the insulin signaling cascade (9).

Insulin can elicit ROS production through enzymatic activation of NADPH oxidases (246–249). Furthermore, insulin-induced receptor tyrosine phosphorylation inactivates the endogenous membrane-associated antioxidant peroxiredoxin I, allowing for increased ROS production (78). Mahadev et al. (246) reported that NADPH oxidase-induced H_2O_2 enhances insulin signaling *via* oxidative inhibition of PTPs. Furthermore, palmitate-induced insulin resistance in rat skeletal muscle occurs through increased activity of PTPs *via* JNK and NF-κB (250), which is reversed 16 h after acute exercise in rats (222). Loh et al. (54) revealed that the elevated H_2O_2 response to insulin in GPx1$^{-/-}$ mouse embryo fibroblasts coincided with elevated PI3K/Akt signaling, which can be suppressed by pretreating cells with ebselen, an NADPH oxidase inhibitor, or the antioxidant *N*-acetylcysteine. Subsequent experiments revealed that elevated H_2O_2 in GPx1$^{-/-}$ mice increased PI3K/Akt signaling and glucose uptake through decreased PTP activity, which was attenuated by the ingestion of *n*-acetylcysteine (NAC) (54). Thus, redox-mediated PTP activity appears to be associated with both positive and negative regulations of insulin signaling and glucose uptake.

Exercise-Induced ROS, SAPK Signaling, and Glycemic Control

Reactive oxygen species are readily induced through contraction of skeletal muscle (251–253). Importantly, contraction of skeletal muscle coincides with increased activation of redox-sensitive SAPK signaling pathways implicated in glucose metabolism (14, 160, 161, 254–256). Therefore, skeletal muscle SAPK signaling has emerged as a potential candidate for the postexercise enhancement of insulin sensitivity (14, 54, 182).

Loh et al. (54) reported that exercise-induced ROS in GPx1 knockout mice coincided with increased phosphorylation of Akt$^{(Ser473)}$ and AS160$^{(Thr642)}$ and enhanced insulin-stimulated glucose uptake 60 min after a single session of treadmill exercise. This beneficial effect on insulin sensitivity was reversed with NAC supplementation, suggesting that redox signaling is not only an important regulator of basal insulin signaling and glucose uptake but also postexercise enhancement of insulin sensitivity. Importantly, GPx1 knockout mice showed similar improvements in insulin sensitivity when measured immediately after exercise, supporting a growing consensus that the effects of postexercise-induced ROS on glycemic control are temporal (14, 222).

One of the first studies to indicate a regulatory role of redox signaling in exercise-induced enhancement of insulin sensitivity in humans was conducted by Ristow et al. (13). It was reported that vitamin C and E supplementation in humans attenuated the 4-week training-induced improvements in insulin sensitivity and gene expression of PGC-1α/β, SOD, GPx1, and CAT (13). Not all studies in humans and rodents have reported impaired exercise-induced improvements in insulin protein signaling and insulin sensitivity with antioxidant supplementation (146, 148, 257). Contradictory findings likely stem from variations in the type of antioxidant compound/s used, the dose used, the timing of supplementation, and the often non-specific and/or ineffective action of antioxidant supplementation for ROS inhibition in humans (81, 193, 194).

Enhanced glucose uptake approximately 4.5 h after one-legged knee extensor exercise in humans is reported to coincide with greater basal and insulin-stimulated p38 MAPK phosphorylation (182), highlighting SAPK signaling as a potential moderator of postexercise glucose metabolism. Trewin et al. (55) reported that NAC infusion attenuated whole-body insulin sensitivity approximately 5 h after exercise. Phosphorylation of p38 MAPK was lower immediately after exercise with NAC infusion; however, phosphorylation was not significantly different to baseline or the placebo after insulin stimulation. However, the null findings for p38 MAPK phosphorylation may be due to the timing of postexercise biopsies, the relatively small effect of NAC on insulin sensitivity (~6% reduction), and that NAC infusion was not maintained during the recovery period and subsequent insulin clamp (55). Interestingly, Parker et al. (14) demonstrated that a bout of high-intensity interval exercise prior to a 2-h euglycemic–hyperinsulinemic clamp in obese middle-aged males elicited greater insulin-stimulated p38 MAPK, JNK, NF-κB, and AS160^{Ser588} phosphorylation, which was associated with improved insulin sensitivity compared to a resting clamp. Equivocal findings in humans may stem from reports that postexercise skeletal muscle SAPK and insulin protein signaling are effected by training status and occur in an exercise intensity and postexercise time course-dependent manner (256, 258).

Berdichevsky et al. (160) reported similar JNK phosphorylation in C2C12 myoblasts and L6 myotubes treated with chronic oxidative stress (1 μM of H_2O_2 for 48 h) and acute oxidative stress conditions (500 μM of H_2O_2 for 3 h). Interestingly, chronic oxidative stress decreased insulin-stimulated Akt$^{(Ser473)}$ phosphorylation, whereas acute oxidative stress enhanced insulin-stimulated Akt$^{(Ser473)}$ and GSK3-α/β phosphorylation. Furthermore, acute oxidative stress exposure in insulin-resistant muscle cells rescues insulin-stimulated glucose uptake through increased IRS1 protein abundance; increased phosphorylation of JNK, Akt$^{(Ser473)}$, Akt$^{(Thr308)}$, and GSK3-α/β; and decreased IRS-1$^{(Ser307)}$ phosphorylation (160). In contrast, Ropelle et al. (222) reported that a single bout of exercise in male rats reverses diet-induced insulin resistance 16 h later *via* attenuation of JNK, NF-κB, and IRS-1$^{(Ser307)}$ signaling. It is possible that acute exercise enhances insulin signal transduction through the transient and immediate increase in ROS and SAPK signaling, which also leads to a delayed increase in antioxidant activity and subsequent attenuation of chronic oxidative stress and sustained SAPK signaling pathways associated with insulin resistance. Certainly, SOD protein content, SOD activity, and total antioxidant status increase and/or remain elevated for up to 16–24 h after exercise (116, 212, 259), whereas lipid-induced postprandial oxidative stress is attenuated (213).

Taken together, previous studies support a potential role for exercise-induced redox-sensitive protein signaling and glycemic control (**Table 2**); however, specific mechanisms remain to be elucidated (**Figure 6**).

Potential Mechanisms Linking SAPK Signaling and Enhancement of Glycemic Control

Modulation of glycogen synthesis by oxidative stress-induced SAPK signaling has been associated with glucose metabolism and regulation (27, 160, 182, 260). Transient stimulation of C2C12 myoblasts with H_2O_2 increases JNK, Akt, and GSK3α/β phosphorylation (160), suggesting the short exposure to exercise-induced ROS may increase postexercise glycogen synthesis and skeletal muscle glucose uptake. Likewise, postexercise enhancement of insulin-stimulated p38 MAPK phosphorylation is associated with postexercise glycogen depletion (182). Chan et al. (261) established that low intramuscular glycogen was associated with greater phosphorylation of nuclear p38 MAPK after 60 min of cycle exercise. In contrast, insulin stimulation of rat skeletal muscle exposed to 1 h of H_2O_2 (~90 μM) exhibits impaired insulin protein signaling, glycogen synthesis, and glucose uptake, despite increased p38 MAPK phosphorylation (27). Diamond-Stanic et al. (260) reported similar findings and proposed that p38 MAPK and GSK3 were unlikely to play a beneficial role in insulin-stimulated glucose uptake. Activation of JNK in skeletal muscle of mice is also associated with increased insulin-stimulated glycogen synthesis *via* the RSK3/GSK3 signaling pathway (262); however, greater postexercise JNK phosphorylation and insulin sensitivity in human skeletal muscle do not coincide with greater insulin-stimulated phosphorylation of GSK3 α/β (14).

TABLE 2 | Summary of key findings from research investigating exercise, redox state, and enhancement of glycemic control.

Reference	Participants/ animals/cells[a]	Exercise stimulus	Redox manipulation	Time point/conditions	SAPK signaling	Glycemic control
Berdichevsky et al. (160)	Myocytes, myoblasts, and/or myotubes	Acute oxidative stress (simulated exercise)	Chronic oxidative stress	Chronic oxidative stress	↑ p-JNK	↓ p-Akt^{Ser473,Thr308}. ↓ glucose uptake
			Acute oxidative stress	Acute oxidative stress	↑ p-JNK	↑ p-Akt^{Ser473,Thr308} and ↑ p-GSK3β. ↑ glucose uptake
				Insulin-resistant cells	↑ p-JNK	↑ p-IRS-1^{Ser312}, ↓ IRS-1 and glucose uptake
				Insulin-resistant cells + acute oxidative stress	Greater ↑ p-JNK	↓ p-IRS-1^{Ser312}, ↑ IRS-1 and glucose uptake
Castorena et al. (51)	Low-fat and high-fat diet fed rats	Swimming exercise		3 h postex. + Ins.	NC p-JNK	NC Akt^{Ser473,Thr308}, IR$^{Tyr1162/1163}$, IRS-1-PI3K ↑ pAS160^{Thr642,Ser588} ↑ Insulin sensitivity Greater ↑ in insulin sensitivity and pAS160^{Thr642,Ser588} in low-fat diet fed rats
Geiger et al. (233)	Male Wistar rats	*In vitro* contraction	p-p38MAPK inhibition	3 h postcontraction	↑ p-p38 MAPK	Not measured
				3 h postcontraction + P38 MAPK inhibition	NC p-p38 MAPK	Not measured
				Post-Ins.	Not measured	↑ glucose uptake
				3 h postcontraction + Ins.	Not measured	Greater ↑ glucose uptake
				3 h postcontraction + Ins. + p38MAPK inhibition	Not measured	Similar glucose uptake as previous condition
Higashida et al. (146)	Male Wistar rats	3-day swimming program or 3 weeks, 6 days/week swimming program	Vehicle or vitamin C + E supplementation	Post 3-day training Post 3-week training		3-day and 3-week training: vitamin C + E: similar ↑ in measures of mitochondrial protein content, ↑ GLUT4, ↑ glucose uptake
Loh et al. (54)	10 wild-type mice 9 GPx1$^{-/-}$ mice	Treadmill exercise	GPx1$^{-/-}$ and *N*–acetylcysteine	Postex. + Ins. 1 h postex. + Ins.		GPx1$^{-/-}$ mice: similar insulin sensitivity GPx1$^{-/-}$ mice: greater ↑ p-Akt^{Ser473} and insulin sensitivity *N*-acetyl.cysteine: attenuated insulin sensitivity
Parker et al. (256)	8 young adults Active Healthy	Crossover design Cycling exercise: SIE: 4 × 30 s all-out sprints; 4.5-min recovery periods HIIE: 5 × 4-min cycling bouts at 75% of W_{max}; 1-min recovery periods CMIE: 30 min at 50% of W_{max}		Postex. 3 h postex.	All ex.: similar ↑ p-JNK, ↑ p-p38 MAPK, ↓ IκBα; SIE: ↑ p-NF-κB; CMIE and HIIE: NC p-NF-κB All ex.: similar ↑ p-JNK, ↑ p-p38 MAPK, ↓ IκBα. NC p-NF-κB	All ex.: NC IRS-1, similar ↓ p-Akt^{Ser473}; SIE: ↑ p-IRS1^{Ser307}, greatest ↓ p-AS160^{Ser588}; HIIE: greatest ↑ p-IRS1^{Ser307}, ↓ p-AS160^{Ser588}; CMIE: ↑ p-IRS1^{Ser307}, NC p-AS160^{Ser588} All ex.: NC IRS-1, similar ↓ p-Akt^{Ser473}; SIE: NC p-IRS1^{Ser307}, NC p-AS160^{Ser588}; HIIE: NC p-IRS1^{Ser307}, ↓ p-AS160^{Ser588}; CMIE: ↑ p-IRS1^{Ser307}, ↓ p-AS160^{Ser588}
Parker et al. (14)	11 middle-aged males Sedentary Obese	Cycling exercise: HIIE: (4 × 4 min at 95% HRpeak; 2-min recovery periods)		Rest trial: 2 h post-Ins. Exercise trial: 1 h postex. 3 h postex + Ins.	↑ p-JNK, ↑ p-p38 MAPK, ↓ IκBα ↑ p-JNK, ↑ p-NF-κB, ↑ p-p38 MAPK, ↓ IκBα Greater ↑ p-JNK, greater ↑ p-NF-κB, greater ↑ p-p38 MAPK Similar ↓ IκBα	↑ p-IRS1^{Ser307}, ↑ p-AS160$^{Ser588, Ser318}$ ↑ p-AS160^{Ser588} Greater ↑ insulin sensitivity and greater ↑ p-AS160^{Ser588} Similar ↑ p-IRS1^{Ser307} and ↑ p-AS160^{Ser318}

(*Continued*)

TABLE 2 | Continued

Reference	Participants/ animals/cells[a]	Exercise stimulus	Redox manipulation	Time point/conditions	SAPK signaling	Glycemic control
Picklo and Thyfault (257)	56 high-fat diet-induced obese Sprague-Dawley rats	Motorized wheel exercise. 12 weeks, 5 times per week	With and without vitamin C + E supplementation	Posttraining		Vitamin C + E: similar improvement in HOMA-IR and OGTT Attenuated measures of mitochondrial protein content
Ristow et al. (13)	40 young males (20 trained and 20 active) Healthy	Biking, running and circuit training. 4 weeks, 5 times per week	Placebo or vitamin C + E supplementation	Posttraining		Vitamin C + E: attenuated ↑ insulin sensitivity, mRNA expression of PPARγ, PGC-1α/β
Ropelle et al. (222)	Male Wistar rats Control (n = 6) Obese (n = 8) Obese + Ex. (n = 8)	Swimming exercise		16 h postex. + Ins.	Compared to control: obese: ↑ p-JNK, ↓ IκBα Compared to obese: obese + Ex.: ↓ p-JNK, ↑IκBα	Compared to both control and obese + Ex.: obese attenuated insulin sensitivity, PI3K, p-IRS-1/2, p-IR. ↑ PTP1B content/activity and p-IRS^{Ser312}
Somwar et al. (181)	Male Wistar rats	*In vitro* contraction	p-p38 MAPK inhibition	Post-Ins. Post-Ins. + p38 MAPK inhibition Postcontraction Postcontraction + p38 MAPK inhibition	↑ p-p38 MAPK and activity Attenuated p38 MAPK activity ↑ p-p38 MAPK and activity Attenuated p38 MAPK activity	Not measured Attenuated glucose uptake Not measured Attenuated glucose uptake
Thong et al. (182)	7 young males Active Healthy	60 min of one-legged knee extension		3 h postex. 3 h postex. + 30 min Ins. 3 h postex + 100 min Ins.	↑ p-p38 MAPK Greater ↑ p-p38 MAPK p-p38 MAPK similar to previous time point	Not measured ↑ insulin sensitivity compared to control leg ↑ insulin sensitivity compared to control leg
Trewin et al. (55)	7 young adults Active Healthy	55 min cycling at 65% VO_{2peak} followed by 5 min at 85% VO_{2peak}	Crossover: *N*–acetylcysteine and saline infusion	Postex. 3 h postex. 3 h postex. + 2 h Ins.	NAC: ↓ p-p38 MAPK Both: NC p-p38 MAPK Both: NC p-p38 MAPK	Both: NC p-Akt^{Thr308,Ser473}, ↑ p-PAS160 Both: ↑ PAS160. NC p-Akt^{Thr308,Ser473} Both: ↑ p-Akt^{Thr308,Ser473}, ↑ p-PAS160 NAC: ↑ insulin sensitivity
Yfanti et al. (148)	21 young males Active Healthy	Intense endurance training program 5 times/week for 12 weeks	Placebo or vitamin C and E supplementation	Posttraining		Vitamin C + E: similar ↑ insulin sensitivity, ↑ Akt, ↑ HXK2, ↑ GLUT4

[a]*Where appropriate sample sizes for animal research are reported.*

Ins., insulin stimulation; NC, no change compared to baseline or control; ↑, significant increase compared to baseline or control; ↓, significant decrease compared to baseline or control; Akt, protein kinase B; AS160, Akt substrate of 160 kDa; CMIE, continuous moderate-intensity exercise; GLUT4, glucose transporter type 4; GSK3, glycogen synthase kinase 3; HIIE, high-intensity interval exercise; HOMA-IR, homeostatic model assessment for insulin resistance; HXK2, hexokinase II; IRS-1/2, insulin receptor substrates 1 and 2; JNK, c-Jun N-terminal kinases; NAC, n-acetylcysteine; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; P38 MAPK, p38 mitogen-activated protein kinases; PI3K, phosphatidylinositol-3 kinase; PPARγ/α/β, peroxisome-proliferator-activated receptor gamma/alpha/beta; PTP1B, protein tyrosine phosphatase 1B; SIE, sprint interval exercise; SAPK, stress-activated protein kinase.

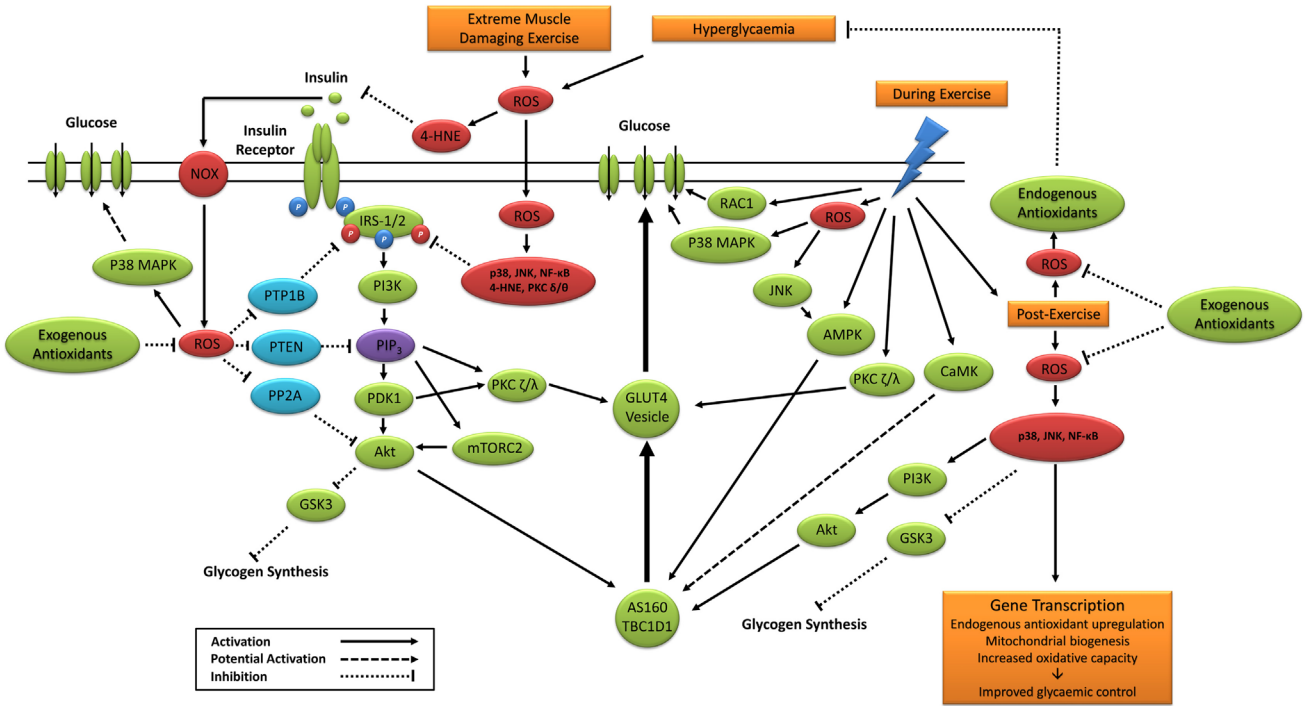

FIGURE 6 | Primary signaling pathways involved in contraction and insulin-stimulated glucose uptake and the potential role of ROS. 4-HNE, 4-hydroxynonenal; Akt, protein kinase B; AMPK, 5′ adenosine monophosphate-activated protein kinase; AS160, Akt substrate of 160 kDa; CaMK, Ca^{2+}/calmodulin-dependent protein kinase; GLUT4, glucose transporter 4; GSK3, glycogen synthase kinase 3; IRS-1/2, insulin receptor substrates 1 and 2; JNK, c-Jun *N*-terminal kinases; mTORC2, mechanistic target of rapamycin complex 2; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NOX, nicotinamide adenine dinucleotide phosphate oxidase; P38 MAPK, p38 mitogen-activated protein kinases; PDK1, phosphoinositide-dependent kinase-1; PI3K, phosphatidylinositol-3 kinase; PIP_3, phosphatidylinositol (3,4,5)-trisphosphate; PKC, protein kinase C; PP2A, protein phosphatase 2; PTEN, phosphatase and tensin homolog; PTP1B, protein tyrosine phosphatase 1B; RAC1, ras-related C3 botulinum toxin substrate 1; ROS, reactive oxygen species.

Other potential pathways include JNK-, NF-κB-, and p38 MAPK-stimulated secretion of the recently identified insulin-sensitizing interleukin-6 (IL-6) (255, 263–265). Carey et al. (264) reported that IL-6 infusion increases insulin-stimulated glucose uptake in humans. Furthermore, IL-6 treatment in L6 myotubes coincides with increased glucose uptake and GLUT4 translocation, likely through AMPK pathways independent of the canonical insulin signaling cascade (264). Importantly, IL-6 secretion is increased following muscular contraction, likely *via* activation of JNK, NF-κB, and/or p38MAPK (261, 266, 267). It has also been reported that p38 MAPK inhibitors, alongside expression of a dominant-negative p38 mutant, impairs insulin-stimulated glucose uptake without reductions in GLUT4 translocation (254). Researchers concluded that p38 MAPK may exert its insulin-sensitizing effect through increased activation of translocated GLUT4 (254), but not all findings are supportive (268) and have yet to be investigated in humans. The reported subcellular redistribution of phosphorylated JNK from the cytoplasm to the nucleus with acute hydrogen peroxide exposure in skeletal muscle cells highlights another potential mechanism for the postexercise insulin sensitizing effect of JNK (160). Future research is warranted to explore the subcellular localization and activation of SAPK proteins after exercise and insulin stimulation in humans.

THE FUTURE OF EXERCISE-INDUCED OXIDATIVE STRESS, ROS, AND REDOX SIGNALING

Early studies, and the majority of current findings, rely primarily on associations and the assumption that increased/decreased ROS and/or markers of oxidative stress are reflective of, or are likely to lead to, increased/decreased redox signaling (91). Certainly, studies inhibiting or increasing ROS have been useful for establishing a relationship between ROS and certain biological outcomes such as glycemic control and exercise adaption (13, 14, 55, 135, 161). However, in the absence of specific redox signaling measurements such as protein cysteine oxidation or S-nitrosylation (162, 269), research studies are limited in their capacity to elucidate specific redox cellular signaling networks that are complex, compartmentalized, and spatiotemporally regulated (195). Future studies utilizing modern redox proteomics are required to establish the reversible and, in some cases, irreversible, redox regulation of kinases, phosphatases, transcription factors, and coactivators, thus establishing the "true" redox signaling role of exercise-induced ROS (195, 270–274). Furthermore, not all ROS are equal in their capacity to exert signaling effects (56). Future studies investigating exercise-induced oxidative stress should therefore strive to identify the specific

ROS involved, which can be achieved through the use of robust techniques such as electron spin resonance, targeted fluorescent probes, and mass spectrometry (252, 275–278).

Despite their non-specificity and/or inability to adequately reflect redox signaling, the measurement of ROS, oxidative stress, and/or antioxidant activity in a biological sample provides insight into the effects of an intervention (e.g., exercise) on redox homeostasis and remains a useful biomarker of overall health and disease (91). As such, a combination of both traditional measures of redox biomarkers, the direct measurement of ROS, redox-sensitive protein signaling, and specific redox proteomics will likely provide a robust investigation of exercise-induced ROS and subsequent redox signaling.

CONCLUSION

Physical inactivity, excess energy consumption, and obesity are associated with elevated ROS production, systemic oxidative stress, and sustained activation of redox-sensitive protein signaling pathways. If left unchecked, this chronic state of physiological stress can lead to insulin resistance, which likely contributes toward the development of cardiometabolic disease. Paradoxically, a single session of exercise transiently increases ROS, oxidative stress, and redox-sensitive protein signaling, yet both acute and regular exercises elicit favorable improvements in glycemic control and skeletal muscle adaptation. It appears that exercise-induced redox-sensitive protein signaling is necessary for adaptation to physiological stress. However, the spatiotemporal interplay between physical activity/inactivity, ROS, PTP activity, SAPK and MAPK signaling, insulin protein signaling, and the subsequent effects on glycemic control and cardiometabolic health remain unclear. Future research would benefit by employing a combination of human primary cell culture, animal research, modern proteomics, and immunohistochemistry/subcellular analysis of human tissue to elucidate the physiological relevance of transient oxidative stress (exercise induced), chronic oxidative stress (physical inactivity/excess nutrition intake), and the role of redox-sensitive protein signaling in human health and disease.

AUTHOR CONTRIBUTIONS

LP, CS, NS, and IL contributed to the conceptualization and overall design of the manuscript. LP drafted the initial version of the manuscript and figures. CS, NS, and IL critically revised the manuscript. All authors approved the final version of the manuscript.

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

frontiers in Endocrinology

MINI REVIEW
published: 02 May 2017
doi: 10.3389/fendo.2017.00097

The Potential Role of Contraction-Induced Myokines in the Regulation of Metabolic Function for the Prevention and Treatment of Type 2 Diabetes

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Skeletal muscle represents the largest organ in the body, comprises 36–42% of body weight, and has recently been recognized as having an endocrine function. Proteins expressed and released by muscle that have autocrine, paracrine, and endocrine bioactivities have been termed myokines. It is likely that muscle contraction represents the primary stimulus for the synthesis and secretion of myokines to enable communication with other organs such as the liver, adipose tissue, brain, and auto-regulation of muscle metabolism. To date, several hundred myokines in the muscle secretome have been identified, a sub-population of which are specifically induced by skeletal muscle contraction. However, the bioactivity of many of these myokines and the mechanism through which they act has either not yet been characterized or remains poorly understood. Physical activity and exercise are recognized as a central tenet in both the prevention and treatment of type 2 diabetes (T2D). Recent data suggest humoral factors such as muscle-derived secretory proteins may mediate the beneficial effects of exercise in the treatment of metabolic diseases. This mini-review aims to summarize our current knowledge on the role of contraction-induced myokines in mediating the beneficial effects of physical activity and exercise in the prevention and treatment of T2D, specifically glucose and lipid metabolism. Future directions as to how we can optimize contraction-induced myokine secretion to inform exercise protocols for the prevention and treatment of T2D will also be discussed.

Keywords: exercise, myokines, muscle, endocrine, diabetes

OPEN ACCESS

Edited by:
Kristian Karstoft, University of Copenhagen, Denmark

Reviewed by:
Martin Whitham, Garvan Institute of Medical Research, Australia
Ashraf Al Madhoun, Dasman Diabetes Institute, Kuwait

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

Received: *24 February 2017*
Accepted: *18 April 2017*
Published: *02 May 2017*

Citation:
Carson BP (2017) The Potential Role of Contraction-Induced Myokines in the Regulation of Metabolic Function for the Prevention and Treatment of Type 2 Diabetes. Front. Endocrinol. 8:97. doi: 10.3389/fendo.2017.00097

INTRODUCTION

Skeletal muscle has recently been identified as an endocrine organ that synthesizes and secretes proteins known as myokines (1). These myokines are involved in autocrine regulation of metabolism in the muscle itself and paracrine/endocrine regulation of other tissues and organs such as the liver, adipose, and brain.

As skeletal muscle represents the largest organ in the body, the influence of myokines on whole-body metabolism is potentially significant (2, 3). As skeletal muscle contraction is likely the primary stimulus for myokine synthesis and secretion, it is plausible that myokines mediate, in part

at least, beneficial adaptations to tissues in response to exercise. Recent research has identified several hundred myokines, a large sub-population of which are specifically induced by contraction (4). However, the specific bioactivity of a vast number of myokines remains largely undescribed and poorly understood. Furthermore, little is known about the role of type, intensity, or frequency of contraction in regulating myokine production and release.

Exercise has long been established as a central tenet to both the prevention and treatment of type 2 diabetes (T2D) (5). Though a number of mechanisms through which exercise confers these metabolic benefits have been well characterized (5), the pluripotency of exercise is not yet fully understood. One such mechanism is *via* cross-talk between tissues stimulated by contraction and release of myokines regulating tissue function. This creates a clear link between exercise and the regulation of whole-body metabolism. There have been several examples of this in recent research, most notably, the role of the contraction-induced myokine IL-6 in mediating skeletal muscle glucose uptake (6–8). These findings generated excitement as to the potential roles of contraction-induced myokines in the prevention of insulin resistance and metabolic diseases such as obesity and T2D. To date, a number of contraction-induced myokines have been identified which play a role in regulating glucose uptake, insulin sensitivity, and fat metabolism, leading factors in the development of T2D (9).

The purpose of this mini-review is to discuss known metabolic roles for contraction-induced myokines that aid in the prevention/treatment of T2D. Future directions in optimizing exercise protocols to maximize the potential of contraction-induced myokines by the type and intensity of exercise and how this informs exercise prescription will also be discussed.

MYOKINES AND METABOLISM

Contraction-induced myokines have been shown to have autocrine, paracrine, and endocrine effects on numerous tissues. In this section, the evidence of contraction as a stimulus for myokine secretion, based on electrical pulse stimulation (EPS) models and/or an increase in circulating concentrations immediately post-exercise, and their effect on metabolic functions affecting the development of T2D in muscle, adipose, and liver will be discussed.

Myokines Regulating Glucose Metabolism

IL-6

Evidence exists for a number of contraction-induced myokines with roles for glucose uptake and insulin sensitivity. IL-6 is most prominent in the literature and has been the focus since the early 2000s of those trying to identify the "exercise factor" through which skeletal muscles communicate to central and peripheral organs (10). IL-6 transcription in skeletal muscle and release to circulation in large volumes in response to contraction was first characterized in 2002 (11). Increased circulating concentrations of IL-6 are known to be affected by both the intensity and duration of contraction in humans (8, 12). Higher intensity and longer duration exercise result in increased circulating concentrations of IL-6 in humans (8, 12). IL-6 release in response to exercise is also dependent on the energy status of the cell, determined by pre-exercise glycogen content, whereby low glycogen content results in a greater release of IL-6 to the energy crisis in the muscle cell during contraction (6). *In vitro* studies demonstrate that IL-6 treatment increases glucose uptake through AMP-activated protein kinase [adenosine monophosphate kinase (AMPK)] and phosphatidylinosotol 3-kinase (PI3K) pathways (13). Carey et al. (7) reported increased insulin-dependent glucose uptake *in vivo* in response to IL-6 infusion. By contrast, Harder-Lauridsen et al. (14) found no increase in glucose uptake during euglycemic hyperinsulinemic clamp with IL-6 infusion in T2D individuals, though there was a reduction in the plasma insulin suggesting increased insulin sensitivity (14). Jiang et al. (15) found a differential effect of IL-6 treatment on primary myotubes from normal glucose tolerant and T2D, suggesting a blunted role of IL-6 on T2D muscle. IL-6 treatment upregulated both insulin-dependent and -independent glucose uptake and glycogen synthesis in healthy myotubes, but this effect was lost in T2D myotubes. This suggests that from a glucose control perspective, the contraction-induced myokine IL-6 is effective in the prevention of T2D but may be ineffective for glucose uptake in patients with existing T2D.

IL-13

IL-13 is released from human primary myotubes *in vitro* and has been demonstrated to have an "insulin-like" effect on glucose metabolism in human muscle by increasing glucose uptake, glycogen synthesis, and glucose oxidation in normal and T2D primary myotubes (15). This "insulin-like" effect is mediated through activation of Akt and PI3K pathways. IL-13 expression is increased in response to strength training in human skeletal muscle (16), but no evidence exists for an increase in plasma IL-13. This suggests the influence of IL-13 on glucose metabolism is localized to the muscle in an autocrine/paracrine manner.

Follistatin-Like-1 (FSTL-1)

Follistatin-like-1 is a secretory myokine of the follistatin family, known to be secreted *in vitro* by C2C12s (murine cell line) (17). Furthermore, Görgens et al. (17) demonstrated FSTL-1 expression and release from human primary myotubes. Interestingly, contraction of primary myotubes by EPS did not induce the secretion of FSTL-1; however, an increase in circulating plasma FSTL-1 in humans is observed following an acute bout of aerobic exercise. *In vitro* incubation of L6 myotubes (rat cell line) in FSTL-1 has been shown to increase glucose uptake in an AMPK- and calcium–calmodulin kinase-dependent manner (18), resulting in increased GLUT4 mRNA expression and translocation to the plasma membrane mediating enhanced glucose control.

Chitinase-3-Like-1 Protein (CHI3L1)

Electrical pulse stimulation of primary human skeletal muscle cells increases CHI3L1 expression and secretion (19). Acute aerobic and resistance exercise increase circulating CHI3L1; however, combined training had no effect, suggesting a transient exercise response. Evidence indicates that CHI3L1 regulates myoblast proliferation, suggesting a role in muscle growth thus affecting

the size and volume of this organ as a "sink" for blood glucose (19). Furthermore, though CHI3L1 is induced by inflammation as well as contraction, it improves glucose uptake and insulin action under pro-inflammatory conditions in human primary skeletal muscle cells through activation of its receptor protease-activated receptor 2 (19). This suggests that CHI3L1 could regulate skeletal muscle glucose uptake under pro-inflammatory conditions observed in obesity and T2D.

IL-15

IL-15 is a known contraction-induced myokine secreted in humans post both aerobic and resistance exercise (20, 21) with similar responses between lean and obese participants (22). IL-15 has an effect on glucose uptake in C2C12 skeletal muscle cells (murine cell line) *via* activation of AMPK (23). Krolopp et al. (24) found a similar increase in glucose uptake with IL-15 treatment, mediated by an enhanced GLUT4 translocation to the plasma membrane. However, in contrast to the findings of Gray and colleagues, GLUT4 translocation was not initiated by activation of AMPK, but rather through the Janus kinase–signal transducer and activation of transcription protein 3 (STAT3) pathway. It is not entirely clear why there is no increase in phosphorylation of AMPK in this study, when using a higher dose of IL-15 (100 vs 1 ng/ml).

IL-8

IL-8 is secreted by primary human myotubes following EPS (25) and circulating IL-8 increases in response to endurance exercise in humans (26, 27). IL-8 is primarily associated with inflammation and angiogenesis; however, Gray and Kamolrat (23) demonstrated *in vitro* an increase in glucose uptake in C2C12s in response to treatment with IL-8 *via* phosphorylation of AMPK. A role for IL-8 in glucose uptake *in vivo* is less clear but may be mediated by increased vascularization, an effect which is lost in muscle from T2D (28).

Fibroblast Growth Factor-21 (FGF-21)

Fibroblast growth factor-21 treatment improves glucose tolerance and insulin sensitivity in the liver of obese Zucker rats (29). FGF-21 treatment has also been demonstrated to lower blood glucose and enhance insulin sensitivity in a diabetic mouse model (30). FGF-21, mediated by activation of Akt, improves glucose uptake in primary human adipocytes, which is enhanced when combined with insulin, reducing the required level of insulin to achieve the same glucose uptake (31). Muise et al. (32) confirmed reduced plasma glucose in WT, HFD, and diabetic mouse models treated with FGF-21 perfusion and identified upregulation of genes associated with several pathways such as glucose uptake, and insulin receptor signaling regulated by FGF-21 in brown and white adipose tissue (WAT) and adipocytes *in vitro*. FGF-21 also increased basal and insulin-stimulated glucose uptake in primary human myotubes by increasing GLUT1 mRNA and translocation to the plasma membrane (33). Circulating concentrations of FGF-21 are increased after an acute bout of endurance exercise in humans (34) and enhanced by higher intensity exercise (35). Short-term training also resulted in increased circulating FGF-21, which was associated with lower fasting glucose (36). Conversely, 3 weeks of sprint interval training results in reduced circulating FGF-21 (37). Similarly, 3 months of combined resistance and aerobic training resulted in a modest decrease in serum FGF-21 in obese women (38). This suggests that an acute bout of exercise leads to a transient increase in FGF-21 but the effect of chronic training is equivocal. Circulating FGF-21 is increased in T2Ds compared to normal glucose tolerant individuals and correlated with fasting insulin and BMI (33). Perhaps, the effect of chronic training is to decrease fasting insulin and adipose mass and thereby reduce circulating FGF-21. The acute increase in FGF-21 post-exercise is likely from muscle with the action of sensitizing muscle, adipose, and liver to insulin to facilitate glucose uptake.

Irisin

Irisin is a controversial candidate, primarily thought to be secreted not only by muscle but also in small amounts by adipose tissue. The main point of contention has been the detection of this myokine in its glycosylated and deglycosylated forms [for review, see Ref. (39)]. Future research should focus on detection by mass spectrometry as per (40); however, the *in vivo* data reported here use the best validated ELISA technique (39). Circulating irisin increases in response to high-intensity interval exercise, resistance exercise, and continuous moderate exercise in both healthy and metabolic syndrome patients (41). Some data suggest a greater increase following resistance compared with aerobic exercise (42). Serum irisin is regulated by exercise intensity, with greater increases following high-intensity exercise (43, 44). By contrast, other research reports an increase in the expression of FNDC5 in human skeletal muscle following 12 weeks of training but a paradoxical decrease in circulating irisin (45). Though synthesized in muscle, it is not clear if irisin is secreted from muscle directly either *in vitro* or *in vivo.* Incubation of L6 myotubes in irisin *in vitro* results in increased glucose uptake in a dose-dependent manner and is mediated by activation of AMPK and ACC (46). Irisin treatment also upregulates expression of PGC-1α4, a specific isoform associated with muscle hypertrophy, in primary myocytes (47). This was accompanied by increased IGF-1 and decreased myostatin expression, suggesting it a role in regulation of muscle growth, thus providing a larger muscle mass to act as a sink for blood glucose. Irisin perfusion in HFD mice resulted in decreased fasting blood glucose and improved glucose and insulin tolerance (48). Furthermore, FNDC5 overexpression in obese and HFD mice led to increased serum irisin resulting in decreased serum fasting glucose and improved glucose tolerance and insulin sensitivity in HFD mice (48).

Brain-Derived Neurotrophic Factor (BDNF)

The effect of resistance training on circulating BDNF remains equivocal. Several studies report no change in BDNF after either acute or chronic resistance training (49–53). By contrast, Yarrow et al. (54) and Coelho et al. (55) report increased plasma BDNF after acute and chronic resistance training. Circulating BDNF increases after both acute and chronic aerobic exercise in healthy participants [for review, see Ref. (56)]. Though a dose response is not apparent, there is evidence to support a greater increase in circulating BDNF following high-intensity exercise (57, 58), although whether muscle was the direct source of BDNF remains

unclear. BDNF mRNA expression is increased by contraction of skeletal muscle cells; however, there is no evidence to show BDNF is secreted by muscle cells following contraction (59). BDNF treatment reduces blood glucose in a diabetic rodent model (60). Yamanaka et al. (61) also found that chronic BDNF infusion improved glucose uptake and metabolism in BAT and muscle of rodents.

Myokines Regulating Fat Metabolism

IL-6

IL-6 infusion stimulates lipolysis and whole-body fatty acid (FA) oxidation in healthy males (62). Similarly, IL-6 treatment in humans results in elevated FA oxidation measured by palmitate oxidation and disappearance rates and a decreased respiratory quotient, peaking 60 min post-infusion (63). Increased whole-body lipolysis is mediated by STAT3 signaling to upregulate skeletal muscle but not adipose tissue lipolysis. Similarly, Petersen et al.(64) found that IL-6 infusion resulted in an increased rate of palmitate appearance and disappearance in human serum of both normal glucose tolerant and T2D patients. *In vitro* experiments confirmed increased lipolysis in adipocytes and FA oxidation in L6 myotubes (60). These data suggest IL-6 plays a beneficial role in fat metabolism through the upregulation of lipolysis in skeletal muscle and an increase in FA oxidation that is maintained in T2D.

IL-15

IL-15 administration to rodents resulted in a 35% decrease in WAT and a 20% decrease in circulating triglycerides, suggesting a role for IL-15 in lipid metabolism (65). Overexpression and oversecretion of IL-15 in a transgenic mouse model resulted in decreased total body and visceral fat (66). Treatment of adipocytes with IL-15 resulted in decreased deposition of lipids (67). Pierce et al. (68) perfused human subcutaneous adipose tissue with IL-15 *via* a microdialysis probe and observed an increase in adipose tissue lipolysis of lean participants. However, this effect was lost in obese participants, whereby, IL-15 perfusion suppressed lipolysis. Interestingly, muscle-derived IL-15, induced by exercise did not have an effect on adipose tissue lipolysis in either lean or obese (68). Therefore, the role of IL-15 in regulating lipolysis in humans remains equivocal and requires further investigation. Little information exists on a role for IL-15 in lipid oxidation; however, Almendro et al. (69) demonstrated an effect of chronic IL-15 administration to rodents on the fate of an exogenous lipid bolus. IL-15 reduced *de novo* lipogenesis in adipose tissue in response to an exogenous lipid load and favored oxidation in muscle and liver *via* the upregulation of FA transport genes. Further evidence for IL-15 and lipid oxidation in both healthy and T2Ds is required.

Brain-Derived Neurotrophic Factor

Brain-derived neurotrophic factor treatment of L6 myotubes and intact *ex vivo* muscle results in increased FA oxidation *via* activation of AMPK (59). Chronic BDNF treatment reduces circulating FAs, total cholesterol, and phospholipids in a diabetic rodent model (60). Chronic intracerebroventricular BDNF

TABLE 1 | Summary of known myokine response to contraction and metabolic action.

Myokine	Secreted from muscle	Electrical pulse stimulation	Increase in plasma/serum	Aerobic exercise	Resistance exercise	Exercise duration effect	Exercise intensity effect	Glucose uptake	Glucose oxidation	Lipolysis	Lipid oxidation	Pathway
IL-6	✓	✓	✓	✓	✓	✓	✓	↑	↑	↑	↑	AMPK, PI3K, STAT3
IL-8	✓	✓	✓	✓		✓		↑				AMPK
IL-13	✓			✓	✓			↑	↑			Akt, PI3K
IL-15	✓		✓	✓	✓			↑		↕	↑	AMPK, JAK–STAT3
BDNF	✓		✓	✓	✓		✓	↑		↑	↑	AMPK
CHI3L1	✓	✓	✓	✓	✓			↑				CHI3L1/PAR-2, p44/42, p38 MAPK, Akt
FGF-21	✓		✓	✓			✓	↑	↑	↕	↑	Akt, p44/42 MAPK
FSTL-1	✓	✓	✓	✓				↑				AMPK, CaMK
Irisin	✓		✓	✓	✓		✓	↑				AMPK
Myonectin	✓		✓	✓							↑	FA transport

✓, positive evidence; ↑, evidence for an increase in metabolic action; ↕, evidence for both an increase and decrease of metabolic action; BDNF, brain-derived neurotrophic factor; CHI3L1, chitinase-3-like-1; FGF-21, fibroblast growth factor 21; FSTL-1, follistatin-like-1; AMPK, adenosine monophosphate kinase; PI3K, phosphatidylinositol 3-kinase; Akt, protein kinase B; JAK, Janus kinase; STAT3, signal transducer and activation of transcription protein 3; PAR-2, protease-activated receptor 2; MAPK, mitogen-activated protein kinase; CaMK, calcium–calmodulin kinase; FA, fatty acid.

administration is also shown to decrease body weight, fat mass, adipocyte size, and serum triglycerides and promote lipolysis (70). Exercise induced increases in plasma BDNF are equivalent in obese and non-obese individuals but are not associated with increases in either whole-body glucose or FA oxidation (71). Further work is required to determine the effect of contraction-induced BDNF on fat metabolism in muscle, adipose, and liver.

Irisin

Irisin treatment of 3T3-L1 adipocytes *in vitro* induces increased gene expression of lipolysis-related genes including adipose triglyceride lipase, hormone-sensitive lipase (HSL), and protein expression of fatty acid-binding protein 4, suggesting irisin has potential to increase lipolysis (72). By contrast, Wang et al. (73) found no effect of irisin on HSL or ATGL protein expression or expression of lipolysis-related genes in 3T3L-1 adipocytes. Irisin perfusion in HFD mice resulted in decreased serum cholesterol, triglycerides, and free FAs (48). FNDC5 overexpression in obese and HFD mice led to increased serum irisin resulting in decreased serum triglycerides and free FAs in obese and HFD mice (48). Irisin treatment of adipocytes resulted in increased expression of UCP-1 and increased energy expenditure. Irisin also induced expression of metabolic genes (CPT-1, PPARα, HSL) and prevented lipid accumulation (74). Irisin treatment of myocytes also elevated FA oxidation suggesting a protective effect against progression of T2D (75).

Myonectin

Myonectin, a member of the C1q/TNF-related protein family, is expressed in skeletal muscle and released to the circulation in response to exercise in animal studies (76). *In vivo* myonectin administration reduced circulating levels of free FAs without altering adipose tissue lipolysis in mice. This reduction in circulating free FAs is purported to occur by an increase in FA uptake upregulated by increased expression of FA transport genes such as CD36, FATP1, Fabp1, and Fabp4 (76).

Fibroblast Growth Factor-21

Fibroblast growth factor-21 treatment of 3T3L-1 adipocytes attenuates lipolysis and expression of perilipin (77) and has also been shown to increase hepatic FA oxidation (78). Chronic FGF21 treatment reduces serum and hepatic triglyceride levels in diet-induced obese mice (79). These data suggest an influential role for FGF-21 in regulation of lipid metabolism.

OPTIMIZING THE MYOKINE RESPONSE FOR THE PREVENTION AND TREATMENT OF T2D

This review has outlined the role of myokines in regulating glucose and fat metabolism as potential mechanisms through which exercise can protect against the onset or progression of T2D. To harness the actions of contraction-induced myokines, we must establish the types, intensity, and volume of contraction required to maximize these regulators to inform future exercise protocols for the prevention and treatment of T2D. **Table 1** summarizes what we currently know with respect to the contraction-induced myokines discussed, in terms of how they are modulated by exercise and their actions in metabolic regulation. The role for aerobic exercise is clear, with evidence for an increase in circulating concentrations post-exercise for all myokines discussed, except IL-13, which appears to be acting in an auto/paracrine manner in response to resistance training only. This aligns with current recommendations that aerobic exercise is the primary component of any regimen in the prevention/treatment of T2D (80). It is logical to expect a dose response to contraction; but so far, few studies have demonstrated an effect or a minimum duration of exercise (12, 27). Similarly, few studies have demonstrated an effect for intensity, with higher intensity exercise generally eliciting a greater increase in circulating myokines (8, 35, 41, 43, 44, 57, 58). Resistance exercise effectively enhances circulating concentrations of the majority of myokines discussed (Il-6, IL-15, BDNF, CHI3L1, irisin) confirming the rationale for inclusion in prevention/treatment protocols.

In order to optimize future exercise prescription and policy to maximize the response and effect of myokines on metabolism, it is clear from this mini-review that there is a need to definitively characterize the following in both healthy and T2D participants: (i) the myokine response to an acute bout of aerobic exercise of varying durations (as low as 10 min); (ii) the myokine response to aerobic exercise of varying intensities; and (iii) the myokine response to resistance exercise of varying volume and intensities. To date, much of the evidence describing the mechanism through which recently identified myokines modulate metabolic function have been characterized using *in vitro* cell models which do not necessarily translate to the *in vivo* human situation. Though this is a necessary preliminary approach, it is important to acknowledge this as a significant limitation when interpreting the findings of the current literature.

Finally, this review has focused predominantly on tissue crosstalk by myokines released to the circulation; however, it is likely that more myokines are secreted post-exercise exclusively to the interstitium where they are exerting a local effect. More work is required to identify the entire *in vivo* contraction-induced secretome by techniques such as interstitial microdialysis. Furthermore, there is a need to establish the bioactivity of contraction-induced myokines for both local and systemic tissues.

AUTHOR CONTRIBUTIONS

BC conceptualized the paper, reviewed the literature, and wrote the manuscript in its entirety.

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