POSSIBLE MECHANISMS TO EXPLAIN ABDOMINAL FAT LOSS EFFECT OF EXERCISE TRAINING OTHER THAN FATTY ACID OXIDATION

EDITED BY: Chia-Hua Kuo, John L. Ivy, Jørgen Jensen, Ahmad Alkhatib and M. Brennan Harris

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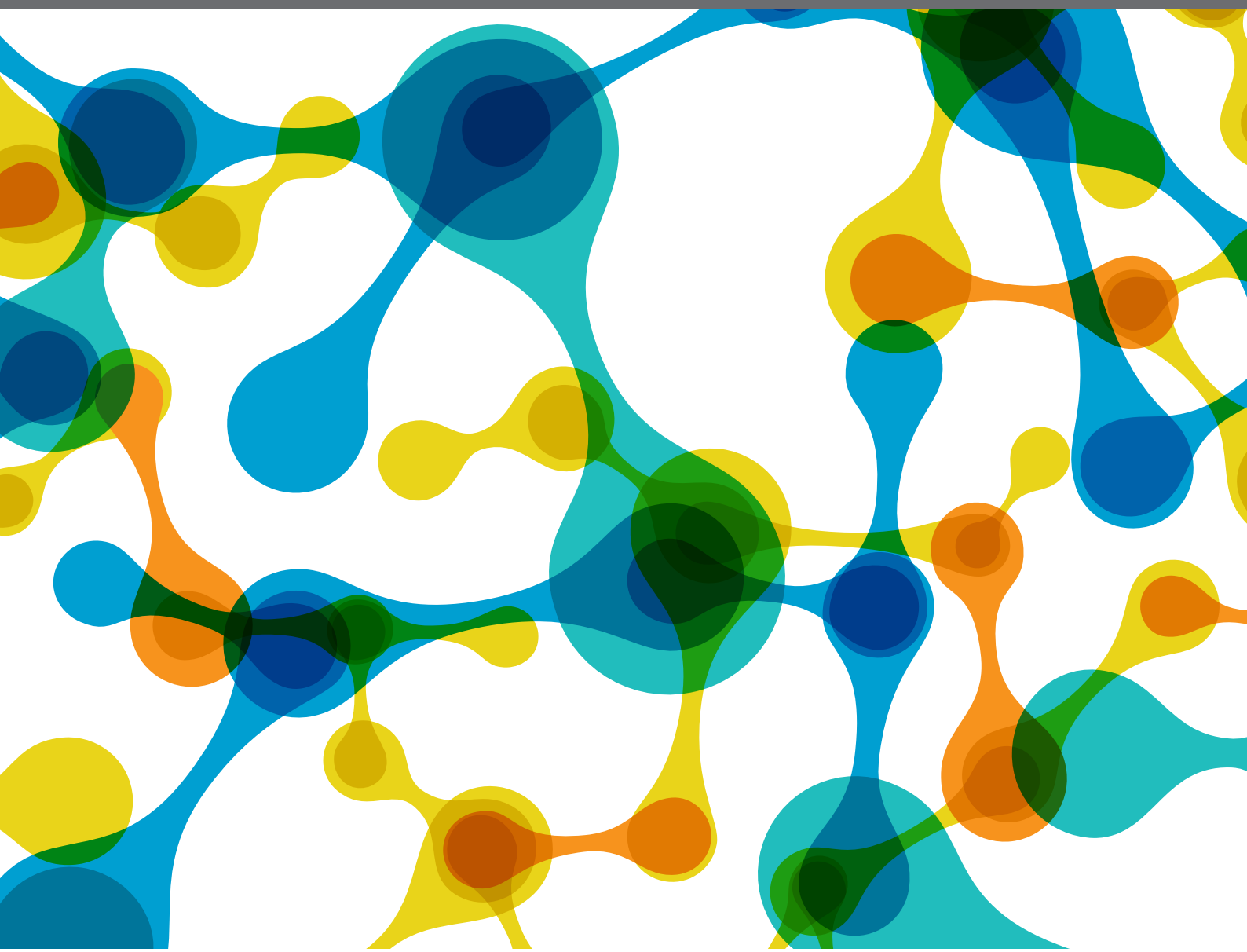

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POSSIBLE MECHANISMS TO EXPLAIN ABDOMINAL FAT LOSS EFFECT OF EXERCISE TRAINING OTHER THAN FATTY ACID OXIDATION

Topic Editors:
Chia-Hua Kuo, University of Taipei, Taiwan
John L. Ivy, University of Texas at Austin, United States
Jørgen Jensen, Norwegian School of Sport Sciences, Norway
Ahmad Alkhatib, University of Taipei, Taiwan
M. Brennan Harris, College of William & Mary, United States

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EDITORIAL
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Editorial: Possible Mechanisms to Explain Abdominal Fat Loss Effect of Exercise Training Other Than Fatty Acid Oxidation

Chia-Hua Kuo[1], *M. Brennan Harris*[2], *Jørgen Jensen*[3], *Ahmad Alkhatib*[4,5] *and John L. Ivy*[6]*

[1] Laboratory of Exercise Biochemistry, College of Kinesiology, University of Taipei, Taipei, Taiwan, [2] Department of Health Sciences, College of William and Mary, Williamsburg, VA, United States, [3] Department of Physical Performance, Norwegian School of Sport Sciences, Oslo, Norway, [4] Laboratory of Exercise Biochemistry, University of Taipei, Taipei, Taiwan, [5] School of Health and Life Sciences, Teesside University, Middlesbrough, United Kingdom, [6] Exercise Physiology and Metabolism Laboratory, Department of Kinesiology and Health Education, University of Texas at Austin, Austin, TX, United States

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Editorial on the Research Topic

Possible Mechanisms to Explain Abdominal Fat Loss Effect of Exercise Training Other Than Fatty Acid Oxidation

Obesity is a major health problem throughout the world, being one of the leading risk factors for type 2 diabetes mellitus, high blood pressure, cardiovascular disease, and premature death (Després and Lemieux, 2006; Ritchie and Connell, 2007). According to the World Health Organization, obesity has tripled over the last 40 years. Thus, recommended methods for weight management have flourished during this time. The fundamental cause for weight gain is an energy imbalance between calories consumed and calories expended. Undeniably, there are a number of factors that can affect caloric expenditure and consumption. However, with strict caloric restriction body mass can be significantly reduced.

Restricting caloric intake alone, however, can result in as much lean mass loss as fat mass loss (Chaston et al., 2007; Weinheimer et al., 2010). Therefore, individuals who are overweight or obese should focus on reducing body fat rather than body mass. In fact, being over fat even when not overweight has greater prevalence and is more strongly related to disease risk than being overweight and lean (Maffetone and Laursen, 2020). Research has shown that combining caloric restriction with an appropriate exercise program can reduce the fat composition of the body while limiting or even preventing the loss of lean mass (Chomentowski et al., 2009; Weinheimer et al., 2010). However, the type of exercise, which is most effective for enhancing fat loss has come into question (Kuo and Harris, 2016).

The removal of body fat by its conversion to carbon dioxide or "fat burning" is the process most described to explain the fat reducing outcome of exercise training. Therefore, it has been hypothesized that the exercise program that maximizes the rate of fatty acid oxidation will result in the greatest reduction in fat mass over time. In general, fatty acid oxidation is maximized at an exercise intensity between 50 and 70% of maximum oxygen consumption (Jeukendrup and Wallis, 2005). With increasing exercise intensity above this threshold level, the rate of fat oxidation declines as the reliance on carbohydrates increases. As a result, low to moderate intensity aerobic training is commonly recommended for weight management programs. Recent research, however, does

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Giuseppe D'Antona, University of Pavia, Italy

***Correspondence:**
John L. Ivy
johnivy@utexas.edu

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not support this recommendation. It has been clearly demonstrated that anaerobic high-intensity intermittent training, which relies predominately on carbohydrates as a fuel source, produces greater body fat reduction than continuous aerobic exercise even when the caloric expenditure is held constant for both modes of exercise (Tremblay et al., 1994; Trapp et al., 2008). Moreover, training under hypoxic conditions, which should limit fatty acid oxidation when compared with normoxic conditions, has been observed to result in a greater reduction in fat mass and greater increase in lean body mass (Chia et al., 2013).

In an insightful review article by Kuo and Harris (2016), they proposed that creating a negative energy balance in fat cells due to competition with skeletal muscle and other body tissues for circulating carbon sources may better explain the fat reducing outcome of exercise than the fat-burning model. In the following series of articles this concept is discussed in depth, as well as possible metabolic and hormonal alterations commensurate with high-intensity exercise training, which could facilitate a competition between fat cells and skeletal muscle for postprandial carbon and nitrogen.

In the opening article, Harris and Kuo present the concept that the primary mechanism underlying the intensity-dependent fat loss effect of exercise is due to a postprandial carbon and nitrogen redistribution to exercise stressed tissues, as opposed to adipose tissue, for fuel replenishment, tissue repair, and adapted responses. They further discuss the possible effects of meal and supplement timing when the nutrient demands of muscle are high to decrease abdominal fat accumulation while facilitating muscle recovery and development.

The relationship between obesity, specifically abdominal obesity, and type 2 diabetes and cardiovascular disease is presented by Kolnes et al. They continue their discussion by comparing the effects of different types of exercise training on energy expenditure and substrate utilization, and the impact these training methods have on adipose tissue function and body composition. This theme is continued by Delgado-Floody et al. in which they present new findings related to the effects of a 16-week high-intensity exercise training program on body composition, blood pressure, cardiorespiratory fitness, and substrate utilization during exercise among overweight prehypertensive and hypertensive patients. Additionally, Puengsuwan et al. report on the effects of a muscle stretching exercise program on overweight and obese middle-age and older adults. They observed a significant reduction in waist circumference and increase in percent fat free mass in their cohort, who trained 5 days per week for 15 weeks. Since muscle stretching is unlikely associated with increased fatty acid oxidation, they propose that this reduction in waist circumference after muscle stretching training was due to a redistribution of carbons from the abdominal region to challenged skeletal muscle.

Hormonal regulation of lipolysis during exercise is reviewed by Laurens et al. They discuss the effects of acute and chronic exercise on abdominal white adipose tissue lipolysis in lean and obese individuals. Of particular interest is the discussion on the regulation of lipolysis by catecholamines, atrial natriuretic peptide, and insulin. Also captivating, is their discussion on the effect contracting skeletal muscle has on adipocyte lipolysis via secretion of myokines such as the newly discovered growth and differentiation factor 15 (GDF15). Growth hormone is also a vital lipolytic hormone with strong influence on central abdominal fat stores (Rudman et al., 1990). Reductions in visceral adipose tissue and insulin resistance have been demonstrated in obese adults with growth hormone therapy (Johannsson et al., 1997; Nam et al., 2001). In the review by Sabag et al., factors contributing to exercise-induced growth hormone response, and how this response influences visceral adipose tissue and cardiometabolic health is evaluated.

With adipocyte hypertrophy, there are substantial changes in cell function, enzyme activity, and the secretion of various adipokines. A reduction in omentin is associated with an increase in metabolic risk factors (De Souza Batista et al., 2007), while an increase in vaspin appears to be an intrinsic compensatory response to insulin resistance, atherosclerosis, and chronic inflammation (Youn et al., 2008; Kobat et al., 2012). The effects of high-intensity interval training on body composition, inflammatory markers, and the adipokines vaspin and omentin in diet-induced obese rats are presented by Costa et al. Another important adipokine is irisin, which possesses anti-obesity and anti-diabetic properties (Boström et al., 2012; Rodríguez et al., 2017). De Oliveira et al. discuss their findings related to the independent and combined effects of diet and exercise on irisin and visceral adiposity. Exercise has also been found to affect carbon distribution by altering the activity of hormone sensitive lipase (HSL). Liu et al. present their findings on the response of HSL to acute and chronic high-intensity intermediate training and moderate-intensity continuous training in adipose tissue of mice.

Diet is also explored in this series of articles. Research indicates that in comparison with low-fat diets, low-carbohydrate or ketogenic diets result in greater weight reduction and a better metabolic profile (Sharman et al., 2004; Volek et al., 2004). On this subject, Kong et al. report on the surprising beneficial effects of a 4-week ketogenic diet on body composition and cardiorespiratory fitness in overweight and obese Chinese females.

Finally, the effect of genotype on exercise-induced weight loss is presented by Cardoso et al. Specifically, they report on the influence of the Pro12Ala polymorphism of the PPARγ2 gene on the body composition of previously inactive participants in response to a 12-week aerobic exercise training program.

In summary, this Research Topic addresses the means by which exercise training reduces fat mass. Specifically, it offers support for the hypothesis that exercise training results in the redistribution of postprandial carbon and nitrogen from adipose tissue to skeletal muscle and other exercise-stressed tissues of the body, and provides several mechanisms by which this could occur. However, much is still unanswered regarding the means by which high-intensity exercise results in fat loss and should be the focus of future research. For example, more information is needed regarding the relative contribution of fat loss from fat cell death and adipocyte fatty acid oxidation after high-intensity exercise. It would be advantageous to determine the

optimal hormonal profile associated with fat loss and the most appropriate exercise and diet program required to achieve such a hormonal profile. It would also be of benefit to identify peptides and nucleotides released from exercising muscle that could potentially induce myogenic differentiation of circulating stem cells, and the impact this could have on exercise-stressed tissue regeneration and development, as well as fat cell metabolism. Such information is ultimately important for developing the most appropriate and efficient exercise programs for reductioning fat mass and treating the numerous metabolic disorders and physical disabilities associated with obesity.

AUTHOR CONTRIBUTIONS

JI wrote the initial draft of the editorial. All authors reviewed and edited the initial draft and agreed with the final version of the editorial.

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Pro12Ala Polymorphism on the PPAR$_\gamma$2 Gene and Weight Loss After Aerobic Training: A Randomized Controlled Trial

*Glêbia Alexa Cardoso[1,2], Darlene Camati Persuhn[3], Mateus Duarte Ribeiro[1,2], Bruno Rafael Virgínio de Sousa[1,3], Klécia de Farias Sena[1,2], Antônio Eduardo Monteiro de Almeida[4], João Modesto-Filho[4], Raquel Suelen Brito da Silva[1,2] and Alexandre Sérgio Silva[1,2]**

[1] Laboratory of Applied Studies in Physical Training to Performance and Health - LETFADS, Department of Physical Education, Federal University of Paraíba, João Pessoa, Brazil, [2] Associate Graduate Program in Physical Education – UPE/UFPB, Department of Physical Education, Federal University of Paraíba, João Pessoa, Brazil, [3] Graduate Program in Nutrition Sciences, Federal University of Paraíba (PPGCN/UFPB), João Pessoa, Brazil, [4] Lauro Wanderley University Hospital – HULW-Federal University of Paraíba – UFPB, João Pessoa, Brazil

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Edited by:
Chia-Hua Kuo, University of Taipei, Taiwan

Reviewed by:
Daniela Caporossi, Foro Italico University of Rome, Italy
Mallikarjuna Korivi, Zhejiang Normal University, China

***Correspondence:**
Alexandre Sérgio Silva
alexandresergiosilva@yahoo.com.br

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The objective of this study was to verify the influence of the Pro12Ala polymorphism of the PPARγ2 gene in response of a training program on the body composition. Sixty-nine previously inactive men and women (32.8 ± 8.2 years) were genotyped and underwent a 12-week aerobic (running/walking) training program (3–5 sessions, 40 – 60 min per session, and intensity between the aerobic and anaerobic threshold) (experimental group n = 53) or were part of the control group (n = 16). They were tested for aerobic capacity (ergospirometry), body composition (DXA), abdomen, waist and hip circumferences and nutritional assessment before and 48 h after the experimental protocol. Two-way repeated measures ANOVA test was used to verify possible differences in variables between the experimental vs. control groups or Pro/Pro vs. Pro/Ala groups, and the Chi-squared test was used to verify the distribution of responders and non-responders according to genotype ($p < 0.05$). Frequencies of 75.5% Pro/Pro (n = 40) and 24.5% Pro/Ala (n = 13) were found, without any occurrence of the recessive homozygote. Body fat reduction was initially confirmed compared to a control group which did not exercise (n = 16; 29.1 ± 8.8 years), so that the exercise group obtained a reduction of −1.3 kg vs. −0.3 kg in the control group (p = 0.03). When they were divided by genotype, there were significant changes in fat mass (−1.3 ± 2.1 kg; p = 0.00), lean mass (0.6 ± 1.5 kg; p = 0.02), fat percentage (−1.3 ± 1.6; p = 0.00), waist circumference (−2.2 ± 2.9 cm; p = 0.00), abdomen circumference (−3.3 ± 3.6 cm; p = 0.00) and hip circumference (−2.7 ± 2.7 cm; p = 0.00) for Pro/Pro genotypes; and fat mass (−1.1 ± 1.7 kg; p = 0.04), fat percentage (−0.9 ± 1.5; p = 0.04), abdomen circumference (−3.9 ± 3.5 cm; p = 0.00) and hip circumference (−1.8 ± 1.8 cm; p = 0.00) for Pro/Ala genotypes, without any group

interaction differences. The Chi squared test revealed no differences in the distribution of responders or non-responders according to genotype. It is concluded that an aerobic training program promotes weight loss, but the Pro12Ala polymorphism in the PPARγ2 gene does not influence the variability of aerobic-induced exercise weight loss.

Keywords: body composition, genetic polymorphism, PPARγ2, aerobic exercise, weight loss

INTRODUCTION

Despite scientific advances, weight loss remains a challenge regardless of intervention strategy. A meta-analysis indicates that interventions with diets and lifestyle changes promote a reduction of around 5 kg after 2–4 years, while pharmacological therapies result in a reduction of 5–10 kg after 1–2 years (Douketis et al., 2005). Meta-analytic studies of the physical training practiced alone (without dietary or pharmacological intervention) indicates weight loss from 0.4 ± 3.3 kg to 2.3 ± 5.5 kg (Johns et al., 2014) or 0.9–2.9 kg (Washburn et al., 2014).

An important individual variability in physical training responses has been noted with people who are good or bad responders and who even acquire body fat after training programs (Donnelly et al., 2003). In a randomized controlled clinical trial of walking/running for 10 months (5 sessions/week, 400 or 600 kcal/session) (Donnelly et al., 2013), only 62.2% of those who spent 600 kcal/session and 45.9% to 400 kcal/session achieved ≥ 5% weight loss compared to baseline, while the other participants did not lose or even gained body weight. Therefore, understanding differences between good and bad responders to training programs can provide important insights for this line of research.

The gamma peroxisome proliferator-activated receptor gene (PPARγ2) may be considered a candidate gene for weight loss. Previous data indicate that this gene is involved in adipocyte regulation, growth and differentiation (Fajas et al., 1997), regulates the expression of numerous genes involved in lipid metabolism, controls the expression of the fatty acid carrier protein, and is predominantly expressed in adipose tissue (Tavares et al., 2007). In fact, this gene is related to obesity and metabolic diseases (Masud et al., 2003; Ereqat et al., 2009; Bozina et al., 2013; Galbete et al., 2013).

The influence of this polymorphism on weight loss is still controversial. Studies have shown that patients with the Ala12 allele of the PPAR gene had greater weight loss in response to a training program (150 min of physical activity/week) in the short and long term (0.63 and 0.93 kg/allele, $p < 0.005$, respectively) (Delahanty et al., 2012). Moreover, diabetic patients with the Pro/Ala + Ala/Ala allele presented a higher body weight reduction when compared to Pro/Pro homozygotes, who underwent exercise intervention (−1.8 ± 1.8 kg vs. −0.3 ± 1.4 kg) (Østergård et al., 2005). On the other hand, women with Pro12Ala polymorphism showed resistance to a dietary intervention (Adamo et al., 2007), in the same way that Korean women with Pro/Ala + Ala/Ala alleles had a significant increase in body mass ($p = 0.01$), BMI ($p = 0.01$), and waist–hip ratio ($p = 0.001$) when compared to the Pro/Pro allele carriers in an intervention with diet and exercise (Kim et al., 2004).

In spite of this, there is no evidence to confirm the hypothesis that this gene acts positively or negatively in the lipolysis or slimming process, since it regulates the expression of numerous genes involved in lipid metabolism. Thus, the objective of this study was to verify the influence of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor gamma 2 (PPARγ2) on the weight loss induced by a continuous aerobic training program.

MATERIALS AND METHODS

This was a controlled and randomized clinical trial with 69 participants, of which 53 were genotyped and involved in an aerobic training program and 16 were part of the control group. In the case, the control group was formed to verify the effectiveness of the physical exercise, so they were not genotyped.

The sample size of this group was determined based on the study by Kim and Jung (2014), in which a similar training program resulted in a reduction of body fat by −2.18 ± 1.96%, and which implied an effect size of 1.11, which in turn generated a minimum sample size of 13 participants for a statistical β power of 0.95. To verify the effectiveness of the training program before starting comparisons between genotypes, a control group was set up that did not perform the training program with 16 people. The distribution of volunteers between the exercise and non-exercise groups was randomized.

Participants were invited through social media ads and pamphlets distributed near the study site, as well as medical clinics and community organizations. The detailed procedures are registered in the Clinical trials under identification NCT03568773.

In order to be eligible, participants had to be adults (ages 20–45 years), under active (<150 min/week of moderate to severe physical activity) as determined by the International Physical Activity Questionnaire (Matsudo et al., 2001), have a BMI of between 25 and 39.9 kg/m^2 for at least 6 months, have not changed more than 5 kg in the last 3 months, did not smoke or consume alcohol (more than two doses/day), did not use medicine, supplements or thermogenic substances which alter the metabolism, and did not have any diseases (diabetes, coronary artery disease or hormonal diseases); for women, to not be menopausal or present any symptoms related to the climacteric period. Those who missed two consecutive weeks or 25% of the physical training program or who began dietary intervention,

physical exercise, or medication during the program period, as well as those who were injured were excluded from the study.

All volunteers who agreed to participate in the study provided written consent after being clarified about procedures and potential risks. The experimental protocol was approved by the Human Research Ethics Committee of the Health Science Center-CCS of the Federal University of Paraíba-UFPB-Brazil, under protocol number 1.981.304.

Study Design

The exercise group completed a 12-week aerobic training program and the control group did a stretching program over the same period. The volunteers underwent ergospirometry (aerobic capacity – VO_2max and anaerobic threshold), Dual-energy X-ray absorptiometry (DXA) (body composition) dual-emission densitometry, abdomen, waist and hip circumference measures, glycemic and lipid profile (blood glucose, triglycerides, total cholesterol, HDL and LDL) and nutritional assessment (also in the sixth week of intervention) before and 48 h after the intervention periods. Each participant also had their buccal mucosa collected during the study for later genotyping.

Aerobic Capacity and Anaerobic Threshold

Aerobic capacity and anaerobic threshold were performed by ergospirometry (Metalyzer 3B - Cortex (Leipzig-Germany) on a treadmill (Centurion-200 Micromed, Brasília – Brazil), with increasing load on a Bruce ramp protocol (8–12 min). Anaerobic Threshold (L1) and respiratory compensation point (L2) were recorded at the test time, which was registered immediately and then calculated for the mean time of 10 s determined at the exponentiation point of CO_2, while VO_2max was determined when the volunteers reached volitional fatigue accompanied by estimated HRmax. The criteria for interrupting the test followed the guidelines of Guazzi et al. (2018).

Body Composition

Body composition was determined by Dual-energy X-ray absorptiometry (DXA) by a full body scan (brand: LUNAR ADVANCE DF + 13.4038 Radiation – GE LUNAR CORPORATION/United States), with the three compartment model (muscle, vital organs and other viscera of the body), fat tissue (amount of body fat) and bone tissue (total skeletal mass), being considered following the guidelines and calibration procedures provided by the manufacturer. In addition, weight, height (for evaluation of BMI) (a scale with coupled stadiometer, Sanny®, São Bernardo do Campo - São Paulo, Brazil) and the abdomen (greater abdomen circumference zone), waist (between the costal border and the iliac crest) and hip (maximum posterior hip extension) circumference measures were taken for analysis (Sousa, 2008).

Blood Collection and Biochemical Measurements

Blood samples were collected in the antecubital vein early in the morning with the volunteer fasting for 10 h. They were then deposited in light-protected test tubes containing EDTA, homogenized by inversion and centrifuged at 3,000 rpm for 10 min. The supernatant was stored at −20°C until analysis. Lipid and Glycemic Profile analyzes were performed on serum samples using commercial kits from the Labtest brand (Minas Gerais, Brazil), following the manufacturer's recommendations and on a Labmax 240 premium automatic analyzer (Lagoa Santa-MG, Brazil).

Nutritional Control

Evaluations were performed before the intervention, in the sixth week and during the last week of intervention through the 24-h recall following a protocol suggested by Dietary Recommendation Intake (DRI) (Gibson, 1990). Three reminders were performed for each of the three evaluations, two of which were for weekdays and one for the weekend. AVANUTRI software version 4.0 (Avanutri & Nutrição Serviços de Informática, Três Rios-RJ-Brazil) was used for caloric and macro and micronutrient calculations. Volunteers were asked to not change their eating habits during the study after the pre-intervention evaluation.

DNA Extraction and Genotyping

Oral epithelial cell samples were obtained with a 3% sucrose wash. DNA extraction was performed according to a previously published method (Aidar and Line, 2007). Genotyping of the Pro12Ala polymorphism (PPARγ2) was performed by the PCR-RFLP technique. The polymorphism was amplified using the primers: (5′-GCCAATTCAAGCCCAGTC-3′-sense and 5′-GATATGTTGGAGAGAGGGTATCAGTGAAGGAATCGCTTTCCG-3′-antisense). Thermal cycling was used as follows: initial denaturation at 94°C for 8 min and 35 denaturation cycles at 94°C for 50 s, annealing at 59°C for 50 s. and an extension at 72°C for 1 min, then final extension at 72°C for 5 min. After digestion with the BstU-I restriction enzyme (Biolabs, New England/United States), a single 270 bp fragment indicated the presence of the Pro/Pro genotype, while three 270, 227, and 43 bp fragments confirmed the presence of the Pro/Ala genotype. Lastly, 15% polyacrylamide gel electrophoresis and stained with silver nitrate was used for this genotypic reading.

Physical Training Protocol

An adaptation protocol consisting of a 3-week treadmill (2 days/week, 20–40 min, intensity < L1 acquired in the ergospirometric test) was performed first. Next, the 12-week training program consisting of fast walking and/or running with intensity between the L1 and the L2 in an open-air environment was implemented as detailed in **Table 1**. There were 3 sessions/week from the first to the fourth week, 40–60 min, intensity = L1, always supervised by the researchers; in the fifth week the intensity increased between L1 and 1/2L2. There was an increase to five weekly sessions from the sixth to the eighth weeks, three of which were supervised by researchers and two in which the volunteers used an application (Endomondo Sports Tracker, version 17.5.1), and sent the report to the researchers. The duration was 60 minutes at this stage, with intensity

TABLE 1 | Aerobic training protocol.

Week	Adaptation	1st	2nd and 3rd	4th	5th	6th to 8th	9th to 12th
Sessions/week	2	3	3	3	3	5	5
Time (min)	20 to 40	40	50	60	60	60	60
Intensity	<L1	L1	L1	L1	L1 a 1/2L2	L1 a 1/2L2	1/2L2 a L2

Subtitle; <L1: below anaerobic threshold; L1 1/2 L2: between anaerobic threshold and half the respiratory compensation point; 1/2 L2 and L2: half of respiratory compensation point and respiratory compensation point.

between L1 and 1/2L2; then the time, volume and weekly frequency remained unchanged from the ninth to the twelfth weeks, and intensity increased to 1/2L2 to L2. Heart rate was continuously monitored in the laboratory sessions by heart rate monitors (Polar®, model FT1, Kempele, Finland). The subjects who were randomized to not participate in the training program (control group) participated in the same intervention period with stretching classes (1 day/week, duration of 60 min).

Statistical Analysis

Data were expressed as mean ± standard deviation, or absolute values. The normality of data homogeneity was initially verified through the Kolmogorov–Smirnov and Levene tests, respectively. Two-way repeated measures ANOVA or Friedman test were used to compare the outcome of the training program between Pro/Pro and Pro/Ala genotypes. The Chi-squared test was performed to verify the genotype influence (Pro/Pro and Pro/Ala) on the variation in the body composition components, categorized as responders (Δ Weight: ≥1 kg; Δ BMI: ≥1 kg/m^2; ΔFat mass: ≥1 kg; ΔLean mass: >0 kg; Δ Fat percentage: ≥1; Δ waist circumference: ≥2 cm; Δ hip circumference: ≥2 cm; Δ abdominal circumference: ≥2 cm) and non-responders (Δ Weight: <1 kg; Δ BMI: <1 kg/m^2; ΔFat mass: <1 kg; Δlean mass: <0 kg; Δ Fat percentage: <1; Δ waist circumference: <2 cm; Δ hip circumference: <2 cm; Δ abdominal circumference: <2 cm) of the experimental group. A linear regression test was used starting with automatic linear modeling which considered the possible influencing variables (age, educational level, average daily sleep time, glycemic and lipid profiles, and nutritional behavior). Data analyzes were performed using the SPSS 20.0 Package (SPSS Inc., Chicago, United States) and a p-value of <0.05 was considered significant.

RESULTS

Of the 53 subjects in the trained group, 75.5% (n = 40) were identified as Pro/Pro and 24.5% (n = 13) with Pro/Ala genotype, with no Ala/Ala appearing in the sample. When the Hardy Weinberg Equilibrium was calculated considering $p > 0.05$, we observed that the study sample is consistent with the expected distribution (p = 0.31). When the Pro/Pro and Pro/Ala groups were compared, they were found to have similar ages, a physical activity level compatible with the insufficiently active classification (IPAQ) (Matsudo et al., 2001), and aerobic capacity between regular and weak according to the American Heart Association (American Heart Association, 1972), with no differences between groups. Likewise, they had similarity for all evaluated body composition components (**Table 2**).

Although inclusion criteria predicted that diabetics could not participate in the study, people with borderline glycemic values were present in the sample, but there were no differences between the exercise and non-exercise groups (**Table 2**).

The Pro/Pro group had higher initial caloric intake, as well as macronutrients (carbohydrates and proteins) in comparison with Pro/Ala, as can be seen in **Table 3**. The evaluations performed in the sixth week and at the end of the protocol indicated that the two groups maintained the same feeding profile in relation to the initial values.

The previous analysis comparing study participants with a sample of people who did not participate in the training program confirmed that exercise promoted increased aerobic capacity in the trained group, which was accompanied by a significant reduction in all body composition components related to obesity and increased muscle mass, in addition to reducing total cholesterol, its LDL fraction and triglycerides, without the same occurring in the group that did not exercise. Glycemia and HDL cholesterol fraction did not change in either group (**Table 2**).

Weight Loss According to Genotype (Pro/Pro versus Pro/Ala)

Figure 1 shows a consistent individual variability in the weight loss response and in the increase of lean mass in the training program. As can be seen in panel C which corresponds to fat mass, there were people who reduced 6.0 kg, but others increased up to 2.7 kg of fat at the end of the training program. This same variability occurred for the other body composition components and are presented in **Figure 1**.

The data shown in **Table 2** indicate a slight weight-loss superiority in volunteers with Pro/Pro genotype, since this group had a significant reduction in fat mass, fat percentage, and abdomen and hip circumferences, in addition to the increase in lean mass. Meanwhile, Pro/Ala genotypes showed no significant reduction in waist circumference and no increase in lean mass. However, the chi-squared test indicated that the studied genotype was not a determinant of greater or lesser weight loss for any of the body composition variables (**Table 4**).

Considering that the Chi-squared test ruled out the genotypic influence on exercise-induced weight loss, we performed a linear regression to verify that other variables could be influential. Other variables such as vitamin C (β = 0.41; p = 0.00), vitamin D (β = 0.33; p = 0.00) and potassium were found to be positively correlated, while gender (β = 0.28; p = 0.00), hours of sleep (β = −0.37;

TABLE 2 | Baseline, variation anthropometric and biochemical characteristics of the participants in the experimental and control groups according to the Pro12Ala Gene PPARγ2 polymorphism.

Variables	Pro/Pro *n* = 40 (*n* = 13 males/*n* = 27 females)			Pro/Ala *n* = 13 (*n* = 2 males/*n* = 11 females)			Total Exercise *n* = 53 (*n* = 15 males/*n* = 38 females)			Non-exercise *n* = 16 (*n* = 3 males/*n* = 13 females)		
	Before	After	Δ	Before	After	Δ	Before	After	Δ	Before	After	Δ
N (%)	40 (75,5)			13 (24,5)			53 (76.8)			16 (23.2)		
Age (years)	33.2 ± 7.5			33.0 ± 9.2			33.1 ± 7.6			29.1 ± 8.8		
PA (min/Week)	75.3 ± 29.4			71.5 ± 32.4			72.6 ± 9.2			69.1 ± 28.4		
Sleep (hours/day)	7.0 ± 1.4			6.0 ± 1.4			6.6 ± 1.5			6.1 ± 2.1		
Glycemia (mg/dL)	96.8 ± 18.8	95.2 ± 20.5	−1.6 ± 13.8	94.9 ± 16.6	92.2 ± 22.0	−2.7 ± 10.3	96.3 ± 18.2	94.5 ± 20.7	−1.9 ± 12.9	90.4 ± 16.0	95.6 ± 19.6	5.2 ± 15.0
Col. Tot. (mg/dL)	199.8 ± 48.8	183.3 ± 37.1*	−16.5 ± 33.0	183.7 ± 41.1	164.1 ± 55.6	−19.6 ± 37.0	195.8 ± 47.1	178.6 ± 42.6*	−17.2 ± 33.7	185.8 ± 35.8	191.7 ± 43.3	5.9 ± 40.8#
HDL (mg/dL)	35.9 ± 8.9	36.5 ± 7.9	0.5 ± 5.9	41.4 ± 10.5	37.7 ± 10.7	−3.6 ± 7.5	37.3 ± 9.5	36.8 ± 8.6	−0.5 ± 6.5	34.6 ± 11.9	35.6 ± 7.6	1.1 ± 8.7
LDL (mg/dL)	131.6 ± 40.9	120.8 ± 33.9*	−10.7 ± 31.9	120.3 ± 34.2	104.7 ± 55.5	−15.6 ± 38.6	128.9 ± 39.4	116.9 ± 40.3*	−11.9 ± 33.3	123.3 ± 29.4	125.4 ± 36.4	2.1 ± 31.1
TG (mg/dL)	158.9 ± 89.3	129.9 ± 71.1*	−29.0 ± 46.9	110.1 ± 47.2	108.0 ± 54.4	−2.1 ± 79.6	146.9 ± 83.4	124.5 ± 67.6*	−22.4 ± 57.0	139.9 ± 75.7	153.6 ± 84.8	13.7 ± 61.5#
VO_2max (mL·kg^{-1}·min^{-1})	29.2 ± 6.7	35.2 ± 9.4	5.9 ± 5.8	27.9 ± 4.6	34.8 ± 10.2	7.0 ± 7.0	28.8 ± 6.0	35.0 ± 9.4	6.1 ± 6.0	23.9 ± 4.6#	24.8 ± 2.7	0.2 ± 2.6#
Weight (kg)	85.8 ± 11.4	84.9 ± 11.9	−0.8 ± 2.7	82.1 ± 10.3	80.9 ± 9.4	−1.1 ± 2.2	85.0 ± 11.3	84.3 ± 11.5	−0.8 ± 2.5	82.7 ± 11.2	83.2 ± 12.1	0.4 ± 2.1
BMI (kg/m^2)	31.5 ± 2.6	31.2 ± 2.9	−0.3 ± 1.2	31.2 ± 3.2	30.8 ± 2.9	−0.5 ± 0.8	31.5 ± 2.9	31.2 ± 3.0	−0.3 ± 1.1	31.0 ± 2.9	31.1 ± 3.2	0.1 ± 0.8
FM (kg)	37.1 ± 5.9	35.8 ± 6.9*	−1.3 ± 2.1	36.2 ± 6.0	35.1 ± 6.5*	−1.1 ± 1.7	36.8 ± 6.0	35.5 ± 6.8	−1.3 ± 1.9	38.4 ± 7.6	38.2 ± 8.4	−0.3 ± 1.4#
LM (kg)	46.0 ± 11.0	46.6 ± 10.7*	0.6 ± 1.5	41.6 ± 7.0	41.9 ± 6.7	0.3 ± 1.2	45.2 ± 10.1	45.8 ± 9.9	0.6 ± 1.5	41.5 ± 6.8	44.5 ± 11.4	2.9 ± 9.5
F%	44.0 ± 7.3	42.8 ± 7.5*	−1.3 ± 1.6	45.7 ± 5.6	44.8 ± 6.2*	−0.9 ± 1.5	44.1 ± 6.8	43.0 ± 7.2	−1.2 ± 1.5	46.7 ± 5.8	46.1 ± 5.9	−0.6 ± 1.3
WC (cm)	94.1 ± 7.5	91.9 ± 7.2*	−2.2 ± 2.9	90.6 ± 8.2	89.6 ± 9.2	−1.0 ± 3.0	93.3 ± 7.9	91.4 ± 8.1	−2.0 ± 2.8	93.8 ± 8.7	93.9 ± 9.8	0.2 ± 3.6#
Abdomen (cm)	105.6 ± 6.6	102.3 ± 7.4*	−3.3 ± 3.6	105.0 ± 8.1	101.1 ± 8.3*	−3.9 ± 3.5	105.3 ± 7.4	101.8 ± 8.0	−3.5 ± 3.6	103.8 ± 10.4	104.2 ± 10.7	0.5 ± 4.7#
HC (cm)	110.5 ± 0.6	107.7 ± 6.2*	−2.7 ± 2.7	112.0 ± 7.0	110.2 ± 6.1*	−1.8 ± 1.8	110.7 ± 6.0	108.3 ± 6.3	−2.5 ± 2.5	111.0 ± 5.1	110.1 ± 7.0	−0.5 ± 3.1#

*Data are means ± SD. N, number of participants; PA, Physical Activity; Col. Tot., total cholesterol; HDL, high density lipoprotein; LDL: low density lipoprotein; TG, triglycerides; BMI, Body Index Mass; FT, Fat Mass; LM, Lean Mass; F%, Fat Percentage; WC, Waist Circumference; HC, Hip Circumference. *Intra-group differences vs. initial values (pairwise Student's t-test). #Between-groups differences (Two-Way ANOVA).*

TABLE 3 | Intake of macronutrients and micronutrients of participants within 12 weeks of intervention.

Nutrition	Pro/Pro			Pro/Ala			*p*
	Baseline	6th week	12th week	Baseline	6th week	12th week	
Energy (kcal)	1947.5 ± 580.0[#]	1924.0 ± 598, 2	1885.1 ± 564.1	1535.3 ± 412.3	1562.8 ± 360.6	1720.6 ± 466.6	0.02
Carbohydrate (g)	253.3 ± 77.8[#]	247.9 ± 84.0	235.8 ± 74.4	203.7 ± 67.7	193.7 ± 47.5	223.6 ± 99.6	0.04
Total fat (g)	63.0 ± 20.6	63.7 ± 25.1	66.7 ± 23.1	51.3 ± 17.0	52.5 ± 20.8	59.2 ± 18.9	
Protein (g)	89.2 ± 41.3[#]	89.8 ± 35.4	88.3 ± 32.8	64.7 ± 15.4	78.9 ± 36.8	73.5 ± 27.9	0.02
SFA (g)	17.3 ± 7.0	18.4 ± 8.7	18.1 ± 6.8	15.0 ± 10.9	15.0 ± 6.1	17.6 ± 8.0	
MUFA (g)	14.8 ± 6.0	14.9 ± 6.5	15.6 ± 6.9	11.2 ± 4.5	13.8 ± 7.9	14.8 ± 5.3	
PUFA. (g)	9.7 ± 5.4	10.2 ± 6.1	9.2 ± 4.5	7.2 ± 3.7	8.6 ± 5.6	10.2 ± 4.8	
Cholesterol (mg)	309.3 ± 212.1	338.6 ± 229.5	312.1 ± 172.6	223.4 ± 52.0	286.4 ± 125.5	295.4 ± 123.2	
Fibers (g)	14.6 ± 7.0	16.4 ± 7.7	15.1 ± 8.4	11.6 ± 4.2	11.5 ± 3.6	11.8 ± 4.7	

Data are means ± SD. SFA, Saturated Fatty Acids; MUFA, Monounsaturated Fatty Acids; PUFA, Polyunsaturated fatty acids. [#]Differences between groups Pro/Pro vs. Pro/Ala, initial values. P < 0.05. (Two-way ANOVA and Friedman's test).

p = 0.00), Magnesium (β = −0.81; p = 0.00), Zinc (β = −0.34; p = 0.00), Iodum (β = −0.04; p = 0.03) and Fibers (β = −0.31; p = 0.03) were negatively correlated. It seems that, when adjusted by gender, these influences have disappeared. Therefore, although some influence was noticed, they were consistency weak for the variables of body composition that were analyzed, especially when adjusted for gender (**Supplementary Table S1**).

DISCUSSION

Discrete weight loss in response to physical training programs has been highlighted in the literature since the middle of the first decade of this century (Douketis et al., 2005; Johns et al., 2014). Although the participants were significantly thinner with the training program, weight loss observed in this study can also be considered clinically discreet (−0.9 ± 2.6 kg, −1.3 ± 2.0 kg, and −1.2 ± 1.6% for body weight, body fat and fat percentage respectively). These data are similar to reviews and meta-analyzes (Johns et al., 2014; Washburn et al., 2014) with physical exercise-induced weight loss which did not analyze the genetic influence. They are also similar to those found in other studies that investigated the influence of PPARγ2 on weight loss (Østergård et al., 2005; Franks et al., 2007; Delahanty et al., 2012).

In addition to corroborating literature on the magnitude of physical exercise, the data from our study corroborate previous literature, indicating significant individual variability in weight loss (Donnelly et al., 2013). The authors of this study showed that only 62.2% of participants, including men and women, achieved weight loss. This individual variability indicates that personal characteristics (which may be physiological, genetic or otherwise) are influential in the weight-loss response to physical training. The influence of the PPARγ2 gene on both obesogenesis and weight loss is still controversial. Regarding obesogenesis, some studies have shown an association of the risk allele with BMI or other variables related to obesity (Danawati et al., 2005; Franks et al., 2007; Gupta et al., 2011; Bhatt et al., 2012), but this association was not found in other population studies (Ereqat et al., 2009; Wang et al., 2014). The data for the influence on weight loss are incipient, while some studies with diet interventions show that Ala allele carriers have dietary resistance (Nicklas et al., 2001; Adamo et al., 2007). On the other hand, Verhoef et al. (2014) demonstrated that Pro/Ala was positively associated with short-term weight loss.

Data related to weight loss in response to training programs until the present study were incipient, but corroborating. In the present study, it was found that the Ala allele had a high affinity for alleles; therefore, our data are controversial regarding the relationship of the PPARγ2 gene with exercise-induced weight loss, as it already occurs in the areas of obesogenesis (Franks et al., 2007; Gupta et al., 2011; Bhatt et al., 2012) and diet-induced weight loss or lifestyle modification (Nicklas et al., 2001; Adamo et al., 2007).

Methodological differences in the interventions of the previous studies are likely to explain the differences in results in our study compared to the three previous studies. In two of them, the intervention consisted of physical exercise in the form of lifestyle modification, so that people started to perform 150 min of physical activity/week (Franks et al., 2007; Delahanty et al., 2012). This differs greatly from our study, in which the intervention was a systematic physical training program. The only study in which a similar protocol was performed was that of Østergård et al. (2005), where participants underwent aerobic training (three times a week, 45 minutes of duration at 70% VO_2 max) for 10 weeks; but the population was constituted by relatives of first-degree diabetics and using a stationary bicycle.

Although our study found no influence of the Pro12Ala polymorphism of the PPARγ2 gene on weight-induced exercise, we presented some practical implications, for research laboratories. We observed that sleep hours and nutritional aspects were shown to be influencers in the promoted weight loss, although without much consistency for the analyzed body composition variables. In any case, the nutritional differences between Pro/Pro and Pro/Ala in our findings could have occurred because the homozygous group had a larger number of men in the sample, although this was not an influencing factor in linear modeling when adjusted for gender.

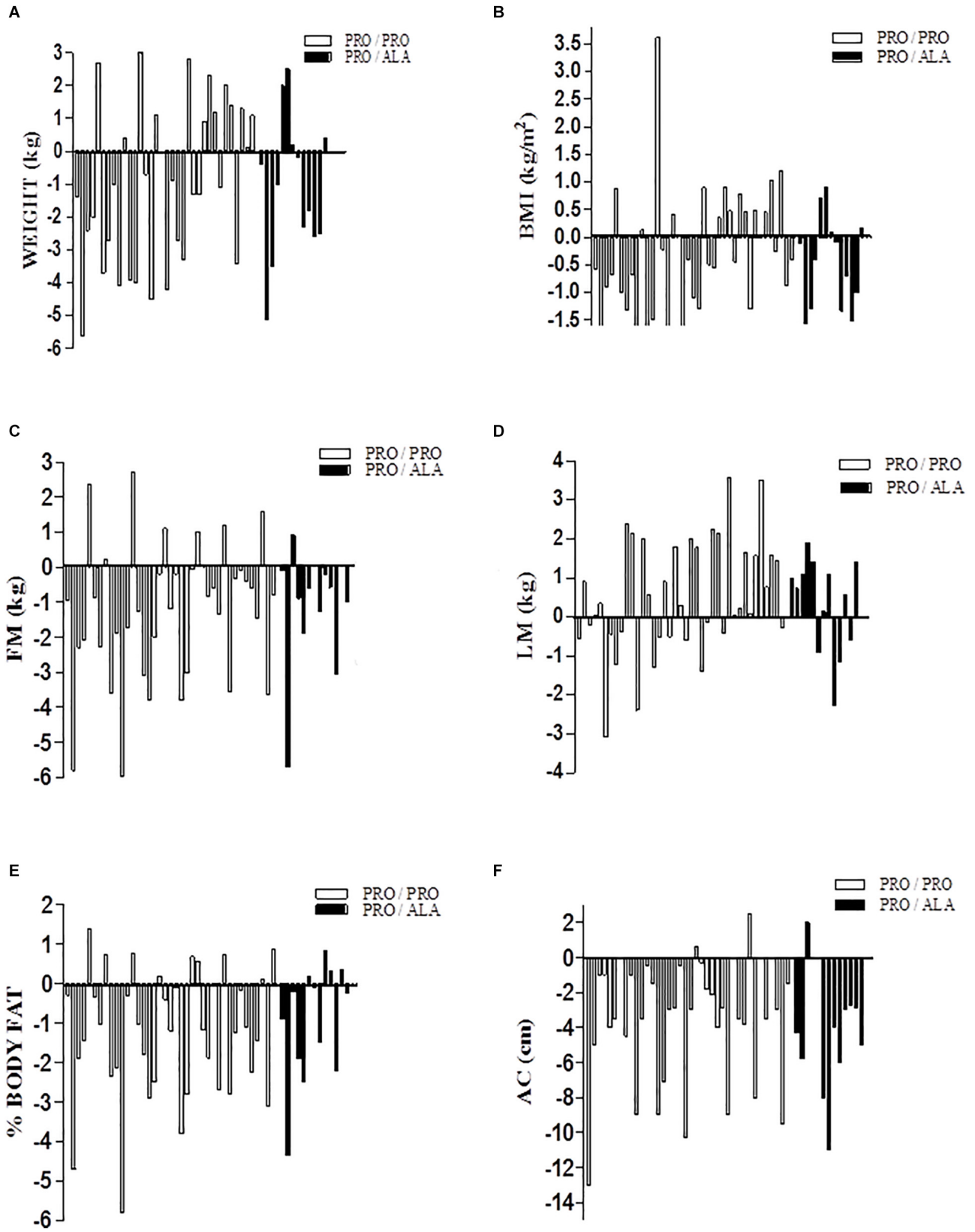

FIGURE 1 | Individual response of body composition to aerobic training between genotypes. Date are: variation of final value minus initial value (Δ variation); **(A)** body weight; **(B)** Body mass index; **(C)** fat mass; **(D)** lean mass; **(E)** body fat percentage; **(F)** abdominal circumference.

This reinforces the multifactorial and complex aspect of weight loss, so that other variables which were not considered in this study such as the metabolic profile, lipolytic or adipogenic hormones, fiber type, and behavioral aspects which have been hypothesized as influencers of weight loss (Boutcher and Dunn, 2009) should be considered in future studies, in which genetics may contribute to elucidate the relationship of discrete exercise-induced weight loss.

TABLE 4 | Association test between PPARγ2 gene polymorphism and responders and non-responders to weight loss training program.

Dependent variables	PPARγ2		Total	*p*
	Pro/Pro *n* (%)	Pro/Ala *n* (%)	*n*	
Δ Weight				
Responders	23 (57.5)	9 (69.2)	32	0.447
Non-responders	17 (42.5)	4 (30.8)	21	
Total	40	13	53	
ΔBMI				
Responders	23 (57.5)	8 (61.5)	31	0.797
Non-responders	17 (42.5)	5 (38.5)	22	
Total	40	13	53	
ΔFM				
Responders	26 (65.0)	10 (76.9)	36	0.414
Non-responders	14 (35.0)	3 (23.1)	17	
Total	40	13	53	
ΔF%				
Responders	31 (77.5)	8 (61.5)	39	0.257
Non-responders	9 (22.5)	5 (38.5)	14	
Total	40	13	53	
ΔLM				
Responders	25 (62.5)	9 (69.2)	34	0.658
Non-responders	15 (37.5)	4 (30.8)	19	
Total	40	13	53	
ΔWC				
Responders	29 (72.5)	9 (69.2)	38	0.821
Non-responders	11 (27.5)	4 (30.8)	15	
Total	40	13	53	
ΔAC				
Responders	33 (82.5)	10 (76.9)	43	0.661
Non-responders	7 (17.5)	3 (23.1)	10	
Total	40	13	53	
ΔHC				
Responders	28 (70.0)	7 (53.8)	28	0.228
Non-responders	12 (30.0)	6 (46.2)	25	
Total	40	13	53	

Date are: frequency of responders (who obtained some weight loss) and non-responders (who did not lose weight or increased any variable related to weight loss. BMI, Body Index Mass; FT, fat mass; LM, lean mass; F%, fat percentage; WC, waist circumference; HC, hip circumference; AC, abdominal circumference. The Chi-squared test (McNemar test) was used to observe the frequency and Fisher's test for values below five.

In addition to the physiological multiplicity, this same perspective must be kept in mind for the multiplicity of genes (besides PAPR) which may be involved in weight loss. In addition, it is necessary to investigate other genes (isolated or associated) which have been demonstrated to be involved in obesity such as FTO (Ben Halima et al., 2018), Melanocortin 4 Receptor - MC4R (Resende et al., 2017), Adenovirus 36 (Zhou et al., 2018) and beta 2 adrenoceptor-ADRB2 (Daghestani et al., 2018).

CONCLUSION

It was demonstrated that Pro12Ala polymorphism in the PPARγ2 gene does not influence the magnitude of the weight loss induced by aerobic training. Other genes, other physiological factors, as well as a larger number of volunteers participating in a training program should be considered in future studies. There must also be at least one group in which Ala/Ala alleles appear within the investigated sample.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Human Research Ethics Committee of the Health Science Center-CCS of the Federal University of Paraíba-UFPB-Brazil, under protocol number 1.981.304. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

GC and AS conceived the idea for the manuscript, agreed on content, contributed to the writing and editing the manuscript, and approved the final draft of the manuscript. DP, MR, BS, KF, AA, JM-F, and RS conceived the editing the manuscript, and approved the final draft of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.00385/full#supplementary-material

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Short-Term Ketogenic Diet Improves Abdominal Obesity in Overweight/Obese Chinese Young Females

Zhaowei Kong[1], Shengyan Sun[2], Qingde Shi[3], Haifeng Zhang[4], Tomas K. Tong[5] and Jinlei Nie[3]*

[1] Faculty of Education, University of Macau, Macao, China, [2] Institute of Physical Education, Huzhou University, Huzhou, China, [3] School of Health Sciences and Sports, Macao Polytechnic Institute, Macao, China, [4] College of Physical Education, Hebei Normal University, Shijiazhuang, China, [5] Department of Physical Education, Hong Kong Baptist University, Hong Kong, China

The purpose of this study was to examine the effects of a short-term ketogenic diet (KD) on body composition and cardiorespiratory fitness (CRF) in overweight/obese Chinese females. Twenty young females [age: 21.0 ± 3.7 years, weight: 65.5 ± 7.7 kg, body mass index (BMI): 24.9 ± 2.7 kg·m^{-2}] consumed 4 weeks of a normal diet (ND) as a baseline and then switched to a low-carbohydrate, high-fat, and adequate protein KD for another 4 weeks. With the same daily caloric intake, the proportions of energy intake derived from carbohydrates, proteins, and fats were changed from 44.0 ± 7.6%, 15.4 ± 3.3%, 39.6 ± 5.8% in ND to 9.2 ± 4.8%, 21.9 ± 3.4%, and 69.0 ± 5.4% in KD. The results showed that, without impairing the CRF level, the 4-week KD intervention significantly reduced body weight (−2.9 kg), BMI (−1.1 kg·m^{-2}), waist circumference (−4.0 cm), hip circumference (−2.5 cm), and body fat percentage (−2.0%). Moreover, fasting leptin level was lowered significantly, and serum levels of inflammatory markers (i.e., TNF-α and MCP-1) were unchanged following KD. These findings suggest that KD can be used as a rapid and effective approach to lose weight and reduce abdominal adiposity in overweight/obese Chinese females without exacerbating their CRF.

Keywords: low-carbohydrate, subcutaneous fat, weight loss, cardiorespiratory fitness, leptin

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Chia-Hua Kuo,
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Andrew McKune,
University of Canberra, Australia
Fiorenzo Moscatelli,
University of Foggia, Italy

***Correspondence:**
Shengyan Sun
sysun@zjhu.edu.cn

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INTRODUCTION

The incidence of obesity and related diseases is increasing rapidly and is a major health challenge faced by both developed and developing countries (Roberts and Barnard, 2005). In contrast to the accumulation of peripheral subcutaneous fat, excessive accumulation of adiposity in abdominal viscera has a more direct association with obesity-related complications, including diabetes, metabolic syndrome, hepatic steatosis, aortic plaque (Neeland et al., 2013), and abnormal activity of the autonomic nervous system (Triggiani et al., 2019). The beneficial effects on metabolic syndrome parameters resulting from visceral fat reduction were greater than those induced by subcutaneous fat reduction (Park and Lee, 2005).

Although the conventional dietary guidelines for weight loss recommend low fat intake and calorie restriction resulting in a negative energy balance (Seagle et al., 2009), accumulating studies

mainly from Western countries have shown that the low-carbohydrate diet approaches are effective in fighting obesity and improving cardiometabolic health (Sharman et al., 2004; Volek et al., 2004a; Brinkworth et al., 2009a; Gu et al., 2013; Sun et al., 2019). Generally, low-carbohydrate diets are considered to contain <100 g/day or <30% of energy from carbohydrates (Sumithran and Proietto, 2008), and very low carbohydrate diets (or called ketogenic diet, KD) are characterized by a daily intake of less than 50 g of carbohydrates (Paoli et al., 2013). It has been revealed that, in comparison with low-fat diets, low-carbohydrate diets resulted in greater weight reduction (Sharman et al., 2004; Volek et al., 2004a) and led to more favorable alterations in blood lipids (Sharman et al., 2004; Brinkworth et al., 2009a) and glucose regulation (Sharman et al., 2004). Undeniably, the evidence regarding the effectiveness of low-carbohydrate diets in weight reduction and cardiometabolic health improvement is strong; however, it is less popular in China. It is noteworthy that people in China and Western countries have different dietary patterns and food preferences (Popkin and Gordon-Larsen, 2004; Zhai et al., 2009); thus, their attitude and acceptance of low-carbohydrate diets may be distinct. Moreover, in limited studies involving the Chinese population, researchers have put an overemphasis on calorie restriction (Gu et al., 2013; Liu et al., 2013; Sun et al., 2019), making it difficult to specify whether the low-carbohydrate diet-induced weight-loss effects are a result of reduction in energy intake or changes in macronutrient proportions. Furthermore, several previous studies pointed out that very low-carbohydrate diets (i.e., KD) seemed particularly effective in reducing subcutaneous and visceral fat mass (Gu et al., 2013; Valenzano et al., 2019) as well as trunk fat mass (Volek et al., 2004a). Therefore, it is necessary to assess whether non-calorie-restriction KD dietary patterns are also useful and feasible for the large overweight/obese population in China.

During KD intervention with strictly restricted carbohydrate content (usually <50 g/day), the typical metabolic changes in individuals without preadaptation are lowering muscle glycogen restoration rates and decreasing glycolytic-enzyme activities (Chang et al., 2017), and higher serum concentrations of non-esterified fatty acids and ammonia following KD may also contribute to central fatigue (Fiorenzo et al., 2020). As a result, several previous studies revealed that the KD intervention might lead to early development of central fatigue (Chang et al., 2017) and further impaired cardiorespiratory fitness (CRF) (Okeeffe et al., 1989; Urbain et al., 2017) or exercise performance (Pilis et al., 2018). However, the findings are not always consistent. After adaption, the aerobic and anaerobic exercise capacity of athletes seems to be unaffected by KD (Zajac et al., 2014; Fiorenzo et al., 2020). CRF is a strong independent indicator for cardiovascular diseases and all-cause mortality (Kodama et al., 2009; Kaminsky et al., 2013). The necessity of evaluating and intervening CRF are highlighted by the American Heart Association to reduce risk factors of cardiovascular disease and promote overall cardiovascular and general health (Kaminsky et al., 2013). Any diet that potentially impair someone's CRF level and the ability to adhere to an exercise regime would be of great concern. Therefore, a maximal incremental exercise test was adopted as one of the main outcome measurements in the present study. The objective was first to examine the efficacy of a 4-week non-calorie-restriction KD on body composition and the impact of KD on CRF in the overweight/obese Chinese females. In addition, serum concentrations of appetite control hormones and inflammatory biomarkers were assessed in this study.

MATERIALS AND METHODS

Subjects

This study was approved by the Panel on Social Science & Humanities Research Ethics of the University of Macau (RC Ref. no. MYRG2017-00199-FED). Recruitment notices, including research purposes, qualifying criteria, and brief research procedures, were released to campus bulletin boards and emails to recruit overweight/obese but healthy women interested in this study. The inclusion criteria were (1) overweight or obese defined as body mass index (BMI) $\geq$ 23 $kg{\cdot}m^{-2}$ (World Health Organization, 2000), (2) between 18 and 30 years old, (3) body weight remained stable in the past 6 months (variation within 5% of body weight), (4) healthy (without any endocrine, metabolic, osteoarticular, gastrointestinal, hepatic, renal, or cardiovascular diseases), (5) sedentary lifestyle (not participant in regular physical activity). Subjects who meet any of the following items were excluded: comfortable drinking alcohol, smokers, participate in structured training programs or specific diet programs at the time of recruitment, have physical barriers to exercise, take any prescribed medicines or nutritional supplements, or have respiratory problems or eating disorders. After the screening process, 24 eligible overweight/obese but healthy young females (19–25 years old) were included. All subjects provided written informed consent before being formally involved. Four subjects withdrew for different reasons; 20 subjects who completed the normal diet (ND) period, KD period, and all three outcome measurements were included in final data analysis.

Experimental Design

This study was performed in the following order: a preparation phase with two nutrition classes, the first measurement of main outcome variables (including anthropometric assessments, blood sampling, and a maximal incremental exercise test) prior to ND, a 4-week ND (28 days), the second outcome measurements after ND, another three nutrition classes about KD, a 4-week KD intervention (28 days), and the last outcome measurements after KD intervention (**Figure 1**).

During the preparation period, subjects received two nutrition classes on how to document food/beverage intake and how to eat within the targeted nutritional goal. Digital scales, food measuring instruments, and detailed instructions and individual consultation were given to all subjects so that they could accurately record the weight and amounts of food/beverage intake. Because the three measures of the main outcome variables were performed within the same phase of each subject's menstrual cycle (i.e., the luteal phase), we asked them to recall and provide their menstrual phases over the past 3 months in the preparation period. Then, the days of outcome measurements were calculated

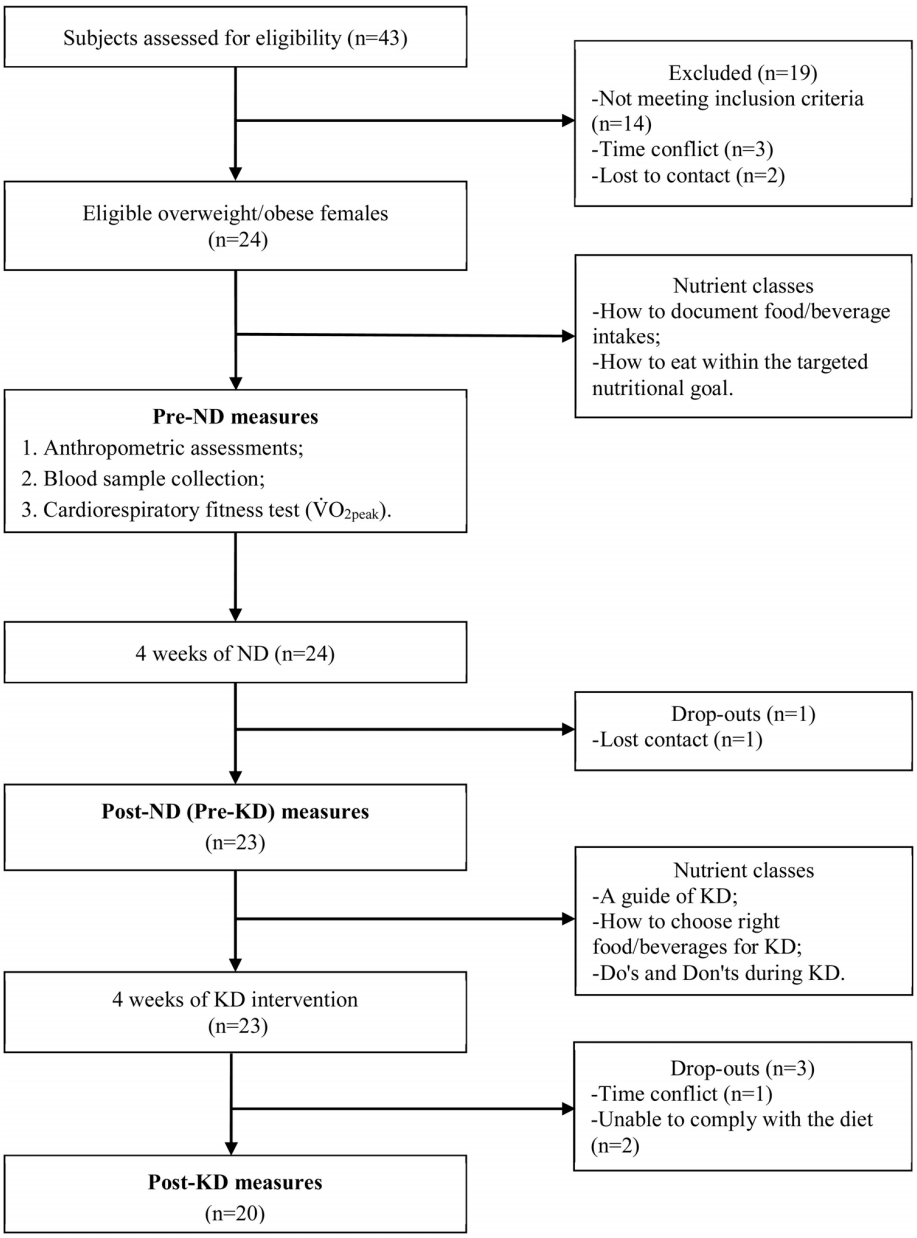

FIGURE 1 | Flow-chart of the study.

individually according to their self-reported menstrual phases, and the menstrual phases for the next 3 months were estimated.

The first measurement of main outcome variables was conducted 2–5 days prior to ND, then a 4-week ND was performed as a control period, and 3-day food diaries (2 weekdays and 1 weekend day) were kept by the subjects during this period. The second outcome measurements were completed within 72–96 h following the last day of ND. Meanwhile, another three nutrition classes on KD were offered, namely a guide to KD, how to choose the right food and beverage for KD, and dos and don'ts during KD. A handout that outlines the main aspects of KD and a specific list of suitable foods, beverages, cooking recipes, and sample meals for KD was also provided. Thereafter, subjects consumed 4 weeks of low-carbohydrate, high-fat KD according to the instruction and kept 3-day diet diaries in the same way as ND. The last outcome measurement was completed between 72 and 96 h following the last day of KD.

Diet Intervention

During the 4-week ND period, subjects maintained their habitual diet and then switched to KD for another 4 weeks, in which they had ∼10% of daily energy intake from carbohydrates (approximately 50 g/d), ∼65% of energy intake from fats, and ∼25% of the rest from protein.

Subjects could choose low-carbohydrate foods/beverages according to their own preferences and were required to have low-carbohydrate foods/beverages and restrict or avoid foods/beverages with high carbohydrate content. Foods/beverages appropriate for KD included all kinds of fat, oils, all kinds of meat (e.g., pork, beef, fowl such as chickens and ducks), eggs, seafood, cheese, non-starchy and green vegetables, nuts/seeds, water, and low-carbohydrate beverages (e.g., green/red tea, black coffee). Although the types of fat from saturated or unsaturated sources were not restricted, we encouraged subjects to add five tablespoons of olive oil to their daily diet. Foods/beverages to avoid during KD included rice, cereals, products made of flour (e.g., bread, noodles, cakes), sugar, desserts, sweets, honey, beans, corns, starchy vegetables, fruits (with the exception of blueberry, lemon, and avocado), milk, yogurt, soft drinks, juices, and alcoholic beverages.

To assure subjects' adherence to KD, we required them to measure urinary ketones every day and record 3-day food diaries (2 weekdays and 1 weekend day) during the experimental period. Reagent strips (UROPAPER, Suzhou First Pharmaceutical Co., Ltd., Suzhou, China) were provided to all subjects for self-assessment of daily urinary ketones, which was performed in the early morning or after dinner (Urbain and Bertz, 2016). The minimum detectable amount of the reagent strip was 10 mg/dL, and the rate of concordance was 95.1% with clinical diagnosis (Ishizaki et al., 2016). Moreover, 3-day food diaries were kept by all subjects for 8 weeks (the 4-week ND period and the 4-week KD period). Thorough instructions on how to estimate portion sizes and record food/beverages intake on food composition tables were given to all subjects in advance. Subjects were asked to report to the laboratory every week to assess changes in body weight and hand in the logbook with food diaries. Energy intake and macronutrient composition were calculated by the same dietician using the nutrition analysis and management system (NRISM, version 3.1, China). Diet compliance was evaluated based on the results of the urinary ketones and food diaries, and subjects received follow-up dietary advice and counseling individually from the dietician. Constant assistance was provided throughout the study; if subjects had any queries, problems, or feedback relating to the experiment, they could contact the researchers via phone, WeChat, e-mail, or meet in person and get answers immediately.

In addition to the targeted intervention, subjects were required to maintain their habitual daily routines and not take extra exercise throughout the study period. Meanwhile, validated pedometers (Yamax SW-200 digiwalker, Japan) were provided to all subjects to assess their daily physical activities. Each subject received a logbook with a calendar to record daily food intake, daily urinary ketone test results, daily physical activities (in steps), and any adverse side effects or symptoms of the intervention.

Measures of Main Outcome Variables

Blood Profiles

Blood samples were collected at the same phase of each subject's menstrual cycle (i.e., the luteal phase) at different measurement time points. Strenuous physical activity, caffeine, and alcohol were prohibited for 48 h before blood sample collection. Subjects arrived at the laboratory at around 7 a.m. under the condition of fasting overnight (>10 h), and 5 ml blood was drawn from the cubital vein by a certificated nurse using a serum separation tube. The blood samples were left for clotting at room temperature for 1 h and then centrifuged at 3000 rpm for 5 min; serum was separated and immediately frozen at −80°C for later analysis.

Leptin, ghrelin, tumor necrosis factor alpha (TNF-α), and monocyte chemoattractant protein-1 (MCP-1) were measured using the EMD Millipore Milliplex MAP immunoassay (Merck KGaA, Darmstadt, Germany). All blood samples were measured in standard procedures in accordance with the manufacturer's instructions (KingMed Diagnostics Co., Ltd., Guangzhou, China), and were conducted at the end of the study to minimize variability.

Anthropometric Assessments

Anthropometric assessments were conducted the same morning after blood samples were taken (without breakfast). Height and weight were measured using a wall-mounted stadiometer and an electronic scale in a standard manner (barefoot and wearing light clothes), and the values were recorded to the nearest 0.1 cm and 0.1 kg, respectively. Body weight (kg) divided by square height (m^2) was calculated as BMI (in $kg{\cdot}m^{-2}$). Waist circumference (WC) was measured at the intermediate position between the upper edge of the iliac crest and the lower edge of the 12th rib while the subject was breathing out gently; hip circumference (HC) was determined as the maximum circumference over the buttocks; the WC and HC values were recorded to the nearest 0.5 cm. Waist-to-hip ratio (WHR) was calculated as WC divided by HC (both in cm). Skinfold thickness measurements were taken on the right side of the body using a Harpenden skinfold caliper (British Indicators Ltd., St Albans, Herts) with the subjects in a standing posture. Three sites were selected for skinfold thickness measurements, including (1) triceps: in the middle between the olecranon and the tip of the acromion, over the midpoint of the muscle belly with the upper arm suspended vertically; (2) anterior superior iliac spine: the highest point of the pelvis that can be touched from the front, the skinfold was lifted along the iliac crest; and (3) thigh: at the midpoint of the groin and knee, on the front side of the thigh. At these three sites, the skinfold was firmly pinched between the thumb and forefinger and gently pulled away from the underlying tissues before the caliper was applied for measurement. The opening width was read off on a scale incorporated in the apparatus and recorded to the nearest 0.1 mm. BF% was calculated using the following equation: BF% = 100 × (4.95/body density−4.5) (Siri, 1956), and body density = $1.0994921-0.0009929 \times (\text{sum}) + 0.0000023 \times (\text{sum})^2 - 0.0001392 \times \text{age}$ (Jackson et al., 1980), where sum refers to the sum of skinfold thickness measured at the above three sites. All anthropometric measurements were performed by the same investigator using the same instrument.

Maximal Incremental Exercise Test

The maximal incremental exercise test was taken to determine the CRF level. After a brief warm-up, subjects started to pedal on an electric-braked cycle ergometer (Monark 839E, Vansbro,

Sweden) with an initial workload of 50 W. The workload was increased by 25 W in 3 min intervals until the subjects reached their volitional exhaustion and then recovered at 25 W for 3 min. The pedaling speed was maintained at 60 ± 5 rpm throughout the test. During the $\dot{V}O_{2peak}$ test, respiratory gases were continuously assessed using a gas analyzer (Vmax Encore System, CareFusion Corp., San Diego, CA, United States). $\dot{V}O_{2peak}$ was calculated as the largest oxygen consumption value averaged over 15 s of the last exercise stage (Rossiter et al., 2006).

Statistical Analysis

Statistical analyses were conducted using the PASW software (Release 22.0; IBM, NY, United States). Prior to the main statistical analyses, the Shapiro–Wilk test was performed to confirm whether the outcome variables were normally distributed. One-way repeated-measures analysis of variance (ANOVA) was performed to detect the differences in body composition; CRF level; blood profiles among the three time points (pre-ND, post-ND, and post-KD); and the differences in dietary energy intake, macronutrient composition, and daily physical activities among the eight time points measured across the study (4 weeks of ND and 4 weeks of KD). A paired-sample *t*-test was used to compare the differences in changes of main outcome variables after ND and KD. Pearson's correlation tests were performed to examine the associations between body composition variables and hormones (i.e., leptin and ghrelin). Partial η^2 values were used to assess the effect sizes of the main and interaction effects; η^2 was considered small if <0.06 and large if >0.14 (Kirk, 1996). Cohen's *d* values were also calculated to evaluate the effect sizes for the difference between variables, which was considered small when *d* was between 0.2 and 0.3, medium when *d* was around 0.5, and large when $d > 0.8$ (Cohen, 2013). Data were presented as means (standard deviations, SDs), and the level of $p < 0.05$ was considered statistically significant.

RESULTS

Diet Compliance, Dietary Compositions, Daily Physical Activities

Urine ketone was introduced as an indicator for diet compliance. During the ND period, urinary ketosis was only detected on 0.2 ± 0.8% of the days, whereas during the KD intervention, urinary ketosis was detected on 97.7 ± 3.9% of the days, suggesting that the subjects had good compliance with the KD. It should be noted that the days' (%) urinary ketones during KD were calculated after excluding the data of the three initial transition days.

The mean daily energy intake during the ND period was 1967 ± 362 kcal, of which carbohydrates, proteins, and fats accounted for 44.0 ± 7.6% (217.2 ± 53.3 g), 15.4 ± 3.3% (75.2 ± 20.8 g), 39.6 ± 5.8% (86.3 ± 19.1 g) of daily energy intake (**Figure 2**). During the KD intervention, the average daily energy intake and the proportions of energy intake derived from carbohydrates, proteins, and fats were 1817 ± 285 kcal, 9.2 ± 4.8% (40.7 ± 21.5 g), 21.9 ± 3.4% (95.4 ± 21.1 g), and 69.0 ± 5.4% (136.4 ± 25.7 g), respectively (**Figure 2**). No changes in daily energy intake were observed in any of the weeks during ND and KD ($p > 0.05$), whereas the macronutrient compositions were significantly changed during the KD intervention when compared to the ND period with higher proportions of protein ($p < 0.01$) and fat ($p < 0.01$) intake and a lower proportion of carbohydrate intake ($p < 0.01$) during KD (data are presented in **Supplementary Table S1**).

Daily physical activities were between 7898 and 8954 steps during the ND period and between 7463 and 8346 steps during the KD intervention. There was no statistical difference on daily physical activities among the eight time points measured throughout the study period (data are presented in **Supplementary Table S2**).

Changes in Anthropometric Parameters, CRF, and Blood Profiles

After KD intervention, the subjects lost 2.9 ± 2.1 kg of body weight ($p < 0.01$, $\eta^2 = 0.686$) and reduced BMI by 1.1 ± 0.7 kg·m^{-2} ($p < 0.01$, $\eta^2 = 0.702$), which remained unchanged during the ND period (**Tables 1**, **2**). The KD intervention also significantly reduced the subjects' WC (−4.0 ± 3.2 cm, $p < 0.01$, $\eta^2 = 0.566$), HC (−2.5 ± 2.3 cm, $p < 0.01$, $\eta^2 = 0.554$), WHR (−0.02 ± 0.03 cm, $p < 0.05$, $\eta^2 = 0.218$), and percentage of body fat (BF%, −2.0 ± 2.2%, $p < 0.01$, $\eta^2 = 0.707$). No differences in CRF level were found between ND and KD at pre- and post-measurements ($p > 0.05$). Circulating leptin level was significantly decreased in response to KD ($p < 0.05$, $\eta^2 = 0.370$), and the concentrations of ghrelin, TNF-α, and MCP-1 were unchanged after 4 weeks of KD intervention (**Table 1**). In addition, we found significant correlations between fasting leptin level and body composition variables before ND ($r = 0.545$–0.796, $p < 0.05$), before KD ($r = 0.510$–0.715, $p < 0.05$), and after KD ($r = 0.480$–0.674, $p < 0.05$, **Table 3**). But, when using the difference values measured before and after the KD intervention, there was no association between KD-induced changes in leptin and body composition ($p > 0.05$). No correlation was found between fasting ghrelin level and body composition variables measured at any time points ($p > 0.05$, **Table 3**).

Adverse Events

During the ND period, there was no feedback on any adverse event, but during the KD period, we received 10 complaints about adverse events from seven subjects; these complaints included fatigue (five complaints), constipation (three complaints), reduced appetite (one complaint), and diarrhea (one complaint).

DISCUSSION

Consistent with findings in Western countries, the present study shows that the 4-week non-calorie-restricted KD dietary approach was also effective in losing weight and reducing abdominal adiposity in Chinese overweight/obese females without impairing their CRF level. In addition, the short-term KD intervention reduced serum leptin concentration, but left unaffected the inflammation biomarkers of TNF-α and MCP-1.

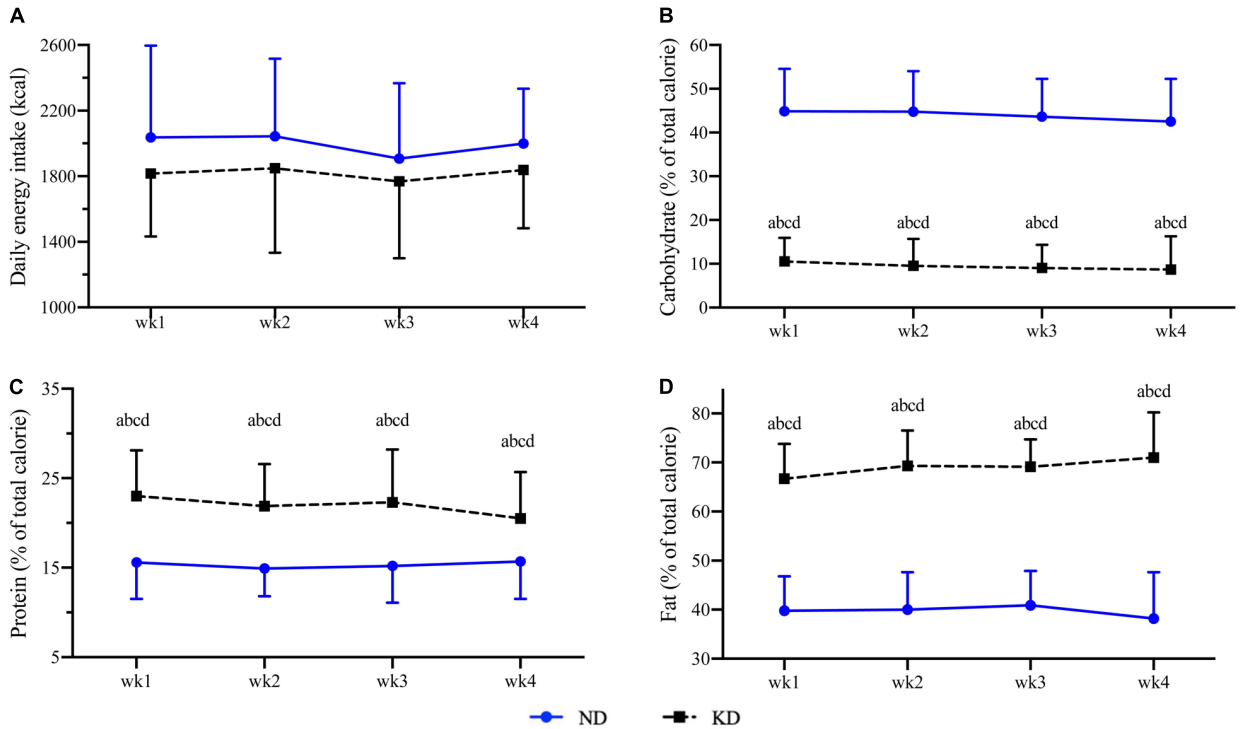

FIGURE 2 | Daily energy intake **(A)**, proportions of carbohydrate **(B)**, protein **(C)**, and fat **(D)** intakes during normal diet (ND) and ketogenic diet (KD). Compared to week 1 of ND at **a** $p < 0.01$; compared to week 2 of ND at **b** $p < 0.01$; compared to week 3 of ND at **c** $p < 0.01$; compared to week 4 of ND at **d** $p < 0.01$.

TABLE 1 | Main outcome variables before and after ND and KD.

	Pre_ND	Post_ND (Pre_KD)	Post_KD	Within-subjects effects		
				F	*p*	η^2
Age (year)	21.0 (3.7)					
Height (cm)	162.1 (5.2)					
Weight (kg)	65.5 (7.7)	65.1 (8.1)	62.1 (7.1)^^**	41.596	0.000	0.686
BMI ($kg \cdot m^{-2}$)	24.9 (2.7)	24.8 (2.8)	23.6 (2.6)^^**	44.688	0.000	0.702
WC (cm)	76.9 (7.7)	77.2 (7.7)	73.6 (6.0)^^**	24.763	0.000	0.566
HC (cm)	100.1 (5.0)	100.0 (4.7)	97.4 (4.3)^^**	23.560	0.000	0.554
WHR	0.77 (0.05)	0.77 (0.05)	0.75 (0.04)^*	5.298	0.009	0.218
BF%	35.2 (3.8)	34.7 (3.9)	32.2 (4.6)^^*	12.075	0.002	0.707
$\dot{V}O_{2peak}$ ($ml \cdot min^{-1}$)	1.67 (0.24)	1.63 (0.22)	1.57 (0.22)	2.177	0.127	0.103
$\dot{V}O_{2peak}$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	25.6 (3.9)	25.4 (4.8)	24.5 (3.1)	1.402	0.258	0.069
Leptin ($ng \cdot ml^{-1}$)	14.5 (10.1)	11.9 (6.8)	6.9 (6.2)^^**	11.168	0.000	0.370
Ghrelin ($pg \cdot ml^{-1}$)	882.0 (703.9)	856.8 (602.7)	785.7 (647.4)	0.535	0.590	0.029
TNF-α ($pg \cdot ml^{-1}$)	5.4 (2.0)	5.0 (2.1)	4.6 (1.5)	1.756	0.186	0.085
MCP-1 ($pg \cdot ml^{-1}$)	137.9 (44.8)	131.3 (41.8)	122.9 (20.0)	0.919	0.407	0.046

*Outcome variables are presented as mean (standard deviation). ND, normal diet; KD, ketogenic diet; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; BF%, percentage of body fat; $\dot{V}O_{2peak}$, peak oxygen uptake; TNF-α, tumor necrosis factor-alpha; MCP-1, monocyte chemoattractant protein-1. Comparison to Pre_ND at ^p < 0.05, ^^p < 0.01, comparison to Post_ND at *p < 0.05, **p < 0.01.*

The overweight/obese females lost an average of 2.9 kg of total body mass after the 4-week KD intervention, corresponding to 1.1 unit of BMI. Also, without calorie restriction, the decrement of body mass was similar to some previous studies that reported ∼2.5 kg weight losses in Western adults after 3–6 weeks of KD administration (Sharman et al., 2002; Volek et al., 2002, 2004b; Urbain et al., 2017; Oneal et al., 2019). In contrast, several other KD studies with restricted calorie intake showed greater weight losses (5.0–8.0 kg) in overweight or obese Chinese adults in response to 8 (Gu et al., 2013) and 12 weeks (Liu

TABLE 2 | Changes in outcome variables after ND and KD.

	ND	KD	ES *(d)*
	Post_ND – Pre_ND	Post_KD – Pre_KD	
ΔWeight (kg)	−0.4 (1.5)	−2.9 (2.1)**	1.42
ΔBMI ($kg \cdot m^{-2}$)	−0.2 (0.6)	−1.1 (0.7)**	1.44
ΔWC (cm)	0.7 (1.5)	−4.0 (3.2)**	1.91
ΔHC (cm)	−0.1 (1.6)	−2.5 (2.3)**	1.23
ΔWHR	0.01 (0.02)	−0.02 (0.03)**	1.22
ΔBF%	−0.6 (1.8)	−2.0 (2.2)	0.68
$\Delta\dot{V}O_{2peak}$ ($ml \cdot min^{-1}$)	0.0 (0.2)	−0.1 (0.2)	0.06
$\Delta\dot{V}O_{2peak}$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	−0.3 (3.8)	−0.9 (3.3)	0.17
$\Delta\dot{V}O_{2peak}$%	−0.8 (14.2)	−1.9 (11.5)	0.09
ΔLeptin ($ng \cdot ml^{-1}$)	−2.7 (7.1)	−5.0 (6.0)	0.36
ΔGhrelin ($pg \cdot ml^{-1}$)	−25.1 (413.3)	−71.1 (405.0)	0.11
ΔTNF-α ($pg \cdot ml^{-1}$)	−0.4 (2.0)	−0.4 (1.9)	0.02
ΔMCP-1 ($pg \cdot ml^{-1}$)	−6.5 (62.1)	−8.4 (38.8)	0.04

*Outcome variables are presented as mean (standard deviation). ND, normal diet; KD, ketogenic diet; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; BF%, percentage of body fat; $\dot{V}O_{2peak}$, peak oxygen uptake; TNF-α, tumor necrosis factor-alpha; MCP-1, monocyte chemoattractant protein-1; Delta (Δ), change from pre- to post-intervention. Δ ND vs. Δ KD at **p < 0.01, Cohen's d value for effect size (ES) when compared to Δ ND.*

et al., 2013; Sun et al., 2019) of KD intervention. Given that no previous studies have compared the weight loss effects of energy-restricted KD to non-energy-restricted KD in the same population, it is difficult to know whether calorie restriction has additional benefits in promoting weight loss. Nonetheless, without changing subjects' habitual calorie intake, the weight reduction after the non-energy-restricted KD intervention in the present study should be mainly ascribed to the changes in macronutrient composition. More than weight loss, we found significant reductions in WC (−4.0 cm, d = 191), HC (−2.5 cm, d = 1.23), and WHR (−0.02, d = 1.22) in the overweight/obese females. This finding supported previous studies that reported that 8 weeks of KD intervention significantly reduced subcutaneous and visceral fat mass in obese but healthy adults (Gu et al., 2013; Valenzano et al., 2019). WC has been recognized as a surrogate indicator of visceral adiposity and is closely associated with cardiometabolic risk (Nazare et al., 2015); thus, marked reductions in WC and WHR may have clinical significance in reducing cardiac and metabolic risks (Neeland et al., 2013; Nazare et al., 2015). Consistently, BF% was decreased by 2.0% as measured using skinfold thickness. These findings illustrate that the KD intervention was not only effective in reducing overall body weight and body fat mass, but it was also beneficial for abdominal fat loss, which is more closely related to cardiometabolic risks.

Higher CRF level is proven to be associated with lower rates of cardiovascular disease events and total mortality (Kodama et al., 2009; Lee et al., 2011; Almallah et al., 2018) regardless of BMI improvement (Lee et al., 2011). However, several studies have reported that KD impaired the CRF level (Okeeffe et al., 1989; Urbain et al., 2017) because the minimal carbohydrate supply during KD intertvention could alter energy metabolism, resulting in reduced muscle glycogen stores and glycolytic-enzyme activities (Chang et al., 2017) as well as central fatigue (Fiorenzo et al., 2020). These metabolic changes limited the energy availability during exercise and, subsequently, impaired the maximal aerobic capacity. Contrary to these studies but supported by others (Brinkworth et al., 2009b; Klement et al., 2013), the CRF level was not changed by the 4-week KD intervention in the present study. These findings suggest that the short-term KD intervention seemed unlikely to affect CRF level, which can be adopted by the overweight/obese Chinese females as a weight-loss diet regime.

An important hypothesis for the mechanism by which KD causes body fat loss could be related to the satiety-increasing effect of higher dietary protein (Weigle et al., 2005; Johnstone et al., 2008). And several studies illustrate that such an effect may be regulated through appetite-mediating hormones, such as leptin and ghrelin (Weigle et al., 2005; Sumithran et al., 2013). Leptin is a hormone mainly synthesized by adipose cells and involved in the regulation of energy balance and fat storage through suppressing hunger (Ahima and Flier, 2000). Previous studies have shown that serum leptin level was declined in parallel with reduced appetite (Weigle et al., 2005; Johnstone et al., 2008; Sumithran et al., 2013) as well as KD-induced reduction in adipose tissue (Boden et al., 2005; Weigle et al., 2005; Sumithran et al., 2013). Although the leptin level was also decreased after KD administration, the KD-induced changes in body composition were not correlated with the

TABLE 3 | The correlation coefficients between the hormones and body composition.

		Weight	BMI	WC	HC	WHR	BF%
Pre_ND	Leptin	0.615**	0.796**	0.545*	0.668**	0.296	0.664**
	Ghrelin	0.176	0.183	0.137	0.271	−0.001	0.041
Pre_KD	Leptin	0.568**	0.715**	0.587**	0.510*	0.516*	0.531*
	Ghrelin	−0.163	−0.165	−0.176	−0.015	−0.294	−0.409
Post_KD	Leptin	0.523*	0.674**	0.433	0.480*	0.213	0.230
	Ghrelin	−0.189	−0.005	−0.143	−0.095	−0.131	−0.164
Delta_KD	Δ Leptin	0.412	0.439	0.373	0.067	0.344	0.217
	Δ Ghrelin	−0.066	−0.140	−0.020	0.401	−0.271	−0.193

*ND, normal diet; KD, ketogenic diet; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; BF%, percentage of body fat; Delta (Δ), change from pre- to post-intervention of KD. *p < 0.05, **p < 0.01.*

alterations in leptin level in the present study, indicating that changes in leptin have less to do with the regulation of body composition during KD. Thus, the decline in the leptin level is more likely a result of the reduced adipose tissue, and there may be other mechanisms rather than leptin pathways that modulate the reductions of body mass and fat mass during KD, for example, pathways relating to fat mobilization. In contrast, the orexigenic hormone, ghrelin, which stimulates appetite, increases food intake and promotes fat storage, was shown to be increased after KD intervention (Boden et al., 2005; Weigle et al., 2005; Sumithran et al., 2013). However, we found a slight but non-significant reduction in ghrelin concentration after KD intervention. Moreover, the daily energy intake was unchanged over the KD period as compared to ND, and this suggests that the overweight/obese females had an unchanged appetite during KD. Therefore, suppressing appetite and spontaneous calorie intake seems to be unable to explain the KD-induced weight reduction and fat mass loss in this study. Other mechanisms, such as alterations in metabolic fuel and insulin level, may also have played important roles in body composition regulation. Under the KD condition in which the carbohydrate intake is drastically reduced, the insufficient metabolic fuel for glucose oxidation forces the body to look for alternative energy sources. Thus, the reliance on metabolic pathways of fat oxidation and gluconeogenesis is increased under KD (Paoli et al., 2013; Ludwig and Friedman, 2014). The main substitute energy source is derived from increased fatty acid oxidation (Paoli et al., 2013). The increased fat oxidation not only promotes the depletion of fat storage in tissues, but also leads to an overproduction of ketone bodies, which can inhibit appetite (Sumithran et al., 2013). Another alternative source of energy is gluconeogenesis from proteins, which is considered an energy-demanding metabolic process that can "waste" an additional 400–600 kcal of calories per day (Fine and Feinman, 2004). Simultaneously, the demand of insulin in assisting glucose uptake is reduced. As a result, fasting insulin level was consistently found to be decreased after KD (Sharman et al., 2002, 2004; Johnstone et al., 2008; Gu et al., 2013; Liu et al., 2013), which further accelerated lipolysis and oxidation of stored and ingested fat (Zammit, 2006). In addition, isocaloric exchange of dietary carbohydrate for fat was found to have decreased respiratory quotient and increase the 24-h energy expenditure in overweight or obese men (Hall et al., 2016). These potential mechanisms may be responsible for the observed losses of body mass and abdominal fat mass in the present study.

Many studies have shown that KD approaches had an anti-inflammatory effect by reducing inflammatory markers, including C-reactive protein, TNF-α, MCP-1, interleukin-6 (IL-6), and IL-8 (Sharman and Volek, 2004; Dansinger et al., 2005; Forsythe et al., 2008; Ratliff et al., 2008), which are generally higher in obesity. However, a 4-week KD intervention did not change proinflammatory cytokines (i.e., C-reactive protein, TNF-α, IL-6) in normal-weight women was also reported (Volek et al., 2003). Consistent with this study, the present study failed to find any influence of KD on the inflammation biomarkers of TNF-α and MCP-1.

By assessing $\dot{V}O_{2peak}$ at pre- and post-measurements, the current study eliminated the concern that KD approaches may impair CRF. Another strength of the present study is that, in addition to the total body mass and overall fat mass, we also assessed changes in abdominal and visceral adiposity in response to KD using surrogate indicators, WC and WHR. Nonetheless, it would be better to assess visceral adiposity accurately using more sophisticated techniques, such as magnetic resonance imaging or dual-energy X-ray absorptiometry. Given that the subjects had difficulties with taking invasive blood ketone tests, we used urinary ketone tests to qualitatively determine whether they were in nutritional ketosis, and this may not be accurate enough. In addition, the intervention duration was relatively short in our study, which limited the interpretation of the long-term weight-loss effects of KD as well as weight maintenance. Finally, this study did not measure the subject's subjective appetite and hormones relating to fat mobilization, making it hard to interpret possible mechanisms. In this regard, future studies would be benefit from evaluating the long-term weight-loss effects of KD and the underlying mechanisms in different populations.

CONCLUSION

Taken together, the 4-week KD intervention led to marked reductions in body mass as well as total and abdominal fat mass without any adverse effect on CRF, suggesting that the KD dietary approaches could also be effective and feasible for the large overweight/obese population in China. Circulating leptin concentration was reduced, but the ghrelin level and energy intake was unchanged in KD. The findings of this study do not seem to support the idea that the weight-loss effect of KD is due to reduced appetite. Further study is required to determine whether the weight-loss effect of KD is mediated through appetite.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Panel on Social Science & Humanities Research Ethics of University of Macau (RC Ref. no. MYRG2017-00199-FED). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SS, ZK, and JN: research design. ZK: funding acquisition. SS and ZK: data collection. SS, QS, and HZ: data analysis and interpretation. SS, ZK, and JN: manuscript drafting. SS, ZK, QS, HZ, TT, and JN: manuscript revision. All authors read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.00856/full#supplementary-material

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Effect of High-Intensity Interval Training on Body Composition, Cardiorespiratory Fitness, Blood Pressure, and Substrate Utilization During Exercise Among Prehypertensive and Hypertensive Patients With Excessive Adiposity

Pedro Delgado-Floody[1], Mikel Izquierdo[2,3,4], Robinson Ramírez-Vélez[2,3], Felipe Caamaño-Navarrete[5], Roberto Moris[1], Daniel Jerez-Mayorga[6], David C. Andrade[7†] and Cristian Álvarez[8]*

[1] Department of Physical Education, Sport and Recreation, Universidad de La Frontera, Temuco, Chile, [2] Navarrabiomed, Complejo Hospitalario de Navarra (CHN)-Universidad Pública de Navarra (UPNA), IdiSNA, Pamplona, Spain, [3] CIBER of Frailty and Healthy Aging (CIBERFES), Instituto de Salud Carlos III, Madrid, Spain, [4] Grupo GICAEDS, Facultad de Cultura Física, Deporte y Recreación, Universidad Santo Tomás, Bogotá, Colombia, [5] Faculty of Education, Universidad Católica de Temuco, Temuco, Chile, [6] Faculty of Rehabilitation Sciences, Universidad Andres Bello, Santiago, Chile, [7] Centro de Investigación en Fisiología del Ejercicio, Universidad Mayor, Santiago, Chile, [8] Quality of Life and Wellness Research Group, Department of Physical Activity Sciences, Universidad de Los Lagos, Osorno, Chile

Regular exercise training is a recognized lifestyle strategy to lower resting blood pressure (BP), but little is known about substrate metabolism in population with high BP. Thus, the purpose of this study was to investigate the effects of 16-weeks of HIIT on body composition, BP, cardiorespiratory fitness by $\dot{V}O_{2max}$, and substrate utilization during exercise among prehypertensive and hypertensive patients with excessive adiposity. We also aimed to test the potential association between changes in cardiorespiratory fitness, substrate utilization during exercise and BP. Forty-two physically inactive overweight/obese participants participated in 16-weeks of HIIT intervention. The HIIT frequency was three times a week (work ratio 1:2:10, for interval cycling: rest period: repeated times; 80–100% of the maximum heart rate). Groups were distributed based on their baseline BP: HIIT-hypertensive (H-HTN: age 47.7 ± 12.0 years; body mass index [BMI] 30.3 ± 5.5 kg/m^2; systolic [SBP]/diastolic BP [DBP] 151.6 ± 10/81.9 ± 4.2 mmHg), HIIT-pre-hypertensive (H-PreHTN: age 37.6 ± 12.0 years; BMI 31.9 ± 5.3 kg/m^2; SBP/DBP 134.4 ± 3.2/74.9 ± 7.0 mmHg), and a normotensive control group (H-CG: age 40.7 ± 11.0 years; BMI 29.5 ± 4.2 kg/m^2; SBP/DBP 117.0 ± 6.2/72.4 ± 4.1 mmHg). Anthropometry/body composition, BP, and metabolic substrate utilization during exercise (fat [FATox], carbohydrate [CHOox] oxidation, respiratory exchange ratio [RER], and $\dot{V}O_{2max}$), were measured before and after the 16-week HIIT intervention. Adjusted mixed linear models revealed a

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***Correspondence:**
Mikel Izquierdo
mikel.izquierdo@gmail.com

†Present address:
David C. Andrade, Pedagogía en Educación Física, Deportes y Recreación, Universidad Mayor, Santiago, Chile

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significant improved in $\dot{V}O_{2max}$ were + 3.34 in the H-CG, + 3.63 in the H-PreHTN, and + 5.92 mL·kg^{-1}·min^{-1}, in the H-HTN group, however, the Time × Group interaction were not significant (p = 0.083). All the exercise types induced similar decreases on SBP (−8.70) in the H-HTN, (−7.14) in the H-CG, and (−5.11) mmHg in the H-PreHTN, as well as DBP levels (−5.43) mmHg in H-CG group (p = 0.032 vs. H-HTN group). At 16-week, no significant correlations were noted for the changes of blood pressure, cardiorespiratory fitness or exercise metabolism substrates outcomes. In conclusion, our results suggest that a 16-week HIIT-intervention improved $\dot{V}O_{2max}$ and blood pressure BP, but these changes are independent of substrate utilization during exercise in normotensive and hypertensive participants with excessive adiposity.

Keywords: obesity, hypertension, blood pressure, metabolic flexibility, cardiorespiratory fitness

INTRODUCTION

Hypertension is the most common primary cardiometabolic disease in several Latin-American countries, with a prevalence of 27.7% in 2019 (Petermann et al., 2017). Not adhere to the international physical activity (PA) recommendations [i.e., 150 min/week of low/moderate PA, or 75 min/week of vigorous PA (O'Donovan et al., 2010)], is one of the most important factors for type 2 diabetes (T2DM) and other cardiometabolic disorders such as arterial hypertension (HTN), and has been associated with comorbidities including obesity and dyslipidaemia [i.e., higher low-density lipoprotein, total cholesterol or triglycerides (Russo et al., 2018)]. HTN is more prevalent in physically inactive populations (Diaz-Martinez et al., 2018), and it has long been recognized that untreated HTN might be linked to overweight/obesity, albuminuria and micro- and macrovascular changes including endothelial dysfunction and heart failure (Yannoutsos et al., 2014). Interestingly, chronic high blood pressure, such as HTN diagnosed, is related to several detrimental vascular effects (i.e., left ventricular hypertrophy, neurogenic dysfunction), which can affect patients systemically (Falqui et al., 2007; Diaz-Martinez et al., 2018). In these patients, and although not clear, the presence of some of these circulatory damage could also potentially have an impact on the normal metabolic and cardiorespiratory performance response during exercise (Neubauer, 2007). Additionally, defects in skeletal muscle lipid metabolism have been found in obese individuals during resting conditions and are associated with insulin resistance and T2DM (Goodpaster et al., 2002). Thus, it is speculative that HTN patients could respond in minor capacity (i.e., after long-term exercise training) than normotensive peers at metabolic behavior during exercise, however, there is limited information.

Exercise training (with characteristics of being controlled and periodized by intensity, volume, frequency and density) is highly recommended as a non-pharmacological treatment for HTN (Pescatello et al., 2015). Exercise training promotes angiogenesis in the skeletal muscles [i.e., the extension of vasculature from pre-existing micro-vessels (Kissane and Egginton, 2019)] and micro-circulation is a relevant adaptive mechanism that can contribute to exercise substrate metabolism and oxygen availability during effort (Hoppeler and Weibel, 1998). Thus, considering these exercise benefits at circulatory system, and given that HTN patients usually show a low micro-circulation (Feihl et al., 2006), there is a potential benefits from exercise training in patients with HTN that have been not at all elucidated.

There is growing evidence to suggest that high-intensity interval training [HIIT, defined as several, brief bouts of high-intensity efforts, usually via cycling/running, interspersed with recovery periods (Gibala et al., 2012)], promote similar adaptations, and in a time-efficient way than to continuous, moderate-intensity training for improving cardiorespiratory fitness (Costa et al., 2018), but with little advantage for improving vascular function (Ramos et al., 2015; Pedralli et al., 2020). Thus, HIIT might have protective effects against the development of HTN (Pescatello et al., 2015; Álvarez et al., 2018), and can lead to the reversal of a clinical diagnosis of HTN to prehypertension [PreHTN], or from PreHTN to normotension, in a relevant proportion of patients (Cano-Montoya et al., 2016; Álvarez et al., 2018). These beneficial cardioprotective effects have been more reported for long-term (i.e., >12 weeks) rather than short-term exercise programs (Jurio-Iriarte and Maldonado-Martín, 2019).

In the context of obesity, as a relevant HTN comorbidity and a target for exercise, fat oxidation rates have been found to be highest during low- to moderate-intensity exercise [i.e., MICT, moderate x watts of power output cycling (Achten et al., 2002)]. Intriguingly, the major mechanisms of adiposity loss associated with HIIT seem to be related to mitochondrial adaptations in skeletal muscle after HIIT, including an increase in mitochondrial biogenesis, and other molecular adaptations at Kreb's cycle (into mitochondria) as increases in proteins citrate synthase, cytochrome oxidase, or at membranes proteins as fatty acid binding protein ($FABP_{pm}$), or fatty acid CD36 (FAT/CD36) among others (Gibala et al., 2012; Astorino and Schubert, 2018). Also, the specific turn-on/turn-off periods in each interval bout in HIIT promote superior hormonal activity [i.e., adrenaline/noradrenaline catecholamines (Boutcher, 2010), and natriuretic peptide (Birjandi et al., 2016)] after exercise than traditional MICT modalities.

In this line, manipulation of exercise intensities (e.g., HIIT combined with resistance training or low- and MICT), however, does not seem to influence whole-body fat oxidation

(Romijn et al., 1995). Also, a higher percentage of body fat does not necessarily translate into a greater FATox during exercise (Friedlander et al., 1999). Interestingly, an increased fat oxidation after circuit-type resistance training interventions has been observed in patients with impaired glucose tolerance, suggesting that other mechanisms may be involved (Eriksson et al., 1998). Thus, there is limited information on the effects of HIIT on blood pressure and metabolic substrate use such as FATox and CHOox in patients with HTN. Obesity is also characterized by skeletal muscle with a reduced transport and/or phosphorylation of glucose, leading to lower rates of fatty acid oxidation at rest, compared with lean insulin-sensitive individuals (Goodpaster et al., 2002). In the same line, the rate of muscle glycogen oxidation during exercise is reduced in obese patients despite rates of plasma glucose uptake that are similar to lean controls. However, little is known regarding patterns of fuel use during exercise in hypertension patients with obesity, and there are no studies that compare the effects of different exercise training methodologies in this population.

Therefore, designing and evaluating an individualized exercise training program on the basis of the pattern of energy substrate utilization for treating or preventing overweight and obesity in HTN patients remains a critical task for exercise scientists. Thus, the purpose of this study was to investigate the effects of 16-weeks of HIIT on body composition, BP, cardiorespiratory fitness by $\dot{V}O_{2max}$, and substrate utilization during exercise among prehypertensive and hypertensive patients with excessive adiposity. Furthermore, we aimed to test the association between potential changes in $\dot{V}O_{2max}$, substrate utilization during exercise, and blood pressure. We hypothesized that BP improvements can be also associated with substrate utilization (i.e., fat and CHOox) during exercise in HTN patients.

MATERIALS AND METHODS

Study Participants

Forty-two (female n = 21; male n = 21) physically inactive adults (non-adherent to 150 min/week of low to moderate physical activity (PA)/week, or to 75 min/week of vigorous PA (O'Donovan et al., 2010), screened by the international physical activity questionnaire (IPAQ) (Serón et al., 2010), with or without hypertension were recruited. Participants were invited to participate in an exercise intervention programme at the Universidad de La Frontera Exercise Laboratory, Chile through open information disseminated by the research center (i.e., social network and email). All participants signed a written informed consent form which complied with the requirements of the last revised Declaration of Helsinki and was approved by the Human Research Ethics Committee of the Universidad de La Frontera, Chile (DI18-0043).

The inclusion criteria were as follows: (i) to have diagnosed clinical stage 1 or 2 hypertension, elevated blood pressure, or to be normotensive (see criteria classification below); (ii) to be adult >18 and <60 years of age (65 years is the retirement age for women in Chile); (iii) participation in physical autonomous daily activities including walking; (iv) medical authorization by a physician to take part in the study, and; (v) body mass index (BMI) $\geq$ 25 kg/m^2 $\leq$ 39.9 kg/m^2. *Exclusion criteria* were; (i) physical limitations (e.g., restricting injuries of the musculoskeletal system such as osteoarthritis, or to be dependent on a third person); (ii) exercise-related dyspnea or respiratory alterations; (iii) chronic heart disease; (iv) altered ECG, and; (v) an adherence rate <80%, in which case data were not included in the final statistical analysis.

A total of 42 patients were included in the final analysis. Participants were allocated to one of the three following groups according to their blood pressure (diagnosed by physician): HIIT-hypertensive (H-HTN: age 47.7 $\pm$ 12.0 years; body mass index [BMI] 30.3 $\pm$ 5.5 kg/m^2; systolic [SBP]/diastolic BP [DBP] 151.6 $\pm$ 10/81.9 $\pm$ 4.2 mmHg), HIIT-pre-hypertensive (H-PreHTN: age 37.6 $\pm$ 12.0 years; BMI 31.9 $\pm$ 5.3 kg/m^2; SBP/DBP 134.4 $\pm$ 3.2/74.9 $\pm$ 7.0 mmHg), and a normotensive control group (H-CG: age 40.7 $\pm$ 11.0 years; BMI 29.5 $\pm$ 4.2 kg/m^2; SBP/DBP 117.0 $\pm$ 6.2/72.4 $\pm$ 4.1 mmHg). The groups were submitted to a 3 $\times$ weekly HIIT program for 16 weeks. The sample size was calculated using the G*Power 3 Software (Faul et al., 2007). We used both delta changes and SD from previous studies of the similar exercise intervention extension, and cohort (patients with obesity), where blood pressure was included (Delgado-Floody et al., 2019). Based on this, with 1 predictive outcome (systolic BP [SD: 6 mmHg]), a moderate effect size (0.60) and a critical t value of 1.73, a total sample size of (n = 10) subjects per group would give a statistical power of 80%, under an alpha error $p < 0.05$. Flow-chart diagram is detailed in **Figure 1**.

Blood Pressure and Anthropometric Measurements

Blood pressure was measured in the sitting position after 5 min rest. Two recordings were made, and the mean of the measurements was used for statistical analysis with an OMRON® digital electronic BP monitor (model HEM 7114, Chicago, IL, United States). There was a 15-min rest interval between readings, as for previous exercise training studies on the cohort profile (Guimaraes et al., 2010). We used the currently published standard cut-off classification for BP of the American Heart Association by Whelton et al. (2018) considering the 4-categories; (i.e., *normal* [SBP < 120; DBP < 80 mmHg], *elevated* [SBP 120–129; DBP < 80 mmHg], *hypertension* stage 1 [SBP < 130–139; DBP 80–89 mmHg] and *hypertension* stage 2 [SBP $\geq$ 140; DBP $\geq$ 90 mmHg]), that is shown in (**Figure 4B**) in continuous and intermittent lines (left side). However, as these criteria have not been updated in the public health systems of some countries, and only for a major contrasting between this current, and the past criteria, we also included the ranges of the past traditional BP classification that included 3-categories; (i.e., normotension [NT], prehypertension [PreHTN], and hypertension [HTN]) from Chobanian et al. (2003), that we denoted in the same (**Figure 4B**) in color boxes, right side, and ranges limits to each category denoted by the same continuous/intermittent lines.

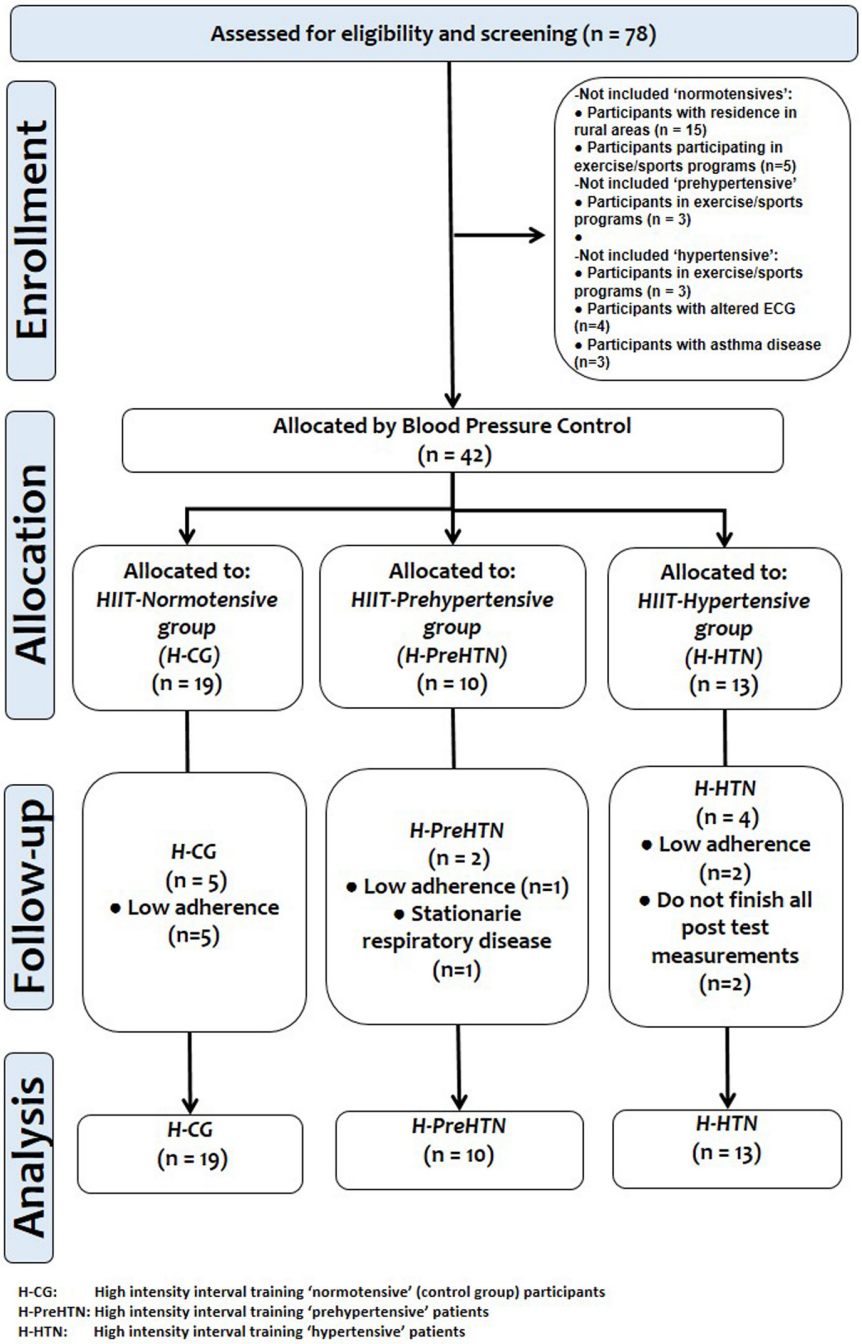

FIGURE 1 | Flow-chart diagram.

Body mass (kg) was measured using a digital bio-impedance scale (TANITA®, model Scale Plus UM – 028, Tokyo, Japan) and height (m) was measured with a SECA® stadiometer (model 214, Hamburg, Germany), with subjects in light clothing and without shoes. Body mass index (BMI) was calculated as the body weight divided by the square of the height (kg/m^2) and was used to estimate nutritional status.

Cardiorespiratory Fitness, Indirect Calorimetry and Substrate Utilization During Exercise Calculations

All participants were also asked to avoid excessive physical activity, especially weight training and high-intensity exercise, to abstain from alcohol, caffeine, adrenergic beverages, consume fat, or nicotine for 8–10 h prior to blood pressure and

anthropometrics measurements. On the morning of day 2, all participants returned to the laboratory between 7 and 9 am (72 h after the last exercise session), in the same order and with the same professional staff as in the baseline assessment. The maximal oxygen uptake ($\dot{V}O_{2max}$) was evaluated using the Åstrand test (Åstrand et al., 2003). The test is progressive, volitional and applied according to sex. The test starts with a rest period of 2 min, followed by 1 min of pedaling on a cycle ergometer (Lode® model Corival, Groningen, The Netherlands) without load, and then the load is increased by 50 watts (for men) or 25 watts (for women) every 2 min, with a pedaling frequency of 60–70 revolutions per minute to achieve maximum cardiorespiratory fitness for estimation of $\dot{V}O_{2max}$ (Mancilla et al., 2014; Olea et al., 2017), where the results were expressed in $mL{\cdot}kg^{-1}{\cdot}min^{-1}$.

We also registered exercise substrate metabolism fat (FATox) and carbohydrate (CHOox) energy substrates] during the test by indirect calorimetry gas analysis (Ultima CPX Medgraphics®, St Paul, MN, United States), to measure oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$), which were used to calculate rates of total lipid and carbohydrate oxidation, and the respiratory exchange ratio (RER). Systemic FAT and CHO oxidation rates were calculated using the stoichiometric equations of Frayn (1983). The relative contributions of FATox and CHOox to energy expenditure were calculated using the following equation (McGilvery and Goldstein, 1983):

$$\% FAT = [(1–RER)/0.29] \times 100$$

$$\%CHO = [(RER–0.71)/0.29] \times 100$$

The criteria for interruption and cessation of the exercise test were as follows: (a) a > 1.1 RER, (b) revolutions per minute (rpm) of ≤ 50 rpm (participants were recommended to exercise between 50–70 rpm), or (c) heart rate stabilization. The gas analyzers were calibrated with a certified calibration gas (4.95% CO_2–95.05% O_2, balance N_2), and the volume transducer was calibrated with a 3-liter calibration syringe (Ultima CPX Medgraphics® , St Paul, MN, United States), before each test by a lab assistant. The study protocol is shown in **Figure 2**.

Exercise Intervention

The HIIT intervention was performed with 1 min of maximum intensity exercise using a magnetic resistance static bicycle (Oxford® Fitness, model BE-2701, Chile) followed by 2 min of passive recovery over the bicycle (i.e., no pedaling), and this was repeated 10 times (Mancilla et al., 2014). The HIIT frequency was three times a week. The intensity of the exercise was calculated by the heart rate (HR) obtained from the calorimeter with a workload of 8, 9, or 10 (high level) on the Borg scale of 1 to 10 of perceived exertion, which was different in terms of load in watts among participants, but of similar intensity by the modified Borg scale [8, 9, or 10 points (Gibala et al., 2012)]. Thus, the subjective intensity of cycling also corresponded to 80–100% of the maximum heart rate in each participant that was correlated with the aforementioned modified Borg scale, at 8–10 points. All participants received 3 previous exercise sessions of familiarization. A total 480 min of effective work and 960 min of pause (passive, no pedaling) were developed in the HIIT intervention.

Statistical Analyses

The study data were processed using SPSS for Windows, program version 23.0 (SPSS® Inc., Chicago, IL) and graphs created using GraphPad Prism 8.0.2 software (GraphPad Software, San Diego, California). The null hypothesis was rejected at a level of significance of $p < 0.05$, and all statistical tests were two-tailed. The data are shown as mean and 95% confidence intervals (CIs) in tables and as least-squares means with 95% CIs in figures. The normal distribution of the data and the equality of variances was checked using the Kolmogorov-Smirnov test and Levene's test, respectively. Analysis of variance or Chi-square test (X^2) test as appropriate were used to analyze differences in outcomes between groups from baseline.

The effect of the intervention was performed using a per-protocol analysis. We used linear mixed-effects modeling for repeated measures over time using $\dot{V}O_{2max}$, blood pressure, and, indirect calorimetry outcomes as the dependent variable and effects for time, group (H-CG, H-PreHTN or H-HTN), and time by group interaction, with age, gender and BMI as covariates and an unstructured covariance matrix. Within the mixed model, we calculated 95% *Cis* and *P* values for 3 pre-specified intergroup contrasts and for change for all continuous variables within each group over time with adjustment for the baseline values, age, gender and BMI as covariates. A Sidack's *post hoc* test was used for multiple comparisons. Eta partial squared for interaction (Time × Group) was assessed by η^2 obtained from the ANCOVA with small ($\eta^2 = 0.01$), medium ($\eta^2 = 0.06$), and large ($\eta^2 = 0.14$) effects defined according to Lakens (2013). Liner regression tests were used in order to investigate the correlations between ΔCRF and ΔBP, and between ΔBP and changes in exercise substrate metabolism outcomes (ΔFATox, ΔCHOox, and ΔRER) adjusted for age, gender and BMI.

RESULTS

Regarding anthropometric measures, there were no significant differences at baseline for the three groups in terms of body mass: H-CG 78.84 (95% CI, 73.35 to 84.41), H-PreHTN 90.70 (95% CI, 76.39 to 105.01) and H-HTN 84.64 (95% CI, 71.41 to 97.88) kg, $p = 0.222$), BMI: H-CG 29.50 (95% CI, 27.49 to 31.51), H-PreHTN 31.97 (95% CI, 28.19 to 35.75) and H-HTN 30.33 (95% CI, 28.82 to 31.84) kg/m^2, $p = 0.439$, or body fat: H-CG 34.25 (95% CI, 31.50 to 37.06), H-PreHTN 35.72 (95% CI, 31.20 to 40.23) and H-HTN 34.33 (95% CI, 30.73 to 37.93) percentage, $p = 0.801$, **Table 1**. There were significant differences in the number/proportion of subjects diagnosed with hypertension at pre-test (13 [100%]) *versus* those at post-test (3 [23.0%]) ($p < 0.001$) (**Table 1**).

There were significant decreases in all groups for body mass: H-CG −1.76 (95% CI, −3.15 to −0.37) kg, $p = 0.032$; H-PreHTN −1.99 (95% CI, −3.91 to −0.07) kg, $p = 0.010$; and H-HTN −2.79 (95% CI, −4.49 to −1.08) kg, $p = 0.002$), however, the Time × Group interaction were not significant ($p = 0.661$) (**Table 1**). There were significant decreases in Δ BMI only in the H-CG group −0.67 (95% CI, −1.27 to −0.06) kg/m^2, $p = 0.041$.

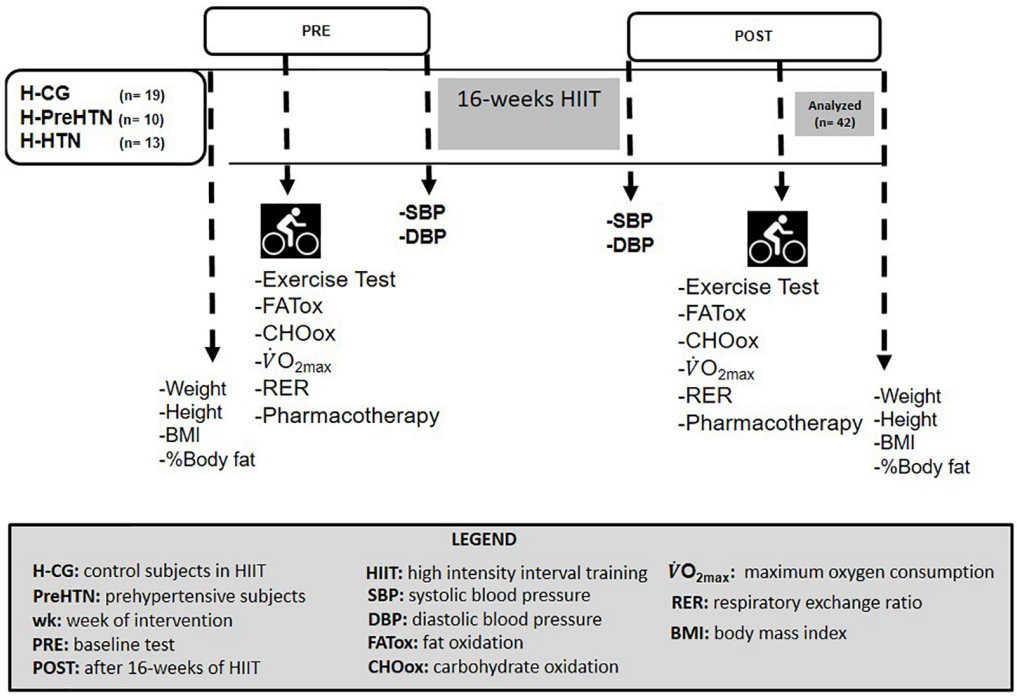

FIGURE 2 | Study protocol.

Body fat (%) decreased in the H-HTN group −1.38 (95% CI, −2.50 to −0.26), $p = 0.015$ (**Table 1**).

Adjusted mixed linear models revealed a significant improved in $\dot{V}O_{2max}$ were + 3.34 (95% CI, 1.91 to 4.76) mL·kg^{-1}·min^{-1} in the H-CG, + 3.63 (95% CI, 1.69 to 5.57) mL·kg^{-1}·min^{-1} in the H-PreHTN, and + 5.92 (95% CI, 4.16 to 7.68) mL·kg^{-1}·min^{-1} in the H-HTN group, however, the Time × Group interaction were not significant ($F_{(2·67)}$, $p = 0.083$, $\eta^2 = 0.13$), **Figures 3A,B**.

At 16-week, RER and CHOox changes were not significantly different from baseline in the H-CG, H-PreHTN, or in the H-HTN group ($p > 0.05$) **Figures 3C,E**. The FATox increases in H-CG + 11.65 (95% CI, 0.02 to 23.33)%, but not in the H-PreHTN + 7.33 (95% CI, −8.04 to 22.71)%, or in the H-HTN group + 8.94 (95% CI, −4,61 to 22, 49)%, **Figure 3D**. However, the Time × Group interaction were not significant ($F_{(0·09)}$, $p = 0.910$, $\eta^2 = 0.01$) (**Figure 3D**).

SBP decreased in the H-HTN −8.70 (95% CI, −16.52 to −0.88) mmHg, H-CG −7.14 (95% CI, −13.10 to −1.18) mmHg, and H-PreHTN −5.11 (95% CI, −9.78 to −0.43) mmHg. No significant intergroup differences were observed ($F_{(0·57)}$, $p = 0.566$, $\eta^2 = 0.03$), **Figures 4A,B**. DBP changes were −5.43 (95% CI, −7.73 to −3.12) mmHg in the H-CG group, and decreased significantly more in the H-HTN group ($p = 0.032$), **Figure 4D**.

There were no significant correlations between the delta changes of blood pressure outcomes (ΔSBP/ΔDBP), the delta changes from the cardiorespiratory fitness ($\Delta\dot{V}O_{2max}$, and ΔRER) and the delta changes for utilization during exercise outcomes (ΔFATox and ΔCHOox), **Figure 5**.

DISCUSSION

The main findings were that 16-weeks of HIIT protocol improved the $\dot{V}O_{2max}$, decreases the body mass and systolic BP especially in the pre-hypertensive and hypertensive patients, independent of changes in exercise substrate metabolism outcomes. Despite slightly greater improvements in some body composition variables or cardiorespiratory fitness, the changes observed in the exercise substrate metabolism were not related to the other BP changes.

The importance of exercise training for prevention and treatment of hypertension is well known (Cade et al., 1984; Guimaraes et al., 2010; Pescatello et al., 2015; Olea et al., 2017). Thus, part of the mechanisms by exercise training decrease blood pressure are explained and mediated by acute transitory mechanisms such as: (a) a reduction in vascular peripheral resistance (Ramírez-Vélez et al., 2019), (b) an increase in nitric oxide (Augeri et al., 2009), (c) a decrease in vasoconstrictors (Low et al., 2007), (d) an increase in shear stress (Birk et al., 2012), (e) a decrease in sympathetic nervous activity (Halliwill, 2001), and potentially other from structural chronic adaptations at vascular (Pedralli et al., 2020). For example, as the HTN patients decreased both SBP and DBP from the (Pedralli et al., 2020), where they also improved vascular structural parameters, from here it is possible to speculate that our results could be related with potential structural modifications. However, as we did not included vascular direct measurements, further research is needed to determine more clear these mechanisms.

TABLE 1 | Characteristics of normotensive, prehypertensive, and hypertensive participants of 16-weeks of high intensity interval training.

Characteristics	Time	H-CG	H-PreHTN	H-HTN	*p* value
N		19	10	13	
Age (y)		40.74 (35.34 to 46.13)	37.60 (29.10 to 46.10)	47.77 (38.40 to 45.94)	$p = 0.105^a$
Anthropometric/Body composition					
Height (cm)		163.74 (160.59 to 166.88)	167.70 (162.20 to 173.20)	165.08 (159.92 to 170.24)	$p = 0.406^a$
Body mass (kg)	Pre	78.84 (73.35 to 84.41)	90.70 (76.39 to 105.01)	84.64 (71.41 to 97.88)	$p = 0.222^a$
	Δkg	−1.76 (−3.15 to −0.37)	−1.99 (−3.91 to −0.07)	−2.79 (−4.49 to −1.08)	$p = 0.661^b$
	p value	**$p = 0.032$**	**$p = 0.010$**	**$p = 0.002$**	
Body mass index (kg/m^2)	Pre	29.50 (27.49 to 31.51)	31.97 (28.19 to 35.75)	30.33 (28.82 to 31.84)	$p = 0.439^a$
	Δkg/m^2	−0.67 (−1.27 to −0.06)	−0.77 (−1.61 to 0.05)	−0.39 (−1.13 to 0.35)	$p = 0.769^b$
	p value	**$p = 0.041$**	$p > 0.05$	$p > 0.05$	
Body fat (%)	Pre	34.25 (31.50 to 37.06)	35.72 (31.20 to 40.23)	34.33 (30.73 to 37.93)	$p = 0.801^a$
	Δ%	−0.71 (−1.63 to 0.20)	−1.17 (−2.43 to 0.09)	−1.38 (−2.50 to −0.26)	$p = 0.642$
	p value	$p > 0.05$	$p > 0.05$	**$p = 0.015$**	
Baseline Blood pressure					
Systolic BP (mmHg)	Pre	117.01 (113.82 to 120.77)	134.40 (132.28 to 136.51)	151.69 (145.49 to 157.89)	**$p < 0.001^a$**
Diastolic BP (mmHg)	Pre	72.42 (70.05 to 74.78)	74.90 (69.82 to 79.97)	81.76 (78,01 to 85.53)	**$p < 0.001^a$**
Substrate utilization during exercise (maximal)					
Fat oxidation (%)	Pre	12.31 (4.81 to 19.81)	31.70 (17.35 to 46.04	26.69 (15.52 to 37.01)	**$p = 0.011^a$**
CHO oxidation (%)	Pre	87.68 (80.18 to 95.18)	68.10 (53.93 to 82.70)	78.58 (62.93 to 83.52)	**$p = 0.011^a$**
Respiratory exchange ratio	Pre	0.98 (0.95 to 1.01)	0.89 (0.85 to 0.94)	0.91 (0.87 to 0.95)	**$p = 0.004^a$**
Baseline Pharmacotherapy¶					
Atenolol, *n* = (50 mg/1 uts. day)			–	13/13	
Losartan, *n* = (50 mg/1–2 uts. day)			1/10	8/13	
Hydrochlorothiazide, *n* = (12.5–25 mg/1 uts. day)			–	6/13	
Atorvastatin, *n* = (10–40 mg/1 uts. day)			5/10	1/13	
Blood Pressure Diagnosed by Group	Pre	19 (100%)	10 (100%)	13 (100%)	
	Post	29 (152.6%)	10 (100%)	3 (23.0%)	**$p < 0.001^c$**

Data are shown in mean and 95% CIs to continuous outcomes, and as (n/percent to categorical outcomes). Groups are described as; (H-CG) HIIT-normotensive control group [Reference group], (H-PreHTN) HIIT-prehypertensive group, and (H-HTN) HIIT-hypertensive group. Bold values denotes significant differences within group, or inter-group at baseline at $p < 0.05$ or less. (¶) Pharmacotherapy is shown according with the proportion of participants per group taking it [e.g., 2 out of 13 (2/13)]. (uts.) Units. [a]Analysis of variance in baseline. [b]Analysis of covariance (ANCOVA) with adjustment for the baseline values, age, gender as covariates (p-value for Time × Group interaction). A Sidack's post hoc test was used for multiple comparisons. [c]Analysis by Chi-square test (X^2) test.

In an exercise study on patients with hypertension (Molmen-Hansen et al., 2012) showed that 12-weeks of HIIT (4 × 4 min at 85–90% $\dot{V}O_{2max}$, walking/running, 38 min total session) or MICT (60% $\dot{V}O_{2max}$, walking/running, 47 min total session) reduced 24-h SBP by Δ −12mmHg (HIIT protocol) or Δ −4.5 mmHg (MICT protocol). Additionally, the authors showed that both exercise modes increased $\Delta\dot{V}O_{2max}$ (HIIT Δ + 5.2 mL·kg^{-1}·min^{-1}, MICT Δ + 1.0 mL·kg^{-1}·min^{-1}), which is in accordance with our HIIT protocol in hypertensive patients (H-HTN Δ + 5.2 mL·kg^{-1}·min^{-1}). Unfortunately, they did not report on exercise metabolism outcomes or exercise substrate changes (Molmen-Hansen et al., 2012). We found that our low exercise-time/session HIIT protocol not only decreased SBP, but also led to relevant clinical changes in the proportion of patients classified as hypertensive in pre-test (13 [31.0%]) compared with post-test (3 [7.1%]) measurements.

A recent study by Soltani et al. (2019), conducted in hypertensive patients, showed that a regimen of only 8-weeks of two HIIT protocols, including a very short HIIT (30s/30s at 80% of $\dot{V}O_{2peak}$, 30 s recovery, ∼40 min total sessions) and a long-duration HIIT (4 min/4 min at 75% of $\dot{V}O_{2peak}$, 4 min recovery, ∼42 min total sessions) protocol reduced SBP by −8.1 and −7.6 mmHg, respectively. Although the authors also reported a reduction in blood and plasma viscosity, and in fibrinogen concentration, no exercise substrates or metabolism outcomes were evaluated (Soltani et al., 2019). The present study, albeit with a longer exercise program (16-weeks), but with ≤ 30 min duration of total exercise per session, showed a superior reduction in SBP of Δ −8.70 mmHg in the H-HTN group and Δ −5.11 mmHg in the H-PreHTN group, but also led to relevant clinical changes in the proportion of patients classified as hypertensive in the pre-test (13 [100%]) as compared with post-test (3 [23.0%]) measurements. This suggests that HIIT is a powerful, non-pharmacological therapy against hypertension progression. Along these lines, an interesting parallel effect has been reported by traditional MICT (∼3 days, ∼40 min session, ∼65% heart rate), where a meta-analysis including 30 studies in HTN patients reported SBP/DBP decreases of −6.9 and −4.9, respectively (Fagard and Cornelissen, 2007). However, in comparison to the low-volume implicated by our HIIT protocol (10 min for training, 20 min rest periods), our exercise modality appears more time-efficient as compared to MICT.

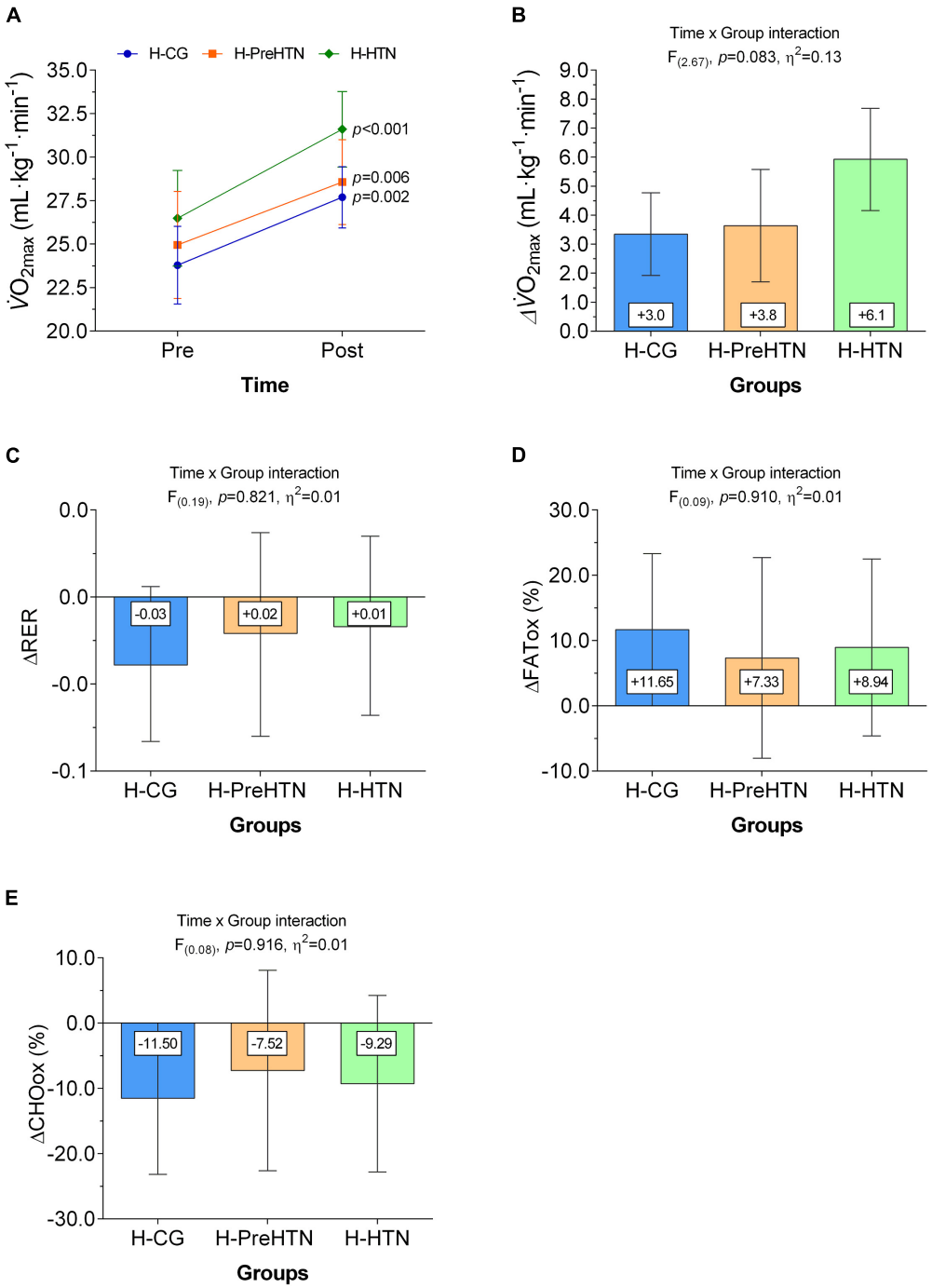

FIGURE 3 | Maximum oxygen consumption ($\dot{V}O_{2max}$), respiratory exchange ratio (RER), FATox and CHOox utilization during exercise measured by indirect calorimetry measured in healthy normotensive, prehypertensive, and hypertensive subjects. Panel **(A)** show absolute values of $\dot{V}O_{2max}$, and Panel **(B)** show $\dot{V}O_{2max}$ in delta values pre-post 16-weeks HIIT intervention. Panel **(C)** show RER peak during exercise in delta values pre-post 16-weeks HIIT intervention. Panel **(D)** shows fat utilization during exercise in delta values pre-post 16-week high-intensity interval training (HIIT) intervention. Panel **(E)** shows CHO utilization during exercise in delta values pre-post 16-week HIIT intervention Groups are described as (H-CG) HIIT-normotensive control group, (H-PreHTN) HIIT-prehypertensive group, and (H-HTN) HIIT-hypertensive group. Within the mixed model, we calculated 95% *CIs* and *P* values for 3 prespecified intergroup contrasts and for change for all continuous variables within each group over time with adjustment for the baseline values, age, gender and BMI as covariates. A Sidack's *post hoc* test was used for multiple comparisons. All results are presented as least-squares means with 95% confidence intervals (CIs).

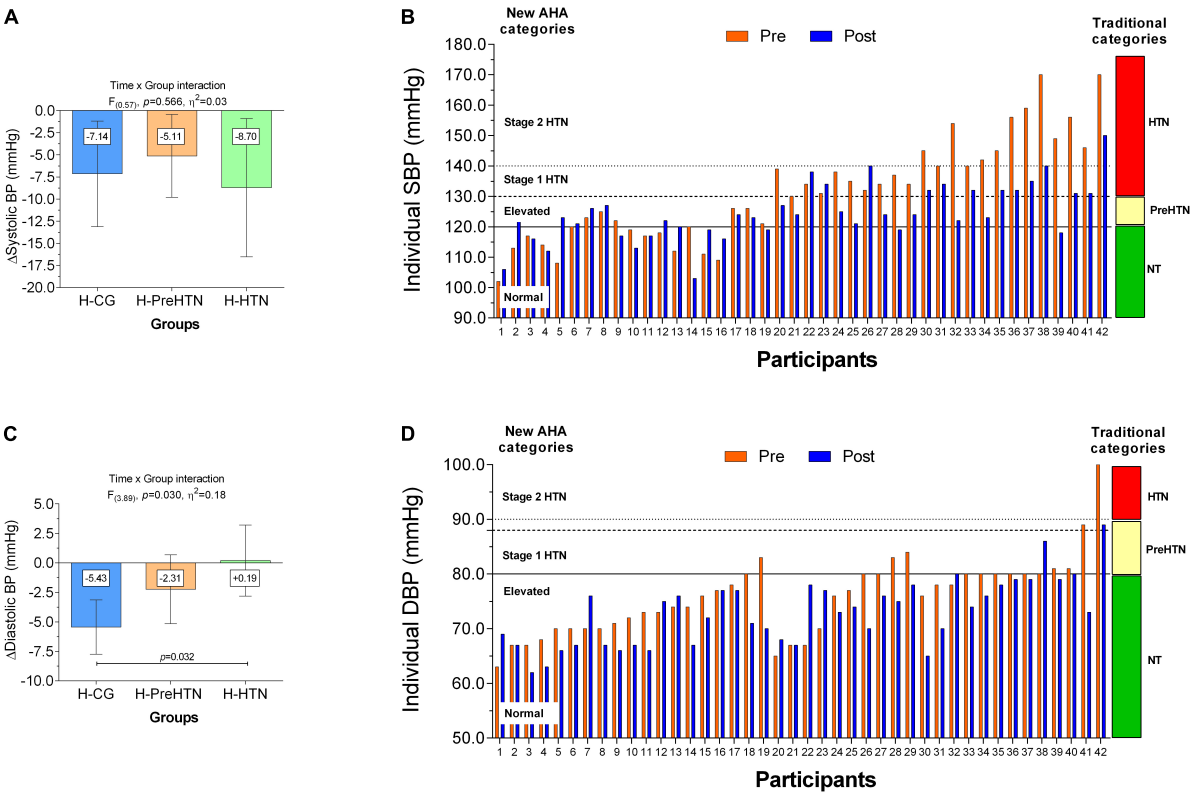

FIGURE 4 | Systolic (SBP) and diastolic blood pressure (DBP) in healthy normotensive, prehypertensive, and hypertensive subjects. Panel **(A,C)** show SBP and DBP delta changes in mean values from pre to post interventions groups. Panels **(B,D)** show show SBP and DBP according with each pre-post individual change, and by the old traditional cut-off point from Chobanian et al. (2003) (continuous, very intermittent, and less intermittent black lines) of blood pressure diagnosed ranged in green, cream, and red boxes, right side **(B,D)**, as well as showing the new cut-off from Whelton et al. (2018) from the AHA 2017, **(B,D)**, left side. Groups are described as (H-CG) HIIT-normotensive control group, (H-PreHTN) HIIT-prehypertensive group, and (H-HTN) HIIT-hypertensive group.

More recently, (Olea et al., 2017) reported that after 24 cycling sessions (8 weeks), using a similar HIIT protocol to ours, all 22 hypertensive patients showed a reduction in systolic/diastolic BP (SBP Δ −27 mmHg and DBP Δ −2 mmHg), which changed their diagnosed clinical baseline from hypertensive to normotensive. The authors similarly reported an increase in $\dot{V}O_{2max}$ of Δ + 3.5 mL·kg^{-1}·min^{-1} in hypertensive patients and Δ + 3.4 mL·kg^{-1}·min^{-1} in normotensive subjects at the end of the HIIT program, although no exercise substrate metabolic outcomes were reported. In an classic study (Cade et al., 1984), reported that, in 101/105 hypertensive patients, a 12-week regimen of traditional endurance continuous exercise (walking 2 miles/day with moderate-to-vigorous intensity) led to a significant decrease in SBP which was similar to our research; nevertheless, it is important to mention that in our study more robust effects were observed in SBP and not in DBP, which could be related to improvements in heart physiology rather than a local effect on blood vessels. However, of the 47 patients from the Cade et al. (1984) study who were receiving hypotensive pharmacological therapy during the pre-exercise period, 24 were able to discontinue all medication. Again, no exercise substrate metabolism or cardiorespiratory fitness outcomes were reported by these authors.

In addition to the results presented here, we have previously reported significant decreases in blood pressure and changes in the initial diagnoses of baseline hypertension or prehypertension patients (Cano-Montoya et al., 2016; Álvarez et al., 2018), but our present study is the first to report both blood pressure changes (as a change in clinical diagnosis), exercise substrate metabolism and cardiorespiratory fitness improvements in a hypertensive cohort. These finding demonstrate that HIIT could be considered an effective therapy against hypertension. In this regard, when HTN patients are under hypotensive pharmacotherapy and are also participating of exercise training, there is of great relevance to evaluate regularly the blood pressure in these patients (i.e., the register of before and after the exercise session) and to do the appropriate delivery to the physicians of this inform. This, due to the beneficial of the exercise adaptations can promote fast regulations (i.e., reductions) in their daily hypotensive dose, where physicians adjust these, according with the blood pressure values of each patient. On the other hand, no relationships were observed when testing the association between potential improvements in cardiorespiratory fitness/exercise substrate metabolism with blood pressure improvements (**Figure 5**). Thus, it is likely that rates of oxidation during exercise conditions or markers of

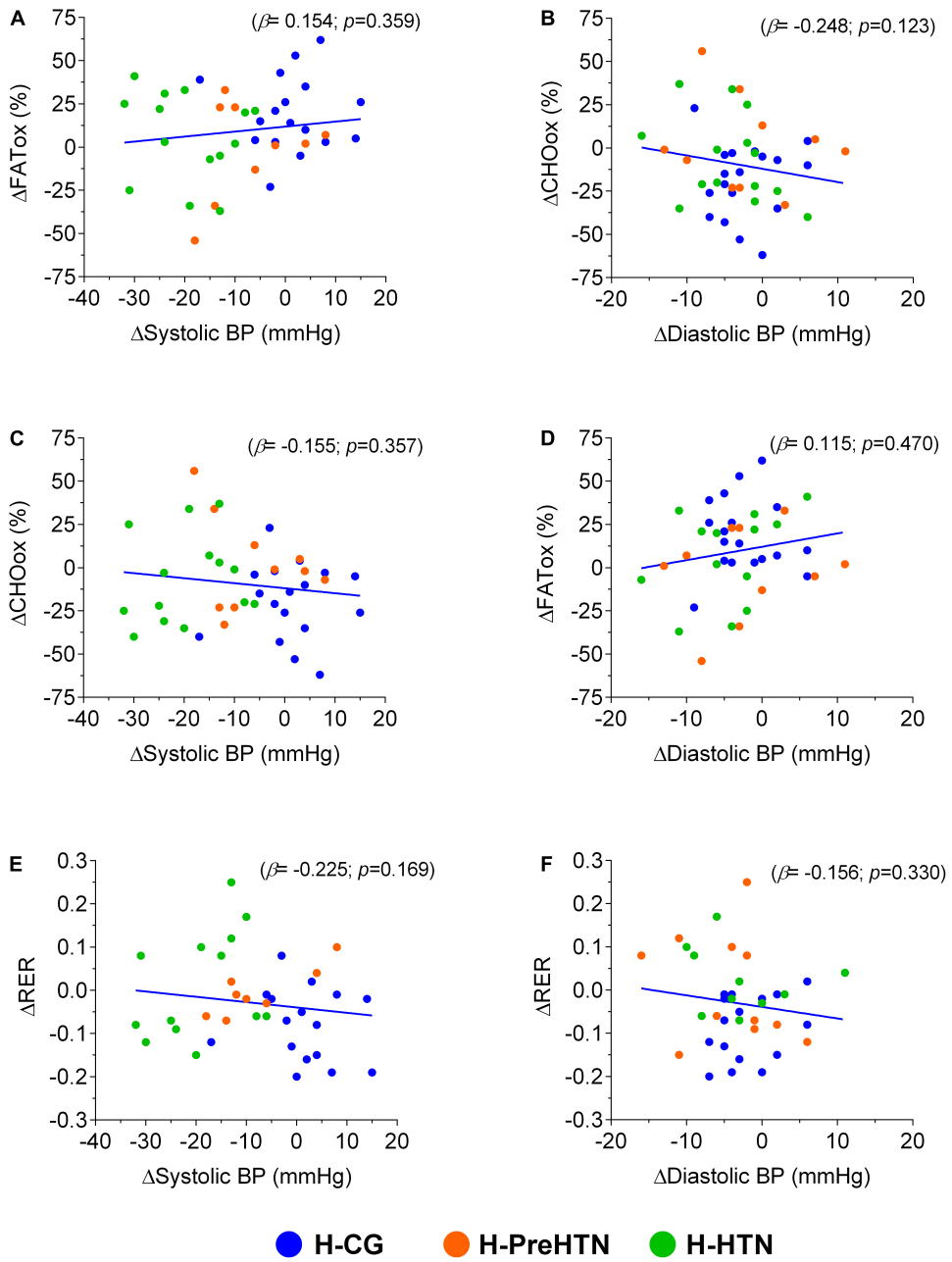

FIGURE 5 | Correlations among the delta of fat (ΔFATox), delta of carbohydrate (ΔCHOox) oxidation, and delta of respiratory exchange ratio (ΔRER) with the delta of systolic (ΔSystolic BP), and diastolic (ΔDiastolic BP). Panel **(A)** show correlation ΔFATox with ΔSystolic BP. Panel **(B)** show correlation ΔCHOox with ΔDiastolic BP. Panel **(C)** show correlation ΔCHOox with ΔSystolic BP. Panel **(D)** show correlation ΔFATox with ΔDiastolic BP. Panel **(E)** show correlation ΔRER with ΔSystolic BP. Panel **(F)** show correlation ΔRER with ΔDiastolic BP. Groups are described as (H-CG) HIIT-normotensive control group, (H-PreHTN) HIIT-prehypertensive group, and (H-HTN) HIIT-hypertensive group. β = Standardized coefficients beta with adjustment for the age, gender and BMI as covariates. Both ΔFATox and ΔCHOox are expressed in %, respiratory exchange ratio (RER).

oxidative capacity may not entirely reflect the capacity of obese persons for fatty-acid or CHO metabolism during exercise (Cao et al., 2019). However, there are numerous indications that obesity is associated with a diminished capacity to oxidize fat (van Baak, 1999). In this line, impairments in the ability to mobilize fatty acids from adipose tissue and to oxidize fatty acids in skeletal muscles have been reported in obese subjects during catecholamine stimulation (through β-adrenoceptors; (van Baak, 1999).

In other hand, FATox or CHOox rate in sedentary, obese/hypertensive subjects after chronic exercise training interventions and utilization during exercise is not well

documented. Also, in obese individuals the findings are controversial. Kempen et al. (1995) showed changes in body composition, energy expenditure, and substrate utilization in obese women after an 8-week combined diet and exercise training programme compared to diet alone. Furthermore, van Aggel-Leijssen et al. (2001) showed that, in obese men, low-intensity training (40% of $\dot{V}O_{2max}$) resulted in an increased total FATox during MICT, which could be attributed to an increase in non-plasma fatty acid oxidation, whereas HIIT (70% of $\dot{V}O_{2max}$) did not affect total FAT. In contrast, Kanaley et al. (1993) found that a 16-weeks aerobic exercise training programme (45% of $\dot{V}O_{2max}$) did not increase exercise FATox in upper- and lower-body obese women, but rather did increase exercise CHOox. Buemann et al. (1992), in a training study (3 × week, 45 ± 60 min of outdoor running and cycling for 3 ± 4 months) in post-obese women no effect on 24 h RER was shown. In agreement with our findings we speculated that a low-intensity exercise programme may be more effective in increasing FAT/CHOox during peak exercise. This is especially relevant because it has been proposed that low-intensity exercise may be more beneficial in improving FATox during exercise, but that HIIT may be more effective in increasing post-exercise FATox (van Aggel-Leijssen et al., 2001).

Several of the aforementioned studies also reported significant changes in body mass in hypertensive patients after HIIT programs. For example, the study by Olea et al. (2017) reported a change (Δ) of −3.5 kg. However, the other above-mentioned studies did not find changes (Cano-Montoya et al., 2018). In the present study, we found significant body mass changes (Δ −2.79 kg), as well as percentage body fat decreases (Δ −1.38%) in the H-HTN group, together with the aforementioned SBP decreases (Δ −8.70 mmHg) in this group. At 16-week, changes in SBP were negatively correlated to changes in $\dot{V}O_{2max}$ (R^2 = −0.444; p = 0.045) only in the H-HTN group. There were no significant changes in by $\Delta\dot{V}O_{2max}$ and the ΔDBP levels (**Supplementary Figure S1**). It is worth noting that a minimum of ∼2 mmHg systolic BP reduction in hypertensive patients is related to a 10% decrease in brain vascular accidents and a 7% decrease in cardiovascular disease (Lewington et al., 2002), suggesting that these results have clinical implications. These changes observed in our study is a significant and clinically relevant finding. In this line, that Kodama et al. (2009) reported that a 1-unit of metabolic equivalents higher level of $\dot{V}O_{2max}$ was associated with a decrement of 13 and 15% in risk of all-cause mortality and cardiovascular disease events, respectively, in healthy men and women.

Limitations and Strengths

This study has six limitations: (i) this was not a randomized control study, but rather an interventional study with pragmatic applications to evaluate the effectiveness of HIIT in real-life routine practice conditions on blood pressure and cardiorespiratory fitness/exercise substrate metabolism in hypertensive patients; (ii) there was a lack of a true control group, but this was not an aim of the study; (iii) the nutritional habits of participants were not evaluated, but we reminded all participants on a weekly basis to maintain their baseline dietary habits; (iv) the IPAQ score was not incorporated, as this was not an aim of the study, and (v) we did not included vascular function test and (vi) the use of indirect calorimetry method for estimating fat and CHOox, since there are other more accurate methods (i.e., isotope tracers or doubly-labeled water). However, the main strength of our study is that we reported an integral approach of blood pressure changes after a time-efficient exercise training as HIIT, reporting results in mean absolute (mmHg), delta (mmHg), as well as at individual changes. We also included clinical frequently used cut-off points for blood pressure classification to each participant for a better understanding.

CONCLUSION

A 16-week HIIT-intervention improved the $\dot{V}O_{2max}$, decreases the body mass and systolic BP especially in the pre-hypertensive and hypertensive patients, independent of changes in exercise substrate metabolism outcomes. Despite slightly greater improvements in some body composition variables or cardiorespiratory fitness, the changes observed in the exercise substrate metabolism were not related to the other BP changes. Because a disturbed muscle FATox or CHOox may be a primary event in the etiology of obesity and/or high blood pressure it is of the utmost importance to know whether, and how, exercise training may compensate for these impairments.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The local Ethics Committee reviewed and approved the study protocol (DI18-0043). All participants signed a written informed consent and the study was developed according the tenets of the Declaration of Helsinki. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PD-F contributed to the conception, organization and oversight of the study, drafting of the analysis plan, writing of the original manuscript draft, and final approval of the version to be published. FC-N, RM, and DJ-M contributed to measurements in the lab, searching the literature and reviewing the manuscript. CÁ and RR-V contributed to the statistical analyses, writing of the original manuscript draft and final approval of the version to be published. RR-V contributed to critical revision of the manuscript and final approval of the version to be published. RR-V, DA, and MI contributed to data analysis and interpretation,

critical manuscript revision and final approval of the version to be published. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.558910/full#supplementary-material

FIGURE S1 | General correlations (panel **A** and **B**), and by group correlations (panel **C** and **D**) among cardiorespiratory fitness (i.e., by O_{2max}) with blood pressure levels (ΔSBP/ΔDBP), following groups training from baseline to 16-weeks post-exercise. Panel **(A)** denotes the general correlation (i.e., all data) of O_{2max} with ΔSystolic BP, panel **(B)** denotes general correlation between O_{2max} with ΔDistolic BP, panel **(C)** denotes by group correlations between O_{2max} with ΔSystolic BP, and panel **(D)** denotes by group correlations between O_{2max} with ΔDiastolic BP. Groups are described as (H-CG) HIIT-normotensive control group, (H-PreHTN) HIIT-prehypertensive group, and (H-HTN) HIIT-hypertensive group. β = Standardized coefficients beta and R^2 = R square, coefficient of determination with adjustment for the age, gender and BMI as covariates.

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Wand Stretching Exercise Decreases Abdominal Obesity Among Adults With High Body Mass Index Without Altering Fat Oxidation

*Punnee Puengsuwan[1], Chia-Hua Kuo[2], Rungchai Chaunchaiyakul[3], Ratanavadee Nanagara[4] and Naruemon Leelayuwat[5,6]**

[1] School of Physical Therapy, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand, [2] Laboratory of Exercise Biochemistry, University of Taipei, Taipei, Taiwan, [3] College of Sports Science and Technology, Mahidol University, Nakhon Pathom, Thailand, [4] Department of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand, [5] Department of Physiology, Faculty of Medicine, Exercise and Sport Sciences Development and Research Group, Khon Kaen University, Khon Kaen, Thailand, [6] Graduate School, Khon Kaen University, Khon Kaen, Thailand

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Edited by:
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***Correspondence:**
Naruemon Leelayuwat
naruemon@kku.ac.th;
naruemon.leelayuwat@gmail.com

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Rationale: We designed a wand-based muscle stretching (WE) exercise program, which has become increasingly popular in physical therapy and has been used for elderly patients with adhesive capsulitis. However, studies regarding the effects of WE training on abdominal obesity and measures of cardiovascular risk factors among overweight/obese adults aged ≥55 years are rare.

Purpose: The objective of this study is to evaluate the effects of a 15-week wand stretching exercise program on waist circumference and cardiovascular risk factors in sedentary adults aged 55–70 years.

Methods: A total of 124 participants were randomly assigned to either participate in wand stretching exercise (WE) over a 15-week period or a control group (n = 62 each). Sixty participants in the WE group (26 overweight and 34 obese) and 51 in the control group (29 overweight and 22 obese) completed the study. The WE program included wand-assisted muscle stretching exercise on both the upper body and lower body for 40 min per day, 5 days per week, whereas the control group maintained their sedentary lifestyle.

Results: No significant improvements were observed in plasma glucose, insulin, and the homeostatic model assessment of insulin resistance (HOMA-IR) after exercise training. Compared with the control group, the WE group had more significant reductions in waist circumference among participants with a body mass index (BMI) < 25 kg/m^2 (−2.6 cm, 95% CI: −4.19 to −0.97 cm, d = 0. 48) and BMI > 25 kg/m^2 (−2.5 cm, 95% CI: −4.1 to −0.9 cm, d = 0.59) (both $P < 0.01$). Furthermore, within groups, a significant increase in % fat free mass was observed after WE training. The basal metabolic rate was slightly increased, but the fat oxidation rate remained unaltered in the WE group. Improvements in low-density lipoprotein cholesterol to high-density lipoprotein cholesterol were minimal

after WE. Significant reductions in high-sensitivity C-reactive protein were observed after WE only for participants with a BMI <25 kg/m^2.

Conclusion: The results suggest redistribution of a carbon source from the abdominal region to challenged skeletal muscle, following prolonged WE training. This abdominal fat reducing outcome of the WE is unlikely to be associated with fatty acid oxidation.

Keywords: aerobic exercise, cardiovascular risk factors, abdominal obesity, waist circumference, energy expenditure

INTRODUCTION

According to the Asian-Pacific cutoff points, overweight is classified as a body mass index (BMI) between 23.0 and 24.9 kg/m^2, and obesity, higher than 25 kg/m^2 (World Health Organization. Regional Office for the Western Pacific, 2000). The correlation between the incidence of cardiovascular disease (CVD) and that of abdominal fat has been reported, particularly among obese subjects (Rexrode et al., 2001; Onat et al., 2004; Nicklas et al., 2006). Other cardiovascular risk factors (CVRF) among obese persons include insulin resistance (Kouvari et al., 2019), dyslipidemia (Syed et al., 2009; Jackisch et al., 2018), and a reduced basal metabolic rate (BMR) (Bhopal and Rafnsson, 2009). The risk of CVD is associated with persistent low-grade systemic inflammation, as indicated by elevated high-sensitivity C-reactive protein (hsCRP) (Pai et al., 2004).

Regular moderate-intensity endurance exercise has been recommended to minimize CVRF (American College of Sports Medicine (ACSM), 2000), including abdominal fat (Rashti et al., 2019; Jiang et al., 2020) and low-density lipoprotein (LDL) (Izquierdo-Porrera et al., 2000), and increase insulin sensitivity, high-density lipoprotein (HDL), and BMR (Morio et al., 1998). The effects of exercise training in lowering persistent systemic inflammation has been confirmed by reports of reduced hsCRP levels (Kohut et al., 2006). However, the beneficial effects of aerobic training on improving insulin sensitivity and glycemic control diminishes with age and is particularly ineffective for those aged ≥55 years (Short et al., 2003). Strength training remains an effective intervention for metabolic outcomes for this age group (Cauza et al., 2005), suggesting that stretching forms of muscle contraction that poses less of a challenge to the cardiopulmonary system is beneficial for metabolic improvement among older individuals. However, the exercise modality employed in the present study is not recommended for those with limited mobility in that age group. In this study, we designed a wand-based muscle stretching exercise (WE) program, which has become increasingly popular in physical therapy and has been used for elderly patients with adhesive capsulitis (frozen shoulder). Importantly, WE is the combination of flexibility, balance, and endurance, which has been recommended for its positive outcomes for individuals (Puengsuwan et al., 2008). With simple and slow movements in all directions, WE will possibly be another handy home-based exercise tool for health promotion in older people. The effects of this mode of exercise on abdominal obesity and CVRF have not been previously evaluated. Obese individuals often developed systemic inflammation with high hsCRP levels, which causes limited joint mobility (Anson et al., 2018). However, studies regarding the effects of WE training on abdominal obesity and measures of CVRF among overweight/obese adults aged ≥55 years are rare. Therefore, in this study, we aimed to investigate whether WE training is an effective intervention that can reduce CVRF for overweight and obese adults. We hypothesized that reductions in waist circumference, CVRF measures, BMR, and hsCRP would be evident among overweight/obese individuals aged ≥55 years, after prolonged WE training.

MATERIALS AND METHODS

Participants

A total of 124 participants, from the urban area of Khon Kaen, Thailand, gave full informed consent after verbal and written explanations of the details of the study. They were recruited via poster and personal contact. The inclusion criteria included those with: (a) a healthy sedentary lifestyle (exercised <30 min twice per week during the previous 12 months); (b) aged between 55 and 70 years; (c) BMI ≥ 23 kg/m^2; and (d) menopause. Participants were divided into two groups; overweight and obese, based on the cutoff point of a BMI of 25 kg/m^2. In particular, Thai participants with BMI >25 kg/m^2 were classified as obese (World Health Organization. Regional Office for the Western Pacific, 2000). Exclusion criteria included non-obesity with normal blood glucose and lipid levels, and blood pressure within the normal range (Grundy et al., 2005) in addition to impaired mobility, hepatic, and renal functions. Participants who regularly engaged in exercise more than twice/week within the previous 3 months were also excluded.

All participants completed a routine medical examination comprising a health questionnaire, physical examination, blood chemistry assessment, and 12-lead electrocardiograph. They were subsequently randomly allocated to either the training or control cohorts with the aid of a computer-generated randomization list that assigned 71 participants to each cohort. This study was approved by the Ethics Committee of Khon Kaen University (HE480102) and conducted in accordance with the 1964 Declaration of Helsinki. A flow diagram of the study as outlined in the Consolidated Standards of Report Trials (CONSORT) statement is shown in **Figure 1**.

Experimental Design and Protocol

A randomized, single-blind, parallel group, two-arm clinical trial was conducted between February 2015 and April 2016 at the School of Physical Therapy, Faculty of Associated Medical

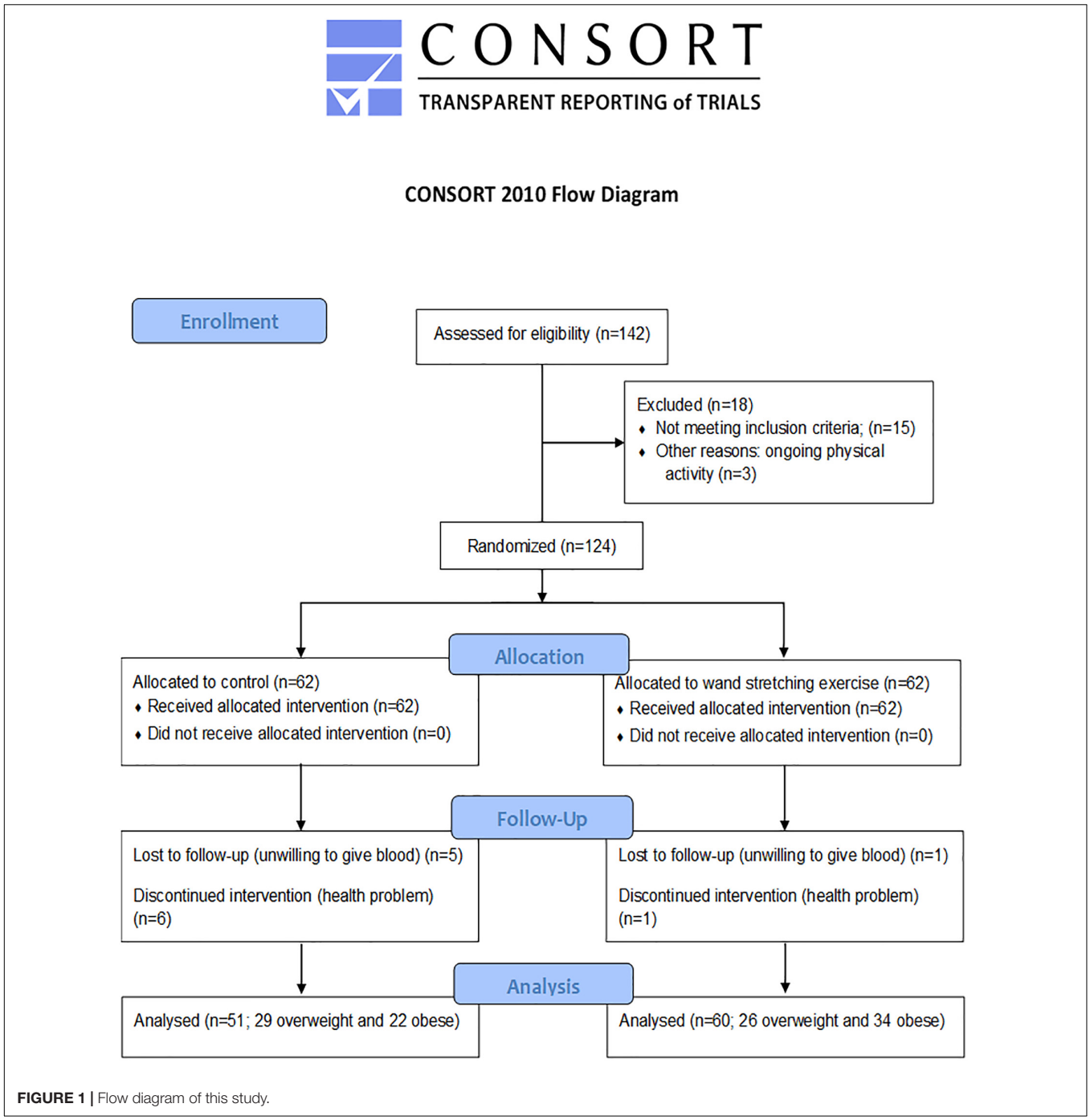

FIGURE 1 | Flow diagram of this study.

Sciences and Department of Physiology, Faculty of Medicine, Khon Kaen University. All participants were screened via medical history, physical examination, electrocardiography, and blood sampling for routine blood chemistry and hematology. Participants were then randomly assigned either to a 15-week period of wand exercise (WE group) or continuation of a sedentary lifestyle (control group) (**Figure 1**).

The WE training consisted of stretching and aerobic exercise 40 min a day, 5 days a week, whereas the control group engaged in their routine activities of daily living without any other regular exercise (<2 days per week). Adherence to the WE was monitored at the laboratory during week 2 and tracked via monthly telephone communication. Before and after 15 weeks, parameters were measured. After a 12-h fast on the day of measurement, participants arrived at the laboratory at 7.30 am and anthropometry and body composition were measured. They then assumed a supine position on a bed and 5-min expired air was collected for analysis of the BMR.

Sample size was calculated using the mean and standard deviation (SD) based on the study of Puengsuwan et al. (2008). Power of the statistical tests was calculated based on different means of abdominal obesity between two dependent groups, using the STATA Version 10 software (StataCorp LLC, United States). At least 62 participants (including 20% dropouts) in each group were required to show significant differences at a 5% significance level, and Cohen's *d* effect size was 0.23. The power (1 - β) was 0.83.

Wand Exercise Program

The WE program comprised a series of exercises, which were all performed in the standing position, while holding a 770 g wand. The exercise included 10 movements of the upper and lower body (**Figure 2**). The movements of the upper body comprised movements of the upper arms and trunk around the waist, including flexion and extension, lateral flexion and rotation of the trunk, and flexion, extension, adduction, abduction, and diagonal flexion and extension of the shoulders. The movements of the lower body comprised flexion, extension, abduction, adduction, and rotation of the hip, and flexion and extension of the knee joints (**Supplementary Figure 1**).

Participants in the training cohort were permitted to adjust the length of the wand to suit their height and arm span. Participants successfully performed all movements of the WE on the first visit. Subsequently, they were asked to repeat these exercises as their home-based program for the next 15 weeks via the teaching media of a video recording. Throughout the 15 weeks, participants in the control cohort did not engage in any regular exercise (<2 days per week). They maintained their usual levels of sedentary physical activity and caloric intake.

Standard Biosecurity and Institutional Safety Procedures

To be familiar with and prevent injury from the WE, they performed the WE during a 20-min session on each of 3 days for the first 3 weeks. After the first 3 weeks, all participants in the training cohort were asked to return to the laboratory for reassessment of the program and adjustment. Then, they performed the WE training by two 20-min sessions per day, 5 days per week for the next 12 weeks. A telephone call was made to each participant every month during the study period to verify their compliance, to report whether there was any injury according to the exercise, and encourage them to maintain their usual levels of daily physical activity (over and above the WE program).

Outcome Measurements

Anthropometry and Body Composition

The weights and heights of all participants were recorded and the BMI was later computed. The waist circumference (WC) was measured midway between the costal margin and the iliac crest at the end of inspiration. Hip circumference (HC) was measured as the greatest value over the buttocks. Fat mass was measured indirectly using the skinfold thickness method. Specifically, skinfold thickness was measured at four sites- the triceps, biceps, subscapular, and suprailiac crest, using a caliper. These measurements were then used to evaluate body fat, applying the equations estimated by Durnin and Womersley (1974). Fat free mass was then calculated based on the BM and fat mass. The coefficients of variation which is widely used to express the precision and repeatability of the measurements of WC, HC, and skinfold thickness were 2.1, 3.2, and 4.8%, respectively.

Basal Metabolic Rate

The BMR was measured, using the technique of indirect calorimetry (Cortex-MetaMax 3x Series, Germany). Calibrations were done prior to each measurement using known gas concentrations and a 3.0-L gas analyzer syringe. Participants were required to refrain from any strenuous exercise for at least 24 h prior to the test and after a night's sleep, and were subjected to a fast (including no breakfast). The BMR was measured via a breathing mask over a period of 30 min, while participants rested quietly in a supine position in an isolated room maintained between 21 and 26°C. Oxygen consumption $\dot{V}O_2$, (L/min) and carbon dioxide production $\dot{V}CO_2$ (L/min) rates were recorded and used to calculate the BMR according to the following formula developed by Weir (1949):

$$\text{BMR (Cal/day)} = [(3.9 \times \dot{V}O_2) + (1.1 \times \dot{V}CO_2)] \times 1.44 \times 4.184 \text{ (kJ/day)} \quad (1)$$

Fat Oxidation Rate

Fat oxidation rate (g/min) was calculated from the expired gas $\dot{V}O_2$ and $\dot{V}CO_2$, according to the following equation developed by Peronnet and Massicotte (1991) (non-protein respiratory quotient):

$$\text{Fat oxidation rate } (\text{g/min}) = (1.695 \times \dot{V}O_2) - (1.701 \times \dot{V}CO_2) \quad (2)$$

Blood Chemistry Analysis

Venous blood samples were obtained after a 12-h overnight fast and analyzed using standard automated laboratory methods at the Laboratory of Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, Thailand. The measurements obtained included fasting blood glucose, serum total cholesterol (TC), HDL and LDL (Roche Integra 800, Basel, Switzerland), and hsCRP concentrations (BN ProSpec, Dade Behring Marburg GmbH, United States). In addition, serum insulin concentration was measured using radioimmunoassay with a commercial kit (I^{125}/RIA) from MP Biomedicals, LLC (Irvine, CA, United States). Insulin sensitivity was determined by the homeostatic model assessment of insulin resistance (HOMA-IR) technique described by Matthews et al. (1985), whereby:

$$\text{HOMA} - \text{IR} = \text{glucose (mmol/L)}) \times \text{insulin } (\mu\text{U/mL})/22.5.$$

Compliance with the target workloads and number of sessions was > 90%.

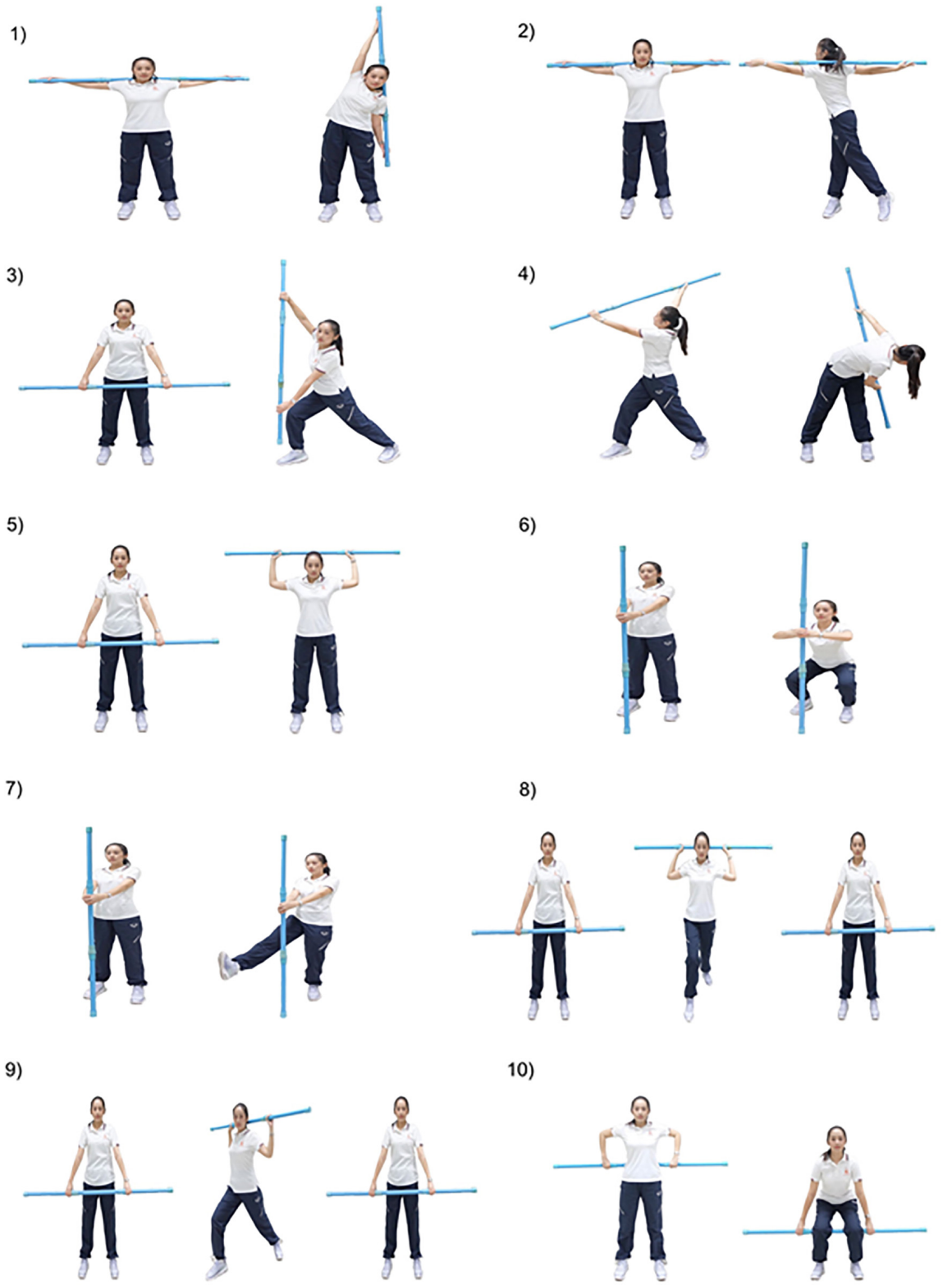

FIGURE 2 | Wand stretching exercise.

Dietary Intake and Physical Activity

All participants were requested to record all dietary intake and physical activities for 3 days (2 weekdays and 1 weekend day) using questionnaires. The dietary intake record was analyzed using a computerized food composition database, the INMUCAL program (Mahidol University, Thailand, 2001) and provided values from dietary components of the participants' meals (carbohydrate, fat, and protein). In addition, participants completed a questionnaire to record the duration and frequency of activities, including occupational work, household activities, sports and leisure activities, and sleeping. Every participant in both cohorts was contacted once every month via telephone calls to verify whether they were maintaining their daily dietary intake and physical activities, including WE records.

Statistical Analysis

The data were tested for normal distribution using the Shapiro–Wilk test. Differences between treatments (control and WE group) and within treatment (before and after experiments) were tested using repeated measures analysis of variance (ANOVA). Bonferroni's *post hoc* test was used to define the pairs with significant differences. A last-observation-carried-forward analysis approach was used to consider the fact that the original 124 participants recruited was reduced to 111 by the end of the study [60 in the WE group (26 overweight and 34 obese) and 51 in the control group (29 overweight and 22 obese)]. Data analysis was performed using STATA: Software for Statistics and Data Science (Version 10)[1]. Statistical significance was achieved with 80% power (β) and a p-value <0.05. Hedges' g was calculated as a measurement of effect size (Rosenthal, 1994). Measurements were expressed as means ± SE for normally distributed data, unless otherwise stated.

RESULTS

Of the 124 participants in the study (62 participants in each group), 60 in the WE group (26 overweight and 34 obese) and 51 in the control group (29 overweight and 22 obese) were fully compliant with the study. In the WE group; two participants withdrew from the study. One withdrew because of health problems unrelated to the exercise training program and the other was unwilling to permit blood sampling. In the control group, 11 participants withdrew from the study because of personal reasons (i.e., unwillingness to provide blood samples and health problems unrelated to the exercise training program).

No differences were noted between the groups in baseline measurements obtained by analysis of anthropometry, respiratory parameters, and blood chemistry. This indicated that the training and control cohorts had similar physiological characteristics facilitated through randomization (**Table 1**). No significant differences were noted in baseline daily dietary intake and energy expenditure between the training and control cohorts ($P < 0.05$) (**Table 2**). However, after completion of the WE program, energy expenditure in the training cohort was significantly increased compared with the control cohort. No differences were observed in these characteristics between participants who remained in the study and those who dropped out. The analysis presented below includes appropriate incorporation of non-compliance data.

[1]https://www.stata.com/

Participants' Compliance With the Exercise Program

Participants completing the study had higher than 90% compliance with the exercise training.

Anthropometry and Body Composition

After completion of the WE program, the average value of WC for participants in the training cohort was significantly lower than that in the control cohort for both overweight ($d = 0.48$) and obese ($d = 0.59$) groups (both $P < 0.01$) (**Tables 3**, **4**). Moreover, those who were overweight in the training cohort had a lower WC/HC ratio than those in the control group ($P < 0.05$, $d = 0.30$) (**Table 3**). Other anthropometry measurements showed no significant change in either cohort (**Tables 3**, **4**). No significant effects of sex or age were observed on any of the parameters.

Basal Metabolic Rate

The WE program induced a significant increase in BMR among the overweight participants alone, compared with the control group ($P < 0.05$, $d = 0.59$) (**Table 3**).

Fat Oxidation Rate

Fat oxidation rates after 15 weeks of WE training and those in the control group showed no significant change in both overweight and obese groups (**Tables 3**, **4**).

Blood Chemistry Analysis

Compared with the control group, overweight participants in the WE group showed a tendency to reduced LDL-C levels ($P = 0.09$, $d = 0.57$, **Table 5**) and LDL-C/HDL-C ratio ($P = 0.08$, $d = 0.37$, **Table 5**). Moreover, within the overweight group, the WE showed significant improvement in the HDL-C, TC/HDL-C, LDL-C/HDL-C, and hsCRP levels after completion of the exercise program (all $P < 0.05$, **Table 5**). In addition, within the obese group, no significant differences were noted in any blood parameters after completion of the study (**Table 6**). For both the training and control cohorts, no differences were noted in any of the blood parameters between the BMI groups (**Tables 5**, **6**).

DISCUSSION

This study proved that completion of a 15-week WE program can significantly reduce abdominal obesity and the WC/HC ratio, and increase BMR in sedentary Thai adults who are overweight. Furthermore, the WE program can reduce abdominal obesity in obese adults.

We hypothesized that the WE could reduce the CVRF, as measured by anthropometry, body composition, blood

TABLE 1 | Baseline characteristics of participants of the control and WE groups.

	Control (n = 51)	WE (n = 60)
Age (year)	61 ± 4	62 ± 4
Sex (F/M)	41/10	50/10
Body mass (kg)	60.2 ± 9.3	60.6 ± 9.3
Height (cm)	155.9 ± 6.2	156.1 ± 6.3
Body mass index (kg/m^2)	25.6 ± 3.1	25.6 ± 2.9
Body fat (%)	32.0 ± 7.0	32.9 ± 6.8
Fat mass (kg)	19.3 ± 5.3	19.9 ± 5.4
Fat free mass (kg)	40.9 ± 6.4	40.7 ± 6.9
Fat free mass (%)	69.0 ± 7.9	67.0 ± 6.9
Circumference (cm)		
Waist	82.0 ± 8.9	82.5 ± 8.9
Hip	97.2 ± 7.0	97.7 ± 6.8
Waist to hip circumference ratio	0.84 ± 0.06	0.84 ± 0.07

Data are expressed as means ± SD. WE, wand stretching exercise.

TABLE 2 | Daily dietary intake and energy expenditure of the control and WE groups before and after 15 weeks.

Daily dietary intake and energy expenditure	Control (n = 51)		WE (n = 60)	
	Before	After	Before	After
Carbohydrate (g)	287 ± 10	268 ± 12	244 ± 9	230 ± 12
Fat (g)	51 ± 3	53 ± 4	50 ± 3	46 ± 3
Protein (g)	78 ± 6	82 ± 4	73 ± 5	75 ± 4
Dietary intake (kJ/day)	7, 163 ± 238	7, 075 ± 230	6, 962 ± 246	6, 815 ± 267$^{\#}$
Energy expenditure (kJ/day)	6, 799 ± 192	6, 832 ± 159	6, 895 ± 213	7, 155 ± 205^{+}

Data are expressed as means ± SE.
WE, wand stretching exercise.
$^{+}$*Significantly different from the control group after 15 weeks, $p < 0.05$.*
$^{\#}$*Significantly different from the energy expenditure after 15 weeks, $p < 0.05$.*

chemistry, BMR, and fat oxidation rate in healthy overweight or obese participants. Therefore, our findings of reduced WC in both groups, and reduced WC/HC ratio and increased BMR in the overweight group, partially confirms our hypothesis.

The WC measurement used in the present study was previously described in the WHO guidelines 2000 (the midpoint between the lower border of the rib cage and the iliac crest). It is accepted as a reliable, feasible measure of abdominal obesity (Rexrode et al., 2001; de Koning et al., 2007) that is convenient for both the practitioner and the general public. The WC has been used previously to assess CVRF in both men and women (Lois et al., 2008) because of the significant association. Thus, it is a useful indicator for exercise recommendations for this clinical population that can lead to reduced risks of future non-communicable diseases (Ortaglia et al., 2020).

Nevertheless, the WC has potential limitations compared with more direct or 3-dimensional measurements obtained using dual X-ray absorptiometry or axial computed tomography imaging (Makimura et al., 2008; Wiklund et al., 2008). Importantly, the coefficient of variation for the measurements of WC was 2.1%, which is acceptable. Thus, use of the WC is both reliable and valid to determine abdominal obesity. Previously however, the measurement protocol reportedly had no influence on any association between the WC and CVD mortality (Ross et al., 2008).

The WC can potentially provide sufficient evidence to confirm the beneficial effects of WE, as observed in the reduced CVRF among overweight and obese participants. Furthermore, the reduction in the WC/HC ratio, which is another potential indicator of abdominal obesity (World Health Organization. Regional Office for the Western Pacific, 2000) confirmed the beneficial effects of WE in reducing abdominal obesity in overweight participants. The reduction of the WC/HC ratio among obese participants may be due to reduced movement at the waist compared with the overweight participants (based on our observations during practice). The greater WC of obese participants made them move less than the overweight participants. Further investigations in a larger cohort and with a longer duration of training is highly desirable in the obese group.

The mechanism underlying the reduction in abdominal obesity in the present study has not been elucidated. One of the expected mechanisms is an exercise-induced increase in the fat oxidation rate, which occurs in muscle mitochondria and is induced by the enzymes citrate synthase and cytochrome C oxidase (Short et al., 2003). However, we observed no significant effects of WE training on the fat oxidation rate. One might argue that we did not measure this at the mitochondrial level. However,

TABLE 3 | Anthropometry, body composition, and BMR in participants with a BMI < 25 kg/m^2 before and after 15 weeks in the control and WE groups.

	Control (*n* = 29)			WE (*n* = 27)			Mean difference (95% confidence interval)	*p*-value[b]	Hedges'
	Before	After	*p*-value[a]	Before	After	*p*-value[a]			
Weight (kg)	64.2 ± 1.49	64.0 ± 1.49	0.54	65.6 ± 1.05	64.7 ± 1.07	0.00	−0.66 (− 1.54 to 0.23)	0.14	0.14
BMI (kg/m^2)	27.1 ± 0.28	27.0 ± 0.33	0.58	27.4 ± 0.26	27.1 ± 0.26	0.00	−0.24 (− 0.62 to 0.13)	0.19	0.30
BF%	35.4 ± 1.29	34.2 ± 1.38	0.25	34.6 ± 1.27	32.9 ± 1.22	0.00	−0.49 (− 2.16 to 1.77)	0.56	0.16
FM (kg)	22.7 ± 0.97	21.9 ± 1.09	0.28	22.6 ± 0.83	21.2 ± 0.82	0.00	−0.59 (− 1.85 to 0.66)	0.34	0.07
FFM (kg)	41.6 ± 1.38	42.0 ± 1.15	0.52	43.1 ± 1.25	43.4 ± 1.21	0.07	0.04 (− 0.91 to 0.99)	0.93	0.37
% FFM	67.3 ± 3.00	68.6 ± 4.00	0.34	66.1 ± 3.00	68.4 ± 2.00	0.0005	0.77 (− 1.70 to 3.20)	0.52	0.35
WC (cm)	86.9 ± 1.63	87.4 ± 1.65	0.35	87.0 ± 0.91	85.0 ± 1.00	0.0003	−2.58 (− 4.19 to − 0.97)	0.002	0.48
WC/HC	0.86 ± 0.02	0.87 ± 0.02	0.01	0.86 ± 0.01	0.85 ± 0.01	0.44	−0.02 (− 0.04 to − 0.004)	0.018	0.30
BMR (kJ/day)	59.8 ± 2.05	56.3 ± 2.65	0.07	58.2 ± 1.87	63.0 ± 1.73	0.006	7.82 (2.99 to 12.65)	0.002	0.59
Fat oxidation rate (g/min)	0.05 ± 0.01	0.07 ± 0.01	0.08	0.06 ± 0.002	0.07 ± 0.002	0.60	0.021 (− 0.073 to 0.102)	0.17	0.11

Data are expressed as means ± SE and mean differences of the subsequent measurement of each variable (adjusted by its baseline) (95% confidence interval); control group (n = 29, 22 females, 7 males) and WE group (n = 27, 23 females, 4 males). Analysis was performed using repeated measure analysis of variance (ANOVA). When a significant difference was observed, a post hoc analysis using the Bonferroni adjustment was performed.

[a] *Test for significant differences within groups.*

[b] *Test for significant differences of subsequent measurements of each variable (adjusted by its baseline) between groups.*

WE, wand stretching exercise; BMR, basal metabolic rate; BMI, body mass index; BM, body mass; BF, body fat; FM, fat mass; FFM, fat free mass; WC, waist circumference; WC/HC, waist to hip circumference ratio.

TABLE 4 | Anthropometry, body composition, and BMR in participants with BMI > 25 kg/m^2 before and after 15 weeks in the control and WE groups.

	Control (*n* = 22)			WE (*n* = 33)			Mean difference (95% confidence interval)	*p*-value[b]	Hedges'
	Before	After	*p*-value[a]	Before	After	*p*-value[a]			
Weight (kg)	73.9 ± 4.28	73.1 ± 4.0	0.55	75.2 ± 3.61	74.8 ± 3.32	0.42	0.59 (− 3.77*to*4.96)	0.74	0.21
BMI (kg/m^2)	31.7 ± 0.49	31.4 ± 0.38	0.57	32.2 ± 0.78	32.1 ± 0.65	0.42	0.45 (− 1.77*to*2.07)	0.51	0.22
BF%	38.2 ± 2.51	38.0 ± 2.48	0.76	36.9 ± 5.78	36.4 ± 5.91	0.08	−0.31 (− 2.58*to*1.97)	0.74	0.26
FM (kg)	28.0 ± 1.66	27.5 ± 1.47	0.64	27.7 ± 4.41	27.2 ± 4.39	0.12	−0.09 (− 3.33*to*3.16)	0.95	0.12
FFM (kg)	45.9 ± 4.28	45.6 ± 4.16	0.40	47.5 ± 4.98	47.6 ± 5.09	0.47	0.55 (− 0.86*to*1.97)	0.36	0.38
% FFM	62.0 ± 1.0	64.0 ± 1.0	0.10	62.0 ± 0.8	64.0 ± 0.7	0.0004	0.08 (− 1.50*to*1.60)	0.92	0.58
WC (cm)	87.0 ± 1.4	87.3 ± 1.2	0.57	86.9 ± 1.0	84.7 ± 1.0	0.0004	−2.5 (− 4.10*to* − 0.90)	0.003	0.59
WC/HC	0.89 ± 0.02	0.88 ± 0.03	0.46	0.92 ± 0.06	0.93 ± 0.06	0.05	0.02 (− 0.05*to*0.09)	0.41	0.02
BMR (kJ/day)	58.8 ± 4.09	62.5 ± 6.41	0.50	72.7 ± 12.84	81.8 ± 10.46	0.13	7.57 (− 14.48*to*29.62)	0.42	0.61
Fat oxidation rate (g/min)	0.06 ± 0.01	0.08 ± 0.01	0.18	0.07 ± 0.002	0.07 ± 0.002	0.92	0.009 (− 0.127*to*0.132)	0.52	0.46

Data are expressed as means ± SE and mean differences of the subsequent measurement of each variable (adjusted by its baseline) (95% confidence interval); control group (n = 22, 20 females, 2 males) and WE group (n = 33, 27 females, 6 males). Analysis was performed using repeated measure analysis of variance (ANOVA). When a significant difference was observed, a post hoc analysis using the Bonferroni adjustment was performed.

[a] *Test for significant differences within groups.*

[b] *Test for significant differences of subsequent measurements of each variable (adjusted by its baseline) between groups.*

WE, wand stretching exercise; BMR, basal metabolic rate; BMI, body mass index; BM, body mass; BF, body fat; FM, fat mass; FFM, fat free mass; WC, waist circumference; WC/HC, waist to hip circumference ratio.

TABLE 5 | Blood chemistry parameters in participants with BMI < 25 kg/m^2 before and after 15 weeks in the control and WE groups.

	Control (n = 29)			WE (n = 27)			Mean difference (95% confidence interval)	p-value[b]	Hedges'
	Before	After	p-value[a]	Before	After	p-value[a]			
FBG (mg/dL)	95.1 ± 2.69	91.6 ± 2.87	0.22	95.4 ± 1.76	94.6 ± 1.66	0.51	2.8 (− 2.1*to*7.6)	0.26	0.50
Insulin (uIU/mL)	10.6 ± 0.66	10.4 ± 0.66	0.94	14.8 ± 0.34	12.3 ± 0.26	0.16	−1.7 (− 14.1*to*14.9)	0.43	0.07
HOMA	3.9 ± 0.49	6.1 ± 2.31	0.32	3.7 ± 0.33	3.9 ± 0.34	0.49	−2.0 (− 5.5*to*1.4)	0.24	0.10
TG (mg/dL)	163.5 ± 41.70	134.6 ± 23.70	0.29	129.6 ± 12.6	113.7 ± 10.80	0.07	−4.0 (− 33.4*to*25.4)	0.79	0.37
TC (mg/dL)	224.9 ± 7.94	222.9 ± 7.62	0.84	215.5 ± 5.81	205.8 ± 5.35	0.07	−13.2 (− 29.9*to*3.6)	0.12	0.49
HDL-C, mg/dL)	57.4 ± 2.70	59.6 ± 3.19	0.37	55.3 ± 2.57	59.6 ± 2.68	0.02	1.7 (− 4.3*to*7.7)	0.57	0.19
LDL-C (mg/dL)	131.5 ± 9.76	136.3 ± 8.13	0.47	130.4 ± 5.98	123.3 ± 5.73	0.17	−12.4 (− 27.0*to*2.3)	0.09	0.57
TC/HDL-C	4.0 ± 0.22	3.8 ± 0.17	0.37	4.2 ± 0.24	3.6 ± 0.18	0.002	−0.3 (− 0.66*to*0.14)	0.19	0.11
LDL-C/HDL-C	2.3 ± 0.16	2.3 ± 0.16	0.75	2.5 ± 0.17	2.2 ± 0.85	0.006	−0.3 (− 0.58*to*0.04)	0.08	0.37
hsCRP (mg/L)	2.3 ± 0.51	1.9 ± 0.53	0.18	2.6 ± 0.37	1.8 ± 0.31	0.004	−0.3 (− 0.99*to*0.44)	0.44	0.03

Data are expressed as means ± SE and mean differences of the subsequent measurement of each variable (adjusted by its baseline) (95% confidence interval); control group (n = 29, 22 females, 7 males) and WE group (n = 27, 23 females, 4 males). Analysis was performed using repeated measure analysis of variance (ANOVA). When a significant difference was observed, a post hoc analysis using the Bonferroni adjustment was performed. [a]Test for significant differences within groups. [b]Test for significant differences of subsequent measurements of each variable (adjusted by its baseline) between groups. WE, wand stretching exercise; FBG, fasting blood glucose; HOMA, homeostatic model assessment; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein.

TABLE 6 | Blood chemistry parameters in participants with BMI > 25 kg/m^2 before and after 15 weeks in the control and WE groups.

	Control (n = 22)			WE (n = 33)			Mean difference (95% confidence interval)	p-value[b]	Hedges'
	Before	After	p-value[a]	Before	After	p-value[a]			
FBG (mg/dL)	101.0 ± 4.34	103.8 ± 8.73	0.71	94.7 ± 5.5	101.3 ± 5.69	0.01	4.8 (− 23.5*to*33.0)	0.68	0.07
Insulin (uIU/mL)	18.2 ± 0.40	17.5 ± 0.4	0.81	15.9 ± 0.2	16.9 ± 0.2	0.55	0.33 (− 22.4*to*31.7)	0.61	0.38
HOMA	6.1 ± 0.77	4.9 ± 0.87	0.29	3.9 ± 0.79	4.7 ± 0.79	0.67	−0.13 (− 4.7*to*4.5)	0.95	0.21
TG (mg/dL)	145.4 ± 34.4	139.4 ± 29.8	0.61	133.0 ± 16.5	97.7 ± 11.9	0.27	−32.4 (− 87.0*to*22.3)	0.19	0.33
TC (mg/dL)	217.4 ± 20.7	223.8 ± 16.4	0.71	175.3 ± 12.2	181.0 ± 14.2	0.56	−19.7 (− 79.2*to*39.8)	0.44	1.11
HDL-C, mg/dL)	58.4 ± 2.9	72.6 ± 22.4	0.54	59.0 ± 7.6	62.0 ± 12.5	0.72	−11.8 (− 91.0*to*67.4)	0.72	0.14
LDL-C (mg/dL)	130.0 ± 15.6	113.0 ± 26.8	0.36	89.7 ± 22.1	99.3 ± 19.8	0.13	35.1 (− 36.2*to*106.8)	0.26	0.28
TC/HDL-C	3.7 ± 0.33	3.9 ± 0.67	0.79	3.1 ± 0.55	3.2 ± 0.83	0.76	0.37 (− 1.3*to*2.0)	0.59	0.24
LDL-C/HDL-C	2.2 ± 0.26	2.2 ± 0.58	0.97	1.7 ± 0.54	1.9 ± 0.72	0.49	0.51 (− 1.1*to*2.1)	0.45	0.15
hsCRP (mg/L)	3.4 ± 1.14	3.9 ± 1.28	0.37	5.1 ± 2.34	4.0 ± 2.41	0.30	−1.6 (− 4.2*to*0.99)	0.18	0.16

Data are expressed as means ± SE and mean differences of the subsequent measurement of each variable (adjusted by its baseline) (95% confidence interval); control group (n = 22, 20 females, 2 males) and WE group (n = 33, 27 females, 6 males). Analysis was performed using repeated measure analysis of variance (ANOVA). When a significant difference was observed, a post hoc analysis using the Bonferroni adjustment was performed.

[a]Test for significant differences within groups.

[b]Test for significant differences of subsequent measurements of each variable (adjusted by its baseline) between groups.

WE, wand stretching exercise; FBG, fasting blood glucose; HOMA, homeostatic model assessment; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein.

we indirectly measured fat oxidation by using the whole-body oxidation rate, as we collected expired gas. The indirect method used in the present study has been shown to be reliable and valid enough to measure the fat oxidation rate (Ghanassia et al., 2006; Prasertsri et al., 2013).

Another possible mechanism of the reduction in abdominal obesity is hydrocarbon redistribution. This hypothesis suggests a negative energy balance in fat cells due to the competition from skeletal muscle for circulating hydrocarbon sources (Kuo and Harris, 2016). The WE was moderate-intensity exercise, which has been shown to reduce abdominal fat (Rashti et al., 2019). However, this mechanism was not confirmed in this study, because muscle mass determined by the fat free mass was increased in the WE group alone without any significant differences in the control group.

A higher intensity WE may significantly improve the fat free mass. Furthermore, the WE under investigation in the present study comprises movements around the waist area, including flexion, extension, rotation, and side flexion. During these movements, all components of the WE involved movement at the waist, with frequent contraction and stretching of the abdominal and back muscles, and this may reduce the WC. This is supported by the study of Lahelma et al. (2019), who found that exercise using a weighted hula-hoop can reduce the WC in overweight subjects. This makes it particularly suited to reducing abdominal obesity. Either a higher intensity or longer duration can increase abdominal muscle mass.

The WE increased the BMR in the overweight participants alone. We do not yet know the reason for this phenomenon because the mass of skeletal muscle, an important tissue associated with the BMR, was not significantly increased. Thus, the increased BMR seems to be unrelated to any increase in muscle mass. This finding is consistent with a previous study that showed an increased BMR with an unchanged fat-free mass after endurance exercise training (Poehlman et al., 1990). They suggested that the variations in maximal oxygen consumption ($\dot{V}O_{2max}$) which is a significant predictor of resting metabolic rate (RMR) may contribute to individual variation in RMR in healthy older men. RMR is a more common measurement which uses less strict criteria than BMR (McMurray et al., 2014). This is comparable with our previous study that showed improved $\dot{V}O_{2max}$ determined by 6-min walk test according to WE training (Puengsuwan et al., 2008). The other explanation may be due to genetic effect since changes in RMR and $\dot{V}O_{2max}$ following the short-term exercise training are genotype dependent. However, the increase in BMR according to WE training could provide beneficial effect on stabilizing body composition to sedentary overweight people. This may permit us to intake higher energy without an increase in body fat mass. BMR is also affected by many factors such as menstrual cycle (Lawson et al., 1987). However, all female subjects in this study were in menopause. Therefore, the high BMR during the luteal phase were not found by this study. Furthermore, the increased BMR in this study may not be attributed to "carry over" effect of the last exercise bout because it was assessed at least 24 h after the last exercise bout. Even after the high-intensity exercise (15–48 h post-exercise), no changes in RMR were found (Devlin and Horton, 1986; Poehlman et al., 1989). Thus, after WE, moderate-intensity exercise, in this study it seems to have no "carry over" effect of the last exercise bout which took around 48 h after.

The WE training group had increased energy expenditure compared with the control group and higher energy intake within the group. This should cause a negative energy balance and should have resulted in body mass reduction. However, the greater energy expenditure in the WE group did not cause any reductions in body mass in either the overweight or obese groups, as compared with the control group. Furthermore, the WE training did not alter body composition, fat, or muscle mass. This is possibly due to our lack of restrictions on the daily diet of the participants. In addition, the difference between energy expenditure and energy intake was not large enough to result in any considerable negative energy balance. Thus, body composition was not significantly changed.

Notably, neither overweight nor obese participants showed significant changes in blood chemistry variables after exercise training. However, we found a tendency in the overweight group to have reduced LDL-C (p = 0.09) and LDL-C/HDL-C (p = 0.08), as compared with the control group. This seems to be consistent with the within-group results, as the WE group showed a significant increase in HDL-C concentration (p = 0.02), and reductions in TC/HDL-C (p = 0.002) and LDL-HDL-C (p = 0.006).

We used 12 weeks of training with 3-week pre-training to prevent injury from starting the WE. During the first 3 weeks, participants performed the WE during a 20-min session on each of three days. After the first 3 weeks, they performed the WE training by two 20-min sessions per day, 5 days per week for the next 12 weeks. At least 150 min per week of moderate-intensity aerobic training is recommended for weight reduction by ACSM (Donnelly et al., 2009). Importantly, several studies have demonstrated that 12 weeks of this exercise training program provided significant beneficial effects on obesity and cardiovascular risk factors such as decreased waist circumference (Saremi et al., 2010), body weight (Schjerve et al., 2008; Seo et al., 2011), fasting glucose level (Seo et al., 2011), lipid profiles (Schjerve et al., 2008; Seo et al., 2011), and diastolic blood pressure (Schjerve et al., 2008; Seo et al., 2011).

This study has several limitations. Firstly, most participants were female (the female vs. male ratio was 4.5 vs. 1) and abdominal obesity is more specific to the male population (Yoo et al., 2010). Therefore, the results of this study cannot be applied to the male population. Secondly, the duration of training may have been too short to improve visceral and subcutaneous fat and muscle compartments. Thirdly, specific assessments of the utilization of visceral and subcutaneous fat compartments, including magnetic resonance imaging, computed tomography, proton magnetic resonance spectroscopy, or muscle biopsy (Sabag et al., 2017), and fat biopsy (Riis et al., 2019) should be considered for more comprehensive information. Further study on either a longer exercise duration or dietary restrictions may help to determine the favorable effects of a WE on all relevant parameters among overweight and obese participants.

CONCLUSION

We have shown that a 15-week WE program comprising 40 min exercises, 5 days per week, produced a significant reduction in WC in a cohort of sedentary middle-aged Thai adults and an increase in BMR in those participants classified as overweight at baseline. This reduction in abdominal obesity and improvement in basal energy expenditure imply a reduced risk of CVD. The WE program is an effective at-home fitness program that requires minimal equipment. It is simple and convenient to apply at home and suitable for the elderly, overweight, or other individuals who may not be so able to participate in commonly offered training programs.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation. Requests to access these datasets should be directed to NL, naruemon@kku.ac.th.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Khon Kaen University (HE480102) in accordance with the 1964 Helsinki Declaration. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

NL conceived the idea for the manuscript, agreed on content, and contributed to the writing and editing of the manuscript. PP collected and analyzed the data and drafted the manuscript. RN did the medical cover. PP, C-HK, RC, RN, and NL contributed to the editing of the manuscript and approved the final draft of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.565573/full#supplementary-material

Supplementary Figure 1 | Wand stretching exercise description.

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Post-exercise Effects and Long-Term Training Adaptations of Hormone Sensitive Lipase Lipolysis Induced by High-Intensity Interval Training in Adipose Tissue of Mice

*Yang Liu[1,2], Gaofang Dong[1,2], Xiaobo Zhao[1,2], Zerong Huang[1,2], Peng Li[1,2] and Haifeng Zhang[1,2]**

[1]*Physical Education College, Hebei Normal University, Shijiazhuang, China,* [2]*Provincial Key Lab of Measurement and Evaluation in Human Movement and Bio-Information, Hebei Normal University, Shijiazhuang, China*

Although studies have proven that high-intensity interval training (HIIT) shows a comparable effect to moderate-intensity continuous training (MICT) on reducing body fat, especially visceral fat, the mechanism is still unclear. Since MICT consumes more fat during exercise, the mechanism of HIIT weight loss may be related to post-exercise effects, long-term adaptive changes, and hormone sensitive lipase (HSL). The objective of this study was to compare the post-effects of acute exercise, long-term adaptive changes on HSL activity, and catecholamine-induced lipolysis between HIIT and MICT. Following a 14-week high-fat diet (HFD), obese female C57Bl/6 mice were divided into acute exercise groups (one time training, sacrificed at rest and 0, 1, and 12 h after exercise, $n = 49$), -L groups (12-week long-term training, 12-h fasting, $n = 21$), and -C groups (12-week training, primary adipocytes were isolated and stimulated by catecholamine *in vitro*, $n = 18$). MICT or HIIT treadmill protocols (running distance matched) were carried out during training. Comparison of acute exercise effects by two-way ANOVA showed no time × group interaction effect, however, a significant increase in HSL-Ser563 (at 0 and 1 h) and Ser660 phosphorylation (at 0, 1, and 12 h) in inguinal (subcutaneous) fat was only observed in HIIT mice ($p < 0.05$ vs. rest), but not in MICT mice. The periuterine (visceral) fat HSL expression and phosphorylation of HIIT mice was similar to or lower than MICT mice. After long-term training, 12-h fasting significantly increased periuterine fat Ser563 phosphorylation in HIIT mice ($p < 0.05$), but there was no change in MICT mice. Under stimulation of catecholamine *in vitro*, isolated primary adipocytes from periuterine fat of long-term HIIT mice showed a higher Ser563 increase than that found in MICT mice ($p < 0.05$). The quantity of triglyceride (TG) lipid bonds (representing lipolysis level) was significantly lower after HIIT than MICT ($p < 0.05$). The results indicate that (1) acute HIIT can induce an increase of HSL phosphorylation in subcutaneous fat lasting at least 12 h, implying longer post-exercise lipolysis than MICT and (2) long-time HIIT has a better effect on improving catecholamine resistance of visceral adipocytes caused by a HFD, which allows fat to be mobilized more easily when stimulated.

Keywords: high-intensity interval training, catecholamine, long-term adaptations, post-exercise effects, lipolysis, hormone-sensitive triglyceride lipase

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Paulo Gentil, Universidade Federal de Goiás, Brazil

***Correspondence:**
Haifeng Zhang
hbnuzhanghaifeng@sina.com

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INTRODUCTION

Obesity is a serious threat to human health and has become an important topic in sports science. Traditionally, moderate-intensity continuous training (MICT) is widely used for reducing body fat because long-duration moderate-intensity exercise increases the total amount of skeletal muscle fat oxidation, while high-intensity exercise can only be maintained by glycogen (Frayn, 2010). However, recent studies suggested that long-term high-intensity interval training (HIIT) may have comparable effects on body fat reduction to MICT (Keating et al., 2017; Viana et al., 2019). Since the absolute value of muscle fat consumption during MICT is higher than during HIIT, it may be a reasonable explanation that the higher fat loss occurs after, but not during, HIIT (Abbasi, 2019). Two potential processes may be involved in this observation: (1) In a recovery period, after each acute exercise session, more fat is consumed due to the post-exercise effect of HIIT (acute effect); or (2) Long-term HIIT can cause adipose tissue adaptive changes, which make it easier to be mobilized and metabolized (sustained effect).

Excessive accumulation of triglycerides (TGs) is a major feature of obesity. TG stored in intracellular lipid droplets of white adipose tissue (WAT) accounts for most of systemic reserves (Braun et al., 2018). The first step of fat decomposition is the hydrolysis of TG (so called lipolysis or fat mobilization) into glycerol and non-esterified fatty acids (NEFA). Lipolysis is important because it is the startup phase for oxidation, gluconeogenesis, and redistribution of fat to skeletal muscle (Thompson et al., 2012; Kuo and Harris, 2016). Lipolysis occurs in a sequential manner from TG, diacylglycerol (DAG), and monoacylglycerol (MAG) to produce three NEFAs and one glycerol. Adipose TG lipase (ATGL) and hormone sensitive lipase (HSL), located on lipid droplet membranes, are key rate-limiting enzymes during this process (Berraondo and Martínez, 2000). The main function of ATGL is to hydrolyze TAG into DAG, while HSL exhibits a wider substrate specificity, which can catalyze the hydrolysis of TAG, DAG, and MAG (Kim et al., 2016). Meanwhile, the lipolysis activity of HSL can be regulated by both extra and intracellular signals such as hormones, sympathetic nerves, and intracellular energy receptors through some site-specific serine phosphorylation (Stallknecht et al., 2001; Thompson et al., 2012; Braun et al., 2018). Wide substrate specificity and integration of extra and intracellular signals suggest that HSL activity can represent lipolysis. Unfortunately, the effects of HIIT on HSL lipolysis activity after exercise, as well as the long-term adaptive changes of adipocyte to HIIT, are still not well-studied.

Catecholamines (Epinephrine, E and Norepinephrine, NE) are well-known as the most important signal to regulate lipolysis and HSL activity (Thompson et al., 2012). Studies showed that lipolysis during exercise mainly depended on the adrenal glands, rather than sympathetic nerves (Stallknecht et al., 2001), and the amount of E and NE secreted by the adrenal glands increased with exercise intensity (McMurray et al., 1987), which is the primary determinant of the plasma catecholamine response (Zouhal et al., 2013). Low-to-medium-intensity exercise can cause a modest increase of circulating catecholamines and HSL lipolysis activity by the β_3 adrenergic receptor (β_3AR)-Gs protein pathway. However, acute high-intensity exercise inhibits HSL activity through the α-adrenergic receptor (αAR)-Gi protein pathway (Frayn, 2010), and a restriction in the adipose tissue blood flow also occurs (Hodgetts et al., 1991), due to excessive catecholamine levels. Therefore, although HIIT can cause a higher increase of blood catecholamines (Bracken et al., 2009), MICT causes higher lipolysis than HIIT during exercise (Horowitz, 2003). Studies confirmed that HIIT could cause higher blood catecholamine levels than MICT, as well as take a longer time to return to a resting level of catecholamines after exercise (Williams et al., 2013; Evans et al., 2016; Verboven et al., 2018). However, although some studies have compared HSL protein expression in muscle and adipose tissue between MICT and HIIT (Samaneh and Nikooie, 2016; Sun et al., 2020), to our knowledge, the catecholamine-mediated HSL phosphorylation and lipolysis were not studied profoundly.

Besides the post-exercise effect, long-term training can also improve the lipolytic capacity of adipose tissue (Romijn et al., 1993). Fasting can activate fat mobilization by the catecholamines-β_3AR pathway to release more NEFA for energy balance. Obesity and high-fat diets (HFDs) can make adipocytes resistant to catecholamines, which makes HSL lipolysis activation more difficult (Gaidhu et al., 2010); however, long-term aerobic training can improve this resistance to catecholamines (Moro et al., 2009). Endurance training can improve the sensitivity of adipocytes to catecholamines and increase HSL phosphorylation in rodents (Snook et al., 2017) and humans (Bertholdt et al., 2018). The mechanism of HIIT reducing fat is often considered to be related to the recovery period after exercise. However, no study had compared whether HIIT can better relieve the catecholamines resistance of adipocytes and improve the fat mobilization ability when compared to MICT.

In summary, the mechanism of a better weight loss effect with HIIT, when compared to MICT, may be related to the higher level of post-exercise HSL lipolysis after single acute training. It may also be related to long-term HIIT improving the sensitivity of adipocytes to catecholamines, which enhances the lipolysis of HSL. Therefore, based on the two potential processes about acute and sustained effect, the purpose of this study was to test two hypotheses: (1) acute HIIT induces stronger HSL phosphorylation than MICT after exercise and (2) long-term HIIT improves the catecholamines resistance of adipocytes caused by HFD, and increases HSL phosphorylation and lipolysis more than MICT. In this study, treadmill training animal model was used to compare the effects of HIIT and MICT on HSL phosphorylation and lipolysis in adipose tissue. These results would provide new evidences to explain the underlying mechanisms about HIIT fat loss effect.

MATERIALS AND METHODS

Study Design

The study design was shown in **Figure 1**. Since it is well-known that the distribution of α and β_3ARs, which receive

catecholamine lipolysis signal, is not exactly same between sexes (Zouhal et al., 2013), and most of our previous HIIT human studies involved young women (Zhang et al., 2017, 2020), for maintaining research continuity, female C57Bl/6 mice (4 weeks old, Vital River Laboratories) were selected as animal models. Mice were housed, one per cage, under controlled temperature conditions (20–24°C) with a 12 h light/ dark cycle. The control group (C, n = 8) was randomly selected and fed standard mice chow, while the remaining mice (as obesity model group, OM) were fed a HFD (60% standard chow, 16% sugar, 5% fat, 18% egg yolk powder, and 1% sodium cholate). The control group was set up as the basis for comparison to determine the effect of a HFD. After 14 weeks, when the body mass of every mouse was at least 10% higher than the mean value of the C group, mice in the OM group were considered to be established as obese animal models. Three separate experiments were executed: (1) An acute exercise experiment to observe post-exercise changes in HSL activity. Forty-nine OM mice were randomly divided into control (HFD-Rest, n = 7), MICT (-0, -1, and -12HR, n = 7 each), and HIIT (-0, -1, -12HR, n = 7 each) groups, and each group was subjected to one-time exercise; (2) A long-term exercise experiment to observe the adipocyte adaptive changes of HSL activation. Twenty-one OM mice were randomly divided into 3 -L groups (HFD-L, MICT-L, and HIIT-L, n = 7 each), which were subjected to long-term exercise. HSL expression and phosphorylation were tested after 12 h of fasting; (3) An *in vitro* catecholamines stimulation experiment to observe catecholamine-induced lipolysis and HSL activation of adipocytes after long-term exercise. Eighteen OM mice were randomly divided into 3 -C groups (HFD-C, MICT-C, and HIIT-C, n = 6 each) for primary adipocyte isolation, and each were subjected to long-term exercise. The quantity of triglyceride bonds and HSL were tested after catecholamines stimulation. The intake of food and water was free, and no statistical difference was found between HFD groups during the study period. Random numbers generated by Excel 14.0 were used to perform the randomizations. All experimental procedures were approved by the Ethics Committee of Hebei Normal University.

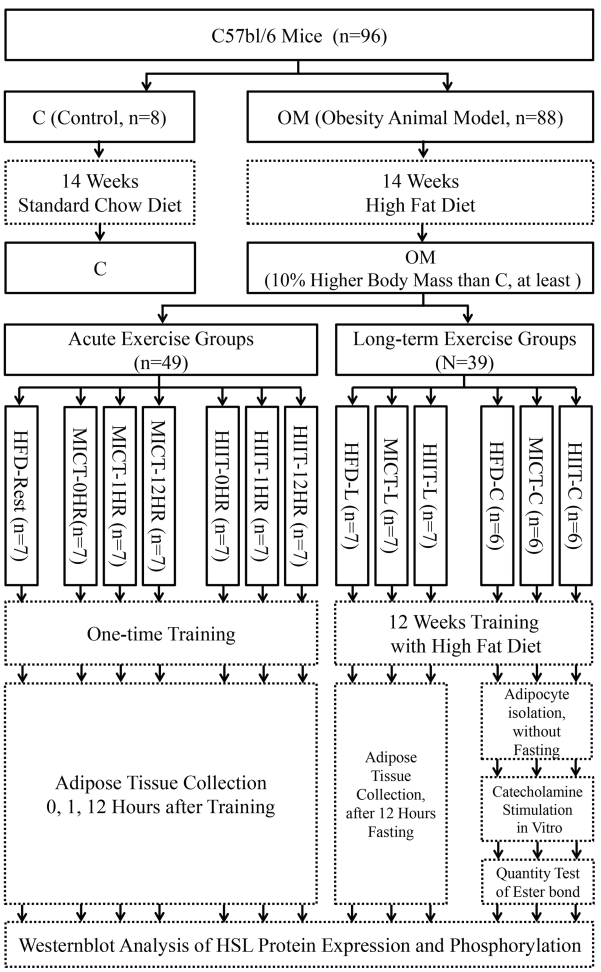

FIGURE 1 | Study design. Experimental groups are shown in solid boxes. Contents of intervention and test are shown in dotted boxes. The suffix "-nHR" means "n hours after acute exercise groups," "-L" means "long-term training groups," and "-C" means "primary adipocyte isolation groups after long-term training."

Exercise Protocol

First, MICT and HIIT mice were adapted to a treadmill for 3 days (10 min/day, 5 m/min, 0°). Training rodents were subjected to a 25° uphill treadmill training once (acute exercise groups) or for 12 weeks (-L and -C groups), while HFD mice rested as a control. HIIT consisted of a series of running for 1 min at maximum speed followed by 1 min of running at medium speed (1–1 min cycle). Running speeds were determined according to an incremental exercise test (IET), based on previous research (Høydal et al., 2007; Pereira et al., 2012; Dehghani et al., 2018). The initial velocity of IET was 6 m/min, after which it increased by 3 m/min for 3 min until exhaustion, which was defined when mice could not keep up with the velocity five times in 1 min despite being physical poked. The maximum running velocity of HIIT equaled the exhaustion speed (ES) of last stage in IET. The interval medium speed of HIIT and the continuous speed of MICT both equaled 55% of ES. One MICT training lasted for 45 min. In order to match the exercise volume, the number of 1–1 min cycles in each HIIT was calculated based on the speeds of MICT to ensure equal running distance. Mice of long-term groups were trained 5 days continuously and given 2 days rest in each week. An IET was conducted every 2 weeks to ensure that running speed would accommodate growth. The running speeds of MICT increased from 13 to 17 m/min and maximum running speeds of HIIT increased from 23 to 31 m/min throughout the training period.

Sample Collection

All mice were sacrificed after anesthesia (pentobarbital sodium, Solarbio, I.P.). Mice of the -0, -1, and -12HR groups were killed at 0, 1, or 12 h, respectively, after one-time training to determine the peak or recovery period of catecholamines

secretion (Williams et al., 2013; Sgrò et al., 2014). After training ended at 12 weeks, mice of -L groups would rest for 48 h, fast for 12 h, and be killed successively. Mice of -C groups would rest for 60 h without fasting and be killed. The inguinal and periuterine (around the uterus) fat pads were used to represent subcutaneous WAT (SCAT) and visceral WAT (VAT). Gastrocnemius samples of -L groups were collected to represent skeletal muscles. Briefly, fat pads and muscle samples were extracted, and fat pads were weighed. Five samples of each -L group were randomly selected for H&E staining. Center pieces were cut-off and fixed with 4% paraformaldehyde to avoid being torn. Rest tissues of -L group and the remaining fat pad tissues from acute exercise groups were frozen in liquid nitrogen and stored in the −80°C refrigerator (DW-86, Haier) for western blot analysis and a muscle TG content test. Samples of -C group were minced and put into tubes containing phosphate-buffered saline (PBS, Solarbio) for primary adipocyte isolation.

Histological Observation of Adipose Tissue

The volume of adipocytes was observed by H&E staining. Briefly, tissues were fixed with 4% paraformaldehyde (Solarbio) for 48 h, embedded in paraffin, and cut into 5 μm sections. After xylene dewaxing and alcohol rehydrating, samples were subsequently stained with hematoxylin (Solarbio) for 5 min, washed with water and 0.6% ammonia, stained with eosin (Solarbio) for 2 min, and dehydrated with alcohol. Finally, sections were sealed with resin (Solarbio) and photographed using an optical microscope. At least, 150 adipocytes per group were analyzed for cell surface areas by ImageJ 1.51, and the investigator was blinded with regard to group allocation. Overall results were represented by representative images.

Primary Adipocyte Isolation and Catecholamine-Induced Lipolysis Test *in vitro*

To observe the adaptive changes after long-term training, we tested the catecholamine-mediated lipolysis ability of adipocytes. NE was selected to represent catecholamines for stimulating adipocyte, since it can be secreted by both sympathetic nerves and adrenal glands. After adipocytes were isolated from fat pads and stimulated by physiological concentrations of NE *in vitro*, the quantity of triglyceride bonds (ester bond, "-O-CO-"), which could be hydrolyzed by HSL, was determined by Fourier Transform Infrared (FTIR) spectroscopy. Each triglyceride molecule contains three triglyceride bonds. The physiological function of HSL is to hydrolyze triglyceride bonds to carboxyl ("-COOH") and hydroxyl ("-OH") and it can convert triglyceride into glycerol and fatty acids; therefore, the reduction of triglyceride bonds represents the ability of HSL to hydrolyze TG bonds. Adipocyte isolation and epinephrine stimulation were carried out according to Gaidhu et al. (2010) and followed by FTIR spectroscopy (Kucuk Baloglu et al., 2017). Briefly, tissues were digested with collagenase (Solarbio) for 30 min at 37°C with gentle agitation, centrifuged (500 *g*, 5 min), washed by collagenase-free PBS (three times), and filtered through a nylon mesh (Jingan Bio). Adipocytes were washed off, collected, and equilibrated for 30 min before the experiment. Isolated cells were transferred to a medium containing fetal bovine serum (10%, Tianhang Biotechnology) and epinephrine hydrochloride (100 nM, Sigma) at 37°C for 75 min. After epinephrine stimulation, the cells were collected, lysed by RIPA (Solarbio), and centrifuged. The lipid layer above the solution was extracted using chloroform (1:1.5). Extraction solutions were mixed with anhydrous sodium sulfate (1:0.05) and allowed to stand for 2 h for dehydration. Samples were scanned on a FTIR spectrometer (Vertex 70, Bruker) over the 370–4,000 cm^{-1} spectral range. A peak near 1,740 cm^{-1} represented the amount of triglyceride bonds present. The area under the curve (AUC) in the range of 1,710–1,770 cm^{-1} was calculated to quantify the presence of triglyceride bonds. Spectral curve drawing and ACU calculation were performed using OriginPro 8 (Origin Lab Corporation). For quality control, three randomly selected samples were measured three times and the obtained Intraclass correlation coefficient was 0.98 (good reliability).

Western Blot Analysis for Adipose Tissue

In order to observe the post-exercise activity and long-term exercise induced adaptation of HSL, protein expression and phosphorylation of VAT, SCAT, and isolated adipocytes were tested after epinephrine stimulation (see details in *in vitro* Lipolysis Test part). Chopped tissue and isolated cells were homogenized with RIPA buffer (Solarbio) supplemented with protease and phosphatase inhibitor (Thermo Fisher Scientific). After the homogenate was centrifuged (4°C, 14,000 *g*, 10 min), the lower layer was extracted and boiled at 98°C for 5 min. Samples were electrophoresed in 12% SDS-PAGE gel and transferred onto polyvinylidene difluoride membranes (Millipore). Primary antibodies were used to detect HSL, HSL-Ser563, and HSL-Ser660 (#4107, #4139, and #4126, Cell Signaling Technology, all by dilution of 1:2,000). Goat anti-rabbit IgG secondary antibody (GB23303, Servicebio, by dilution of 1:5,000) was used for luminescence. Actin (primary antibody No. AP0060, Bioworld Tech, by dilution of 1:5,000) was used as the loading control. The ECL (Solarbio) excited luminescence was collected and analyzed by the gel imaging system (Fusion Fx5-xt, VILBER LOURMAT). Overall results were represented by representative bands.

Test of Skeletal Muscle TG Content

Triglyceride content of skeletal muscle was tested using the GPO-PAP enzyme method. Chopped muscle samples were homogenized with RIPA buffer (Solarbio) and tested using TG kits (Prod No. A110-1-1, Jiancheng Bioengineering Institute). For quality control, seven randomly selected samples were measured two times and the obtained Intraclass correlation coefficient was 0.77 (good reliability).

Statistical Analyses

Data are presented as means ± SD. Differences in body mass, fat pad weight, muscle TG, spectral AUC, HSL protein expression,

and phosphorylation of -L group were analyzed using a one-way ANOVA. Differences of HSL in acute exercise groups were analyzed with a two-way ANOVA (time and training type as two factors). Main effect and least significant difference (LSD) *post hoc* tests were used to compare treatments when no significant interaction was found. Statistical significance was set at $p < 0.05$. According to the data of fat pad mass in previous experiments, the minimum sample size is 4 ($\alpha = 0.05$, power = 0.8).

RESULTS

HSL Protein Expression and Phosphorylation 0–12 h After Acute Training

To compare the post-exercise effects of MICT and HIIT on lipolysis activity of HSL, we observed the protein expression and phosphorylation of Ser563 and Ser660 at 0, 1, and 12 h after one-time acute exercise (**Figures 2A–F**). Except for the significant increase of VAT in MICT-0HR group, HSL expressions at 0–12 h after acute exercise were not significantly different from sedentary mice (HFD-Rest), regardless of VAT or SCAT (**Figures 2A,D**). VAT ser563 of MICT-0HR, HIIT-0HR, and MICT-1HR significantly increased when compared to HFD-Rest. MICT groups overall had significantly more VAT ser660 than HIIT, while only MICT-0HR had significantly more compared to the resting group. Unlike VAT, HSL phosphorylation, both Ser563 and Ser660, of SCAT in HIIT groups was significantly higher than that found in MICT groups, while no difference was found between MICT groups and the resting group, with the exception of MICT-12HR for Ser660. For Ser563 and Ser660, HSL phosphorylation in HIIT groups was significantly higher than the resting group, except at 12 h for Ser563. To summarize, in SCAT, HSL phosphorylation in HIIT mice was higher than in MICT mice, but in VAT, the phosphorylation in HIIT mice was lower than in MICT mice at 1 h after exercise.

Changes in Body Mass, Fat Pad Weight, and Adipocyte Morphology After 12 Weeks of Training

To compare the effect of catecholamines resistance by HIIT and MICT, OM mice were first fed a HFD for 14 weeks to establish obese animal models. After 14 weeks, the average

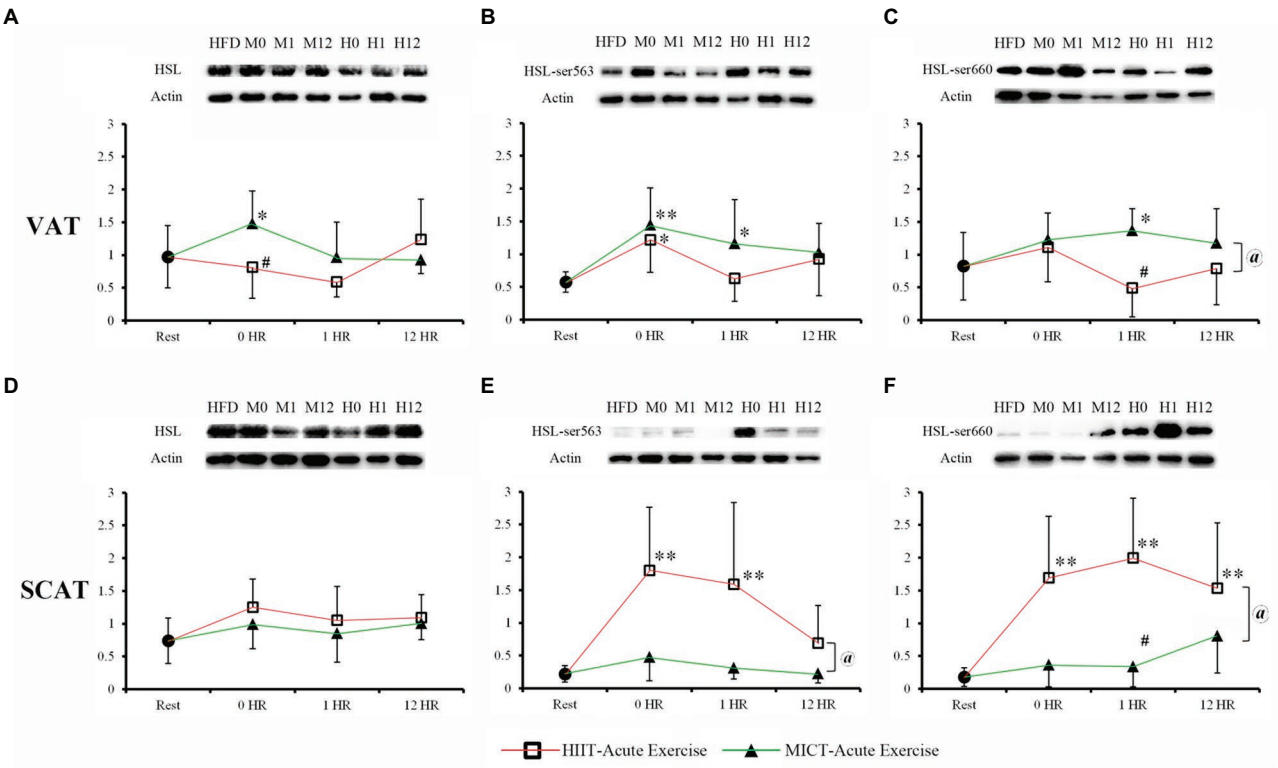

FIGURE 2 | Hormone sensitive lipase (HSL) protein expression and phosphorylation of visceral WAT (VAT; **A-C**) and SACT **(D-F)** after one-time acute exercise. High-fat diet (HFD) or rest: HFD-Rest group, set as baseline without training; M0, M1, and M12: moderate-intensity continuous training (MICT)-0HR, -1HR, and -12HR; H0, H1, and H2: high-intensity interval training (HIIT)-0HR, -1HR, and -12HR; $^{*}p < 0.05$ vs. rest; $^{**}p < 0.01$ vs. rest; $^{\#}p < 0.05$ HIIT vs. MICT; and @Main effect of the training type (MICT vs. HIIT) $p < 0.05$, when no significant interaction between two factors (training type and time point) was found. Values of p between HFD-Rest and acute exercise groups were calculated by one-way ANOVA, and values of p between MICT and HIIT were calculated by 2 × 3 two-way ANOVA. Bands shown are representative for overall results.

body mass of OM was 20% higher than the control group (standard chow diet), even the lightest mouse of OM was 12% higher (**Figure 2A**). To compare the weight loss effects of HIIT and MICT, 21 mice were randomly selected from the OM group and divided into HFD-L, MICT-L, and HIIT-L for 12 weeks of training (**Figure 1**). No body mass difference between three groups before training was found (**Figure 2A**).

After 12 weeks of exercise, the average body mass of HIIT-L and MICT-L was 22 and 15% lower than HFD-L (sedentary, both $p < 0.01$), respectively, while no significant difference was found between HIIT-L and MICT-L (**Figure 3A**). Inguinal fat weights of HIIT-L and MICT-L, as well as periuterine fat weights, were significantly lower than HFD-L ($p < 0.05$ or $p < 0.01$, **Figure 3B**). Periuterine (but not inguinal) fat weights of HIIT-L were lower than MICT-L ($p < 0.01$, **Figure 3B**). H&E staining showed that both visceral and subcutaneous adipocyte sizes of exercise groups were lower than HFD-L and SCAT adipocyte sizes of HIIT-L were lower than MICT-L ($p < 0.05$ or $p < 0.01$, **Figures 4A–H**). These results suggest that long-term training protocols can reduce body mass, fat weight, and adipocyte sizes, and HIIT showed stronger visceral fat mass reducing effect compared to MICT.

Skeletal Muscle TG Content

To test if weight loss with high-intensity exercise is due to the redistribution of hydrocarbon sources between fat and skeletal muscle, we tried to examine the TG content increase of skeletal muscle and decrease of fat pad weight in the -L group. After long-term training, TG of MICT-L and HIIT-L was significantly lower than HFD-L. HIIT-L showed a tiny higher trend than MICT-L, but without significant difference ($p = 0.062$, see **Figure 5**).

HSL Protein Expression and Phosphorylation During Fasting After 12 Weeks of Training

To compare the effects of long-term HIIT and MICT on fat mobilization, we observed HSL protein expression and phosphorylation of -L groups during fasting (**Figures 6A–C**). In VAT, after 48 h resting and 12 h fasting, Ser563 phosphorylation of HIIT-L was significantly higher than HFD-L and MICT-L (**Figure 6B**), even though HSL expression and Ser660 phosphorylation were similar among these groups. No significant differences were found in SCAT. This suggests that with the same hunger stimulation, HSL-Ser563 of VAT adipocytes can be phosphorylated easier after long-term HIIT.

HSL Phosphorylation and Triglycerides Bonds Quantity of Isolated Adipocytes After Incubation With Epinephrine

To determine if long-term HIIT is more effective than MICT at improving HSL-mediated fat mobilization, we compared Ser563 and triglyceride bonds of adipocytes with epinephrine stimulation (to imitate fasting *in vitro*). We found that fasting-induced HSL-Ser563 phosphorylation of VAT significantly more in the HIIT-L group. **Figure 7A** shows a complete FTIR spectrum, including a peak near 1,740 cm^{-1} representing the quantity of triglyceride bonds. Peaks of all triglyceride bonds are overlapped and most peak heights of HIIT-C are lower than the average

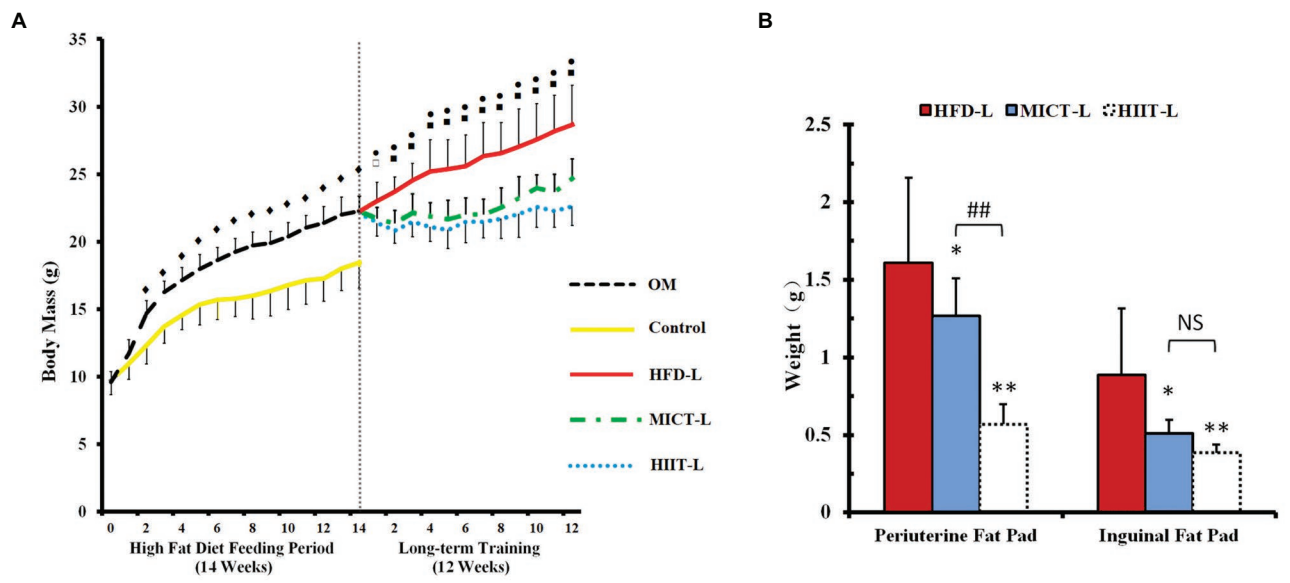

FIGURE 3 | Body mass **(A)** and fat pads weights **(B)** of long-term exercise groups. Periuterine fat pad represents visceral fat, while inguinal pad represents subcutaneous fat. Data are mean ± SD. **(A)** $^{\blacklozenge}p < 0.01$ OM vs. C, $^{\bullet}p < 0.01$ HIIT-L vs. HFD-L, $^{\circ}p < 0.05$ MICT-L vs. HFD-L, $^{\blacksquare}p < 0.01$ MICT-L vs. HFD-L, and no significant difference of body mass between MICT-L and HIIT-L. **(B)** $^{*}p < 0.05$ vs. HFD-L, $^{**}p < 0.01$ vs. HFD-L, $^{\#\#}p < 0.01$ MICT-L vs. HIIT-L, and NS, no significant difference.

height of HFD-C and MICT-C, in both VAT and SCAT (**Figures 7B,C**). ANOVA of AUC showed both VAT and SCAT of HIIT-C were significantly lower than HFD-C ($p < 0.05$ or $p < 0.01$, **Figures 7D,E**), and VAT of HIIT-C was also lower than MICT-C ($p < 0.01$, **Figure 7D**). Ser563 of HIIT-C was significantly higher than in HFD-C and MICT-C and was similar to changes after fasting in VAT. In SCAT, both Ser563 of MICT-C and HIIT-C were higher than in HFD-C, although they were not significantly different (**Figure 7E**).

DISCUSSION

This study compared HIIT and MICT for acute and sustained effects involved in the regulation of weight loss and lipolysis. The major findings of this study were that: (1) one-time acute HIIT can increase HSL lipolysis activity of SCAT more than MICT after exercise and (2) compared with MICT, long-term HIIT can increase visceral adipocytes lipolysis sensitivity to catecholamines, and increase HSL-Ser563 phosphorylation of VAT during fasting or catecholamines stimulation. Incidentally, we found that the TG content of skeletal muscle in HIIT mice showed tiny higher trend ($p = 0.062$) than that of MICT mice after long-term training, but not lower. It suggested that increased lipolysis caused by HIIT during a recovery period or fasting may cause redistribution of TG from adipose tissue to muscle for increasing energy substrate storage, which may be a kind of "training paradox" (Russell, 2004). However, since no statistical difference was found, future studies are needed to explore this phenomenon.

Previous studies focused on comparing the protein expression of HSL in muscle or adipose tissue between MICT and HIIT (Samaneh and Nikooie, 2016; Sun et al., 2020), and very few studies have compared the HSL phosphorylation and lipolysis during fasting or after catecholamine stimulating. Our findings may explain why HIIT has comparable subcutaneous and visceral fat reducing effects, even though the fat oxidation rate of high-intensity training is lower than moderate-intensity training during exercise.

HIIT Has Similar Body Mass Controlling Effects and Better Visceral Fat Reduction Effects Than MICT

From the perspective of the energy substrates proportion, MICT has the highest fat consumption rate during exercise. However, some human studies have shown that HIIT can achieve a similar fat loss effect than MICT and be more time efficient (Keating et al., 2017; Viana et al., 2019). Since visceral fat accumulation is a risk factor for a number of metabolic diseases, researchers have also observed the effectiveness of HIIT and MICT in reducing visceral fat in human trials. Some human studies found that HIIT reduced visceral fat similarly to MICT (Zhang et al., 2017; Tong et al., 2018), while others showed that HIIT can reduce more visceral fat (Maillard et al., 2016; Zhang et al., 2020). Animal studies have shown similar results. Wang et al. (2017) showed that of mice fed a HFD, the HIIT group had a lower body fat percentage than the MICT group. Similarly, it has been shown that HIIT could significantly reduce the abdominal and epididymal fat mass in Zucker rats (a genetic model of obesity) and HFD-fed SD rats, when compared to MICT (Kapravelou et al., 2015; Shen et al., 2015; Maillard et al., 2019). The present results showed similar results to some previous studies. In 12 weeks of training, HIIT and MICT mice lost a similar amount of body mass and inguinal fat weight; however, the periuterine fat weights of HIIT groups were lower than MICT groups. This data indicate that long-term HIIT has a similar effect on controlling fat accumulation as MICT and could reduce more visceral fat.

The mechanisms, by which HIIT can reduce body fat equally and visceral fat mass more efficiently than MICT, are still unclear. This study focused on HSL, a key enzyme for fat mobilization and catecholamine-induced lipolysis. Here, we explain this mechanism with the post-effect of acute exercise and adaptive changes of long-term training of fat mobilization caused by HIIT.

The Post-exercise Effect of HIIT and MICT: More HSL Lipolysis Activation of Subcutaneous Fat 0–12 h After Acute Exercise

The mechanism of HIIT reducing fat is often considered to be related to the recovery period after exercise (Gentil et al., 2020). A frequently proposed viewpoint is that HIIT can cause higher excess post-exercise oxygen consumption (EPOC) during a recovery period, which makes the total energy expenditure higher than during MICT. Some studies support this view (Malatesta et al., 2009), while others found HIIT caused EPOC similar to that of MICT (Moniz et al., 2019). Another viewpoint is that because HIIT consumes large amounts of glycogen during exercise, fat is oxidized during the recovery period to resynthesize glycogen (Kiens and Richter, 1998). The third viewpoint is that TGs are redistributed from adipose tissue to muscle because of the stronger stimulation of muscle with HIIT, a process related to the "endurance paradox" (Kuo and Harris, 2016). Although the mechanisms underlying these three viewpoints are different, either the increase of EPOC, synthesis of glycogen, or the redistribution of TG requires higher fat mobilization to provide sufficient NEFA. This study attempted to test the regulation of post-exercise HSL lipolysis and expected to provide helpful evidence for the above viewpoints.

Due to the wide substrate specificity and integration of extra and intracellular signals, the activity of HSL could be regarded as a representative of fat mobilization. HSL can be activated by many signals to catalyze the hydrolysis of TAG, DAG, and MAG, through site-specific serine phosphorylation of Ser563, Ser565, and Ser660 (Kim et al., 2016). Firstly, lipolytic hormones transported through blood, such as catecholamines, growth hormone (GH), and atrial natriuretic peptide (ANP) can bind to the membrane receptors of adipocytes and activate the cAMP-PKA or cGMP-PKG pathways and phosphorylate Ser563 and Ser660 to increase the lipolysis activity of HSL. Secondly, sympathetic nerve endings in adipose tissue can also secrete NE and promote lipolysis of HSL. Thirdly, AMPK, an important intracellular energy receptor, can inhibit HSL activity through Ser660 phosphorylation (Stallknecht et al., 2001; Thompson et al., 2012; Braun et al., 2018). It is reasonable to assume that HIIT can also cause a greater post-effect increase in HSL-Ser563 and

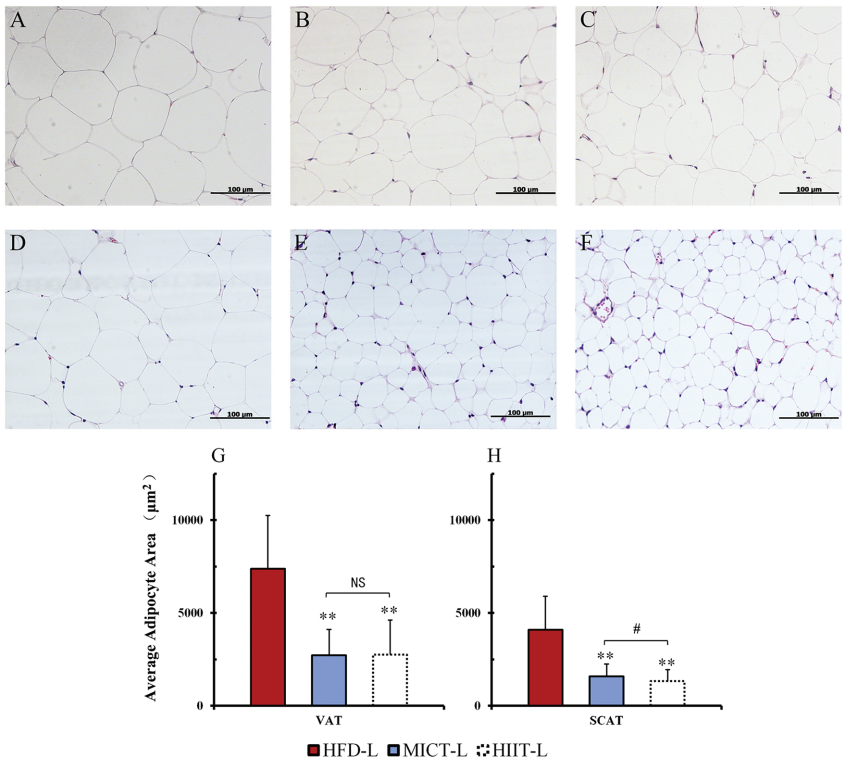

FIGURE 4 | Adipocyte sizes of different fat depots. Periuterine adipocytes (VAT) of HFD-L **(A)**, MICT-L **(B)**, and HIIT-L **(C)**, as well as inguinal adipocytes (SCAT) of HFD-L **(D)**, MICT-L **(E)**, and HIIT-L **(F)** were shown by H&E staining; Average adipocyte area **(G,H)**; $^{**}p < 0.01$ vs. HFD-L, $^{\#}p < 0.05$ MICT-L vs. HIIT-L, and NS, no significant difference. Images shown are representative for overall results.

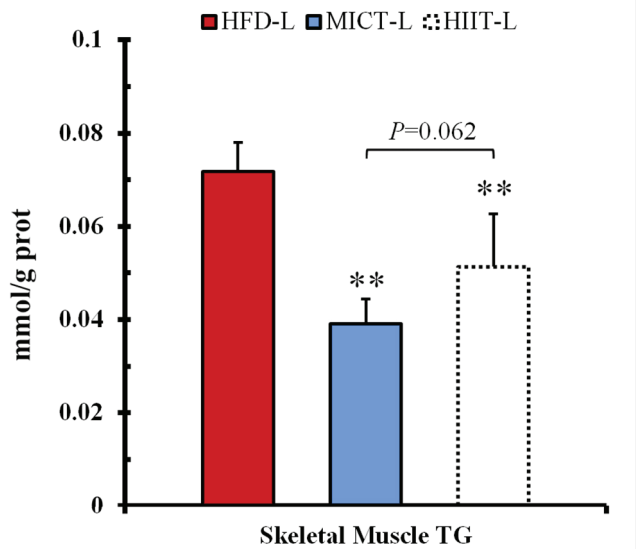

FIGURE 5 | Skeletal muscle triglyceride (TG) content. $^{**}p < 0.01$ vs. HFD-L. Values of *p* were calculated by one-way ANOVA.

Ser660 phosphorylation and lipolysis, since intensity is a more important factor than duration of altering catecholamines responses to exercise (Zouhal et al., 2008, 2013). HIIT had been shown to cause high levels of catecholamines secretion within 1–3 h after acute exercise (Williams et al., 2013; Evans et al., 2016; Verboven et al., 2018) and the elevation of catecholamines caused by MICT can return to a resting level within 0–1 h, while the increase caused by HIIT did not recover for 1–3 h (Williams et al., 2013; Sgrò et al., 2014). In our study, HSL expression and phosphorylation were recovered to resting level after 12 h in both MICT and HIIT group, except SCAT Ser660 phosphorylation of HIIT mice. Two-way ANOVA analysis showed that there was no significant interaction effect between training type and time, indicating that the post-exercise change trends of HSL expression and phosphorylation in HIIT groups and MICT groups were similar over time. However, the results of VAT ser660, SCAT ser563, and SACT ser660 (**Figures 2C,E,F**) showed significant main effects of training types, indicating that HIIT and MICT may have different effects on post-exercise HSL phosphorylation except VAT ser563.

Because visceral fat is more sensitive to catecholamines than subcutaneous fat (Davies et al., 1974), we also assumed that highest HSL phosphorylation after acute exercise should be observed in visceral fat of HIIT mice. Unexpectedly, at 0–1 h after exercise, MICT increased HSL expression and phosphorylation of both Ser563 and Ser660 of VAT, while HIIT did not. At 0–1 h after exercise, HIIT increased HSL phosphorylation of SCAT (but not VAT) by 5–10-fold, while MICT did not. Even after 12 h, the SCAT Ser660 phosphorylation of HIIT mice was still

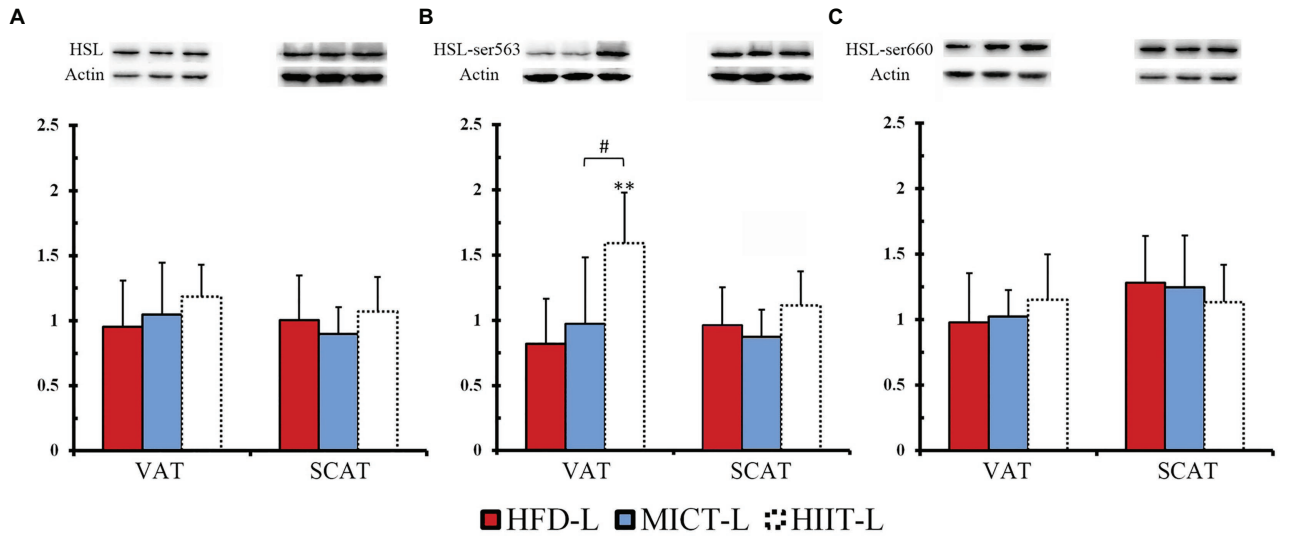

FIGURE 6 | HSL protein expression **(A)**, phosphorylation of ser563 **(B)** and ser660 **(C)** with 12 hours of fasting and after 12 weeks of MICT or HIIT. $^{**}p < 0.01$ vs. HFD-L; $^{\#}p < 0.05$ HIIT-L vs. MICT-L. Values of p were calculated by one-way ANOVA. Bands shown are representative for overall results.

higher than resting level. This indicated that MICT might activate HSL lipolysis of VAT during exercise (**Figure 2A**), which lasted more than 1 h after acute exercise (**Figure 2C**), while HIIT may have longer post-effect on HSL lipolysis of SVAT (**Figures 2E,F**).

Our study showed that MICT can activate HSL lipolysis 0–1 h after exercise and this was consistent with previous studies (Watt et al., 2006; Ogasawara et al., 2010); however, few studies have compared the post-effects of HIIT and MICT. Unexpectedly, HIIT activated HSL of SCAT, but not VAT, which was more sensitive to catecholamines. The inhibition of αAR probably played a key role during this process. It should be noted that the regulation of lipolysis by blood catecholamine concentration has two sides: low-to-medium-intensity exercise can cause a modest increase in catecholamines, which activates HSL phosphorylation and lipolysis through the β_3AR-Gs protein pathway. The lipolysis rate reaches the maximum at about 65% VO_2max (Horowitz, 2003). However, when the catecholamines concentration is too high, the αAR-Gi protein pathway is activated, thereby inhibiting HSL phosphorylation and lipolysis (Frayn, 2010). During the first hour after exercise, HIIT caused excessively high concentrations of catecholamines, which inhibited HSL of VAT by αAR; however, because the sensitivity of SCAT was lower, high concentrations of catecholamines caused by HIIT did not activate αAR of SCAT, and still activated β_3AR and caused a post-exercise increase of HSL lipolysis.

Another problem is that the high blood catecholamines concentration caused by acute HIIT can only last for 1–3 h. It is unclear why HSL-Ser660 of SCAT in the HIIT group remains 5-fold higher than the resting level at 12 h after acute exercise. Another study had found that the level of phosphorylation in HSL was still very high 3 h after MICT (Ogasawara et al., 2010), even though the catecholamines concentration had recovered at this time point. Some studies have also shown that lipolysis continues to increase 24 h after acute exercise (Magkos et al., 2009). These results indicate that the blood catecholamines concentration was not the only regulatory signal for post-exercise HSL lipolysis. Lipolysis activity of HSL is also regulated by other signals such as sympathetic nerves and natriuretic peptides (Braun et al., 2018), and the increase in Ser660 at 12 h after acute HIIT may due to these long-lasting signals.

In summary, HIIT can induce stronger post-exercise HSL phosphorylation in SCAT, which may explain why HIIT and MICT have similar effect on reducing subcutaneous fat. However, the post-effect of HSL on VAT by HIIT is weak and could not explain the phenomenon that HIIT can reduce more visceral fat than MICT.

Adaptation Changes to Long-Term Training: HIIT Can Improve the Catecholamines Resistance of Adipocytes Caused by a HFD and Increase HSL Lipolysis Activity

Another hypothesis that might explain the fat loss by HIIT, besides acute effects after exercise, are the adaptive changes of adipose tissues caused by long-term training (sustained effects). A HFD can cause catecholamines resistance in adipocytes, which makes lipolysis more difficult. As a result, it is difficult to hydrolyze TG in adipocytes to glycerol and NEFA during fasting or exercise. Gaidhu et al. (2010) found that, although the cAMP-PKA pathway was not affected in mice fed a HFD for 8 weeks, HSL was difficult to be phosphorylated under physiological catecholamines concentrations, indicating that a HFD reduced the sensitivity of adrenergic receptors of adipocytes to catecholamines. Exercise intervention studies have shown that MICT can improve this resistance and increase HSL activity of both humans and rodents (Zouhal et al., 2008; Snook et al., 2017; Bertholdt et al., 2018), and compared with MICT, long-term HIIT could increase HSL

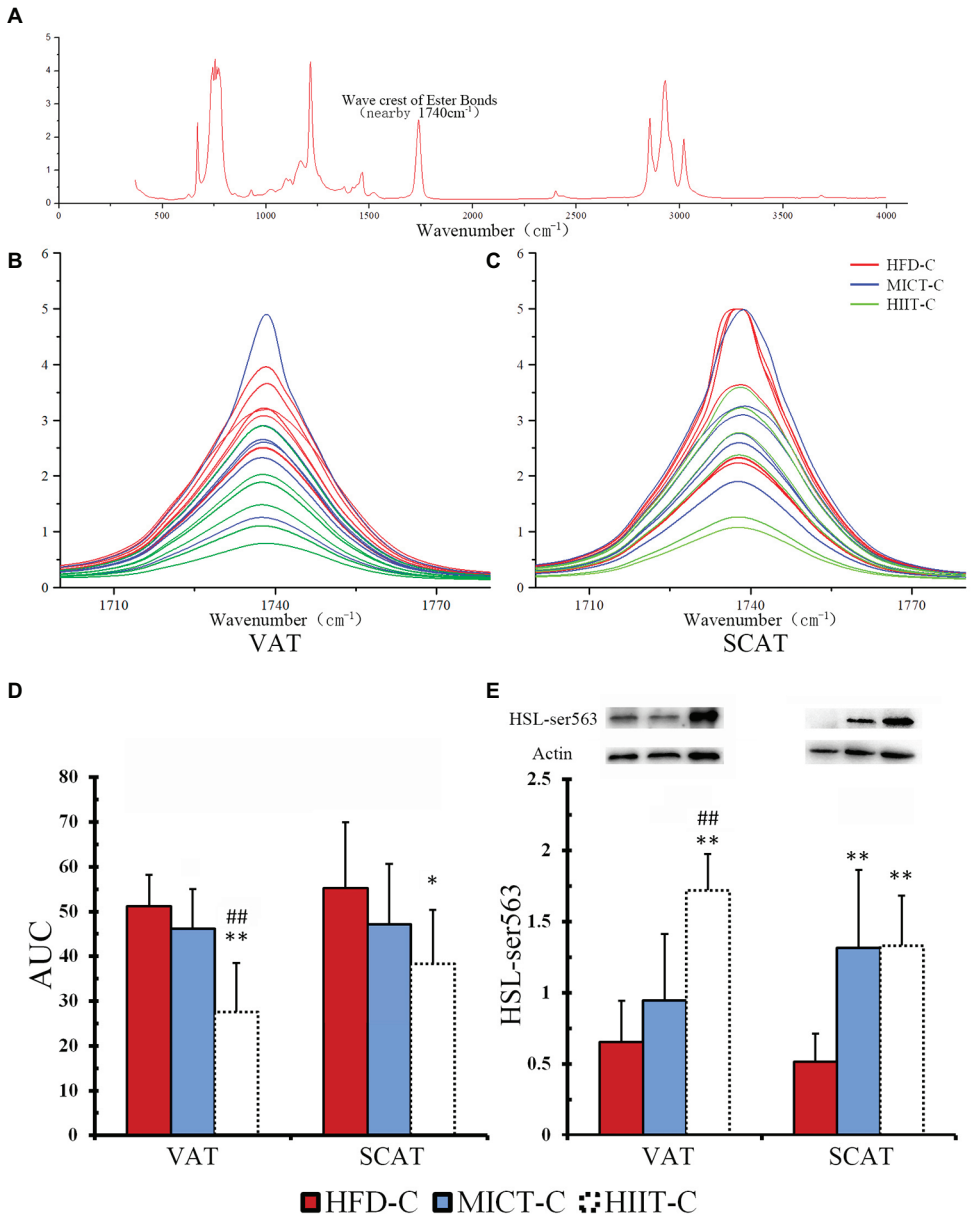

FIGURE 7 | A complete FTIR spectrum **(A)** is shown, including a peak nearby 1,740 cm^{-1} representing the quantity of triglyceride bonds. Peaks of VAT **(B)** and SCAT **(C)** are overlapped to show the difference between HFD-C, MICT-C, and HIIT-C. The area under the curve (AUC), representing the quantity of triglyceride bonds. Differences are shown in the amount of triglyceride bonds **(D)** and HSL-Ser563 phosphorylation **(E)** between HFD-C, MICT-C, and HIIT-C after physiological concentration of epinephrine stimulation (simulated fasting *in vitro*). $^{*}p < 0.05$ vs. HFD-C; $^{**}p < 0.01$ vs. HFD-C; and $^{\#\#}p < 0.01$ HIIT-C vs. MICT-C. Bands shown are representative for overall results.

expression higher in visceral fat of aged rats (Sun et al., 2020). However, there are few studies comparing the differences between HIIT and MICT in improving catecholamines resistance.

We assumed that long-term HIIT improved catecholamines resistance better than MICT. When mice in the HIIT group were exercising or starving, adipocytes could release more NEFA under catecholamines stimulating, and the body could use more NEFA instead of glucose to maintain energy balance. The data from the -L and -C groups of this study supported this hypothesis. After 12 weeks training, the -L mice fasted for 12 h. At this time point, HSL-Ser563 phosphorylation of VAT in the HIIT-L group increased significantly, which did not happen in the HFD-L or MICT-L groups. This means that with the same fasting period, visceral fat HSL lipolysis in HIIT mice was stronger. To further verify whether higher HSL lipolytic activity is associated with improved catecholamines resistance, visceral and subcutaneous adipocytes of -C groups were isolated after 12 weeks of training without fasting and

stimulated by catecholamines of the same physiological concentration. We found that HSL-Ser563 phosphorylation of VAT in the HIIT-C group was significantly higher than in the MICT-C group, while the phosphorylation of SCAT was similar in both groups. The FTIR spectrum data showed that the triglyceride bonds of VAT in HIIT-C groups were lower than in MICT-C groups, indicating that lipolysis was more intense. These data indicated that long-term HIIT improved HFD-induced catecholamines resistance of VAT more than MICT. Better catecholamines sensitivity of VAT means stronger lipolysis under external stimuli, which explains why long-term HIIT reduced more visceral fat than MICT in this experiment. It should be noted that a recent study compared the phosphorylation of visceral fat HSL in a resting state (without fasting) after long-term HIIT and MICT, and found no difference between the two protocols (Maillard et al., 2019). This suggested that long-term HIIT only improves the sensitivity of VAT to catecholamines, rather than HSL lipolytic activity when in a resting state without fasting or exercise. In summary, -L and -C groups showed that visceral fat HSL of long-term HIIT mice is more likely to be activated by fasting or catecholamines stimulation because it causes more TG to be hydrolyzed and more NEFA is released for fat oxidation, gluconeogenesis, or redistribution to skeletal muscle. This phenomenon may be an important mechanism to explain why HIIT can reduce more visceral fat than MICT.

Further, based on the adaptive changes caused by HIIT in this study, two interesting questions could be raised: (1) was the higher muscle TG content of HIIT mice compared to MICT related to the endurance paradox in this study? High levels of intramyocellular TG correlate with insulin resistance in both obese and diabetic patients, but the "good" high muscle TG increases of endurance athletes do not cause insulin resistance, which called "endurance paradox" (Russell, 2004). Is it a "beneficial" change that mice with long-term HIIT gain higher muscle TG content than MICT? and (2) Is HIIT better at preventing obesity rebound? Previous studies have suggested that exercise cessation after MICT appeared to increase adipose accumulation (Del Vecchio et al., 2020), while HIIT showed stronger sustained effects on promoting enzymes activity associated with glycolysis and beta-oxidation pathways than MICT (Gentil et al., 2020). Like other sustained effects, could the better improvement of HIIT on sensitivity of adipocyte lipolysis imply better obese rebound prevention effect? Future studies on these questions may help improve the fat loss theory of HIIT.

Limitations

Our study had several limitations. Firstly, due to the large workload of a long experimental period (14-week to establish obese mice and 12-week for long-term training) and numerous experimental groups and animals, the normal diet groups were not set to verify whether the effects of HIIT and MICT on improving lipolysis were only seen in obese or HFD-fed mice. Secondly, due to the lower protein content of fat than other tissues, the quantity of proteins that could be extracted after primary adipocyte isolation and stimulation, were not sufficient for HSL protein expression and Ser660 phosphorylation testing, so only Ser563 of adipocytes in -C groups was tested. This choice was based on results of -L groups after fasting, as only Ser563 was significantly different between groups. Finally, it should be noted that there are indeed species-differences in the effect of sympathetic nerve and hypothalamic-adrenal axis on promoting lipolysis (Braun et al., 2018), whether the results of this study are applicable to humans remains to be verified by future studies.

Conclusion

The present study indicates that an acute HIIT could promote HSL phosphorylation of subcutaneous fat lasting at least 12 h which is longer than MICT, implying HIIT had a longer post-exercise effect on subcutaneous fat lipolysis than MICT. Long-time HIIT was confirmed to have a better effect on improving HFD-induced catecholamines resistance of visceral adipocytes. These effects allow fat to be easier mobilized by fasting, exercise, or other stimulation, which means that more NEFA can be released for energy balance. This study laid the foundation for explaining the mechanism by which HIIT has the same or better weight loss effect than MICT. Future works should explore whether enhanced fat mobilization by HIIT can cause more oxidation, gluconeogenesis, or redistribution of fat to reduce weight.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

The animal study was reviewed and approved by Ethics Committee of Hebei Normal University.

AUTHOR CONTRIBUTIONS

YL and HZ designed the study and analyzed and interpreted the data. YL, GD, XZ, and ZH collected the data. YL drafted the manuscript. HZ and GD revised the manuscript. All authors contributed to the article and approved the submitted version.

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Influence of Acute and Chronic Exercise on Abdominal Fat Lipolysis: An Update

*Claire Laurens[1,2], Isabelle de Glisezinski[1,2,3], Dominique Larrouy[1,2], Isabelle Harant[1,2] and Cedric Moro[1,2]**

[1] INSERM, UMR 1048, Institute of Metabolic and Cardiovascular Diseases, Obesity Research Laboratory, Toulouse, France, [2] Paul Sabatier University, Toulouse, France, [3] Department of Physiological Functional Explorations, Rangueil University Hospital, Toulouse, France

Exercise is a powerful and effective preventive measure against chronic diseases by increasing energy expenditure and substrate mobilization. Long-duration acute exercise favors lipid mobilization from adipose tissue, i.e., lipolysis, as well as lipid oxidation by skeletal muscles, while chronic endurance exercise improves body composition, facilitates diet-induced weight loss and long-term weight maintenance. Several hormones and factors have been shown to stimulate lipolysis *in vitro* in isolated adipocytes. Our current knowledge supports the view that catecholamines, atrial natriuretic peptide and insulin are the main physiological stimuli of exercise-induced lipolysis in humans. Emerging evidences indicate that contracting skeletal muscle can release substances capable of remote signaling to organs during exercise. This fascinating crosstalk between skeletal muscle and adipose tissue during exercise is currently challenging our classical view of the physiological control of lipolysis, and provides a conceptual framework to better understand the pleotropic benefits of exercise at the whole-body level.

Keywords: adipose tissue, lipolysis, fatty acids, exercise, weight loss

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***Correspondence:**
Cedric Moro
Cedric.Moro@inserm.fr

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INTRODUCTION

Physical exercise is one of the most effective lifestyle interventions to fight against many chronic diseases and in particular obesity and type 2 diabetes. Health benefits of exercise are achieved through an improvement of energy metabolism and glucose homeostasis. These benefits are sustained over the long term through an improvement of body composition induced by muscle hypertrophy and fat mass loss (Ross and Bradshaw, 2009; Peterson et al., 2011; Stoner et al., 2016; Evans et al., 2019; Hsu et al., 2019; Viana et al., 2019). Importantly, even if exercise training interventions usually have a very modest impact on body weight, a consistent observation is a reduction of waist circumference and visceral white adipose tissue (WAT) mass, and is therefore an efficient strategy to reduce cardiometabolic risk in obese individuals (Wewege et al., 2017).

During an exercise bout, skeletal muscle relies on both fatty acids (FA) and glucose as fuels to sustain muscle fiber contraction. When exercise is performed at high intensity and for a short duration, muscle cells primarily rely on glucose and muscle glycogen as fuels, which is mainly released from muscle and liver glycogen stores. However, if the exercise is performed at a moderate intensity and for a longer duration, FA will become the main source of energy to sustain muscle contraction. Indeed, FA oxidation in muscle is dependent of FA supply from different sources: FA released through lipolysis of triacylglycerols (TG) stored within WAT, from circulating very low-density lipoproteins-TG (VLDL-TG), from intramyocellular triacylglycerols (IMTG) and

potentially TG stored within inter/intra-muscular adipose tissue (IMAT). The contribution of VLDL-TG to whole-body lipid oxidation varies from 5 to 10% at rest and seems to be marginal during exercise (Wolfe et al., 1985; Kiens and Lithell, 1989). Fatty acids-derived from IMTG and peripheral WAT are thus by far the major sources of lipid fuels during exercise (Horowitz, 2003). Their relative contribution to exercise energy expenditure is influenced by a number of factors such as exercise intensity, duration, and training status (Horowitz and Klein, 2000). Low to moderate intensity exercise, ranging from 25 to 65% of maximal oxygen consumption (VO_2max), is associated with a 5 to 10-fold increase in whole-body lipid oxidation compared to rest (Romijn et al., 1993). A large part of the increase in FA availability is supplied by WAT lipolysis, which increases by 2–4 times (Romijn et al., 1993; Klein et al., 1994; Krauzova et al., 2018). In this review, we will discuss the effects of acute and chronic exercise on abdominal WAT lipolysis in lean and obese individuals.

EFFECT OF ACUTE EXERCISE ON ADIPOSE TISSUE LIPOLYSIS

Main FA Sources

Numerous studies have demonstrated a tight link between lipolysis and FA oxidation during exercise. Indeed, a positive correlation has been observed between lipolytic rate measured *in vitro* in isolated adipocytes and whole-body resting FA oxidation in healthy individuals (Imbeault et al., 2000). In addition, a strong positive relationship has been described between subcutaneous abdominal WAT lipolysis and whole-body FA oxidation measured during an exercise bout in endurance-trained subjects (Moro et al., 2014). Furthermore, the adipose triglyceride lipase (ATGL) activity is increased during exercise in WAT from lean and obese individuals (Petridou et al., 2017).

The abdominal WAT is made of two main fat compartments, subcutaneous WAT (SCAT) on one side, and visceral WAT on the other side. Exercise mostly activates lipolysis in SCAT, as only 5–10% of circulating long-chain FA are released from visceral adipose tissue in lean subjects (Horowitz, 2003; Nielsen et al., 2004). Abdominal SCAT lipolytic response is dependent on both exercise intensity and duration (Horowitz, 2003). In addition, it has been suggested that subcutaneous abdominal lipolysis is greater than gluteo-femoral lipolysis both in men and women, and that men have a relative "resistance" to norepinephrine-mediated lipolysis due to higher adipocyte content of alpha2-adrenergic receptors that inhibits lipolysis (Leibel and Hirsch, 1987; Jensen and Johnson, 1996; Moro et al., 2007). However, in these studies, the relative lipolytic rate measured *in vitro* on isolated adipocytes, *in situ* through microdialysis, and *in vivo* using A–V differences appeared similar between men and women, thus indicating that the greater lipid mobilization observed during exercise in women is mostly accounted for by a higher subcutaneous fat mass compared to men.

Main Lipolytic Hormones and Factors

The activation of SCAT lipolysis during exercise can be attributed to an increase in plasma catecholamines concentration, which stimulates beta-adrenergic receptors on the adipocyte plasma membrane leading to the intracellular activation of the hormone-sensitive lipase (HSL; Horowitz, 2003). However, we have previously shown that local infusion of the beta-blocking agent propranolol in the SCAT only partially inhibits exercise-induced lipolysis (Moro et al., 2004; Verboven et al., 2018). The 60–70% residual lipolysis was found to correlate with plasma atrial natriuretic peptide (ANP) concentration (Moro et al., 2004, 2008). The role of ANP in exercise-induced lipolysis was then further confirmed during repeated bouts of endurance exercise in lean healthy and obese individuals (Moro et al., 2006; Koppo et al., 2010). Thus, besides the well-known role of catecholamines on exercise-induced WAT lipolysis, the increase of plasma ANP, along with the decrease of plasma insulin (Moro et al., 2007), in relation to exercise intensity actively contribute to enhance adipocyte lipolysis during exercise. Interestingly, when exercise is performed the day after an exercise-bout when muscle glycogen stores are still low, lipolysis is increased compared with the same exercise performed after a resting day, in elite cyclists (Moro et al., 2014). Strikingly, this observation could not be explained by the aforementioned classical lipolytic agents, therefore suggesting that other factors may participate in the activation of WAT lipolysis during exercise (Moro et al., 2014). Recent evidence indicate that proteins secreted by muscle fibers during contraction, the so-called myokines, could activate WAT lipolysis in humans. Indeed, interleukin-6 (IL-6) was the first myokine to be discovered and IL-6 plasma levels were increased in response to an acute exercise bout (Pedersen et al., 2001; Reihmane and Dela, 2014). A recent clinical study has demonstrated that IL-6 is required to reduce visceral adipose tissue mass in response to exercise training (Wedell-Neergaard et al., 2019). However, the role of IL-6 in the activation of WAT lipolysis is still a matter of debate, as IL-6 acute treatment does not activate adipocyte lipolysis *in vitro* (Trujillo et al., 2004). In addition, an acute elevation of IL-6 *in vivo* was described to increase whole-body lipolysis due to a rise in muscle FA release, while WAT lipolysis remained unchanged (Wolsk et al., 2010). Irisin is another myokine that has been described to increase WAT lipolysis through an indirect mechanism involving WAT browning (Bostrom et al., 2012). However, altthough some experiments performed in rodents suggest that exercise-released myokines may activate WAT browning (Stanford et al., 2015), the relevance of such mechanism in humans remains controversial (Norheim et al., 2014; Lehnig and Stanford, 2018).

More recently, we identified a novel myokine secreted by contracting human primary skeletal muscle cells, called growth and differentiation factor 15 (GDF15), which enhances adipocyte lipolysis *in vitro* (Laurens et al., 2020). Furthermore, GDF15 was also secreted following both high-intensity or moderate-intensity exercise in humans *in vivo*, and recombinant GDF15 protein was able to activate lipolysis in subcutaneous WAT explants (Laurens et al., 2020).

White adipose tissue has also been described to produce soluble factors that may act in a paracrine/autocrine fashion to sustain lipolysis during exercise such as interleukin-15 (IL-15).

It has been demonstrated that IL-15 can be produced by SCAT during a one h-cycling exercise, which is known to increase WAT lipolysis. In addition, resting IL-15 secretion correlates with SCAT lipolysis, and an infusion of IL-15 through microdialysis activates SCAT lipolysis in lean subjects while it suppresses lipolysis in obese subjects (Pierce et al., 2015). However, no correlations were observed between IL-15 secretion and lipolysis during exercise. Thus, whether IL-15 contributes to exercise-induced lipolysis is still debated and warrants further investigations.

Exercise Intensity

The relative contribution of FA utilization during an exercise bout depends on its intensity. White adipose tissue lipolysis increases from low to moderate intensities and decreases at high intensity (Romijn et al., 1993). Indeed, when exercise is performed at high intensity, glucose is the major energy substrate to rapidly fuel the contracting muscle. However, as exercise intensity decreases, a switch occurs and lipids become the major energy substrate (i.e., crossover concept) (Brooks and Mercier, 1994). The concept of "Fatmax" has then been taken up by Jeukendreup and colleagues to describe the exercise intensity, expressed as a percent of VO_2max, eliciting the maximal reliance on fat as fuel oxidized in skeletal muscle (Jeukendrup and Wallis, 2005). At this intensity, half of the FA oxidized by muscle fibers are supplied by WAT lipolysis, the remaining part being intracellularly provided by IMTG pools. Fatmax value differs for each individual and mostly depends on body weight, diet, sex, and training status (Jeukendrup and Wallis, 2005). For instance, the Fatmax has been measured at 48% of VO_2max in a large cohort of lean sedentary individuals, while it was around 65% in endurance trained subjects (Achten et al., 2002; Jeukendrup and Wallis, 2005). Interestingly, Fatmax was found to be lower in men than in women (45% vs 52% of VO_2max, respectively) (Jeukendrup and Wallis, 2005). As stated earlier, the greater lipid oxidation at a given exercise intensity in women is accounted for by a higher lipid mobilization at a same relative exercise intensity due to a higher subcutaneous fat mass. Furthermore, Fatmax is lower in obese than in lean individuals (Perez-Martin et al., 2001). However, even if Fatmax has been widely used in exercise-based weight-loss programs, this concept has also raised some criticisms. First, Fatmax is highly dependent on diet and nutritional state, as the body relies more on carbohydrates (CHO) as fuel when they are highly available such as in postprandial conditions. Second, FA oxidation rate is similar in a large range of exercise intensities, usually from about 45 to 75% of maximal aerobic capacity, and thus does not differ much from the peak value (i.e., Fatmax value). Third, the amount of FA burned throughout 24 h not only depends on FA oxidized during exercise but also during the post-exercise recovery period, especially when exercise is performed at high intensity. Finally, Fatmax is a rate of FA oxidation, but the total amount of FA utilized is dependent on energy expenditure and high intensity exercise elicits the largest energy expenditure. Thus, training at Fatmax intensity may not confer further weight-loss benefit than other training interventions performed at higher exercise intensities.

Exercise Duration

The contribution of FA to fuel the contracting muscle also depends on exercise duration. Studies from different groups have shown that FA oxidation gradually increases during a prolonged exercise bout while CHO oxidation decreases (Ravussin et al., 1986; Klein et al., 1994). This goes along with an increase of lipolysis with exercise duration (de Glisezinski et al., 2003; Lafontan et al., 2008). Interestingly, it has been shown that the activity of muscle HSL decreases during a prolonged exercise bout (Watt et al., 2003). This is a consequence of the increased uptake of circulating FA by muscle fibers, which in turn decreases lipolysis and oxidation of IMTG stores. The increase of WAT lipolysis is mostly due to the increase of plasma levels of pro-lipolytic hormones during prolonged exercise. Indeed, catecholamines secretion increases as a function of exercise duration. This increase is more pronounced for epinephrine than for norepinephrine, probably due to a slightly lower glycemia (de Glisezinski et al., 2003) and to the fact that norepinephrine secretion is mostly impacted by exercise intensity (Leuenberger et al., 1993). In line with this observation, we have previously demonstrated that epinephrine is the main beta-adrenergic agent contributing to exercise-induced lipolysis in SCAT (de Glisezinski et al., 2009). We have shown that this increase of adipocyte lipolysis is not only dependent on the beta-adrenergic stimulation by catecholamines but also on the reduction of plasma insulin level and the increase of plasma ANP (Arner et al., 1990; Moro et al., 2004). For instance, ANP plasma level was found to be particularly high after running a marathon, and could participate in the activation of WAT lipolysis to compensate the acute elevation of energy demand during long-distance running (Niessner et al., 2003).

Finally, the whole energy expenditure elicited by exercise has also to be taken into account when considering the contribution of FA burned in response to exercise, as a high percentage does not always reflect a large amount of FA burned if the energy expenditure elicited by the exercise bout is low. Exercise energy expenditure is linked to both exercise intensity and duration.

Impact of Osbesity

Importantly, we and others have observed that exercise-induced SCAT lipolysis is lower in obese subjects than in non-obese subjects (Stich et al., 2000; Mittendorfer et al., 2004; Ross and Bradshaw, 2009). This was attributed to a higher sensitivity of anti-lipolytic alpha2-adrenergic receptors and a lower sensitivity of pro-lipolytic beta-adrenergic receptors in obese subjects (Stich et al., 2000). However, due to the higher fat mass in obese versus non-obese individuals, plasma FA concentration was higher in obese individuals both at rest and during exercise (Stich et al., 2000). In addition, the expression of the ANP clearance receptor NPRC is higher in adipocytes from obese subjects than in lean healthy individuals, and could participate in a lower lipolysis activation in response to ANP secretion during exercise (Dessi-Fulgheri et al., 2003; Kovacova et al., 2016; Ryden et al., 2016). Thus, while basal lipolytic rate is higher in obese vs non-obese subjects, exercise-induced lipolysis is reduced in obese subjects.

This adaptive response in obesity could be seen as a protective mechanism to avoid excessive release of FA into the bloodstream during an exercise bout.

INCREASED LIPOLYSIS DURING POST-EXERCISE RECOVERY

The relationship between exercise intensity and FA oxidation, and therefore FA release from WAT lipolysis, is not as straightforward as initially thought. The role of FA as nutrients during post-exercise recovery has been described in a recent review by Bente Kiens' group (Lundsgaard et al., 2020). Briefly, even if high-intensity exercise (i.e., performed at an intensity over 75% of the subject's maximal aerobic power) elicits a low FA oxidation rate during the exercise bout, the post-exercise FA utilization is higher than after a low intensity exercise bout (Pillard et al., 2010). This greater FA oxidation after a high-intensity exercise bout is mainly reflected by a decrease of the respiratory quotient (Marion-Latard et al., 2003) and appears independent of energy expenditure during 6 h post-exercise. This is a consequence of the preferential use of CHO to replenish muscle glycogen stores which have been depleted during the high-intensity exercise bout, which favors FA as major fuels during 24–48 h after the exercise bout (Tremblay et al., 1994; Kiens and Richter, 1998). We have previously shown in isolated adipocytes that, after a long-duration exercise bout, the WAT displays an increased responsiveness to beta-adrenergic lipolytic agents, which may participate to the increased FA availability during the recovery period (Harant et al., 2002). Strikingly, this increase in post-exercise FA consumption is more pronounced in men than in women (Henderson et al., 2007). In addition, using stable isotope-labeled palmitate infusion, Magkos et al. (2009) have observed that the exercise-induced increase of FA utilization during the post-exercise recovery period is greater in subjects with a low resting plasma FA availability and is greater after an exercise resulting in high energy demand. Interestingly, it has been demonstrated that post-exercise lipolysis is stimulated in the SCAT by an increase of plasma growth hormone level, which is secreted by somatotropic cells during the exercise bout (Enevoldsen et al., 2007). A recent study performed in mice also evoked a role of IL-6, a myokine secreted by skeletal muscle fibers during exercise, in the regulation of WAT lipid metabolism during the exercise recovery (Knudsen et al., 2017).

In summary, it appears critical to consider the post-exercise recovery period to fully assess the impact of different exercise modalities on FA utilization and thus body weight loss.

EFFECT OF EXERCISE TRAINING ON ADIPOSE TISSUE LIPOLYSIS

Exercise training improves FA mobilization during an exercise bout. Indeed, it has been shown that FA appearance rate (Ra) in the blood is higher in endurance trained subjects compared to sedentary controls (Coggan et al., 2000). Exercise training affects both the sensitivity of WAT to catecholamines, but also their secretion during exercise, which is reduced in response to a given absolute workload after training (Kjaer et al., 1987; Riviere et al., 1989; Arner, 1995). Transversal studies performed on SCAT adipocytes have suggested that beta-adrenergic sensitivity is higher in trained subjects than in sedentary controls (Crampes et al., 1986; Crampes et al., 1989; Riviere et al., 1989). In addition, longitudinal studies have demonstrated that endurance training improves the beta-adrenergic lipolytic response of isolated adipocytes in obese subjects (De Glisezinski et al., 1998a; Moro et al., 2009).

Furthermore, exercise training improves ANP responsiveness in obese subjects, but it is yet unclear whether this is due to an increase of ANP plasma concentration or to an increase of ANP receptors on the adipocyte cell surface (Moro et al., 2005). Indeed, we were able to show through *in situ* microdialysis experiments in SCAT of young overweight men, that 4 months of aerobic training improve both beta-adrenergic and ANP lipolytic responses (Stich et al., 1999; Moro et al., 2005). Finally, insulin concentration decreases with training status but the impact on WAT lipolysis is partly counterbalanced by an improvement of WAT insulin sensitivity with exercise training (Polak et al., 2005; Riis et al., 2019). Strikingly, even if exercise-induced lipolysis is higher in trained subjects, plasma FA concentration is lower both at rest and during exercise (Crampes et al., 2003; de Glisezinski et al., 2003). This could be explained by an increase of FA utilization by skeletal muscle in trained subjects. Indeed, the amount of both resting and exercise-induced FA oxidation is higher after a training program, resulting in an increased oxygen consumption (de Glisezinski et al., 2003). The improvement of exercise-induced lipolysis observed in endurance-trained obese subjects also seems to be partially due to a reduction of the anti-lipolytic effect of alpha2-adrenergic receptors in the SCAT, which may be a consequence of a lower epinephrine plasma levels, the main alpha2-adrenergic ligand. Indeed, the anti-lipolytic activity of alpha2-adrenergic receptors was reduced after endurance training in lean and obese subjects (De Glisezinski et al., 2001; Richterova et al., 2004). Interestingly, similar adaptations of WAT lipolytic response have been found after a resistance training program in obese individuals (Polak et al., 2005).

Finally, it has been observed that the exercise intensity which elicits the higher lipolytic rate is increased with exercise training (Perez-Martin et al., 2001; Achten et al., 2002). Thus, while the maximal FA utilization is reached at intensities of 30% of maximal aerobic power in sedentary subjects, it is achieved around 65% in trained individuals. This means that the total amount of FA mobilized during an exercise bout is higher in trained subjects because both energy expenditure and the percentage of FA used are increased. In addition, high intensity training elicits a gain of muscle mass which impacts basal metabolic rate and thus may increase energy expenditure and consequently impact FA oxidation during resting periods and body weight loss (Heydari et al., 2012; Osawa et al., 2014; Schubert et al., 2017; Batrakoulis et al., 2018).

Altogether, these data suggest that an exercise training program combining high-intensity and moderate intensity exercise bouts could optimize daily FA utilization and optimize body weight loss in overweight or obese individuals.

IMPACT OF DIET AND TIME OF THE DAY ON EXERCISE-INDUCED LIPOLYSIS

Carbohydrates availability influences exercise-induced lipolysis. Indeed, glucose ingestion during an exercise bout reduces SCAT lipolysis and partially inhibits FA oxidation (De Glisezinski et al., 1998b). Exercising in the fasting state has been shown to increase FA oxidation and whole-body lipolysis in healthy subjects (Vicente-Salar et al., 2015; Andersson Hall et al., 2016; Hansen et al., 2017). This appears to be a compelling approach to achieve maximal fat utilization during exercise. Interestingly, a recent study has shown that exercising after a high-protein breakfast has similar effects on lipolysis than exercising in the fasting state (Saghebjoo et al., 2020). Furthermore, volunteers fed for 5 days with a high-fat diet display a higher WAT lipolytic rate during exercise than people fed with a CHO-rich diet, which can be explained by a higher catecholamine response and lower insulinemia (Suljkovicova et al., 2002).

Numerous review articles have described the impact of time of the day on exercise efficiency, but very few focused on lipid metabolism and WAT lipolysis (Chtourou and Souissi, 2012; Seo et al., 2013; Dollet and Zierath, 2019; Parr et al., 2020). A few studies have shown that exercise performed during the evening elicits a higher reliance on lipids compared to exercise performed during the morning (Aoyama and Shibata, 2020). In addition, a crossover study performed in young men has demonstrated that an endurance exercise bout performed during the evening enhances plasma epinephrine, IL-6 and plasma FA levels compared to the same exercise performed during the morning, thus suggesting that evening exercise is the most effective to achieve high rates of WAT lipolysis (Kim et al., 2015). However, data are still scarce and future studies should be performed to fully address this question.

CALORIE RESTRICTION AND EXERCISE INDUCED WEIGHT LOSS

Many studies have shown that calorie restriction is more efficient at reducing body weight than exercise training, and that combining exercise training with a caloric restriction intervention confers a slight additional benefit to achieve weight loss compared to calorie restriction alone (Miller et al., 1997; Swift et al., 2018). However, exercise has an important role in body weight maintenance after weight loss (Swift et al., 2018). Indeed, calorie restriction-induced weight loss increases WAT sensitivity to lipolytic stimuli produced during exercise (Mauriege et al., 1999). Furthermore, exercise protects against loss of lean body mass during calorie restriction, and avoids a drop of resting metabolic rate (Chomentowski et al., 2009).

Therefore, even if combining exercise to a calorie restriction intervention does not achieve further weight loss than calorie restriction alone, exercise potentiates visceral fat mass loss and a sustained improvement of body composition (You et al., 2006), and prevents from the well-described "yo-yo" effect of dieting.

CURRENT GAPS IN RESEARCH

There are many additional questions that remains to be answered to fully understand the impact of exercise on WAT lipolysis and body composition. Indeed, future studies should aim at identifying unknown lipolytic factors secreted during exercise, such as myokines and potentially micro-RNAs released in extracellular vesicles in response to muscle contraction (Whitham et al., 2018). Understanding the complex inter-organ crosstalk during exercise will pave the way to new areas of research and could lead to the discovery of new molecular players with a potential therapeutic role.

Finally, research efforts should also focus on refining exercise training modalities to achieve a maximal and sustained improvement in body composition, especially in overweight or obese individuals. Assessing the combination of time-restricted eating patterns with exercise training sessions performed during the fasting state could be an attractive approach to potentiate fat mass loss.

CONCLUSION

Collectively, there is little debate that exercise training facilitates abdominal weight loss in overweight and obese individuals. Chronic exercise has largely demonstrated its ability to facilitate weight loss during calorie restriction and maintenance of long-term weight loss. A number of studies suggest that combining moderate and high intensity exercise can provide additional benefits on weight loss, at least in part, by favoring higher rates of energy expenditure during exercise and greater FA oxidation rates during post-exercise recovery. Although canonical lipolytic systems and hormones have been studied in detail during the past 30 years, more recent studies uncovered a muscle-adipose tissue crosstalk mediated by myokines regulating WAT lipolysis. However, much remains to be discovered. With the discovery that contracting muscles can produce myokines capable of remotely targeting organs, including WAT, our current knowledge will likely be challenged in the next few years.

AUTHOR CONTRIBUTIONS

CL and CM wrote and revised the manuscript. IG, IH, and DL edited and revised the manuscript. All authors contributed to the article and approved the submitted version.

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High-Intensity Interval Training Does Not Change Vaspin and Omentin and Does Not Reduce Visceral Adipose Tissue in Obese Rats

*Leandro Ribeiro Costa[1], Cynthia Aparecida de Castro[2], Diego Adorna Marine[1], Fernando Fabrizzi[3], Vanessa de Oliveira Furino[1], Iran Malavazi[4], Fernanda de Freitas Anibal[2] and Ana Cláudia Garcia de Oliveira Duarte[1]**

[1] Department of Physical Education and Human Motricity – DEFMH, Biological and Health Sciences Center – CCBS, Federal University of São Carlos – UFSCar, São Carlos, Brazil, [2] Department of Morphology and Pathology – Biological and Health Sciences Center – CCBS, Federal University of São Carlos – UFSCar, São Carlos, Brazil, [3] Faculty of Philosophy, Sciences and Letters of Penápolis-Brazil, Penápolis, Brazil, [4] Department of Genetics and Evolution – Biological and Health Sciences Center – CCBS, Federal University of São Carlos – UFSCar, São Carlos, Brazil

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***Correspondence:**
Ana Cláudia Garcia de Oliveira Duarte
anaclau@ufscar.br

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This study aimed to determine the expression of omentin and vaspin, inflammatory markers, body composition, and lipid profile in diet-induced obese rats and high-intensity interval training (HIIT). Forty Wistar rats were divided into four groups: untrained normal diet, trained normal diet (T-ND), untrained high-fat diet (Unt-HFD), and trained high-fat diet (T-HFD). For the animals of the Unt-HFD and T-HFD groups, a high-fat diet was offered for 4 weeks. After that, all the animals in the T-ND and T-HFD groups were submitted to HITT, three times per week, for 10 weeks (2 weeks of adaptation and 8 weeks of HIIT). Muscle (gastrocnemius), liver, epididymal adipose tissue, retroperitoneal adipose tissue, visceral adipose tissue (VAT), and serum were collected to analyze TNF-α, IL-6, PCR, IL-8, IL-10, IL-4, vaspin, and omentin. A body composition analysis was performed before adaptation to HIIT protocol and after the last exercise session using dual-energy X-ray absorptiometry. Omentin and vaspin in the VAT were quantified using Western blotting. The results showed that, when fed a high-fat diet, the animals obtained significant gains in body fat and elevated serum concentrations of vaspin and blood triglycerides. The HIIT was able to minimize body fat gain but did not reduce visceral fat despite the increase in maximum exercise capacity. Moreover, there was a reduction in the serum levels of adiponectin, IL-6, and IL-10. Finally, we concluded that, although the training protocol was able to slow down the weight gain of the animals, there was no reduction in visceral fat or an improvement in the inflammatory profile, including no changes in omentin and vaspin.

Keywords: vaspin, omentin, visceral adipose tissue, high-intensity interval training, body composition, obesity, high fat diet

INTRODUCTION

Obesity is related to a wide range of diseases, such as arterial hypertension, diabetes mellitus type II, some types of cancer, and non-alcoholic hepatic steatosis. These comorbidities can mostly be attributed to metabolic and endocrine alterations occurring in the adipose tissue, from its expansion (Hwang et al., 2015; Shie et al., 2015). Researchers showed that adipocyte hypertrophy results in an abnormal function of the cell, and this remodeling could lead to an alteration in the secretion of metabolites such as adipokines (leptin, adiponectin, vaspin, and omentin), causing adipocyte death, local hypoxia, and influx of fatty acid (Choe et al., 2016).

One of the strategies adopted to mitigate this low-grade chronic inflammation is to reduce body fat, resulting in higher circulating levels of anti-inflammatory cytokines, such as adiponectin, associated with a reduction in pro-inflammatory characteristics. Thus, physical exercise has been shown to be an efficient strategy (Gleeson et al., 2011). Physical exercise has anti-inflammatory characteristics, offering a protective effect against diseases associated with chronic low-grade inflammation present in obesity, reducing the levels of inflammatory cytokines and increasing anti-inflammatory properties (Hermsdorff and Monteiro, 2004; Petersen and Pedersen, 2005; Ghoshal, 2015).

Among the training protocols available, high-intensity interval training (HIIT) has achieved an increase in popularity. HIIT alternates periods of high intensity with active or passive intervals and is a time-efficient strategy suitable to improve cardiorespiratory fitness, reduce cardiometabolic risks, and improve fat oxidation, leading to significant weight loss in obese and overweight populations (Alahmadi et al., 2011; Heydari et al., 2012; Alahmadi, 2014). HIIT generates physiological adaptations including the elevation of mitochondrial content, maximal aerobic capacity, and generation of hypertrophy in skeletal muscle (MacInnis and Gibala, 2017; Robinson et al., 2017). In addition to these benefits, the use of HIIT in obese populations can lead to changes in the inflammatory profile by reducing inflammatory cytokines while increasing the anti-inflammatory properties due to the reduction in body weight and visceral adiposity (Steckling et al., 2015).

This reduction in visceral obesity has been the target of studies since it can cause changes in the expression of important adipokines. Over the last decades, many adipokines have been discovered and are of special interest to researchers for improving the condition of obesity, diabetes, and low-grade inflammation, such as omentin and vaspin. Omentin is adipokine produced by the stromal–vascular fraction of visceral adipose tissue (VAT) and, in low concentrations, by subcutaneous adipose tissue (Yang et al., 2006; De Souza Batista et al., 2007). It has been suggested in the literature that production occurs under glucose and insulin regulation (Komiya et al., 1998) and is modified in several pathological situations, such as obesity and insulin resistance (Kuperman et al., 2005). Due to this fact, a reduction in omentin levels is associated with an increase in metabolic risk factors, suggesting its use as a negative biomarker for obesity (De Souza Batista et al., 2007).

On the other hand, vaspin is a member of the serine protease inhibitor family (Hida et al., 2005; Nakatsuka et al., 2012) and is highly expressed by VAT in obesity conditions as well as subcutaneous adipose tissue (Shaker and Sadik, 2013) and in low quantities by skeletal muscle and liver (Körner et al., 2011; Goktas et al., 2013). However, although the mechanisms of action of vaspin are poorly understood, it is proposed that its action may represent a compensatory mechanism in metabolic abnormalities induced by obesity (Barraco et al., 2014; Proença et al., 2014). Thus, a better understanding of the adjacent mechanisms of exercise in the secretion of adipokines can define more effective strategies to control obesity and co-morbidities.

Therefore, based on the pathophysiological aspects associated with obesity, this study aimed to determine the expression of omentin and vaspin, inflammatory markers, body composition, and lipid profile in diet-induced obese rats and HIIT.

MATERIALS AND METHODS

Ethics and Experimental Groups

The experimental protocol lasted 18 weeks. The experimental procedures in this study conformed to the Committee on Animal Research and Ethics (no. 3963080116) from the Federal University of São Carlos (UFSCar). Adult Wistar male rats (n = 40, ≅300 g) were housed in groups (n = 4 to 5/cage) with a temperature-controlled environment (22–24°C), humidity of 50–60%, reversed 12/12-h light/dark cycle (lights on at 6 pm), and water and food *ad libitum*. After 4 weeks of acclimatization (90 days), the rats were randomly divided into two groups: normal diet (ND; n = 20) and high-fat diet (HFD; n = 20), and they were fed for 8 weeks. Then, the animals were randomly distributed into four experimental groups (n = 10): untrained normal diet (Unt-ND), trained normal diet (T-CD), untrained high-fat diet (Unt-HFD), and trained high-fat diet (T-HFD).

Diets

The normal fat diet (in pellet form), containing 4.8% total fat, was used as control diet in the NFD group, as previously reported (Estadella et al., 2004; de Castro et al., 2017, 2019). The palatable high-fat diet was prepared with standard rat chow plus peanuts, milk chocolate, and sweet biscuits in a proportion of 3:2:2:1 (Estadella et al., 2004; de Castro et al., 2017, 2019). All components were powdered and mixed to form pellets. This diet is composed of 20% fat (**Table 1**) and was standardized by Estadella et al. (2004); since then, it has been used to induce obesity phenotype in Wistar rats (de Castro et al., 2017, 2019). The nutritional composition of the diet was analyzed by CBO Laboratories of Analyzes Ltda., Valinhos-SP, Brazil. It is worth mentioning that the use of the term high-fat diet is due to the increase in fat due to the standard diet that has 4% fat *vs* 20% (HFD), being efficient in the study of obesity (Bruder-Nascimento et al., 2013; Moreno-Fernández et al., 2018; Li et al., 2020).

TABLE 1 | Nutritional composition of the diet.

	HFD	ND
Energy value (cal/g)	4,665.00	3,854.00
Moisture and volatiles (%)	14.72	12.47
Fat (%)	20	4.80
Carbohydrates (%)	32.90	39.23
Proteins (%)	18.12	22.81
Fibers (%)	2.97	5.82
Minerals (%)	3.29	6.87
Potassium (%)	0.60	1.26
Calcium (%)	0.52	1.2[a]
Sodium (%)	0.14	0.22
Vitamin A (UI/kg)	1,149.00	25,000.00[a]
Vitamin D3 (UI/kg)	836.90	4,000.00[a]
Vitamin E (mg/kg)	229.94	80.00[a]
Maltose (%)	Undetectable	Undetectable
Free xylose (%)	Undetectable	Undetectable
Free glucose (%)	0.12	Undetectable
Free fructose (%)	0.17	Undetectable
Sucrose (%)	12.49	1.65
Lactose (%)	1.88	Undetectable
Free galactose (%)	Undetectable	Undetectable
Raffinose (%)	0.30	0.74

ND, normal diet; HFD, high-fat diet.
The undetectable values were below the threshold of quantification (<100 mg/kg, 0.1%).
[a]*Nutritional information obtained from the manufacturer.*

Body Mass and Food Intake Measurement

The body mass (BM) was measured once a week, and food intake was measured every 2 to 3 days, between 8 and 12 h. Diet intake was calculated by the difference in weight between the amount of food offered and the amount of food remaining.

HIIT Protocol

Adaptation

The animals were adapted to a treadmill for 2 weeks. The animals of the training group ran on the treadmill between 10 and 20 m/min. In order to simulate a similar environment to training, the untrained animals were also placed on the treadmill so that they could adapt.

Maximum Exercise Capacity

After the adaptation, a maximum exercise capacity (MEC) test was performed. The animals started to run on the treadmill at 6 m/min with 25% incline for 5 min, with an increase of 0.5 m/min every 2 min until the maximum speed was obtained. As a criterion for determining exhaustion, the interruption was the moment when the animal was no longer able to run by increasing the speed of the treadmill (Wisløff et al., 2001; Høydal et al., 2007).

HIIT Protocol

The HIIT protocol consisted of three exercise sessions per week for 8 weeks. The training was preceded by a 5-min warm-up, with the animals running at 40% of the MEC and then the alternation between high intensity for 4 min (85–95% of MEC) and recovery for 3 min (40–50% of MEC), with a maximum of six intermittent intervals. Every 2 weeks, another incremental test to determine the MEC was carried out again to adjust the intensities of the exercise (Haram et al., 2009; Kemi et al., 2015; Songstad et al., 2015). Throughout the procedure, electric shocks were not used as a form of stimulation.

Experiment and Sample Collection

The animals were euthanized by decapitation using a guillotine after 8 h of fasting. The trained animals were sacrificed 48 h after the last exercise session. VAT, epididymal (EPI) adipose tissue, retroperitoneal (RET) adipose tissue, brown adipose tissue (BAT), liver, gastrocnemius muscle, and serum were collected, dissected, weighed, and stored in a freezer at −80°C for posterior biochemical and morphometric analyses.

Dual-Emission X-Ray Absorptiometry

Body composition evaluation was performed before adaptation to the HIIT protocol and after the last exercise session. The animals were anesthetized with ketamine (40 mg/kg) and xylazine (5 mg/kg; IACUC) and were later placed in prone position for them to be scanned using the dual-energy X-ray absorptiometry (DXA)–dual-range emission densitometry (Lunar iDXA 200368 GE® instrument, Lunar, WI, United States). BM, body fat, fat mass, and fat-free mass values were obtained. Image analyses were performed using the Encore 2008, 12.20 GE, HEALTHCARE.

Western Blotting to Determine Omentin and Vaspin

Omentin and vaspin in the VAT were quantified using Western blotting. The tissues were processed to obtain the total protein extract using an extraction buffer [sodium dodecyl sulfate (SDS), 0.1% (p/v); Triton, 1% (v/v); Tris–HCl, pH 7.5, 50 mM; NaCl, 150 mM; EDTA, 15 mM; EGTA, 5 mM; NaF, 100 mM; and $Na_2P_2O_7$, 10 mM] as well as protease inhibitors (Complete-Mini Roche® 1×). The concentration of protein was quantified using Lowry's colorimetric method (1951). The crude protein extracts for each experiment were submitted to SDS–polyacrylamide gel electrophoresis (12%) and Tris-glycine buffer 1 × (Laemmli's method) using a vertical electrophoresis tank (BioRad). The proteins were then transferred from the gel to the nitrocellulose membrane (0.45 μm, BioRad) in a submerged transfer procedure according to the manufacturer's protocol. Membrane blockage was done with Tris-buffered saline with 0.1% Tween® 20 (TBST) 1 × containing 9% of milk powder for 4 h at room temperature. The membranes were then incubated, overnight at 4°C, with the primary antibody anti-omentin (1-1000, sc-104334, and Santa Cruz®) and anti-vaspin (1-1000, sc-79815, and Santa Cruz®) TBST 1 × containing 5% of milk powder. The membrane was incubated with a secondary anti-goat IgG-HRP antibody: (1-3000, sc-2020) in TBST 1×, immunodetection was performed using a chemiluminescence kit (ECL Prime, GE Healthcare®, Life Sciences). The blot image was acquired using the Chemidoc

(BioRad®) equipment. Protein concentrations were normalized by using GAPDH diluted 1:10,000 (Abcam®) in VAT. All the membranes were normalized using an intra-membrane control.

Quantifications of Cytokines and Adipokines

The quantifications of omentin, vaspin, TNF-α, IL-6, IL-8, IL-10, C-reactive protein, and adiponectin were performed from serum and determined by enzyme-linked immunosorbent assay (ELISA) method following the specifications corresponding to the kits. For the cytokine analyses, such as IL-4, IL-10, IL-6, and TNF-α, OptEIA (BD Biosciences®) kits were used; for the IL-8, PCR and adiponectin analyses, DuoSet ELISA kits were used (R&D Systems®); for the omentin and vaspin analyses, EIA-OME and EIA-VAP (RayBiotech®) kits were used. The concentrations of the samples were calculated from the titration curve of the cytokine patterns, and the final concentrations were expressed in pg/ml or ng/ml depending on the kit.

Statistical Analysis

All statistical analyses were performed using the Sigma Stat Software (version 3.5). Data normality was verified by the Kolmogorov–Smirnov test; equality of variance (Levene's method) and non-parametric tests were used when the data did not present normal distribution and/or equality of variance. Comparisons among the groups were made using two-way ANOVA. Tukey multiple-comparison test was used when the two-way ANOVA test detected a statistical difference. Independent *t*-test was used for comparisons between two independent groups. The level of significance was set at 5% ($p < 0.05$).

RESULTS

Body Mass and Food Consumption

The animals fed on a high-fat diet presented significantly higher BM than their respective controls at the end of the experiment. The Unt-HFD and T-HFD groups presented lower caloric intake when compared to the Unt-ND and T-ND groups, respectively, (**Table 2**). In addition, the Unt-HFD group showed significantly higher triglyceride values when compared to the Unt-ND group.

Maximum Capacity of Exercise

The training variables are presented in **Table 3**. The MEC at the beginning of the training protocol was significantly different between the T-ND and T-HFD groups, 9.1% lower in the T-HFD group. At the end of 10 weeks, this training capacity was higher in relation to the first MEC test, showing an improvement of this variable for the animals trained with HIIT, but there was no difference between the T-ND and T-HFD groups in the post-exercise condition (**Table 3**). The same behavior occurred with the variable time to exhaustion, but the distance covered was greater in T-ND compared to T-HFD group. Interestingly, the MEC in the T-ND group was 228%, and in the T-HFD group it was 235%; there was no difference in the post-exercise. Therefore, it showed 16% improvement compared to T-ND.

Body Composition

Figure 1A shows that, from the fourth week, the Unt-HFD group weight was significantly higher than the untrained normal diet group (Unt-ND). After the experimental protocol, it was found that training was not able to promote significant changes in the T-HFD group. However, it presents 5.72% lower value for this variable than the Unt-HFD group. The animals' fat-free mass, assessed by DXA, was higher in all groups after 10 training weeks, but there was no significant change between groups for this parameter considering only the end of training (**Figure 1B**).

Regarding adipose mass (**Figure 1C**), it was observed that, at the end of the diet-induced obesity (pre-exercise), the Unt-HFD and T-HFD groups showed higher values when compared to the respective control groups (Unt-ND and T-ND). After 10 weeks of training, a similar pattern was observed as the groups that were fed a high-fat diet (Unt-HFD and T-HFD) showed higher values of body fat compared to the Unt-ND and T-ND groups. However, the T-HFD group showed significantly lower body fat values compared to the Unt-HFD.

Relative Weights of Depots

The relative weights of visceral, RET, and EPI abdominal fat depots and BAT were higher in the groups fed a high-fat diet when compared to groups fed a normal diet. The exercise, as observed in **Table 4**, was not able to cause changes in these fat depots. The Unt-HFD and T-HFD groups showed lower relative weights of the hepatic and muscular tissues when compared to the Unt-ND and T-ND groups, without significant changes between the HFD groups. The diet led to a reduction in the relative weight of the hepatic and muscle tissues since the Unt-HFD group had lower values than the Unt-ND and T-ND, and those of the T-HFD group were less than those of the T-ND. For these tissues, the proposed exercise model was also not able to cause significant changes.

Cytokines

In **Figure 2C**, it can be observed that serum IL-6 was reduced in the T-HFD group when compared to the Unt-HFD and T-ND groups. Besides that, there were no significant changes due to exercise and diet in the values for cytokines CXCL-8, PCR, and TNF-α (**Figures 2A,B,D**). It is shown that adiponectin decreased in the trained T-HFD compared to the Unt-ND and T-ND (**Figure 3C**). Besides that, serum IL-10 is reduced in the T-HFD compared to the T-ND group and Unt-HFD group (**Figure 3A**). Serum IL-4 showed no statistical differences between the groups (**Figure 3B**).

Vaspin and Omentin

The vaspin serum concentration increased upon obesity induction, but it was not changed after the HIIT protocol (**Figure 4A**). In VAT, the values of vaspin were not altered either by the diet or by the exercise (**Figure 4C**). Considering the response of omentin in the experimental groups, no statistical differences were found in both serum (**Figure 4B**) and VAT (**Figure 4D**).

TABLE 2 | Body mass gain and food intake.

Groups	Body mass (g)			Caloric intake (kcal/day)	Triglycerides (mg/dl)
	Initial	Final	Body mass gain		
Unt-ND	280.90 ± 15.50	527.80 ± 43.88[d]	246.90 ± 36.85	121.98 ± 2.84	34.51 ± 10.04
T-ND	273.80 ± 23.69	497.40 ± 31.74[d]	223.6 ± 23.32	118.44 ± 2.67	30.96 ± 8.09
Unt-HFD	277.40 ± 19.54	619.00 ± 77.94[ad]	341.90 ± 72.42[a]	114.89 ± 3.59[a]	50.88 ± 11.73[a]
T-HFD	282.50 ± 14.57	584.70 ± 49.51[bd]	302.20 ± 56.18[b]	109.87 ± 3.34[b]	42.08 ± 9.09

Unt-ND, untrained normal diet (n = 10); T-ND, trained normal diet (n = 10); Unt-HFD, untrained high-fat diet (n = 10); and T-HFD, trained high-fat diet (n = 10).
Data presented as mean ± SD ($p < 0.05$).
[a]*vs. Unt-ND.*
[b]*vs. T-ND.*
[d]*Initial vs. final.*

TABLE 3 | Variables of exercise.

Variables		T-ND	T-HFD
Pre-exercise	MEC (m/min)	13.74 ± 0.87	12.5 ± 0.95[a]
	Time to exhaustion (min)	28.66 ± 1.65	25.77 ± 3.15[a]
	Distance covered (m)	393.78 ± 1.43	322.12 ± 2.99[a]
Post-exercise	MEC (m/min)	31.36 ± 3.63[b]	29.25 ± 2.67[b]
	Time to exhaustion (min)	27.00 ± 6.60	20.20 ± 10.15[b]
	Distance covered (m)	846.70 ± 24.00[b]	590.85 ± 27.10[ab]
Δ of MEC (%)		228	235

Unt-ND, untrained normal diet (n = 10); T-ND, trained normal diet (n = 10); Unt-HFD, untrained high-fat diet (n = 10); and T-HFD, trained high-fat diet (n = 10).
Data presented as mean ± SD ($p < 0.05$).
[a]*vs. T-ND.*
[b]*Pre-exercise vs. post-exercise in the same group.*

DISCUSSION

This study reports the response to omentin and vaspin of Wistar rats fed or not with a high-fat diet and under HIIT. We expected that HIIT could cause metabolic adaptations in adipose tissue, promoting changes in the concentration of omentin and vaspin in obese animals. However, we do not confirm these results. Although the training protocol was able to slow down the weight gain of the animals, there was no reduction in visceral fat or an improvement in the inflammatory profile.

In the present study, the high-fat diet induced an increase in body weight, serum triglycerides, all visceral depots and organs evaluated, and fat mass. We also highlight that, although there were no differences between most cytokines, there was an increase in vaspin in obese animals, which has been used as a biomarker of adiposity, and a reduction of adiponectin in HFD animals. These data together show the effective induction of obesity, supporting the studies already described in the literature (Estadella et al., 2004; Duarte et al., 2008; Sene-Fiorese et al., 2008; Speretta et al., 2012, 2016; Suk and Shin, 2016; Diogo et al., 2020). In addition, the diet promoted a decrease in the food intake of these animals. This behavior was observed in previous investigations and is justified by the increased caloric density of the high-fat diet, which results in a greater satiety of the animals when compared with the commercial chow diet (Estadella et al., 2004; Zambon et al., 2009; Rocha et al., 2016).

Given the small participation of HIIT in slowing down the gain of adipose mass, BM, and triglyceridemia of animals fed a high-fat diet, we believe that these adjustments may be related not only to exercise (Sene-Fiorese et al., 2008; Speretta et al., 2012; Ramos-Filho et al., 2015; MacInnis and Gibala, 2017) but also to the increase in the supply of fats provided by the type of diet. We consider that the energy expended by the animals during the exercise equalized the excessive energy consumption offered in the high-fat diet, thus avoiding a greater accumulation of BM.

Our data reinforced the lipogenic activity generally observed from the consumption of high-fat diet since the animals had an increased rate of lipid anabolism, resulting in the high accumulation of fat in the depots EPI, RET, and VAT (Estadella et al., 2004; Sene-Fiorese et al., 2008; Speretta et al., 2012). Besides the increase in abdominal adipose tissue, the diet caused an increase in the BAT, which was already expected since diets rich in fat are able to elevate the thermogenic activity of BAT, leading to a greater synthesis of UCP1. As a consequence, there is an increase in the weight of this tissue (LeBlanc and Labrie, 1997; Estadella et al., 2004). In addition, an increase in liver lipids was observed, probably due to an increase in lipogenesis, or a decrease in beta oxidation (Gauthier et al., 2015). Surprisingly, although the high-fat diet effectively promoted obesity, the liver weight was reduced, which was not commonly observed by other studies using the same type of diet (Duarte et al., 2008; de Castro et al., 2017). This finding can be partially explained by the increase in hepatic triglyceride concentrations (Sene-Fiorese

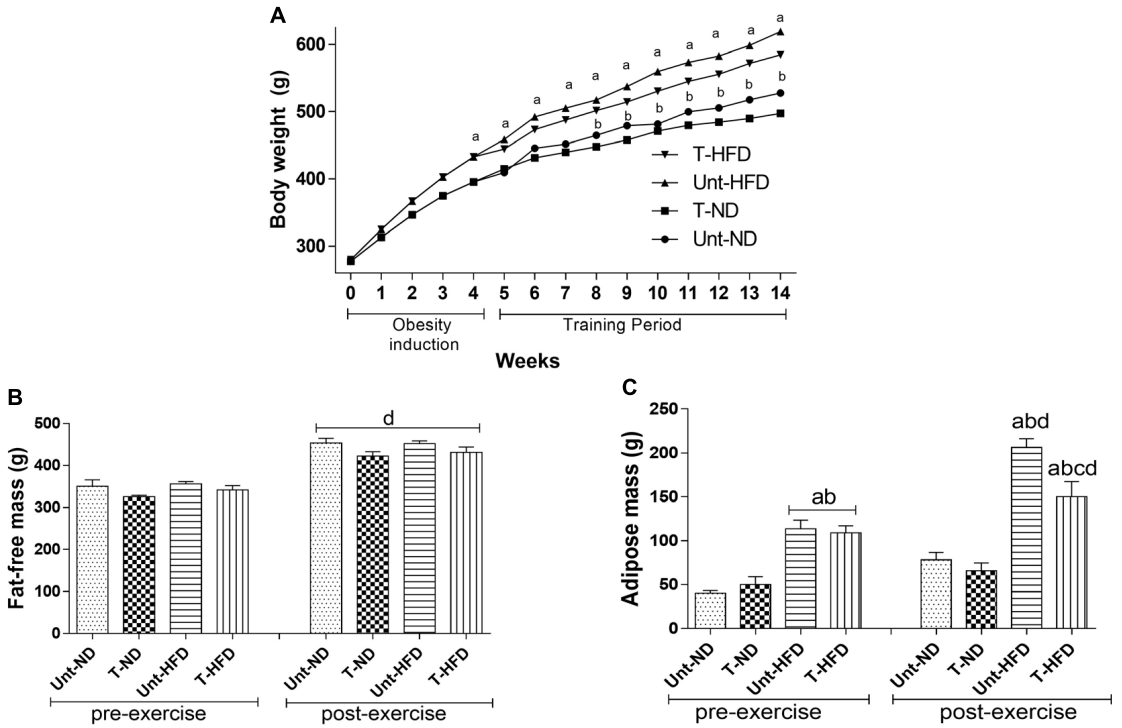

FIGURE 1 | Body composition. **(A)** The evolution of body weight gain, **(B)** fat-free mass pre- and post-exercise, and **(C)** adipose mass pre- and post exercise. Unt-ND, untrained normal diet (n = 10); T-ND, trained normal diet (n = 10); Unt-HFD, untrained high-fat diet (n = 10); and T-HFD, trained high-fat diet (n = 10). Data presented as mean ± SD ($p < 0.05$). [a]*vs.* Unt-ND; [b]*vs.* T-ND; [c]*vs.* Unt-HFD; and [d]pre-exercise *vs.* post-exercise.

et al., 2008). This accumulation displaces the predominance of fatty acids as the main energy substrate in organic reactions to the detriment of hepatic glycogen. In turn, the reduction of glycogen levels in the liver, as observed in this study, may be associated with lower liver weight since glycogen carries water molecules for its transport, and this would significantly increase the weight of the liver (Zambon et al., 2009; Gauthier et al., 2015).

It is important to mention that exercise capacity and adaptations have also been measured indirectly through distance covered and time to exhaustion (Kemi et al., 2015). Both animals that were fed a standard diet (228%) and a high-fat diet (235%) achieved increases in MEC, showing evidence of the adjustments promoted by the proposed exercise model as previously described (Kemi et al., 2015). However, the effects of HIIT on adipose depots (EPI, RET, VAT, and BAT), liver, and muscle proved to be inefficient since the weights of these tissues were not changed by HIIT but only by diet. These results suggest a possible mobilization of free fatty acids from other depots of white adipose tissue during exercise, such as the subcutaneous tissue (Maillard et al., 2019; Motta et al., 2019).

As the exercise did not cause changes in the abdominal fat depots, we already expected that HIIT would also not change the concentration of omentin and vaspin in VAT and circulation. However, an interesting fact was that HIIT was able to reduce the serum levels of adiponectin, IL-10, and IL-6. This dynamic, which was presented by the anti-inflammatory molecules because of the physical exercise used, seems to be related to the mobilization of different fat depots as well as different tissues that secrete these cytokines such as the muscle itself (Pedersen and Febbraio, 2012).

The secretion of inflammatory cytokines is altered in obesity as a compensatory way to mitigate the deleterious effects resulting from obesity (You and Nicklas, 2008; Stefanyk and Dyck, 2010; Golbidi and Laher, 2014). Concerning omentin and vaspin, it was observed in the present study that the training was not able to promote changes in its production in all groups, regardless of the type of diet. These data have been observed previously in our research group (de Castro et al., 2019). The positive values of omentin in their work were observed only in diabetic animals (type 2) submitted to aerobic exercise when compared to combined and resistance exercise. Because it does not mobilize visceral fat, it can be hypothesized that the exercise model proposed in our study was not able to bring about changes in omentin and vaspin within the proposed time. Thus, we assume that the mobilization of these adipokines may be more related to visceral adipose depots.

To confirm the predominance of secretion by adipose tissue, we evaluated the serum and protein expression of adipokines in VAT. We observed that vaspin, but not omentin, was responsive to the induction of obesity, which increases its serum concentration considering the expansion of fat deposits, as previously reported (Shaker and Sadik, 2013; Dimova and Tankova, 2015). Adipocytokines such as omentin and vaspin may be involved with inflammation and have different

TABLE 4 | Relative weight of abdominal fat depots and organs (g/100 g body weight).

Groups	Abdominal fat depots			BAT	Liver	Muscle
	EPI	RET	VAT			
Unt-ND	1.39 ± 0.10	1.35 ± 0.15	0.91 ± 0.08	0.04 ± 0.005	2.71 ± 0.19	0.47 ± 0.03
T-ND	1.25 ± 0.14	1.23 ± 0.21	0.68 ± 0.06	0.05 ± 0.005	2,78 ± 0.18	0.48 ± 0.03
Unt-HFD	2.70 ± 0.14[a]	2.99 ± 0.23[a]	1.81 ± 0.17[a]	0.08 ± 0.006[a]	2.32 ± 0.33[a]	0.40 ± 0.05[a]
T-HFD	2.79 ± 0.14[b]	3.22 ± 0.20[b]	1.65 ± 0.10[b]	0.08 ± 0.008[b]	2.38 ± 0.17[b]	0.42 ± 0.04[b]

Unt-ND, untrained normal diet (n = 10); T-ND, trained normal diet (n = 10); Unt-HFD, untrained high-fat diet (n = 10); T-HFD, trained high-fat diet (n = 10); EPI, epididymal adipose tissue; RET, retroperitoneal adipose tissue; VAT, visceral adipose tissue; and BAT, brown adipose tissue.
Data presented as mean ± SD ($p < 0.05$).
[a]*vs. Unt-ND.*
[b]*vs. T-ND.*

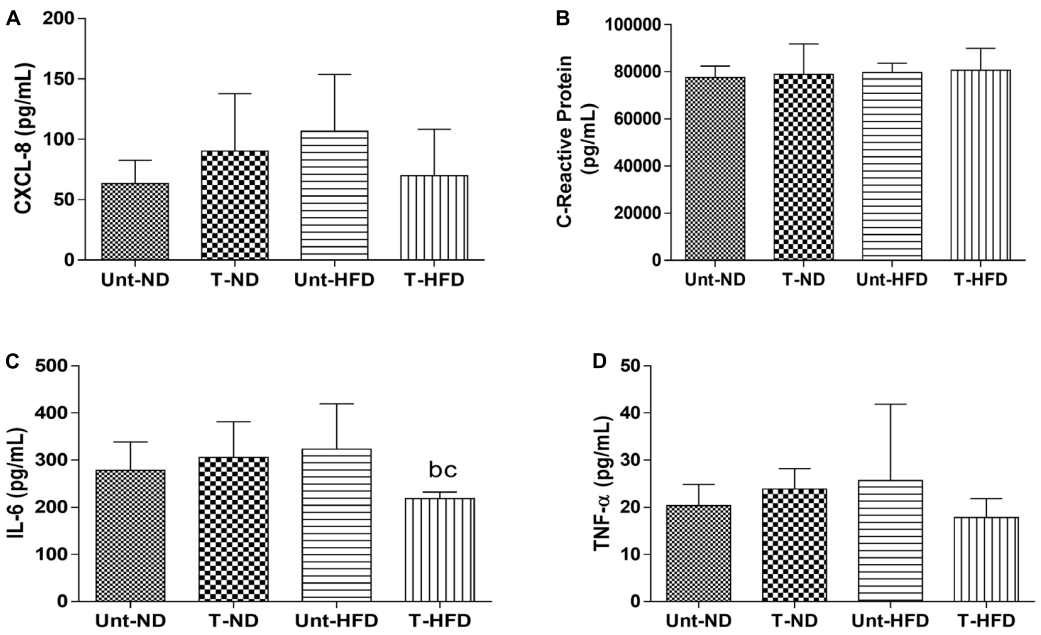

FIGURE 2 | Serum concentrations of pro-inflammatory cytokines. **(A)** CXCL-8: interleukin 8, **(B)** PCR: C-reactive protein, **(C)** IL-6: interleukin 6, and **(D)** TNF-α: tumor necrosis factor alpha. Unt-ND, untrained normal diet (n = 10); T-ND, trained normal diet (n = 10); Unt-HFD, untrained high-fat diet (n = 10); and T-HFD, trained high-fat diet (n = 10). Data presented as mean ± SD ($p < 0.05$). [b]*vs.* T-ND; and [c]*vs.* Unt-HFD.

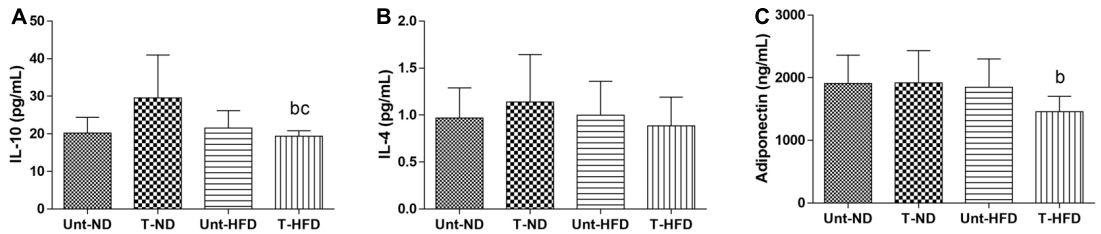

FIGURE 3 | Serum concentrations of anti-inflammatory cytokines. **(A)** IL-10: interlecucin-10, **(B)** IL-4: interleukin-4, and **(C)** adiponectin. Unt-ND, untrained normal diet (n = 10); T-ND, trained normal diet (n = 10); Unt-HFD, untrained high-fat diet (n = 10); and T-HFD, trained high-fat diet (n = 10). Data presented as mean ± SD ($p < 0.05$). [b]*vs.* T-ND; and [c]*vs.* Unt-HFD.

expressions in eutrophic and obese individuals (Hida et al., 2005; de Castro et al., 2019). These adipokines have been studied as pathological biomarkers because they relate to insulin resistance (Flehmig et al., 2014). Thus, we monitored adipose tissue expansion by the values of serum vaspin in high-fat diet animals. However, despite finding an increase in the expression

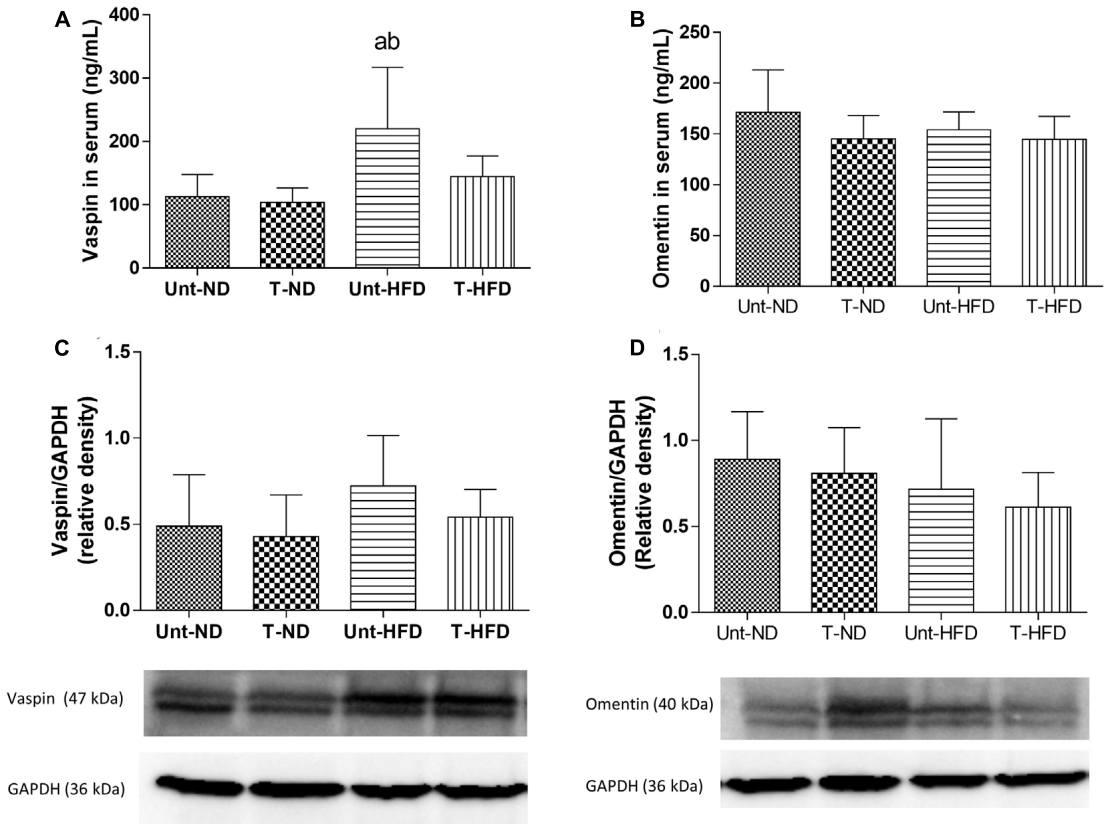

FIGURE 4 | Behavior of serum adipokines and adipose tissue. **(A)** Serum vaspin concentration, **(B)** serum omentin concentration, **(C)** Western blot representative of vaspin (47 kDa) in visceral adipose tissue (VAT), and **(D)** Western blot representative of omentin (40 kDa) in VAT. Unt-ND, untrained normal diet (n = 10); T-ND, trained normal diet (n = 10); Unt-HFD, untrained high-fat diet (n = 10); and T-HFD, trained high-fat diet (n = 10). Data presented as mean ± SD ($p < 0.05$). ^{a}*vs.* Unt-ND; ^{b}*vs.* T-ND.

of vaspin mRNA in obese women compared to eutrophic women, no positive correlation with obesity was observed (Auguet et al., 2011). In general, studies with vaspin still show controversial results as some point to the lack of a relationship between the serum levels and obesity and the distribution of fat, while others demonstrated a positive correlation (Blüher, 2012). Moreover, the hypothesis that vaspin may be directly related to the consumption of excess lipids is not ruled out, thus triggering possible insulin resistance, arteriosclerosis, and heart problems (Derosa et al., 2013; Szkudelska et al., 2014). The increase in serum vaspin without changing its expression in VAT may be due to a greater contribution from the subcutaneous adipose tissue, as previously shown (Jung et al., 2011; Shaker and Sadik, 2013; Weiner et al., 2019).

Regarding omentin, these results were not expected since earlier data showed evidence that omentin can be significantly reduced in obese rats when compared to non-obese rats (De Souza Batista et al., 2007; Feng et al., 2013; Proença et al., 2014). There is no consensus in the literature regarding the concentration of omentin in patients with obesity because their response has not yet been fully elucidated (Derosa et al., 2013; Escoté et al., 2017; Aliasghari et al., 2018). Just as HIIT slowed down fat gain, but this loss was not seen in abdominal fat depots, we suggest that this exercise may have mobilized more subcutaneous fat, which did not reflect the change in adipokines omentin and vaspin.

The importance of these adipokines lies in the fact that they can influence adipocytes and other tissues in an autocrine or paracrine manner, affecting multiple metabolic processes such as regulating eating behavior, insulin sensitivity, inflammation, and immunity (Escoté et al., 2017). Thus, obesity is directly associated with low-grade chronic inflammation, as the expression of proinflammatory cytokines (IL-6, IL-8, PCR, and TNF-α) is shown to be increased in this pathology. Studies show that there is a close link between cytokines from obesity and the development of other chronic diseases (de Leal and Mafra, 2013; Mraz and Haluzik, 2014). However, in the present study, no inflammatory condition was observed in animals that were fed the high-fat diet. It is believed that this situation was due to the short time of exposure of the animals to the high-fat diet and that the accumulation of fat was not sufficient to cause changes in the production of such adipokines with pro-inflammatory characteristics (Rocha et al., 2016). This was contrary to other studies found in the literature (Tzanavari et al., 2010; Jung and Choi, 2014).

Even with this slight inflammatory activation caused by the diet, it is noteworthy that HIIT in obese animals reduced the levels of IL-6, IL-10, and adiponectin. It is known that the stress promoted by physical exercise is linked to an increase in catecholamine discharge and that the catecholamine receptors present in macrophages have great importance in modulating the inflammatory response (Figueiredo et al., 2017). In the condition of obesity, a high-fat diet could lead to an increase in catecholamines, which, in turn, *via* cAMP response element-binding protein, would suppress the expression of adiponectin (Liu and Liu, 2009). Thus, it can be considered that the lower values of adiponectin and IL-10 in trained animals may also mean that the training was intense for the metabolic condition of obese animals.

The effects of HIIT in the systemic inflammatory profile are controversial since serum levels may arise not only from adipose tissue but also from muscle, liver, and others. Studies show that this reduction in adiponectin in obese individuals may contribute to the susceptibility to viral lung infections and the severity of these infections in obese individuals (Salvator et al., 2020). Despite the benefits of adipokine in protecting against metabolic diseases such as obesity and diabetes (Jortay et al., 2012; Martinez-Huenchullan et al., 2020), it is important to note that there are different adiponectin isoforms with functions that are not entirely clear, and exercise seems to regulate each isoform differently (Gerosa-Neto et al., 2016; Martinez-Huenchullan et al., 2018). Further studies are needed to verify the role of different exercise modalities in circulating adipokines and cytokines.

The potential limitations include failure to assess the thermogenic effects of HIIT on whole-body fat metabolism (often done by direct or indirect calorimetry or oxygen uptake and carbon dioxide gas exchange measurement). However, exercise performance improvement in both HFD and NFD animals suggests the negligible thermogenic effects of HIIT on adipose tissues. Still HIIT fat loss thermogenic effects and adipose tissue mobilization need further investigation. Another potential limitation is that, although in this study we used a lower fat (20%) content than other studies to induce obesity, previous results showed that our diet was effective to promote obese phenotype, including augmentation in body adiposity, body weight, weight gain, total mass, and visceral depots (Estadella et al., 2004; Duarte et al., 2008; Sene-Fiorese et al., 2008; Oishi et al., 2018).

In summary, we suggest that vaspin and omentin are not responsive to HIIT in obese and eutrophic animals, although the training protocol was able to retard the weight gain, with no change in visceral abdominal fat and no improvement in the inflammatory profile. Further studies are needed to explore the molecular mechanisms involved in the expression of omentin and vaspin in response to exercise.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The experimental procedures in this study conformed to the Committee on Animal Research and Ethics (n°. 3963080116) from the Federal University of São Carlos (UFSCar).

AUTHOR CONTRIBUTIONS

LC, AD, CC, and FF helped conceive the design, performed the analyses, analyzed the data, and wrote the first draft of the manuscript. IM and FA performed other data analyses and helped to draft the manuscript. DM, VF, LC, and CC helped conceive the design and supervised the experimental trials and training sessions. LC, DM, VF, CC, FA, and FF helped with data analyses and helped draft the manuscript. DM, CC, FF, and AD interpreted the study results and edited the manuscript. LC, DM, VF, CC, FF, and AD helped conceive the design, helped with the data analyses, provided funding for the study, and helped draft the manuscript. All the authors have read and approved the final version of the manuscript and agreed with the order of presentation of the authors.

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OPINION
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Growth Hormone as a Potential Mediator of Aerobic Exercise-Induced Reductions in Visceral Adipose Tissue

Angelo Sabag[1]*, *Dennis Chang*[1] and *Nathan A. Johnson*[2]

[1] NICM Health Research Institute, Western Sydney University, Westmead, NSW, Australia, [2] School of Health Sciences, Faculty of Medicine and Health, The University of Sydney, Camperdown, NSW, Australia

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INTRODUCTION

Obesity remains one of the leading causes of death worldwide and is a well-known risk factor for a myriad of non-communicable diseases including diabetes, cardiovascular disease, and a variety of cancers (Wolf and Colditz, 1998; Frühbeck et al., 2013). While the relationship between obesity and cardiometabolic risk is well-established, the location of adipose tissue, particularly in the abdominal region, is considered a greater predictor of metabolic dysfunction than total fat mass (Kahn et al., 2006). Central obesity, characterized by the excess accumulation of adipose tissue in the abdominal region, is strongly and independently correlated with metabolic syndrome and is assessed clinically through the measurement of waist circumference (Shen et al., 2006). Central adiposity can be further subcategorized into abdominal subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) (Snel et al., 2012). While the relationship between SAT and cardiometabolic risk remains equivocal, VAT has been established as a unique pathogenic fat depot. VAT acts as an endocrine organ by secreting adipocytokines and other vasoactive substances (Kanaya et al., 2004) and is associated with cardiometabolic risk independent of body mass index (BMI) or total body adiposity (Fox et al., 2007; Pak et al., 2016). Consequently, it is important to identify new, as well as further develop existing therapies to improve the management of obesity.

A landmark study in 1990 showed that exogenous growth hormone (GH) administered to older healthy males led to significant improvements in total body adiposity and lean body mass (Rudman et al., 1990). Since then, the results from further studies have shown that GH therapy can improve VAT, circulating lipid levels, and insulin resistance in adults with obesity and/or diabetes (Johannsson et al., 1997; Nam et al., 2001). Although studies like these highlighted the potential utility of GH therapy for the amelioration of age-related declines in metabolic function and body composition, further studies identified various side effects of GH therapy such as an increased likelihood of soft tissue edema, joint pain, carpal tunnel syndrome, gynecomastia, and diabetes (Liu et al., 2007). Consequently, exogenous GH therapy became typically reserved for individuals with GH deficiencies resulting from hypothalamic/pituitary disease (Clemmons et al., 2014). Despite this, there has since been increasing interest in identifying therapies, including lifestyle interventions, that increase physiologic GH release and action.

Exercise and diet modification are cornerstone therapies for the management of obesity-related disease. Interestingly, pooled data from clinical trials show that while exercise is less effective than diet modification for body weight loss, it appears to elicit superior reductions in VAT (Verheggen et al., 2016). This finding may partly be explained by exercise-induced changes in lipolytic hormones, such as GH, during and after exercise, which seem to target VAT (Berryman and List, 2017). Acute exercise has been shown to temporarily increase GH release in an intensity-dependent

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Zhaowei Kong,
University of Macau, Macau
Ashril Yusof,
University of Malaya, Malaysia
Jinfu Wu,
South China Normal University, China

***Correspondence:**
Angelo Sabag
a.sabag@westernsydney.edu.au
orcid.org/0000-0002-0195-7029

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manner (Godfrey et al., 2003), and such responses appear to be mediated by cardiorespiratory fitness (CRF) (Holt et al., 2001). However, the degree to which temporal exercise-induced changes in GH release and action improve VAT is unclear and warrants further investigation. Furthermore, although both aerobic and resistance exercise elicit a GH response, the relative contribution of aerobic exercise on GH response and action arguably is less clear. Consequently, this article will evaluate the various factors that contribute to aerobic exercise-induced GH response and how these changes influence VAT and cardiometabolic health more broadly.

SOMATOTROPIC AXIS

The somatotropic axis is a primary regulator of metabolism and consists of GH and insulin-like growth factors (IGF-I and -II), and their associated carrier proteins and receptors, which are further regulated by nutritional status and hormones such as ghrelin and insulin (Renaville et al., 2002; Savastano et al., 2014). GH is secreted at the anterior pituitary gland in a pulsatile manner and is primarily regulated by hypothalamic neuropeptides GH-releasing hormone (GHRH) and somatostatin, which stimulate and inhibit GH secretion, respectively (Vijayakumar et al., 2011). GH affects multiple systems within the body and is the primary secretagogue of IGF-I, which itself is a regulator of GH secretion (Ohlsson et al., 2009).

GH is a potent anabolic hormone that plays a significant role in lipid metabolism at various sites including the liver, skeletal muscle, and adipose tissue (Dehkhoda et al., 2018). During periods of fasting or stress, GH promotes the use of lipids as the primary fuel source in order to preserve carbohydrates and protein stores (Lewitt, 2017). In the liver, lipid uptake and production are increased through the phosphorylation of sterol regulatory element-binding proteins (SREBP-1a) and by increased lipoprotein lipase (LPL) expression (Vijayakumar et al., 2011). In addition to this, GH also indirectly increases fatty acid oxidation and activates the adenosine monophosphate-activated protein kinase (AMPK) pathway (Vijayakumar et al., 2011).

While GH is a powerful regulator of lipid metabolism, its role varies depending on the target site. For example, although GH has lipogenic effects within the liver, the opposite occurs in adipose tissue, particularly VAT, where GH elicits lipolytic effects through the suppression of LPL activity (Stanley and Grinspoon, 2015). During exercise or fasting, GH stimulates the release of free fatty acids (FFAs) into circulation where they are transported to various organs, including myocytes where they may be repackaged as triglycerides or undergo β-oxidation in the mitochondria. While it is recognized that GH also elicits various effects on glucose and protein metabolism, exercise-induced alterations in physiologic GH appear to primarily affect lipid metabolism (Kanaley et al., 2004).

OBESITY

The bidirectional relationship between central obesity and impaired GH secretion has been widely reported despite being scantily understood (Stanley and Grinspoon, 2015; Lewitt, 2017). Increased ectopic fat, such as VAT and intrahepatic triglyceride, contributes to insulin resistance and may affect the feedback control system of the somatotropic axis, resulting in a cascade of metabolic impairments (Savastano et al., 2014). Interestingly, physiologic increases in GH secretion through fasting or exercise contribute to increases in circulating FFAs; however, these do not lead to metabolic impairments due to various mechanisms, such as concurrent increases in skeletal muscle fatty acid uptake and oxidation (Huang et al., 2020). Interestingly, a study by Stokes et al. (2008) showed that FFA levels may also regulate GH *via* a negative feedback control, as nicotinic acid-mediated suppression of lipolysis, and consequently reduced circulating FFAs, led to a significantly greater GH response. This finding may help further explain why individuals with obesity and reduced CRF, who on average have elevated levels of FFAs (König et al., 2003; Boden, 2008), exhibit impaired GH secretion and action, as their ability to uptake and oxidize FFAs is reduced (Kim et al., 2000).

The obesity phenotype shares considerable overlapping risk factors with adult GH deficiency such as increased serum low-density lipoprotein cholesterol (Cordido et al., 2010) and inflammation (Utz et al., 2008), thereby making it difficult to decouple the cause from the effect. However, it is likely that impaired GH secretion is an acquired transient defect that occurs prior to the onset of obesity, as previous reports showed that following 2 weeks of overeating, GH levels were decreased while body weight remained unchanged (Cornford et al., 2011). Importantly, not all adult GH deficiency is caused by obesity-inducing behaviors, as adults with hypothalamic or pituitary diseases also exhibit suppressed GH production and increased central adiposity. A known therapy for improving many of the aforementioned risk factors is energy restriction *via* diet modification. In fact, a previous study involving 18 adults with obesity and 18 age- and sex-matched controls showed that defects in GH secretion were ameliorated to near-normal function following significant diet-induced weight loss (Rasmussen et al., 1995). Other lifestyle interventions such as exercise have been shown to alter physiologic GH response; however, as mentioned previously, this response has been shown to be blunted in people with obesity, including childhood obesity (Oliver et al., 2012). These data suggest that obesity-inducing behaviors and obesity itself both contribute to altered GH release and function beyond normal age-related declines.

AEROBIC EXERCISE

Regular aerobic exercise enhances the body's ability to transport and oxidize FFAs during exercise at varying work rates (Van Tienen et al., 2012). This is also true for individuals with impaired fatty acid oxidation, such as those with obesity (Kim et al., 2000) and diabetes (Ghanassia et al., 2006; Van Tienen et al., 2012), and it has been suggested that these improvements may be mediated through exercise-induced increases in mitochondrial and fatty acid transporter content, carnitine shuttle activity (Melanson et al., 2009), and cardiorespiratory fitness (Kujala et al., 2019). In fact, low CRF may be a greater predictor of metabolic dysfunction than VAT (Kim et al., 2014), and as such, improving CRF has emerged as a therapeutic target for individuals with

obesity-related disease, which may also serve to reverse GH-related impairments.

As mentioned earlier, GH promotes lipolysis within adipose tissue and increases mitochondrial oxidative capacity (Short et al., 2008). Although obesity blunts exercise-induced GH response, CRF, which in part reflects muscle oxidative capacity, appears to be a greater determinant of exercise-induced GH secretion than obesity (Holt et al., 2001). Therefore, at least in theory, improving CRF in the absence of weight loss should still yield beneficial effects of GH secretion. However, despite exercise and GH eliciting similar effects on adipose tissue and lipid metabolism, it is unclear whether exercise-induced improvements in central adiposity are mediated by concurrent changes in physiologic GH response or if the improvements in central adiposity and GH response are simply independent by-products of exercise adherence. As GH responses to acute and chronic exercise are affected by a variety of factors such CRF, exercise volume, and exercise intensity (Frystyk, 2010), further research is required to determine optimal exercise prescriptions for the amelioration of somatotropic dysfunction.

Acute aerobic exercise has been shown to increase GH levels, and these changes have been shown to be strongly associated with exercise intensity and volume, a function of exercise duration and frequency (Pritzlaff et al., 1999). In fact, current available evidence suggests that exercise-induced GH responses may only be elicited at or above specific exercise volume and intensity parameters. For example, Felsing et al. (1992) showed that in order to increase circulating GH, healthy adult men needed to exercise for a minimum of 10 min above lactate threshold. Similarly, Gilbert et al. (2008) showed that healthy adult men performing 30 min of aerobic exercise at 70% VO_{2peak} elicited greater GH response than when performing a single bout of 30-s sprint on a cycle ergometer. While the majority of research has been undertaken in men, a study by Sauro and Kanaley (2003) also showed that exercising at 75% VO_{2peak} for a minimum of 10 min is sufficient to increase GH response in healthy young women. These findings highlight that while exercise intensity does appear to influence GH levels acutely, sufficient exercise volume may also be required.

Currently, it is unclear whether regular aerobic exercise can increase the GH-response to acute exercise. A study by Sasaki et al. (2014) reported that following 4 weeks of high-intensity interval training (HIIT) or moderate-intensity continuous training (MICT), the magnitude of GH response to exercise did not increase from pre-intervention measures in sedentary but otherwise healthy men for both interventions. Furthermore, neither HIIT nor MICT improved whole-body fat mass, liver fat, and intramyocellular lipid content. Further findings from a recent randomized controlled trial involving young women with obesity showed that when compared to energy-matched MICT, HIIT or supramaximal aerobic exercise led to greater VAT reduction but not greater changes from pre-intervention levels in serum GH measured immediately or 4 h after exercise (Zhang et al., 2021). In fact, all groups showed elevated GH responses to exercise; however, only the higher-intensity interventions decreased VAT, suggesting that other factors likely contributed to these improvements in this cohort.

While there are limited data pertaining to the effects of regular exercise on chronic changes in 24-h GH release and function, a study involving untrained eumenorrheic women showed that regular aerobic exercise above lactate threshold led to a two-fold increase in 24-h GH secretion (Weltman et al., 1992). A recent study involving young and middle-aged men showed that regular concurrent resistance and aerobic exercise led to significantly increased GH levels at rest (Sellami et al., 2017); however, whether these changes influence 24-h GH release remains unknown. Another limitation of the GH and exercise literature is that most studies are undertaken in younger to middle-aged adults. Consequently, it is unclear whether these findings translate to older adults or those with more severe metabolic abnormalities. A recent review involving three studies showed that regular exercise resulted in negligible effects on GH in older adults (Sellami et al., 2019). As the studies included in the review were limited to small samples and did not include stand-alone aerobic exercise interventions, further research is required to determine whether exercise-induced changes in physiologic GH improve age- and obesity-related disease in elderly cohorts. Furthermore, there is limited data pertaining to the effects of chronic exercise on basal GH or 24-h GH release; therefore, we are unable to comment on whether any such changes would increase total energy expenditure or fat oxidation rates.

Although the precise mechanisms driving exercise-induced improvements in cardiometabolic outcomes remain a matter of scientific debate, given the congruent metabolic effects of exercise, particularly aerobic exercise, and GH, it could follow that the behavior itself and ensuing endocrine and metabolic changes both improve cardiometabolic health, with the latter providing somewhat of an additive, but perhaps not essential, effect (**Figure 1**). Like GH, exercise exerts potent lipolytic effects, particularly on VAT (Tsiloulis and Watt, 2015). Furthermore, exercise has been shown to elicit significant improvements in ectopic fat in the absence of weight loss (Sabag et al., 2017, 2020), thus indicating that these improvements are mediated through mechanisms other than simple energy expenditure. As exercise-induced GH response occurs in an intensity-dependent manner, and appears to be mediated by CRF, this may explain why HIIT can lead to similar improvements in waist circumference and VAT than in higher-volume MICT despite requiring less time and expending less energy (Keating et al., 2015). While the current evidence remains circumstantial, future research exploring the relative contribution of somatotropic change in exercise-related metabolic improvements could have far-reaching implications, including advancing current exercise prescription practices for the management of cardiometabolic health.

CONCLUSION

Based on the current available literature, it appears that exercise-induced reductions in VAT are mediated by multiple factors, which may include acute and chronic exercise-induced change in GH levels. This could be due to the similar lipolytic effects of

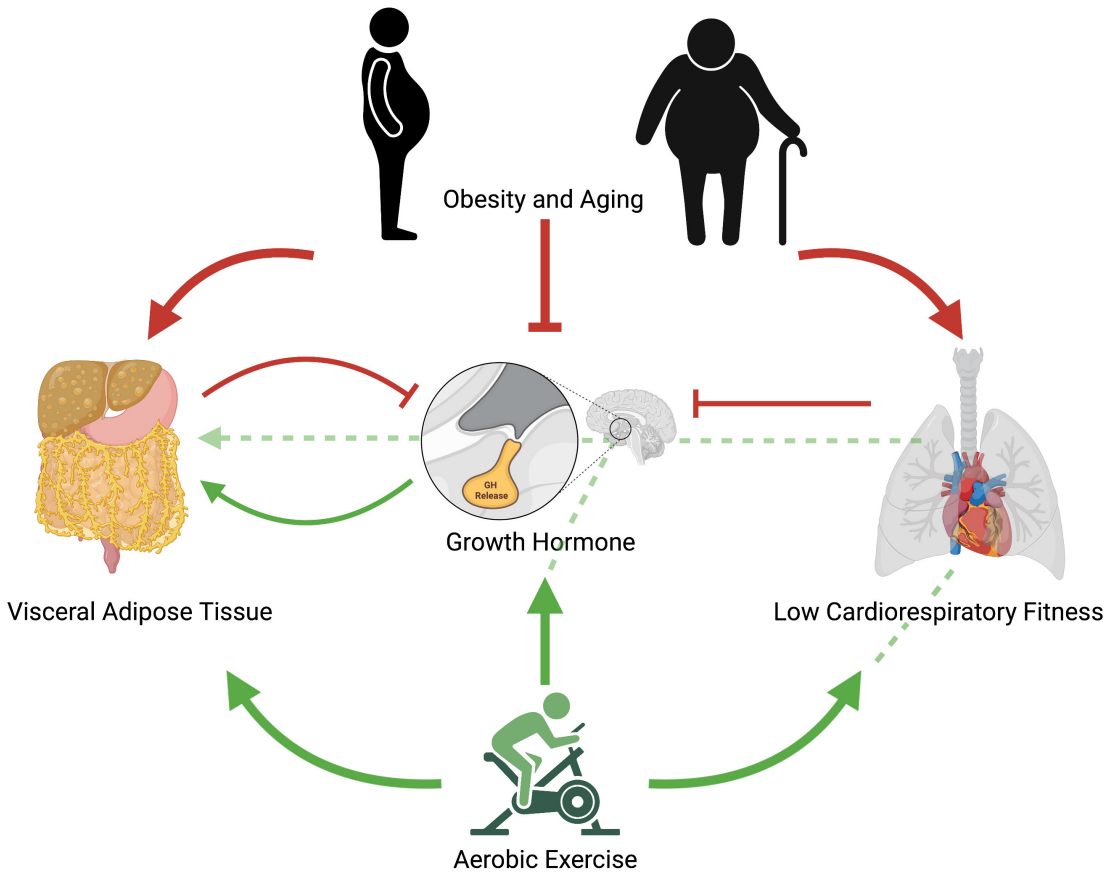

FIGURE 1 | Proposed mechanistic pathway for aerobic exercise induced changes in growth hormone release and ensuing reductions in visceral adipose tissue. Red arrows represent negative effects; green arrows represent positive effects; unbroken red lines represent inhibitory effects; broken green lines represent potential positive pathways of exercise-induced improvements in cardiometabolic health. Obesity and aging contribute to increased visceral adipose tissue, reduced cardiorespiratory fitness, and impaired growth hormone release. Low cardiorespiratory fitness further contributes to impaired growth hormone release as does visceral adipose tissue. Regular aerobic exercise reduces visceral adipose tissue, increases growth hormone release, and increases cardiorespiratory fitness. Regular aerobic exercise may also indirectly improve visceral adipose tissue through increases in cardiorespiratory fitness, which lead to improved growth hormone response and ensuing interactions in visceral adipocytes. Acute aerobic exercise elicits growth hormone release, which increases lipolysis of visceral adipose tissue.

both GH and exercise on VAT. Furthermore, because CRF plays a significant role in GH response, partaking in regular exercise may ameliorate age-related reductions in GH response and action.

As exercise has been shown to ameliorate obesity-related CRF and other cardiometabolic impairments, exercise should be incorporated into routine care for the treatment of adult-onset GH deficiency and associated metabolic perturbations. Importantly, increasing CRF and weight loss concurrently through exercise and dietary modification may yield greater restoration of GH function than either intervention on its own; however, there is limited evidence to confirm this. Consequently, further research is required to elucidate the relationship between somatotropic changes and exercise-induced cardiometabolic improvements.

AUTHOR CONTRIBUTIONS

The conception and drafting of this article was led by AS. NJ and DC contributed to the drafting and critical revision of the article. All authors approved the submitted version.

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Scientific Challenges on Theory of Fat Burning by Exercise

M. Brennan Harris[1] *and Chia-Hua Kuo*[2]*

[1] Department of Health Sciences, College of William and Mary, Williamsburg, VA, United States, [2] Laboratory of Exercise Biochemistry, College of Kinesiology, University of Taipei, Taipei, Taiwan

Exercise training decreases abdominal fat in an intensity-dependent manner. The fat loss effect of exercise has been intuitively thought to result from increased fat burning during and after exercise, defined by conversion of fatty acid into carbon dioxide in consumption of oxygen. Nevertheless, increasing exercise intensity decreases oxidation of fatty acids derived from adipose tissue despite elevated lipolysis. The unchanged 24-h fatty acid oxidation during and after exercise does not provide support to the causality between fat burning and fat loss. In this review, alternative perspectives to explain the fat loss outcome are discussed. In brief, carbon and nitrogen redistribution to challenged tissues (muscle and lungs) for fuel replenishment and cell regeneration against abdominal adipose tissue seems to be the fundamental mechanism underlying the intensity-dependent fat loss effect of exercise. The magnitude of lipolysis (fatty acid release from adipocytes) and the amount of post-meal carbon and nitrogen returning to abdominal adipose tissue determines the final fat tissue mass. Therefore, meal arrangement at the time when muscle has the greatest reconstruction demand for carbon and nitrogen could decrease abdominal fat accumulation while increasing muscle mass and tissue repair.

Keywords: resistance training, fat loss, intensity, carbon and nitrogen redistribution theory, fatty acid oxidation, aerobic training, fat burner, obesity

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Pradeep R. Varadwaj, The University of Tokyo, Japan

***Correspondence:**
Chia-Hua Kuo
kch@utaipei.edu.tw; kuochiahua@gmail.com

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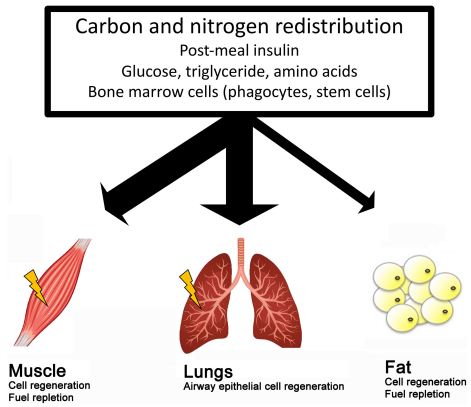
THE SCIENTIFIC CHALLENGES

Exercise training decreases abdominal fat, in which high-intensity exercise produces more prominent fat loss than low and moderate intensity exercise (Vissers et al., 2013; Viana et al., 2019). Fat burning is a classic theory to describe the abdominal fat-reducing outcome of exercise training. This theory is built on the intuition that exercise as an energy consuming behavior will increase fatty acid oxidation from abdominal fat stores compared with sedentary condition, and thus accounts for the fat loss outcomes of exercise training (Abbasi, 2019). Increased lipolysis with elevated circulating fatty acids together with increased oxygen consumption during exercise seems to favor this explanation (Romijn et al., 1993; Mora-Rodriguez and Coyle, 2000). However, the absolute energy contribution from plasma fatty acids (assuming all from adipose tissue) decreases as exercise intensity increases (from 25 to 85% VO_{2max}) and is consistent with decreased tissue fatty acid uptake during exercise (Romijn et al., 1993). Furthermore, increased energy expenditure, especially during high intensity exercise, comes from fuel stored in skeletal muscle (mostly glycogen), not adipose tissue (fatty acids) (Romijn et al., 1993). Neither aerobic exercise nor resistance exercise increases 24-h fatty acid oxidation (Melanson et al., 2002).



GRAPHICAL ABSTRACT | Exercise decreases abdominal fat mass, especially at high intensity. This outcome is not causally associated with fat burning, but better explained by carbon and nitrogen redistribution. Since abdominal fat tissue constantly releases fatty acids into circulation under post-absorptive condition with natural cell deaths, exercise diverts more post-meal carbon and nitrogen to muscle for energy repletion and cell regeneration after phagocytosis and stem cell homing. This in turn leads to concurrent fat mass loss and muscle mass gain. Respiratory ventilation during high-intensity aerobic exercise amplifies the competition for post-meal carbon and nitrogen against adipose tissues.

A number of clinical studies divulges paradox between fat burning and fat loss outcome. A 15-weeks sprint training depending primarily on anaerobic metabolism effectively decreases abdominal fat, whereas moderate-intensity exercise training depending on aerobic metabolism with similar energy expenditure (60% VO_{2max} consuming ∼200 kcal, three times per week) failed to decrease body fat in young women (Trapp et al., 2008). Similarly, no fat loss effect was observed following 12-weeks of aerobic training at both low-intensity (40% VO_{2max}) and moderate-intensity (70% VO_{2max}) among obese men (∼350 kcal, three times per week) (Aggel-Leijssen et al., 2002). Therefore, an alternative theory to explain the fat loss outcome of exercise should be explored in order to provide robust scientific basis for designing effective fat loss training regimens.

Lipolysis appears to be more relevant with fat loss than fatty acid oxidation. Exercise increases plasma epinephrine levels at high intensities (Mora-Rodriguez and Coyle, 2000). Epinephrine stimulates lipolysis and inhibits the esterification of triglycerides via adrenergic receptors of adipocytes (Reilly et al., 2020), leading to release of free fatty acid from adipose tissue into circulation (Urhausen et al., 1994). Long-term adrenergic stimulation (i.e., clenbuterol and ractopamine) has been shown to decrease fat mass and increase muscle mass without changes in food intake and body temperature (Page et al., 2004). Abdominal adipocytes show much higher lipolytic response to epinephrine than gluteal adipocytes, which may partly explain the commonly observed abdominal fat loss response to high-intensity exercise training (Wahrenberg et al., 1989; Thompson et al., 2012).

The physiological significance of the enhanced release of fatty acids from lipolysis without the corresponding increase in fatty acid oxidation during and after exercise remains unclear. However, a proposed role of adipocyte-derived fatty acids in tissue repair has been recently described elsewhere (Shook et al., 2020). Fatty acids (e.g., eicosapentaenoate, linoleate, α-linolenate, γ-linolenate, and arachidonate) have been found to accelerate wound healing (Ruthig and Meckling-Gill, 1999). In addition, vascular structure formation can be enhanced by fatty acids, which is mediated by increasing reactive oxygen species and activating endothelial NOS synthase (Taha et al., 2020). Both findings implicate a possible role of elevated fatty acid concentrations in the repairing mechanism of exercise-induced tissue damage.

BASIC ASSUMPTION OF FAT BURNING THEORY

The first basic assumption of fat burning theory is that fat cell death has no role in fat loss. However, this assumption is unlikely valid since fat cells are continuously dying and regenerating throughout our life. Approximately 8.4% of subcutaneous abdominal adipocytes are renewed annually with an average half-life of 8.3 years in human adults (Spalding et al., 2008). Abdominal fat mass is determined by the balance of fat cell death and regeneration of adipose tissue, which is influenced by exercise (Allerton et al., 2021). Acute adrenergic stimulation has been reported to induce fat cell death (Kim et al., 2010). The balance between fat cell death and regeneration is also strongly influenced by plasma insulin concentrations, which varies with exercise habit, meals, and sleeping fast. Lowering insulin for 2 weeks causes a massive fat loss >70%), associated with the death of adipocytes and endothelial cells in adipose tissues (Géloën et al., 1989). Lowering physical activity increases plasma insulin concentration and waist circumference without an observable change in body weight (Chen et al., 2006). In a contrast, high-intensity exercise lowers fasting and post-meal insulin levels while increasing the insulin sensitivity of exercised muscle (Ivy et al., 1999; Rice et al., 1999; Trapp et al., 2008), which partly explains the decreases in fat mass and increases in muscle mass among training individuals.

The second basic assumption of the fat burning theory is that muscle and fat cells are not interconvertible in a human body. However, we could not preclude the possibility that the fat mass loss concurrent with muscle mass gain after exercise training is associated with conversion between muscle and fat progenitor cells, derived from circulating bone marrow stem cells. Conversion from muscle satellite cells to an adipogenic lineage contributes the development of obesity and muscle mass loss in animals (Durschlag and Layman, 1983; Scarda et al., 2010). Glucose and reactive oxygen species (ROS) also stimulate the adipogenic conversion from muscle-derived stem cells (Aguiari et al., 2008). Both plasma glucose and ROS elevate with age and weight growth (Ho et al., 2019; Wang et al., 2019). However, exercise training lowers plasma glucose (Colberg et al., 2010) and ROS (Vinetti et al., 2015) in animals and humans. Circulating myokines released from contracting muscle also suppress adipogenesis and stimulate myogenesis (Barra et al., 2012; Ma et al., 2019). As a result, exercise appears to, at the very

least, attenuate the conversion of muscle to fat and may instead activate the conversion of fat to muscle.

Further evidence of this mechanism comes from the wide array of exosomes (containing nucleic acids or peptide) released from exercising skeletal muscle implicating the crosstalk between muscle and fat tissues. Adipose tissues are a major source of circulating exosomes containing a variety of mediators, which may influence muscle development (Thomou et al., 2017; Ying et al., 2017). Some nucleic acid molecules encapsulated in the extracellular vesicles may play a role in the interconvertibility between fat and muscle progenitor cells. For example, muscle contraction induces acute increases of miR-21 into circulation (Xu et al., 2016). This molecule has been shown to inhibit proliferation of human adipose tissue-derived mesenchymal stem cells and high-fat diet-induced obesity (Kim et al., 2012). In addition, circulating miR-130 level has been found lowered in obese women and exercise stimulates release of miR-130 from skeletal muscle into circulation which inhibits adipogenesis (Lee et al., 2011).

The third assumption of the fat burning theory is that the increased carbon and nitrogen demands for airway epithelial cells regeneration in lungs does not contribute to fat loss during and after exercise. However, the possibility that the fat loss effect of high-intensity aerobic training due to competition for carbon and nitrogen between lungs and adipose tissues cannot be excluded (Leibacher and Henschler, 2016; Saat et al., 2016). The lungs are strong competitors for bone-marrow stem cells (main source of muscle and adipose progenitor cells) which is required for cell regeneration of peripheral tissues. Greater than 60% of bone marrow derived stem cells are used by the lungs (Rochefort et al., 2005) for regenerating the short-lived airway epithelial cells (Murphy et al., 2008; Rawlins and Hogan, 2008). This suggests a much higher demand of the lungs for carbon and nitrogen against other tissues. Acute airway epithelium damage induced by acute ventilations during aerobic exercise significantly increases phagocyte infiltration to the lungs (Adams et al., 2011; Leibacher and Henschler, 2016; Combes et al., 2019). This also induces cell regeneration following phagocytic clearance of unhealthy cells in airway epithelial lining in a way similar to muscle inflammation (Su et al., 2005). Massive consumption of bone marrow immune cells and stem cells by the lungs may explain why high-intensity aerobic exercise has greater magnitude of fat loss effect compared with resistance exercise (Willis et al., 2012).

ALTERNATIVE THEORY

During unfed conditions, visceral adipose tissues continuously releases fatty acids into circulation (Coppack et al., 1990), together with normal turnover of adipocytes and endothelial cells in adipose tissues (Spalding et al., 2008). Therefore, post-meal carbon and nitrogen returning to fat cells determines the abdominal fat mass (Coppack et al., 1990; Chen et al., 2006). Skeletal muscle is a competitor for the post-meal carbon and nitrogen (Ivy et al., 1988) and therefore decreases post-meal carbon and nitrogen returning to adipose tissue. Increasing muscle demand at the time when post-meal nutrients are supplying into circulation can minimize the substrates returning to adipose tissue. This concept is supported by animal and human studies in which providing meal immediately after resistance training results in greater magnitude of muscle mass gain and fat mass loss compared with the condition of detaching mealtime away from the workout (Suzuki et al., 1999; Cribb and Hayes, 2006).

Studies employing dual energy X-ray absorptiometry have also provided solid support for the carbon and nitrogen redistribution effect of exercise training by the evidence of concurrent increases in lean body mass and decreases in fat mass (Cribb and Hayes, 2006; Abbasi, 2019; Kemmler et al., 2021). This nutrient redistribution effect remains noticeable in sarcopenic elderly aged above 70 years and above (Kemmler et al., 2020) and perimenopausal women (Coll-Risco et al., 2019). It is likely that the decreased abdominal fat accumulation after exercise training is associated with increased muscle regeneration attracting more postprandial carbon and nitrogen to challenged muscle tissue against abdominal adipose tissues (Huang et al., 2017; Tidball, 2017). Interventions that promote muscle growth have been shown to decrease fat mass (Mcpherron and Lee, 2002; Leong et al., 2010). Muscle regeneration during inflammation is known to contribute muscle mass gain (St Pierre and Tidball, 1994). High-intensity exercise causes immune cell infiltration into challenged muscle to eliminate senescent cells (Huang et al., 2017; Yang et al., 2018). The inflammation process includes stem cell homing, proliferation, and differentiation after phagocytosis by infiltrated immune cells in challenged muscle tissues (Tidball, 2017; Wu et al., 2019). The increased reconstruction demand of exercised muscle may partly explain the disparity in the development of muscle and adipose tissues after exercise training.

Lipoprotein lipase (LPL) attached on the surface of endothelial cells in capillary lumen determines relative partition of circulating triglycerides to muscle and adipose tissues after meals. The molecular size of triglyceride carried by chylomicron and VLDL is too large to transport across cell membrane of adipocytes from blood unless it is locally hydrolyzed by LPL in the adipose and muscle tissues. Relative LPL expression in adipose tissue and muscle tissues thus determines the daily distribution of circulating triglycerides (chylomicron and VLDL) partitioning into adipose tissues and skeletal muscle after meals. This ratio is substantially influenced by exercise training, in which trained women have relatively higher (∼8 times) muscle-to-adipose tissue LPL ratio compared to their untrained state (Simsolo et al., 1993). This suggests that exercise training favors postprandial triglyceride partitioning into skeletal muscle rather than adipose tissue.

CONCLUDING REMARKS AND FUTURE PERSPECTIVE

Fatty acids (from lipolysis) are continuously released from abdominal adipose tissue into the circulation and fat cells are continuously dying in normal human adults. The size of adipose

tissue is determined by the magnitude of nutrient competition from muscle and lungs for cell regeneration and energy replenishment after exercise. This is varied by types of exercise (aerobic or resistance exercise). Despite the fact that lower exercise intensity relies more on fatty acid oxidation, high-intensity exercise training (anaerobic in nature) provides a superior abdominal fat loss effect than low- and moderate-intensity exercise training. Given the fact that exercise does not increase 24-h fatty acid oxidation during and after exercise training, the carbon and nitrogen redistribution theory is more suitable to explain the abdominal fat loss outcome of exercise training than fat burning theory. This reasonably explains why low- and moderate-intensity exercise often fail as strategies for fat loss despite the greater percentage of fatty acid oxidation compared with high intensity exercise. Studies on inter-tissue communication during exercise (such as muscle-derived extracellular vesicles) for post-meal carbon and nitrogen redistribution are promising and may provide useful application to normalize body composition and prevent obesity. Furthermore, the role of fatty acids on repairing post-exercise damage deserves further investigation. More data are needed to support the carbon and nitrogen redistribution theory on fat loss effect of exercise.

AUTHOR CONTRIBUTIONS

Both authors contributed significantly to this work.

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Dietary Intervention, When Not Associated With Exercise, Upregulates Irisin/FNDC5 While Reducing Visceral Adiposity Markers in Obese Rats

Vanessa de Oliveira Furino[1], João Manoel Alves[1], Diego Adorna Marine[1], Marcela Sene-Fiorese[1], Carla Nascimento dos Santos Rodrigues[1], Cristina Arrais-Lima[2], Stela Márcia Mattiello[2], Cynthia Aparecida de Castro[3], Ricardo Carneiro Borra[4], Marina Campos Rocha[4], Iran Malavazi[4] and Ana Cláudia Garcia de Oliveira Duarte[1]*

[1] Department of Physical Education and Human Motricity – DEFMH, Biological and Health Sciences Center – CCBS, Federal University of São Carlos – UFSCar, São Carlos, Brazil, [2] Department of Physiotherapy – DFisio-Biological and Health Sciences Center – CCBS, Federal University of São Carlos – UFSCar, São Carlos, Brazil, [3] Department of Morphology and Pathology-Biological and Health Sciences Center – CCBS, Federal University of São Carlos – UFSCar, São Carlos, Brazil, [4] Department of Genetics and Evolution-Biological and Health Sciences Center – CCBS, Federal University of São Carlos – UFSCar, São Carlos, Brazil

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Ahmad Alkhatib, University of Taipei, Taiwan

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***Correspondence:**
Vanessa de Oliveira Furino
vafurino@hotmail.com

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Obesity is an epidemic disease and the expansion of adipose tissue, especially visceral fat, promotes the secretion of factors that lead to comorbidities such as diabetes and cardiovascular diseases. Thus, diet and exercise have been proposed as an intervention to reverse these complications. An adipocytokine, known as irisin, mediates the beneficial effects of exercise. It has been proposed as a therapeutic potential in controlling obesity. In view of the above, this paper attempts to determine the modulation of irisin, visceral adiposity and biochemical markers in response to dietary intervention and aerobic exercise. To do this, 52 diet-induced obese male *Wistar* rats were divided into the following four groups: high-fat diet and exercise (HFD-Ex); HFD-Sedentary (HFD-Sed); chow-diet and exercise (CD-Exercise); and CD-Sed. The exercise-trained group performed a treadmill protocol for 60 min/day, 3 days/week for 8 weeks. Body mass (BM), body fat (BF), fat mass (FM), and fat-free mass (FFM) were analyzed. Mesenteric (MES), epididymal (EPI), and retroperitoneal (RET) adipose tissue was collected and histological analysis was performed. Biochemical irisin, triglycerides, glucose, insulin and inflammatory markers were determined and, FNDC5 protein expression was analyzed. In this study, the diet was the most important factor in reducing visceral adiposity in the short and long term. Exercise was an important factor in preserving muscle mass and reducing visceral depots after a long term. Moreover, the combination of diet and exercise can enhance these effects. Diet and exercise exclusively were the factors capable of increasing the values of irisin/FNDC5, however it did not bring cumulative effects of both interventions. Prescriptions to enhance the obesity treatments should involve reducing visceral adiposity by reducing the fat content in the diet associated with aerobic exercise.

Keywords: body composition, endurance training, visceral adipose tissue, high-fat diet, obesity, irisin/FNDC5

INTRODUCTION

Obesity is the result of an increase in the intake of a high-fat and high-carbohydrate diet associated with low levels of physical activity (Romieu et al., 2017). A chronic state of positive energy balance, derived from this condition, promotes the unhealthy expansion of visceral and subcutaneous adipocytes, inducing a remodeling of adipose tissue (Bray et al., 2017; Schoettl et al., 2018). A growing body of evidence shows that obesity-related comorbidities are influenced by adipocyte distribution and more specifically visceral fat. Progressive enlargement of visceral adipose tissue (VAT) causes alterations to mitochondrial oxidative function, increases lipolytic activity induced by catecholamine, and the secretion of pro-inflammatory cytokines leading to chronic inflammation and subsequent dysfunction bioenergetics and structural changes in adipocytes (Wajchenberg, 2000; Kusminski et al., 2016). This dysfunction is associated with an array of metabolic complications, such as type 2 diabetes and cardiovascular disease (Bray et al., 2017; Schoettl et al., 2018).

Due to the high remodeling capacity of white adipose tissue (WAT) and endocrine functions, preserving healthy WAT function and decreasing adiposity, especially visceral fat, has been considered an attractive approach for the treatment or prevention of metabolic disorders related to obesity (Verheggen et al., 2016; Kahn et al., 2019). Thus, exercise and diet have been proposed as non-pharmacological strategies for VAT management (Verheggen et al., 2016). Indeed, diet plays an important role in weight reduction, recent data has shown that exercise is a predominant factor in the regulation of VAT when compared to a hypocaloric diet (Verheggen et al., 2016). Visceral fat reduction is associated with a decrease in body mass (BM) and fat mass (FM), improvement of glucose homeostasis, lipid profile, and reduction of an inflammatory state (Verheggen et al., 2016; Chait and den Hartigh, 2020). The mechanism involved in this regulation is not completely understood, but it can be suggested that the muscle considering high energy demand, secretes factors that stimulate the thermogenesis of WAT (Rodríguez et al., 2017).

Among the secreted factors, irisin is a novel exercise-induced adipomyokine cleaved of FNDC5 (fibronectin type III domain-containing protein 5), a transmembrane protein expressed in muscle (Boström et al., 2012). It is believed that after secretion into the circulation, soluble irisin binds to a recently identified irisin receptor, integrin αV/β5, that induces a thermogenic program (Kim et al., 2018). The transcription of thermogenic genes regulates the mitochondrial activity and increases energy expenditure, transdifferentiating white adipocytes to brown-like phenotype adipocytes (Cheng et al., 2021). Besides, studies have also shown that irisin facilitates glucose uptake by skeletal muscles, increases insulin sensitivity of tissues, stimulates mitochondrial biogenesis and oxidative metabolism, improving the metabolic profile (Boström et al., 2012; Rodríguez et al., 2017) and attenuates the expression of obesity-related inflammatory markers (Lu and Li, 2020; Luo et al., 2020). Since then, several studies show that short-term aerobic exercises (Anastasilakis et al., 2014; Aydin et al., 2014) and long-term exercises (Boström et al., 2012; Kim et al., 2016) upregulate FNDC5 and irisin levels in humans and animals. Although the main source of expression is exercise/muscle, VAT and subcutaneous adipose tissue (SAT) also secretes irisin in a reduced amount against different nutritional states (Frühbeck et al., 2020). For instance, obesity seems to positively regulate the concentrations of irisin in adipocytes and muscles to respond to an uncommon metabolic condition (Park et al., 2013; Crujeiras et al., 2014; Pardo et al., 2014; Sahin-Efe et al., 2018). However, a negative correlation has already been observed between circulating levels of irisin, BMI, and the amount of adipose tissue (Bonfante et al., 2017; Grygiel-Górniak and Puszczewicz, 2017) and reductions in plasma concentrations in patients with morbid obesity (Frühbeck et al., 2020).

Finally, in addition to the aforementioned factors that interfere with the circulation of irisin, among the adipose tissue compartments, there are differences in the protein secretion of each depot. There is strong evidence indicating irisin is upregulated in VAT, but not in SAT in human adipose tissue (Frühbeck et al., 2020), justified by the specific characteristics of these compartments (Roca-Rivada et al., 2013). It is even suggested that the thermogenic capacity of SAT is conferred by irisin (Arhire et al., 2019).

Many studies have compared irisin/FNDC5 in VAT and SAT, but very little is currently known about the responses among the different visceral compartments. The VAT is a heterogeneous tissue and the epididymal (EPI), mesenteric (MES), and retroperitoneal (RET) depots regulate energy metabolism uniquely (Wronska and Kmiec, 2012; Schoettl et al., 2018). For this reason, we consider that irisin is modulated in a particular way with each depot and this difference results in specific metabolic adaptations during the obesity process. Therefore, understanding the responses of each depot, as well as specific factors secreted by each tissue, is relevant.

Part of the low efficiency in the management of obesity is due to the knowledge gap that still persists in factors that act in the regulation of BM and FM. Thus, additional studies are needed to explore the factors secreted by the adipose and muscular tissue that promote corrective and physiological actions in the obesity process and in response to the strategies for the treatment of obesity. In view of the above, this paper attempts to determine the modulation of irisin and inflammatory markers, visceral adiposity parameters and depots in response to dietary intervention and moderate-intensity exercise. We hypothesize that exercise training exclusively increases the secretion of irisin and improves dyslipidemia, improves glucose homeostasis, reduces inflammatory markers and the effects can be intensified from a shift from a high-fat diet (HFD) to a chow diet (CD).

MATERIALS AND METHODS

First Intervention (Diet-Induced Obesity)

Experimental protocols were approved by the Ethics Committee on the Use of Animals (no.7631210617) at the Federal University of São Carlos (UFSCar). As shown in **Figure 1**, the experimental protocol lasted 16 weeks. Adult male *Wistar* rats ($n = 66$; ≅ 300 g) were housed (n = 3–4/cage) in a temperature-controlled environment (23 ± 1°C) and humidity (50–60%) on a reversed

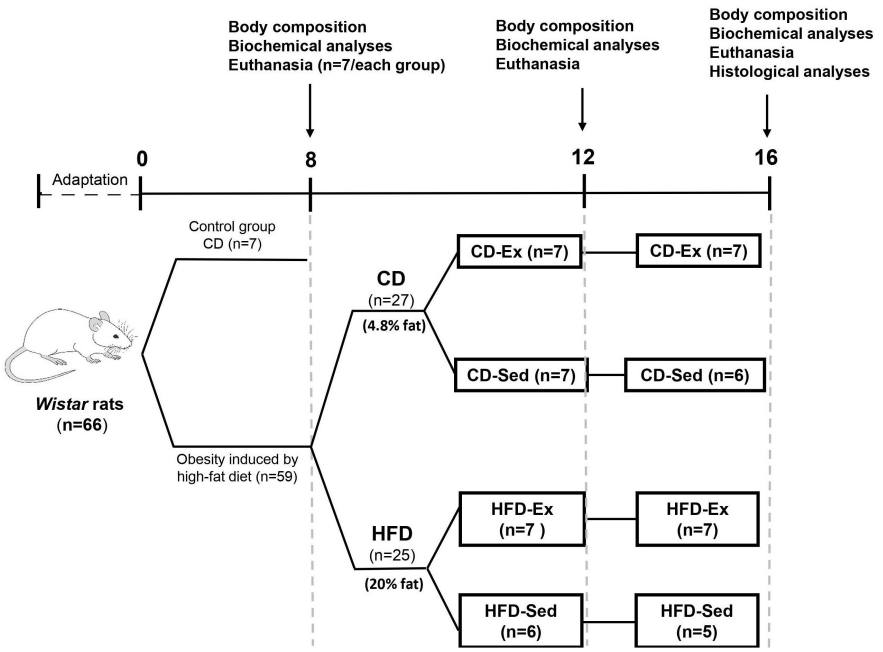

FIGURE 1 | Schematic representation of the study design. HFD: high-fat diet; CD: chow diet; HFD-Ex: high-fat diet and exercise; HFD-Sed: sedentary high-fat diet; CD-Ex: chow diet and exercise; CD-Sed: sedentary chow diet.

12/12 h light/dark cycle (lights on at 6 pm) with food and water *ad libitum*.

After adaptation, rats were randomly divided to be fed one of the following diets over 8 weeks: CD (n = 7) or HFD (n = 59). At the end of the first intervention (**Figure 1**), 7 animals of the CD group and 7 animals of the HFD group were euthanized to assess the efficiency of the HFD in inducing obesity and for further analysis. The remaining 52 animals in the HFD group proceeded to the subsequent intervention (training and dietary intervention).

Second Intervention (Training and Dietary Intervention)

After 8 weeks on HFD, 52 animals were randomly divided into four groups (**Figure 1**): high-fat diet and exercise (HFD-Ex, n = 14), sedentary high-fat diet (HFD-Sed, n = 11), and two groups that switched to CD; chow diet and exercise (CD-Ex, n = 14) and sedentary chow diet (CD-Sed, n = 13) for 8 more weeks. After 4 and 8 weeks during the second intervention, 5–7 animals from each group were euthanized after a 12-h fast by guillotine decapitation between 8 am and 12 pm. The trained animals were sacrificed 48 h after the last physical activity to assess body composition and biochemical parameters.

Diets

The CD (in pellet form) was provided by Agromix (Jaboticabal, SP, Brazil) that contained (100 g) 23% protein, 39% carbohydrates, 4.8% total fat, and 6% fiber. The palatable HFD consists of standard chow diet, peanuts, milk chocolate, and sweet biscuit at a proportion of 3:2:2:1. All components were powdered and mixed to form pellets (Estadella et al., 2004). The diet contains 18% protein, 20% fat, 33% carbohydrate, and 3% fiber (100 g). The caloric densities were 4.66 kcal/g for the palatable high fat diet and 3.85 kcal/g for the chow diet (IKA 5000, CBO, Valinhos, Brazil). The complete description of the macronutrients and vitamins was previously described (Costa et al., 2021).

Training Protocol

Exercise Maximum Capacity Assessment

All animals were adapted to the treadmill (10–15 min/day; 6 to 10 m/min) for 5 days before beginning the exercise training protocol. At the end of the adaptation and in order to determine the running speed for the training protocol, the exercise intensity was estimated by the total distance covered and the maximum speed obtained in the maximum test protocol (Brooks and White, 2018). The progressive effort test on the treadmill comprised increments at a speed of 2 m/min every 2 min until the maximum speed was obtained (Souza et al., 2018). The exhaustion time (in min) and the maximum speed (m/min) were determined as 100% of the exercise capacity and used to determine the intensity of the training sessions (**Table 1**). During the procedure, an electric shock was not used as a form of stimulation.

Treadmill Training Protocol

The training protocol consisted of running sessions on a treadmill adapted for rats, containing six individual lanes separated by bays made of acrylic, always between 8 am and 12 pm corresponding to the dark cycle of the animals. The aerobic training protocol had a frequency of 3 weekly sessions for 8 weeks, lasting 60 min

TABLE 1 | Maximal incremental exercise testing.

	Baseline		Week 4		Week 8	
	HFD-Ex	CD-Ex	HFD-Ex	CD-Ex	HFD-Ex	CD-Ex
V_{max} (m/min)	25.7 ± 0.6^{a}	21.5 ± 0.3	30.6 ± 0.7^{b}	30.1 ± 1.0^{b}	33.1 ± 0.6^{b}	$34.8 \pm 1.1^{b,c}$
Δt (min)	19.6 ± 0.8^{a}	15.6 ± 0.6	$26.9 \pm 0.5^{a,b}$	26.0 ± 1.1^{b}	29.3 ± 0.6^{b}	$30.7 \pm 0.9^{b,c}$
Δs (m)	508.0 ± 30.0^{a}	337.7 ± 16.1	825.2 ± 31.2^{b}	798.9 ± 64.0^{b}	972.6 ± 36.6^{b}	$1076.0 \pm 65.8^{b,c}$

*HFD-Ex: high-fat diet and exercise; CD-Ex: chow diet and exercise; V_{max}: maximum speed; Δt: elapsed time; Δs: distance covered. The results are presented as means ± SEM. *$p < 0.05$ vs. CD.*

[a]*vs. CD-Ex in the same week.*

[b]*vs. baseline in the same group.*

[c]*vs. week 4 in the same group.*

per session, at an intensity of 50–80% of the maximum speed obtained in the progressive effort test, with a slope of 0%. Each training session was divided into three parts, 10 min for warm-up (50–60% of V_{max}), 40 min for the main part (65–80% of V_{max}) and 10 min of gradual speed reduction (50% of V_{max}).

Body Composition and Food Intake Measurement

The BM was measured every 4 weeks, between 8 am and 12 pm. Diet intake was calculated by the difference in weight between the amount of food offered subtracting the amount of food remaining. The energy intake per rat (kcal/week/rat) was calculated as: food consumption × Et (Et is the total energy of the diet which is 4.665 kcal/g in HFD and 3.854 kcal/g) [adapted from Gong et al. (2016)].To assess body composition, the fed animals underwent anesthesia using an intra-peritoneal injection with ketamine (80 mg/kg) and xylazine (32 mg/kg), before euthanasia. Rats were later placed in prone position to be scanned using the DXA-Dual Range Emission Densitometry-between 8 am and 12 pm (Hologic Inc., Bedford, MA, United States). Thus, the body fat (BF), FM, fat-free mass (FFM) were obtained. Image analysis was performed using the QDR 4500 software (Hologic®).

Experiments and Sample Collection

Visceral adipose tissue (EPI, RET, and MES) and gastrocnemius were dissected and weighed. The blood was obtained immediately after decapitation. Then, the head was removed and the neck was placed in a funnel attached to a collection tube without anticoagulant. The collection tube remained at RT for approximately 30 min until coagulation. After that, the samples were centrifuged at 1,500 × *g* for 10 min at 4°C to obtain the serum. The serum was then collected and placed in 500 μl aliquots in the Eppendorf and frozen at −80°C for further analysis.

Glucose and Insulin Assessment

Blood glucose was measured using an Accu-Check glucometer (Roche Diagnostic, Indianapolis, IN, United States), after 12 h of fasting, immediately before euthanasia. A puncture was performed in the caudal vein of the animal to obtain blood. A drop of blood was placed on the edge of the test strip. After the capillary was filled, the device automatically showed the blood glucose value. The insulin was analyzed through an ELISA assay from the serum obtained after euthanasia. The insulin kit (ER1113) was purchased by Fine Biotech Co., Ltd. (Wuhan, China) and the test was performed according to the manufacturer's instructions. The HOMA-IR index (model for assessing insulin resistance homeostasis) is a method that is based on plasma glucose and insulin and has been used to define insulin resistance (Matthews et al., 1985). To do this, the HOMA-IR index was calculated using the formula: fasting insulin (ng/ml) × fasting glycemia (mg/dl)/405 (Roza et al., 2016).

Lipid Profile

Levels of high-density lipoproteins (HDL) (Ref. K015-Bioclin) and triglycerides (TG) (Ref. K117-Bioclin) were determined using the colorimetric enzymatic method (Bioclin, Belo Horizonte, Brazil), after 12 h of fasting. To do this, the serum was collected as previously described.

Obesity-Related Inflammatory Markers

To assess the serum concentration of inflammatory and anti-inflammatory markers, an ELISA analysis was performed. The cytokines IL-1β (ab255730) and leptin (ab100773) were purchased by Abcam® (Cambridge, United Kingdom and the cytokine IL-10 (no.555134) was purchased by BD Biosciences Pharmingen (San Diego, CA, United States) and, the myokine irisin (MET-5089) was purchased by Cell Biolabs (San Diego, CA, United States). The test was performed according to the manufacturer's instructions.

Protein Extraction and Western Blotting Analyses

Tissues were lysed with extraction protein buffer [SDS 0,1% (p/v); Triton 1% (v/v); Tris–HCl pH 7,8; 50 mM; NaCl 150 mM; EDTA 15 mM; EGTA 5 mM] as well as protease inhibitors (Complete-Mini Roche 1×). Equal amounts of each protein sample (60 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride (PVDF) membranes (GE HealthCare, Marlborough, MA, United States). After blocking the membranes with 5% skim milk in TBST, the membranes were incubated with anti-FNDC5 antibody (1:1,000; ab174833, Abcam) and anti GAPDH (ab181602, Abcam/MAB5718, R&D System). Then, the membranes were incubated for 1h at room temperature with an appropriate secondary antibody: horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (1:2,500, sc-2004, Santa Cruz);

anti-rabbit (1:10,000, sc2357, Santa Cruz); anti-mouse (1:5,000, sc516102, Santa Cruz). Each protein band was visualized using an Amersham ECL Advance Western Blotting Detection Kit (GE Healthcare).

Histopathological Analysis of Visceral Depots

For histopathological analysis, the EPI, RET and MES tissues were fixed in 10% formalin for 48 h. Dehydration was performed in increasing alcohol baths (90%, 100% I, 100% II, 100% III, and 100% IV) for periods of 60 min, diafinized in Xylol baths (50% Xylol I, 50% Xylol II, and 50% Xylol III) and finally embedded in paraffin (Merck Milipore). Tissues were cut into 5-μm sections and stained with HE. The images were digitized on a histological slide scanner (Panoramic Desk, 3DHISTECH Ltd., Hungary). To analyze the morphometry of the adipocyte images, five fields from each sample were collected with a 20× magnification (Panoramic Viewer) for quantification. The files were analyzed for the adipocyte area by the Adiposoft plugin (v. 1.15) from ImageJ Fiji (v 2.0.0). The Adiposoft plug-in was equalized with a diameter between 25 and 200 microns according to the program calibration. For each condition, a sample of $n = 5$ was selected and 100 fat cells per animal were evaluated (Ferland et al., 2020).

Statistical Analysis

All statistical analyses were performed using Graph-Pad Prism Version 8.0 and R. To verify if the data followed a normal distribution, the Kolmogorov–Smirnov test was performed in each dataset. Data are presented as the mean ± SEM. At the first intervention, comparisons between groups were performed using a two-tailed Student's *t*-test or Mann–Whitney *U* test depending on the normality of the data. The effect of diet and exercise and the interaction of training × diet, from the second intervention, was analyzed using two-way ANOVA. When ANOVA was not indicated, we used ANOVA-ART (aligned rank transform ANOVA) (Elkin et al., 2021). The Dunn or Tukey test (depending on the normality of the data) for *post hoc* analysis was performed to assess multiple comparisons. Spearman's correlation coefficient was used to analyze the correlations between all study variables and the interpretation was performed according to Mukaka (2012). The criterion for statistical significance was $p < 0.05$ (two-tailed), using *p* values adjusted for multiple comparisons by false discovery rate (FDR). The data are presented as mean ± standard error (SEM).

RESULTS

High-Fat Diet Promoted Obese Phenotype by Increasing Body Mass, Visceral Adiposity, and Serum Irisin Concentration

The body composition parameters at the end of the first intervention are described in **Table 2**. After 8 weeks of diet-induction obesity, a slight increase of ∼20% was observed in the BM of HFD animals. Additionally, all the visceral depots (EPI, RET, and MES) were significantly elevated in the HFD group compared to CD. As expected HFD had higher BF than CD animals. Regarding the biochemical parameters, irisin and glucose registered increased values in the HFD group. Irisin showed an almost threefold increase in the HFD group when compared to the CD group and on the other hand, FNDC5 was increased in the CD group compared to HFD. None of the cytokines, insulin and HOMA-IR differed significantly between the CD and the HFD group.

Epididymal Adipose Tissue Responds Later Than Mesenteric and Retroperitoneal Depots to Diet and Exercise Intervention

As shown in **Figure 2A**, at the beginning of the first intervention (4 weeks), the animals in the CD-Sed group (440.6 ± 13.1 g, $p < 0.001$) showed reduced BM when compared to the HFD groups. However, after 8 weeks, a significant difference was observed in the two CD groups when compared to the two HFD groups.

Visceral fat depots were also assessed during the experimental protocol, as represented in **Figures 2B–D**. We observed a similar response from RET and MES depots to diet and training interventions. In the fourth week, the CD groups had a reduction in RET and MES compared to the HFD groups. At the end of the 8th week, CD-Ex, CD-Sed and HFD-Ex showed a reduction in RET and MES compared to the HFD-Sed group. As shown in **Figure 2B**, no differences in EPI tissue mass were found after 4 weeks of intervention. However, after 8 weeks, the HFD-Ex group and the two CD groups showed a reduction in relation to HFD-Sed (2.95 ± 0.54 g/100 g BM).

Food and energy consumption was also assessed throughout the period and are shown in **Figures 2E,F**. There was no difference in food and energy consumption.

Regarding the diameter of the adipocytes (**Figure 3**), a similar behavior was observed in the RET and MES depots. The lowest values of the EPI diameter were observed in the CD-Ex animals (44.49 ± 3.19 μm) when compared to all other groups. In RET, the highest values of diameter were observed in the animals HFD-Sed (92.95 ± 4.84 μm) in relation to all groups. In the MES tissue, the largest area record was observed in the animals in the HFD-Sed groups (75.81 ± 8.83 μm) in relation to the groups fed the standard diet.

Following the characterization of Verboven et al. (2018) adipocytes were classified as small (< 50 μm), medium (50–69 μm), large (70–89 μm), and very large (>90 μm) (Verboven et al., 2018). The HFD-Sed animals had in the three visceral deposits large adipocytes (>75 μm), however in the RET tissue, the adipocytes were classified as very large. The animals in the HFD-Ex group had mean adipocytes in the three compartments, similar to the diameter of the CD-Sed animals. CD-Sed animals also showed small adipocytes in the MES tissue. The CD-Ex group, on the other hand, showed a reduction in all adipocytes and only adipocytes classified as small and medium were observed.

TABLE 2 | Body composition of diet-induced obesity for 8 weeks.

Parameters	CD (n = 7)	HFD (n = 7)	P
BM (g)	509.1 ± 13.1 (6.8)	613.7 ± 13.7 (5.9)	<0.0001*
BF (%)	12.53 ± 0.71(15.1)	22.47 ± 1.33 (15.7)	<0.0001*
FFM (g)	445.0 ± 10.6 (6.3)	475.4 ± 8.0 (4.4)	0.0417*
FM (g)	64.0 ± 4.4 (18.2)	138.3 ± 10.5 (20.0)	<0.0001*
MES (g/100 g BM)	0.71 ± 0.06 (23.7)	1.73 ± 0.17 (26.0)	0.0001*
RET (g/100 g BM)	1.00 ± 0.11 (29.9)	2.13 ± 0.20 (25.2)	0.0004*
EPI (g/100 g BM)	1.14 ± 0.11 (26.9)	2.40 ± 0.24 (26.4)	0.0006*
Glucose (mg/dl)	100.9 ± 3.7 (9.8)	115.7 ± 3.2 (7.3)	0.0107*
Insulin (ng/ml)	0. 28 ± 0.04 (37.2)	0.33 ± 0.04 (31.8)	0.2593
HOMA-IR	0.07 ± 0.01 (34.4)	0.10 ± 0.01 (31.1)	0.0855
TG	180.6 ± 24.2 (35.5)	236.2 ± 38.2 (42.8)	0.2423
HDL	41.8 ± 7.2 (45.5)	69.1 ± 8.7 (33.5)	0.0326*
Irisin (ng/ml)	3.02 ± 0.50 (43.5)	8.20 ± 2.3 (73.7)	0.0472*
IL-1β (pg/ml)	209.7 ± 19.0 (23.9)	201.6 ± 24.2 (31.8)	0.6200
IL-10 (pg/ml)	378.0 ± 23.9 (16.7)	351.6 ± 60.9 (45.9)	0.6940
Leptin (pg/ml)	145.8 ± 44.0 (79.8)	226.8 ± 74.5 (86.9)	0.3674
FNDC5 (relative density)	1.42 ± 0.01(18.2)	1.10 ± 0.06 (15.8)	0.0166*

*CD: chow diet; HFD: high-fat diet BM: body mass; BF: body fat; FFM: fat-free mass; FM: fat mass; MES: mesenteric adipose tissue; RET: retroperitoneal adipose tissue; EPI: epididymal adipose tissue; HOMA-IR: Homeostatic model assessment; IL-1β: interleukin-1 beta; IL-10: interleukin-10. Results are means ± SEM (%CV). *p < 0.05 vs. control (p-values refer to the Mann–Whitney U test or Student t-test depending on the normality of the data).*

Short-Term Diet Intervention Reduces Visceral Adiposity and Fat-Free Mass, While Short-Term Exercise Only Increases Fat-Free Mass in Animals With Improved Metabolic Profile

To determine the effects of diet and exercise on adiposity, BF and FM were assessed by DXA (**Figure 4**). FM and BF had a similar response. After 4 weeks, CD-Sed and CD-Ex had lower BF and FM than HFD-Ex and HFD-Sed. At the end of the experiment (8 weeks), the CD-Ex and CD-Sed remained with reduced values compared to the HFD-Sed group (BF: 26.73 ± 3.00%; FM: 187.6 ± 28.16 g, $p < 0.05$). The short-term effects of interventions in FFM (4 weeks) (**Figure 4D**) showed that the lowest values were observed in the CD-Sed group (403.30 ± 10.81 g) compared to the HFD-Ex, HFD-Sed and CD-Ex groups. At the end of the experiment, the only difference was observed in the CD-Sed group compared to the CD-Ex group.

After 4 weeks, the gastrocnemius muscle (**Figure 4E**) was elevated in the HFD-Ex and CD-Sed groups compared to the HFD-Sed and CD-Ex/HFD-Sed groups, respectively. After 8 weeks, the same results were repeated and the HFD-Sed group had a lower gastrocnemius mass compared to the HFD-Ex and CD-Sed group, and the CD-Ex group had a reduced gastrocnemius mass compared to the CD-Sed and HFD-Ex.

The Exclusive Diet Was the Most Important Factor to Mitigate the Biochemical and Inflammatory Markers Related to Obesity

The glycemic and lipid profile was also assessed in this study (**Figure 5**). There were no significant differences in serum insulin levels between the groups after 4 and 8 weeks (**Figure 5A**). Both control diet groups showed a significant reduction in glycemia compared to the HFD groups after 4 and 8 weeks of intervention (**Figure 5B**). We also evaluated the HOMA-IR (**Figure 5C**) index between the groups. We observed that significant differences appeared only after 4 weeks of intervention. The HFD-Sed group (0.09 ± 0.01) showed higher values compared to the CD-Ex and HFD-Ex groups.

Regarding lipid fractions, TG levels (**Figure 5D**) increased in the HFD groups after 4 and 8 weeks of intervention. The HFD-Sed group (56.11 ± 2.22 mg/dl) presented the highest values in the fourth week, compared to the HFD-Ex, CD-Sed and CD-Ex groups. In the eighth week, the CD-Sed group (45.14 ± 2.55 mg/dl) had the lowest values, which were significantly different from the HFD-Sed and HFD-Ex groups.

Regarding the HDL fraction (**Figure 5E**), in the fourth week, it was observed that the CD-Sed animals (24.31 ± 3.38 mg/dl) registered the lowest values and were significantly different from HFD-Ex and HFD-Sed. The CD-Ex group (31.71 ± 4.87 mg/dl) also showed a reduction in values compared to the HFD-Sed group but did not differ from the HFD-Ex group. After 8 weeks, the response of HDL was similar to the response of the fourth week, the CD-Sed group (33.42 ± 2.70 mg/dl) showed the lowest values in relation to the animals of the groups HFD-Sed, HFD-Ex, and CD-Ex.

Diet and Exercise Exclusively Were the Most Important Factors in the Modulation of Irisin/FNDC5

Changes in irisin were observed only after 4 weeks of intervention, but only in the CD groups (**Figure 6A**). The CD-Sed group (13.57 ± 3.00 ng/ml) registered higher values than the CD-Ex group (5.81 ± 2.30 ng/ml), which registered the lowest

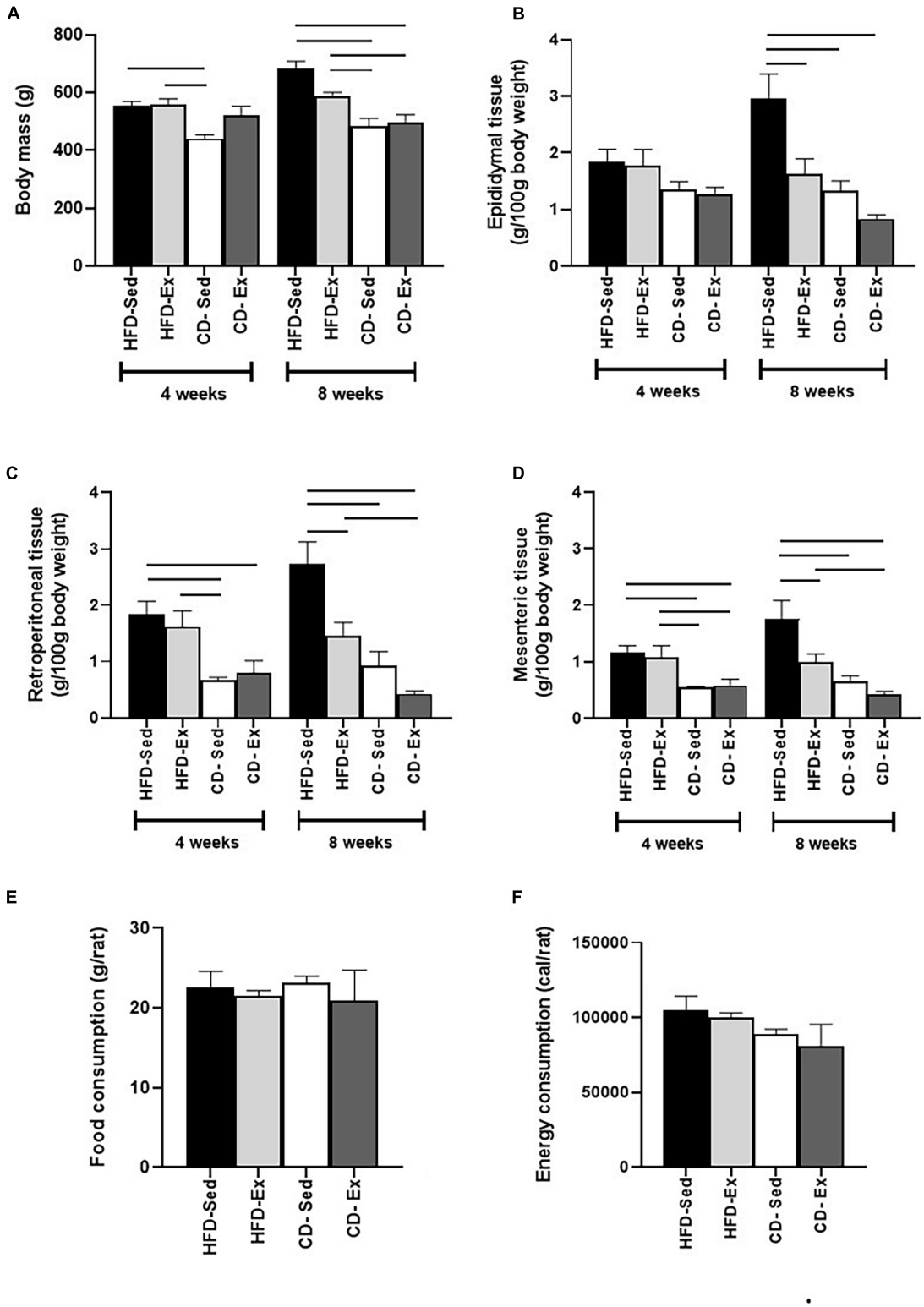

FIGURE 2 | Effects of diet and exercise on body mass and visceral depots. **(A)** Body mass was assessed by DXA every 4 weeks. **(B)** Epididymal adipose tissue of male *Wistar* rats. **(C)** Retroperitoneal adipose tissue of male *Wistar* rats. **(D)** Mesenteric adipose tissue of male *Wistar* rats. **(E)** Food consumption of *Wistar* rats after 8 weeks of intervention. **(F)** Energy consumption of *Wistar* rats after 8 weeks of intervention. HFD-Ex: high-fat diet and exercise; HFD-Sed: sedentary high-fat diet; CD-Ex: chow diet and exercise; CD-Sed: sedentary chow diet. The bars represent the significant differences between the groups indicated. Results are means ± SEM ($p < 0.05$).

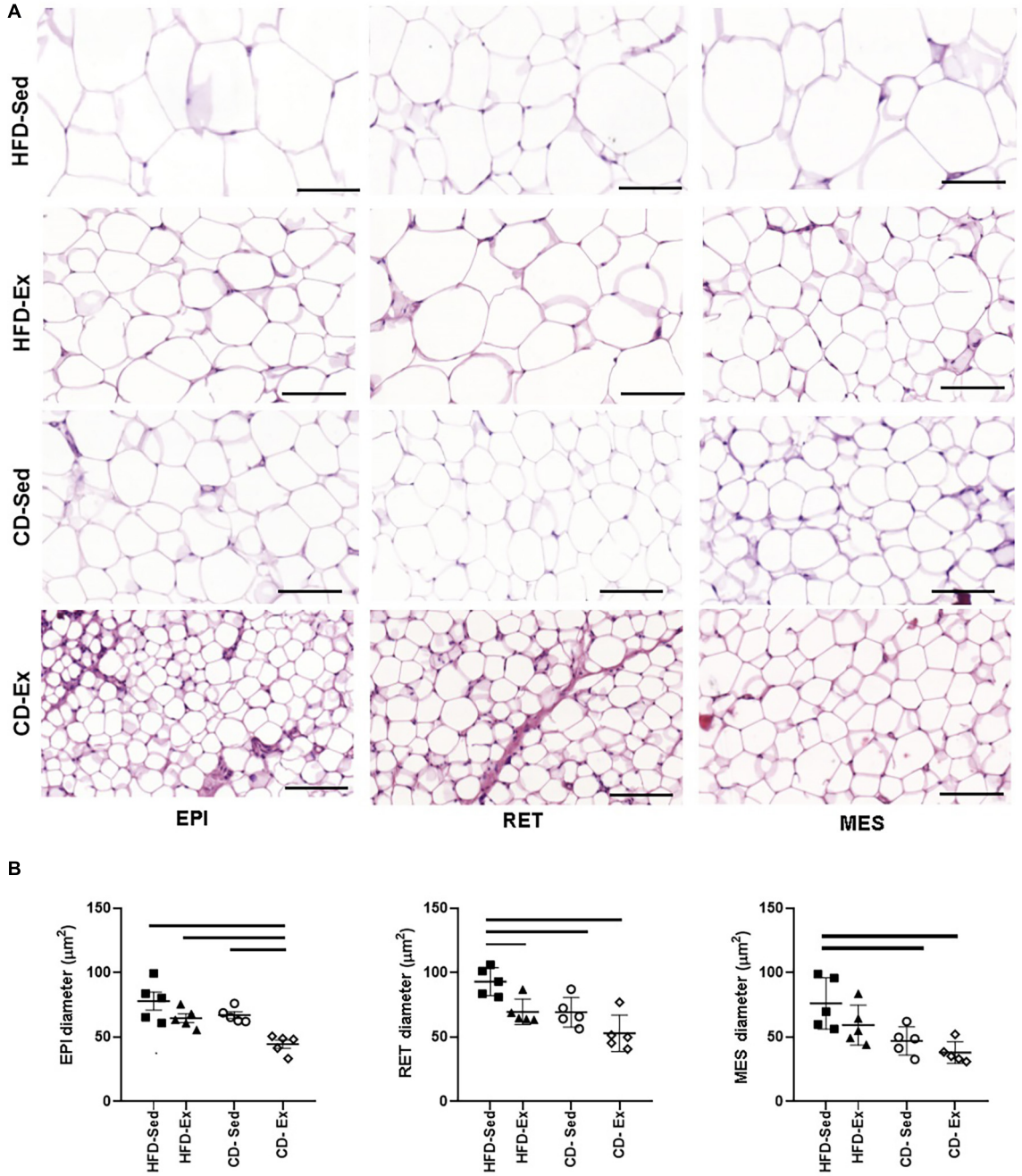

FIGURE 3 | Photomicrograph of adipose tissue after 8 weeks of dietary intervention and training. **(A)** Representative images of adipose tissue showing hematoxylin and eosin staining using 20× (100 μm) objective, light-field microscopy. **(B)** Diameter of EPI, RET and MES adipocytes; RET: retroperitoneal adipose tissue; MES: mesenteric adipose tissue. HFD-Ex: trained high-fat diet group; HFD-Ex: high-fat diet and exercise; HFD-Sed: sedentary high-fat diet; CD-Ex: chow diet and exercise; CD-Sed: sedentary chow diet. The bars represent the significant differences between the indicated groups. The results are presented as means ± SEM ($p < 0.05$).

serum concentrations. Interestingly, after 8 weeks there were no significant differences between groups.

Serum IL-1β values were determined by ELISA (**Figure 6B**). Interestingly, 4 weeks after the intervention, reduced cytokine values were observed in the HFD-Sed group (359.3 ± 58.5 pg/ml) compared to the HFD-Ex and CD-Ex groups. In addition, the CD-Sed group (391.0 ± 39.6 pg/ml) also showed a reduction in IL-1β compared to the HFD-Ex and CD-Ex groups (**Figure 6B**). However, at week 8, the animals in the HFD-Sed group (948.2 ± 85.6 pg/ml) registered the highest values compared to the other groups. The IL-10 values (**Figure 6C**) showed that there was no significant difference between the groups after the

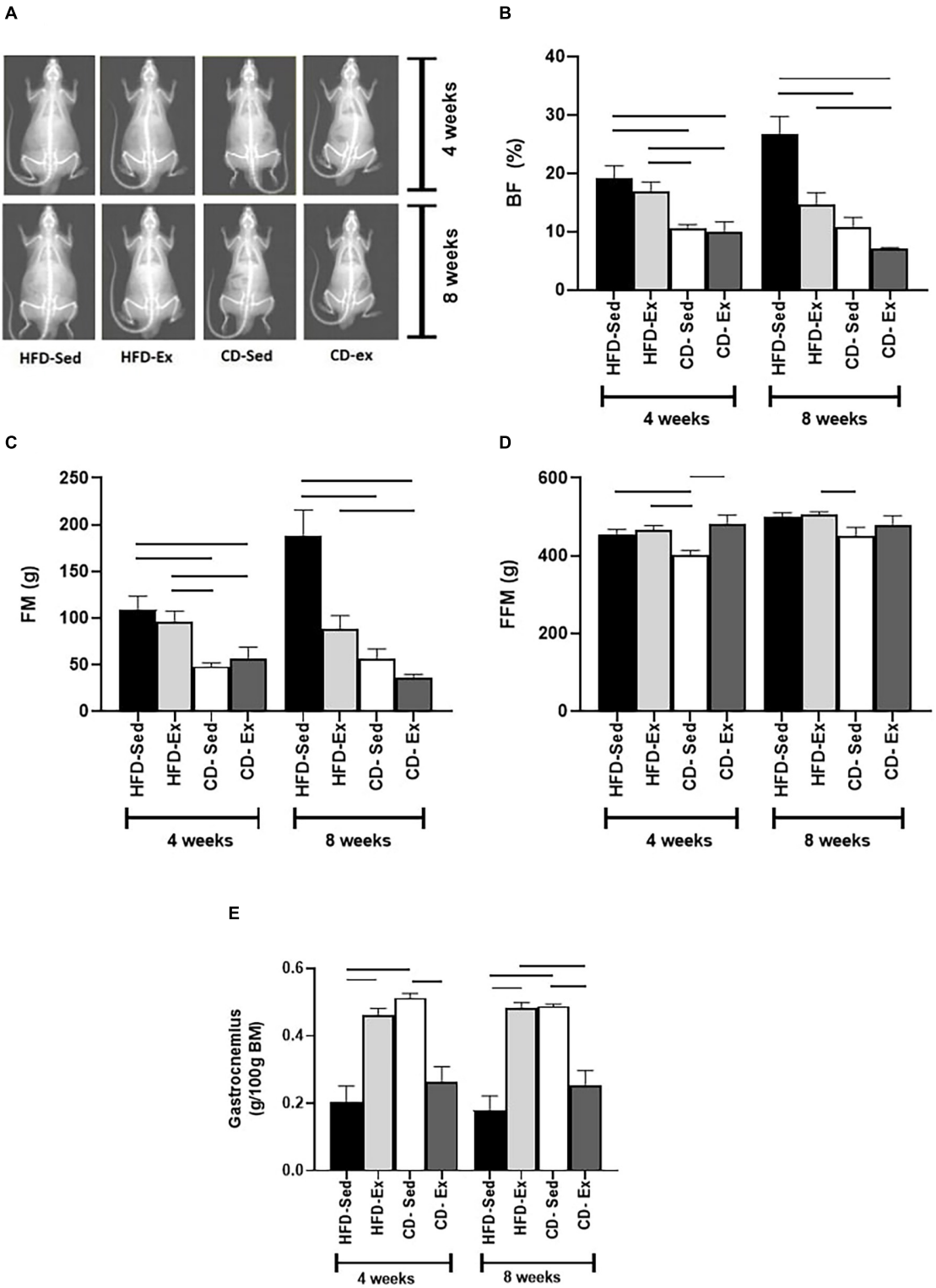

FIGURE 4 | Effects of diet and exercise on body composition. **(A)** DXA images of high-fat diet (HFD) and chow diet (CD) groups. **(B)** Body fat (%) was assessed after 4 and 8 weeks. **(C)** Fat mass was determined at weeks 4 and 8. **(D)** Fat-free mass was registered every 4 weeks. **(E)** Gastrocnemius mass was assessed at 4 and 8 weeks. The bars represent the significant differences between the groups indicated. HFD-Ex: high-fat diet and exercise; HFD-Sed: sedentary high-fat diet; CD-Ex: chow diet and exercise; CD-Sed: sedentary chow diet. The bars represent the significant differences between the indicated groups. Results are means ± SEM ($p < 0.05$).

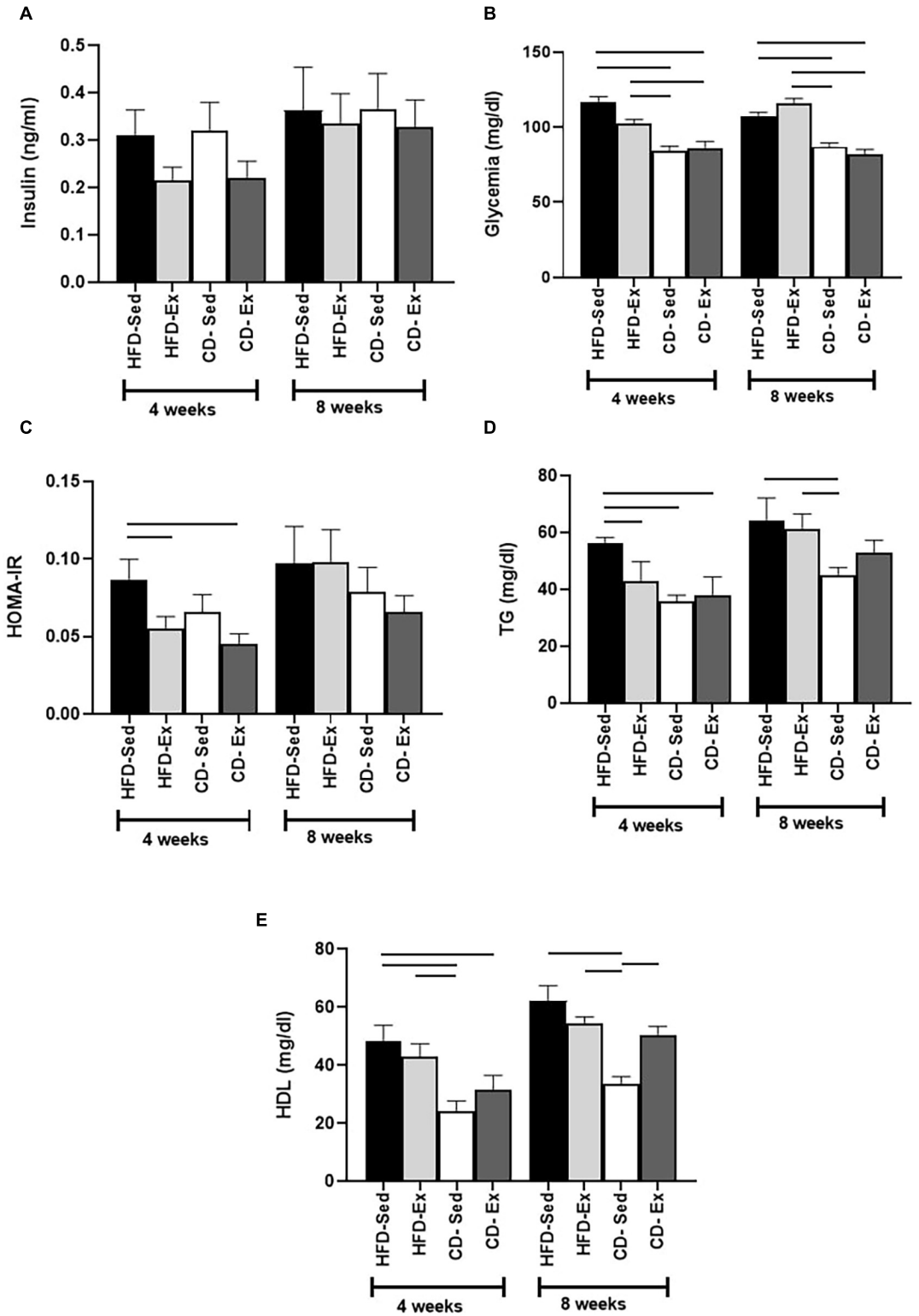

FIGURE 5 | Effects of diet and exercise on body composition on biochemical parameters. **(A)** Serum insulin concentration after 4 and 8 weeks of dietary and training interventions. **(B)** Blood glucose after 4 and 8 weeks of dietary and training interventions. **(C)** HOMA-IR index after 4 and 8 weeks of dietary and training interventions. **(D)** Serum triglycerides levels after 4 and 8 weeks of dietary and training interventions. **(E)** High-density lipoproteins (HDL) levels after 4 and 8 weeks of dietary and training interventions. HFD-Sed: sedentary high-fat diet; CD-Ex: chow diet and exercise; CD-Sed: sedentary chow diet. The bars represent the significant differences between the groups indicated. Results are means ± SEM ($p < 0.05$).

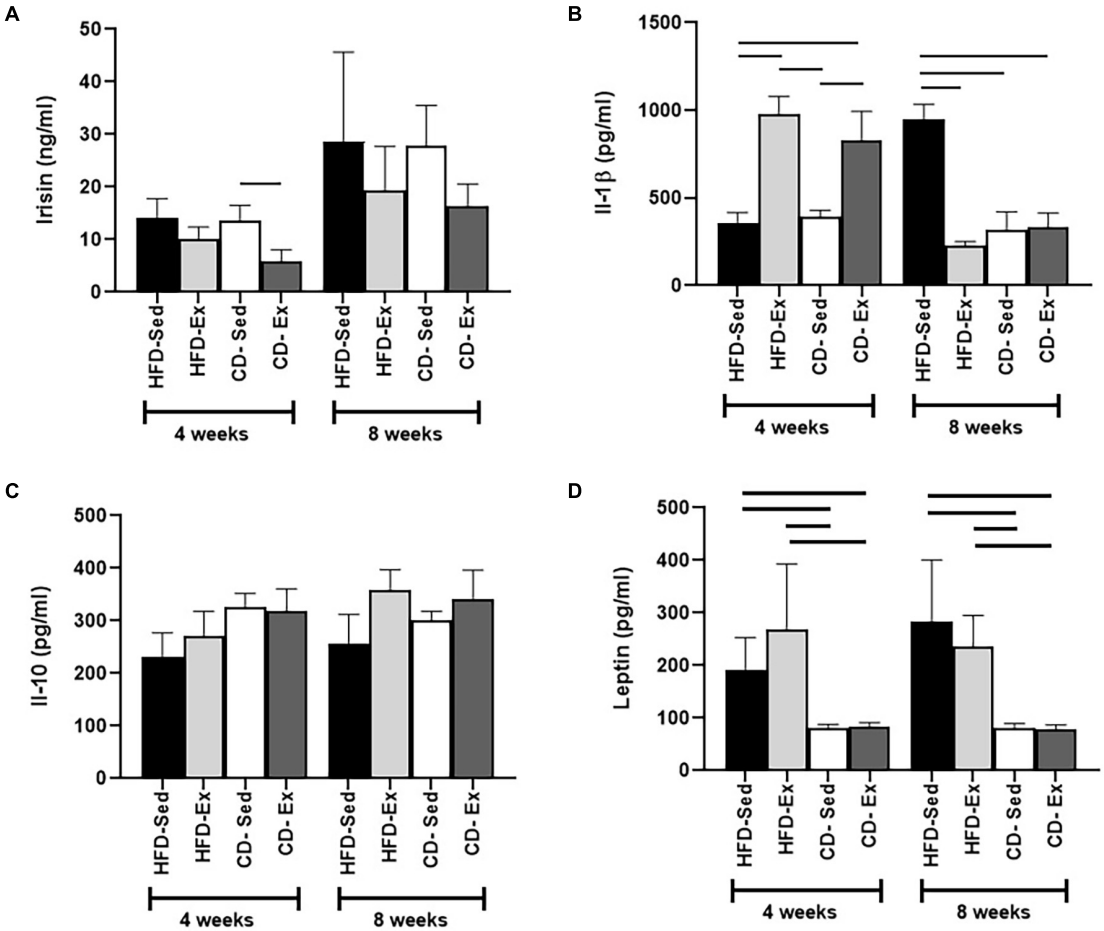

FIGURE 6 | Determination of serum levels of adipocytokines during the experiment. **(A)** Serum irisin concentration after 4 and 8 weeks of dietary and training interventions. **(B)** Interleukin 1-beta (IL-1β) was evaluated every 4 weeks. **(C)** Levels of interleukin 10 (IL-10) were determined after 4 and 8 weeks. **(D)** Serum leptin levels after 4 and 8 weeks.

4 and 8 weeks of intervention. In the initial weeks (4 weeks) and after 8 weeks of intervention, the leptin values showed the same behavior. Both control diet groups showed a significant reduction in serum leptin in relation to the HFD groups after 4 and 8 weeks of intervention (**Figure 6D**).

Protein expression was assessed in the gastrocnemius muscle after 4 and 8 weeks of dietary intervention and training (**Figure 7**). After 4 weeks, higher values were observed in the CD-Sed group (1.45 ± 0.14) compared to all other groups HFD-Sed, HFD-Ex, and CD-Ex. No significant differences were observed after 8 weeks of intervention.

To verify whether there was a correlation among the metabolic parameters, the body composition and irisin/FNDC5, a heatmap was designed and Spearman's correlation was performed and analyzed (**Figure 8**). After the 4 weeks of intervention, there was a positive correlation in the animal HFD-Ex between irisin and FM (r = 0.786/p = 0.048); in CD-Sed animals between irisin and TG (r = 0.811/p = 0.035); between irisin and HOMA-IR (r = 0. 786/p = 0.048) and between FNDC5 and leptin (r = 0.857/p = 0.024). In CD-Ex animals, a negative correlation was observed between irisin and HDL (r = −0.857/p = 0.024). After 8 weeks, significant correlations were found in the CD-Sed group, a positive and strong correlation between irisin and BM (r = −0.943/p = 0.017) and a strong and negative correlation between irisin and TG (r = −0.899/p = 0.028); and between the FNDC5 and MES diameter (r = −1,000/p = 0.017). A strong positive correlation was observed between FNDC5 and EPI (r = 0.786/p = 0.048).

DISCUSSION

Diet and physical inactivity play a major role in the genesis of obesity and irisin/FNDC5 modulation. Here we report that the diet was effective in promoting an obese phenotype after 8 weeks, including augmentation in body adiposity, BM, and visceral depots. These findings support previous studies that used a similar composition to induce obesity after six (Oishi et al., 2018) and eight weeks (Estadella et al., 2004). In this study, 8 weeks after the introduction of HFD an increase in

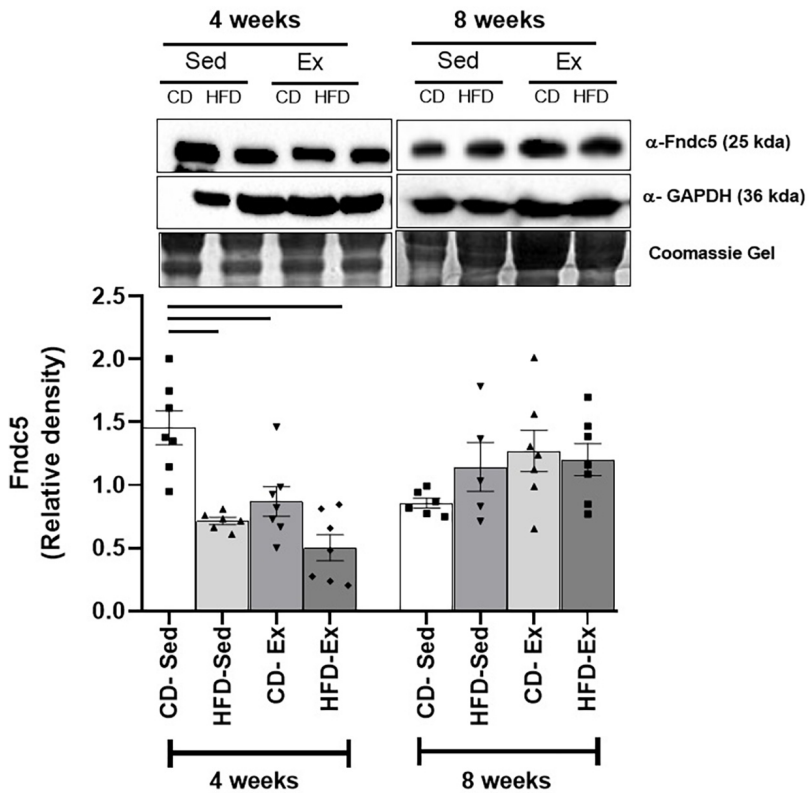

FIGURE 7 | Protein expression of FNDC5. The presence of a 25 kDa band was detected in the gastrocnemius with anti-FNDC5 antibodies after 4 and 8 weeks of dietary intervention and training; GAPDH was used as a loading control. HFD-Ex: high-fat diet and exercise; HFD-Sed: sedentary high-fat diet; CD-Ex: chow diet and exercise; CD-Sed: sedentary chow diet. The bars represent the significant differences between the indicated groups. The results are presented as means ± SEM ($p < 0.05$).

irisin secretion was noted. This observation supports the theory that irisin plays a compensatory role during metabolic disorders, such as obesity, impaired glucose homeostasis and insulin resistance (Guilford et al., 2017). Interestingly, contrary to the values of irisin, the expression of FNDC5 in the gastrocnemius muscle was shown to be significantly elevated in the diet intervention group. Although the findings show that the highest expression of FNDC5 occurs through muscle (in physiological or pathological conditions), it has been reported that irisin is partly secreted by VAT in Frühbeck et al. (2020), Kirat et al. (2021). This suggests that the origin of FNDC5, which increased the circulating irisin values in HFD animals, may be of adipose origin.

Long-term exposure to HFD resulted in the obesity phenotype, with an increase in all visceral depots, BF and FM. The dietary intervention was responsible for affecting most of the parameters evaluated. The exercise had an influence on FFM on short-term and, visceral adiposity in long-term intervention. In contrast, irisin/FNDC5 was affected by diet and exercise exclusively, but not by their combination. Diet and training were proposed as interventions in obese rats in this study to observe the modulation of irisin/FNDC5. We demonstrated that after 4 weeks, exercise was the most important factor in reducing irisin secretion. Moreover, high-fat diet and exercise were the most important factors in reducing FNDC5 secretion. The association between chronic consumption of HFD has been explored in the literature and there is no consensus on the findings. In humans, irisin was highly and positively related to BMI, BM and BF (Park et al., 2013; Crujeiras et al., 2014; Pardo et al., 2014; Fagundo et al., 2016; Sahin-Efe et al., 2018). However, in rodents, several effects have been reported. Quiñones et al. (2015) showed that Sprague-Dawley mice that received HFD for 10 weeks did not detect any significant difference in irisin levels compared to control (Quiñones et al., 2015). Kang et al. (2019) showed that Sprague-Dawley mice that received HFD for 16 weeks presented a reduction in irisin levels at the end of the experiment (Kang et al., 2019). The same findings were reported by Lu et al. (2016) after 24 weeks of HFD and Yang et al. (2015) in C57BL/6 mice after 12 weeks of HFD (Yang et al., 2015; Lu et al., 2016). Subsequently, data showed that this decline did not influence the BM and glucose that were increased during the experiment. In these animals, the reduction was probably due to adipose FNDC5 since the concentrations of irisin in the skeletal muscle remained unchanged. Positive associations between irisin and obesity were demonstrated by Guilford et al. (2017), who reported that mRNA *Fndc5* in adipose tissue was significantly higher in HFD compared to counterparts (Guilford et al., 2017). Moreover, they were reinforced by Kazeminasab et al. (2018) who

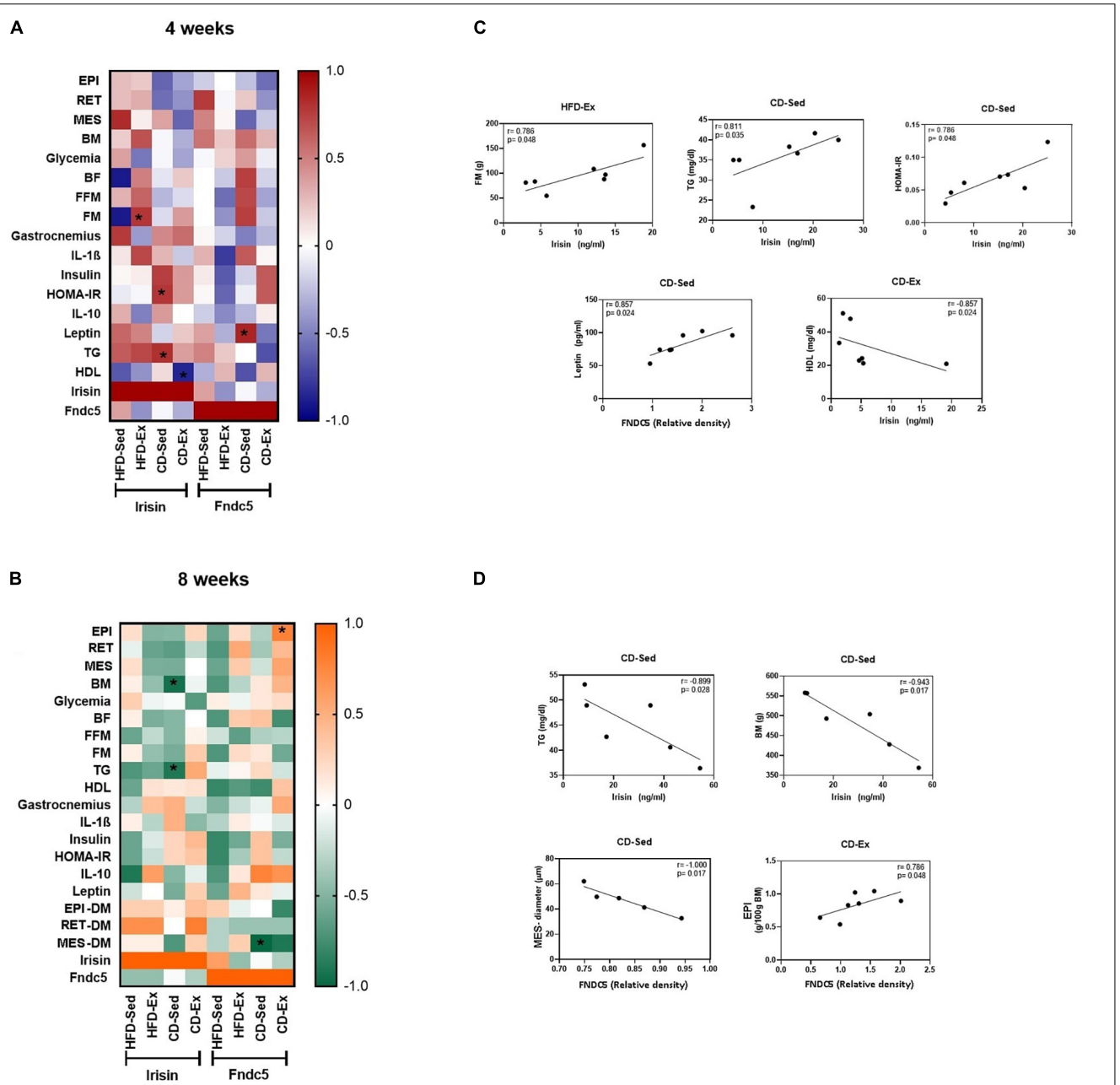

FIGURE 8 | Spearman's correlation coefficient between irisin/FNDC5, body composition and biochemical parameters after 4 and 8 weeks of intervention. **(A)** Heatmap of Spearman's correlation coefficient between irisin/FNDC5, body composition and biochemical parameters after 4 weeks of intervention. **(B)** Heatmap of Spearman's correlation coefficient between irisin/FNDC5, body composition and biochemical parameters after 8 weeks of intervention. **(C)** Spearman's correlations that were significant between irisin/FNDC5 and the study variables after 4 weeks of intervention. **(D)** Spearman's correlations that were significant between irisin/FNDC5 and the study variables after 8 weeks of intervention. EPI: epididymal adipose tissue; RET: retroperitoneal adipose tissue; MES: mesenteric adipose tissue; BM: body mass; BF: body fat; FFM: fat-free mass; FM: fat mass; HOMA-IR: Homeostatic model assessment; TG: triglycerides; HDL: high density lipoprotein; IL-1β: interleukin 1-beta; IL-10: interleukin 10; AR: area; DM: diameter. HFD-Ex: high-fat diet and exercise; HFD-Sed: sedentary high-fat diet; CD-Ex: chow diet and exercise; CD-Sed: sedentary chow diet. Individual values were plotted on the graphs. Statistical significance: $p < 0.05$.

observed the highest irisin secretion in obese mice, compared to non-obese mice (Liu et al., 2017). In line with the earlier findings in rodents, we did not observe an increase in the expression of irisin/FNDC5 in animals fed a HFD, but only in CD-Sed animals. In addition, a significant improvement in glycemic and lipid profile, besides a reduction in visceral adiposity was observed. Thus, we can attribute the highest secretions of irisin to a more favorable glycemic/adipose profile. This observation does not support the theory that irisin plays a compensatory role during metabolic disturbances such as obesity, impairments in glucose

homeostasis, and insulin resistance (Roca-Rivada et al., 2013; Guilford et al., 2017).

Thus, blood glucose was assessed in this study. Our data showed evidence of a significant increase in blood glucose in both the HFD groups after 4 and 8 weeks when compared to CD animals. However, these changes are not due to exercise-training exclusively. Irisin appears to play an active role in improving glucose homeostasis and higher levels suggest a therapeutic potential in the control of comorbidities associated with insulin resistance (Moreno-Navarrete et al., 2013). *In vitro*, irisin increases glucose uptake by muscle cells by p38 AMPK pathway promoting the proliferation of β cells (Boström et al., 2012; Lee et al., 2015; Yang et al., 2015). Moreover, irisin prevents apoptosis of pancreatic β cells resulting from persistent hyperglycemia, through negative regulation of pro-apoptotic proteins (Liu et al., 2017). Data *in vivo* confirmed that irisin increased glucose uptake by stimulating GLUT4 translocation in skeletal muscle cells of HFD-mice treated with exogenous irisin (Huh et al., 2014; Lee et al., 2015). Obese and diabetic/non-diabetic individuals that had increased secretion of irisin may be due to an attempt to improve glucose uptake and to prevent hyperglycemia (Perakakis et al., 2017). The response of reduced levels of glycemia in the diet intervention group that showed improvements in serum blood glucose levels, reinforcing the associations of glycemic profile and the action of irisin/FNDC5. However, this statement is only relevant in the short term and is not supported in the long-term interventions, therefore it may be a transitory mechanism and needs further investigations.

Maintenance of BM through diet and exercise training is beneficial in the prevention of metabolic disturbances associated with obesity (Fagundo et al., 2016; Verheggen et al., 2016). Initially, irisin was reported as an exercise-inducible myokine capable of mediating the beneficial effects of exercise and increasing thermogenesis, which contributes to maintaining energetic homeostasis (Boström et al., 2012). Since then, diverse endurance exercises have evaluated irisin circulation in humans and animals. Circulating levels of irisin are increased in individuals involved in exercise-induced activities and progressively reduced in those less active and sedentary (Arhire et al., 2019). Several studies show that short-term aerobic exercises (Anastasilakis et al., 2014; Aydin et al., 2014) and long-term exercises (Boström et al., 2012; Kim et al., 2016) upregulate FNDC5 and irisin levels in humans and animals promoting pleiotropic effects. Contradictory to those findings, some human studies failed to confirm the response of *Fndc5* mRNA and irisin by exercise (Hecksteden et al., 2013; Norheim et al., 2014; Hew-Butler et al., 2015). These discrepancies can be explained by the variability between species, exercise intensity, frequency, session duration, nutritional status, and training protocol (Fatouros, 2018). Recent data showed that in humans, training is often carried out that includes moderate to high intensity running (60–90%) with durations between 3 and 21 weeks of 2–3 days a week and showed controversial results (Fatouros, 2018). Our findings did not support the initial hypothesis that irisin is upregulated during exercise. Our data corroborate with human studies and here we show that irisin and FNDC5 were reduced in exercised animals compared to sedentary animals after 4 weeks of intervention, only in the diet intervention group. Part of our findings can be explained because the gastrocnemius muscle of the exercised control animals had a significantly reduced mass in relation to their non-exercised counterparts, however there was no significant correlation between these two variables. On the other hand, these discrepancies can be explained by the variability between species. In humans, frequently the protocols used to evaluate irisin and which failed to show an increase in circulating irisin were performed 2–3 times a week, while most animal studies used it 5 times a week (Fatouros, 2018). Therefore, our findings may reflect the chosen protocol that mimicked human training conditions. Our studies are corroborated by previous findings that show that in treadmill protocols, the results were not so unanimous. Continuous exercise and HIIT protocols increased serum irisin values compared to sedentary controls (Khalafi et al., 2020); however, some data showed that irisin was not changed after 8 weeks of training (Kazeminasab et al., 2018).

Diet plays an essential role in the genesis of obesity and metabolic syndrome, but the composition of the diet does not seem to directly interfere in the secretion of irisin. Anastasilakis et al. (2014) showed that the total caloric or macronutrient intake: carbohydrates, proteins, fats and fibers are not related to irisin. Nonetheless, De Macedo et al. (2017) demonstrated that mice fed high-fat (20% fat), high-carbohydrate (80% carbohydrate) diets for 60 days, had less expression of FNDC5 and irisin in the soleus muscle when compared to standard diet and high-protein diet (31% protein), respectively. In our findings, the diet exclusively seems to determine the serum concentrations of FNDC5 and, high levels were observed in CD-Sed animals compared to the groups fed a HFD. Our data are not supported by previous studies. In both human and mice showed that irisin/FNDC5 is decreased in response to a hypocaloric diet and caloric restriction (Crujeiras et al., 2014; Varela-Rodríguez et al., 2016). It is important to highlight that although we did not perform caloric restriction, a common feature of these interventions is the reduction of visceral adiposity, which was demonstrated here in this study, and which may have contributed to the increase in the secretion of irisin. The hypocaloric diet when not combined with exercise effectively induces weight loss but also reduces FFM (Willoughby et al., 2018). Since irisin is mostly released by muscle, a reduction in FFM may impair irisin secretion (Arhire et al., 2019). Supporting this hypothesis, a positive correlation between irisin and FFM has been reported (Stengel et al., 2013; Pardo et al., 2014). Our data partially corroborate the above, because CD-Sed animals showed a FFM reduction, however we can associate the high FNDC5/irisin values of CD-Sed animals with an elevation in gastrocnemius mass, as mentioned. Thus, the low levels of FNDC5 observed in HFD-Sed animals, can be attributed to a lower gastrocnemius mass. Low levels of circulating irisin have been reported in individuals with loss of muscle strength and atrophy (Chang et al., 2017). It is important to highlight that recent findings have shown that there is a significant interaction between the FNDC5 genotype and the state of sarcopenia in patients with non-alcoholic liver disease (Gao et al., 2020). Thus, irisin is a potential biomarker for muscle dysfunction and can help in the early

diagnosis of sarcopenia and muscle changes associated with age (Chang et al., 2017).

Skeletal muscle represents the main source of secretion, with an expression of ∼72% of the total circulating levels of the protein (Boström et al., 2012). Additionally, FNDC5/irisin is secreted by adipocytes and is modulated in a manner dependent on the location of the fat depots (Roca-Rivada et al., 2013). Visceral depots appear to be greater contributors to circulating levels of irisin in rodents than subcutaneous adipocytes. VAT is a heterogeneous tissue with significant differences between regional depots. EPI adipocytes have more mitochondria, higher cytochrome oxidase, citric synthase activities and higher respiration rate than inguinal depots. RET adipose tissue had higher mRNA levels of lipolysis and lipogenesis-related genes than inguinal and mesenteric adipose tissue (Deveaud et al., 2004; Wronska and Kmiec, 2012; Chusyd et al., 2016; Schoettl et al., 2018). Most studies address the secretion of irisin against subcutaneous and visceral depots. This research investigated the relationship between irisin and different visceral compartments. Our findings indicate that after 4 weeks, circulating irisin was increased in CD-Sed animals and occurred synergistically to the reduction of visceral depots. However, we did not observe a significant correlation between irisin and BF, EPI, RET and MES after 4 weeks. Furthermore, the morphometry of visceral depots did not show a correlation with irisin/FNDC5 after 4 weeks. This may suggest that protein is mostly secreted by muscle tissue instead of adipose tissue even in obese rats.

We observed in the CD-Sed group, an increase in FNDC5 that was positively correlated with leptin. Our findings corroborate with previous studies that showed that leptin positively regulated FNDC5 expression in murine C2C12 myocytes and stimulated baseline myogenesis and lower mRNA expression of factors related to muscle atrophy (Rodríguez et al., 2015). In addition, in the CD-Sed group, an increase in serum irisin was observed, which was positively correlated with the HOMA-IR index. These results have been observed previously in obese men and women (Fukushima et al., 2016), in men and women independent of nutritional status (Park et al., 2013) and in patients with kidney diseases (Ebert et al., 2014). In contrast, in female children irisin correlated negatively with the HOMA-IR index (Al-Daghri et al., 2016), as well as in obese men (Moreno-Navarrete et al., 2013). Park et al. (2013) also showed that metabolic syndrome indicators were positively correlated with irisin, even when adjusted for BMI and BF. Our data corroborate the findings and there was a positive correlation between irisin and TG in the CD-Sed animals. This same correlation has already been observed in overweight non-diabetic individuals, however the correlation was weak and the other anthropometric markers related to the risk of cardiovascular events were negatively related to irisin (Tang et al., 2019). Moreover, in the CD-Ex animals of this study, a negative correlation between irisin and HDL was observed, previously reported by Huh et al. (2012) in obese and non-obese individuals (Huh et al., 2012). Although HDL values are not significantly elevated in CD-Ex animals compared to CD-Sed, there is a tendency for this increase, which may have contributed to the reduction of circulating irisin in these animals. Together these data reinforce that the values of irisin/FNDC5 are regulated differently both in the presence of exercise and nutritional status. We showed that the positive relationship with the inflammatory marker and TG and negative with HDL is only significant while the nutritional status of the animal is with reduced adiposity, since the same findings were not confirmed in animals fed a sedentary HFD.

International guidelines often recommend combining exercise and low-calorie diets to treat obesity (Verheggen et al., 2016; Kahn et al., 2019). Thus, it is relevant to understand the combined and isolated effects of each intervention, on visceral adiposity, and on irisin. The results of this investigation showed that diet exclusively induced beneficial changes most of the parameters related to visceral adiposity both short and long term, and had no effect on the serum concentrations of irisin, but downregulated FNDC5 expression. In 4 weeks, the diet reduced most of the parameters related to body adiposity, and this effect was enhanced in 8 weeks. In addition, the diet reduced the mass of all visceral depots after 4 and 8 weeks of intervention. On the other hand, the training independently reduced visceral depots only after 8 weeks of intervention. Regarding the body composition parameters evaluated, training did not have an effect on any of those. Contrary to what was hypothesized, although the combination of exercise and diet reduced the adiposity and inflammatory parameters of obesity, these effects were not enhanced by this combination but there is a tendency for that. Therefore, we reinforce the importance of combining the diet associated with exercise in the control of obesity, especially in the preservation of muscle mass. It is important to emphasize that the combination promoted effects superior to exercise in body composition, but did not overcome the effects of diet.

There are some limitations that should be noted when interpreting these results. First, our study sample size was relatively small and some data showed very heterogeneous values, limiting our power to detect differences between groups. Secondly, we were unable to identify the detailed source of irisin secretion into the visceral depots due to the absence of expression protein in the tissue sample. Lastly, though the scope of this current study was the interactions between irisin and visceral depots, it is important to highlight that muscle and adipose tissue are the major but not the only source of irisin in the body. We did not study SAT or heart–two other significant sources of irisin. These tissues have an important influence on the metabolism of adipocytes and may be responsible for part of the effects presented in that study.

In conclusion, considering the metabolic repercussions of obesity, VAT has been the focus of several studies that aim to attenuate the metabolic repercussions of obesity. Thus, new myokines, such as irisin, which actively participate in thermogenic regulation have been investigated. In this study, the diet was the most important factor in reducing visceral adiposity in the short and long term, followed by the combination of exercise and diet. Exercise was also important, as it preserves lean muscle mass and reduces visceral depots, after the diet. And diet and exercise exclusively were the factors capable of increasing the values of irisin, however, it did not bring cumulative effects of both interventions. Prescriptions to enhance the obesity treatments should involve reducing visceral adiposity

as the focus of planning. To do this, reducing the fat content in the diet and aerobic exercise should be included as an initial treatment strategy. Furthermore, in addition to monitoring the classic biomarkers associated with obesity, such as blood glucose and HDL, irisin should also be evaluated as an early metabolic marker of obesity and FNDC5 as a marker of sarcopenia.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Experimental protocols were approved by the Ethics Committee on the Use of Animals (no. 7631210617) at the Federal University of São Carlos (UFSCar).

AUTHOR CONTRIBUTIONS

VF, DM, AD, MS-F, JA, and CC helped to conceive the design, analyzed the data, and wrote the first draft of the manuscript. VF, DM, AD, JA, CA-L, SM, MS-F, RB, CC, CR, IM, and MR performed the other data analysis and helped to draft the manuscript. VF, DM, JA, MS-F, AD, and CR helped to conceive the design, and supervised the experimental trials and training sessions. VF, CC, AD, MS-F, CR, DM, JA, RB, IM, and MR interpreted the study results and edited the manuscript. VF, CC, AD, CA-L, SM, MS-F, CR, DM, JA, RB, IM, and MR helped to conceive the design, assisted with data analyses, provided funding for the study, and helped to draft the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Effect of Exercise Training on Fat Loss—Energetic Perspectives and the Role of Improved Adipose Tissue Function and Body Fat Distribution

*Kristoffer Jensen Kolnes[1], Maria Houborg Petersen[1], Teodor Lien-Iversen[2], Kurt Højlund[1] and Jørgen Jensen[3]**

[1] Steno Diabetes Center Odense, Odense University Hospital, Odense, Denmark, [2] Department of Internal Medicine, Randers Regional Hospital, Randers, Denmark, [3] Department of Physical Performance, Norwegian School of Sport Sciences, Oslo, Norway

In obesity, excessive abdominal fat, especially the accumulation of visceral adipose tissue (VAT), increases the risk of metabolic disorders, such as type 2 diabetes mellitus (T2DM), cardiovascular disease, and non-alcoholic fatty liver disease. Excessive abdominal fat is associated with adipose tissue dysfunction, leading to systemic low-grade inflammation, fat overflow, ectopic lipid deposition, and reduced insulin sensitivity. Physical activity is recommended for primary prevention and treatment of obesity, T2DM, and related disorders. Achieving a stable reduction in body weight with exercise training alone has not shown promising effects on a population level. Because fat has a high energy content, a large amount of exercise training is required to achieve weight loss. However, even when there is no weight loss, exercise training is an effective method of improving body composition (increased muscle mass and reduced fat) as well as increasing insulin sensitivity and cardiorespiratory fitness. Compared with traditional low-to-moderate-intensity continuous endurance training, high-intensity interval training (HIIT) and sprint interval training (SIT) are more time-efficient as exercise regimens and produce comparable results in reducing total fat mass, as well as improving cardiorespiratory fitness and insulin sensitivity. During high-intensity exercise, carbohydrates are the main source of energy, whereas, with low-intensity exercise, fat becomes the predominant energy source. These observations imply that HIIT and SIT can reduce fat mass during bouts of exercise despite being associated with lower levels of fat oxidation. In this review, we explore the effects of different types of exercise training on energy expenditure and substrate oxidation during physical activity, and discuss the potential effects of exercise training on adipose tissue function and body fat distribution.

Keywords: exercise, high intensity interval aerobic training, fat loss, adipose tissue function, inflammation, type 2 diabetes, obesity

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Angelo Sabag, Western Sydney University, Australia

***Correspondence:**
Jørgen Jensen
jorgen.jensen@nih.no

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INTRODUCTION

Obesity, and in particular abdominal obesity, increases the risk of several diseases, including type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), non-alcoholic fatty liver disease (NAFLD), polycystic ovarian syndrome (PCOS), severe COVID-19 disease, and certain types of cancer (Kopelman, 2000; Stefan et al., 2021). The prevalence of obesity worldwide has increased

dramatically over the last few decades (Forouhi and Wareham, 2014). Increased energy intake and insufficient physical activity result in a positive energy balance, the main cause of weight gain that subsequently leads to obesity. Despite its simple equation, energy intake vs. output, obese people find it difficult to maintain a negative energy balance over time, highlighting the need to find methods that can achieve a better metabolic outcome.

Lifestyle adaptation is the primary intervention for managing obesity and its related diseases (Wadden et al., 2012). Weight loss can be achieved by increasing energy expenditure and/or reducing calorie intake. Energy expenditure during physical activity depends on the type of exercise as well as its intensity and duration. Theoretically, the energy content in 1 kg of fat allows a person weighing 70 kg to run approximately 125 km (Frayn, 1983; Litleskare et al., 2020). In reality, however, humans need to run much further to oxidize 1 kg of fat because carbohydrate oxidation contributes to energy expenditure. Protein oxidation is determined by protein intake, while amino acid oxidation has a minimal effect on total energy expenditure during exercise, and is therefore not included in the calculations in this review (Tarnopolsky, 2004).

Indirect calorimetry is used not only to measure cardiorespiratory fitness (VO_{2max}) but also to provide estimates of the energy expenditure and whether the substrate utilized is derived from fat or carbohydrate oxidation (Frayn, 1983). During low-to-moderate intensity exercise (30–65% of VO_{2max}), fat is the major source of energy, and this type of exercise can be maintained for several hours. In contrast, carbohydrates become the principal energy source during high-intensity exercise (>85% of VO_{2max}), which can rarely be maintained for more than half an hour, except by elite endurance athletes (Romijn et al., 1993). Additionally, a very low rate of fat oxidation is observed during high-intensity exercise (Romijn et al., 1993; Andersson Hall et al., 2016). These observations underlie the belief that exercise training with low-to-moderate intensity and of long duration is the best way to lose fat mass. However, high-intensity short-duration exercise training has become increasingly popular due to its time-related effectiveness (Keating et al., 2017).

High-intensity interval training (HIIT) and sprint interval training (SIT) are among the most popular and most studied high-intensity training regimens. Here, we use the nomenclature described in the review by MacInnis and Gibala (2017). HIIT is defined as a near-maximal effort, often performed as bouts of 2–6 min of work at 85–95% of maximal heart rate (MHR) with 2–3 min of rest between bouts. SIT is defined as a maximal or supramaximal effort and is often performed as all-out bouts of 30 s or less, with 2–5 min of rest between bouts. Moderate-intensity continuous training (MICT) consists of continuous exercise at lower intensities. Regarding the ability of the different exercise regimens to reduce visceral adipose tissue (VAT), HbA1c, and fasting glucose, HIIT and SIT seem to have effects that are at least similar to those of MICT in both healthy and diabetic subjects (Burgomaster et al., 2008; Fealy et al., 2018; Søgaard et al., 2018; Winding et al., 2018; Sabag et al., 2020). HIIT and SIT also efficiently reduce total fat mass, despite carbohydrates being the predominant source of energy during the exercise bouts (Trapp et al., 2008; Kuo and Harris, 2016). As the amount of fat lost after a relatively long period of HIIT and SIT is disproportionately larger than the estimated utilization within the HIIT or SIT sessions, the mechanisms explaining this fat-reducing effect are of interest (Kuo and Harris, 2016).

Fat is predominantly stored in adipocytes in various depots throughout the body. Fat localization is a strong predictor of T2DM, NAFLD, and CVD (Mooney et al., 2013). Abdominal obesity, especially increased visceral fat, is associated with an increased risk of the above-mentioned diseases (Karpe and Pinnick, 2015), rendering it an important target in attempts to improve metabolic health. In addition, obesity-related insulin resistance is associated with impaired insulin-mediated inhibition of lipolysis in adipose tissue (AT), resulting in an increased efflux of free fatty acids (FFAs) into the blood and, consequently, ectopic lipid deposition (Snel et al., 2012). Impaired insulin-mediated inhibition of lipolysis is further exacerbated by AT dysfunction (Crewe et al., 2017). Evidence suggests that dysfunctional AT promotes systemic low-grade inflammation, fat overflow, and, hence, ectopic lipid deposition, which further contributes to insulin resistance (Virtue and Vidal-Puig, 2010). Although AT dysfunction is improved by weight loss, the specific impact of exercise training on the former is still unclear (Murphy et al., 2017). Nevertheless, it is thought that the exercise-mediated improvement in metabolic health—including increased insulin sensitivity—may involve improved AT function, comprising an increased ability to store and oxidize fat, reduced fat overflow, and decreased systemic low-grade inflammation (Park et al., 2014). This may drive a reduction in abdominal fat levels, including VAT, and reduce ectopic lipid deposition in the liver and skeletal muscle, as well as in other organs.

Here, we review the effect of exercise training on body fat, focusing on energy expenditure and fat metabolism. The mechanisms explaining the benefits of high-intensity exercise training on AT function and metabolic flexibility, despite minimal β-oxidation during the training sessions, are also discussed.

ENERGY EXPENDITURE IN THE HUMAN BODY

During evolution, movement was necessary for humans to obtain food for survival. Although movement to obtain food and flee from predators remains a prerequisite for animals living in the wild, this is no longer true for most humans, most of whom have an increasingly sedentary lifestyle. Most people exercise to maintain fitness and prevent obesity rather than moving to hunt and gather food (Jensen and O'Rahilly, 2017). Furthermore, pre-prepared food is cheap, energy-dense, and abundant. Humans easily eat more calories than they can expend (O'Rahilly, 2016). As we are still adapted for a life as hunter–gatherers, these significant lifestyle changes have resulted in an increasing prevalence of obesity.

To create a framework for understanding energy expenditure, we introduce and briefly describe some relevant terms. Total daily energy expenditure (TDEE) is the total amount of energy used

in a day and is measured using the doubly labeled water (DLW) method (Westerterp, 2017). TDEE depends on resting energy expenditure (REE) and activity-related energy expenditure (AEE). REE is the energy used during complete rest and AEE refers to the energy used during daily activity and/or exercise. AEE and REE are usually measured by indirect calorimetry.

Precise calculations of energy intake require carefully controlled experimental conditions. The correct collection of such data is difficult in population studies as people normally underreport food intake and overestimate activity when questionnaires are used to address energy balance (Fogelholm et al., 2006; Stubbs et al., 2014). TDEE depends on the REE, free-living activities, and the amount of physical activity performed, collectively called AEE. A fraction (∼10%) of the TDEE can be explained by the thermic effect of food (TEF) (Westerterp, 2004). Even though cardiorespiratory fitness is a predictor of the TEF, the effect of exercise on the TEF remains uncertain (Calcagno et al., 2019). Accordingly, the TEF will not be included in the calculations in this review. The gold standard for measuring total energy expenditure is the DLW method (Westerterp, 2017). This technique is expensive and time-consuming, and it takes 1–3 weeks to collect good data. Energy expenditure can also be measured by direct calorimetry, which measures the amount of energy lost as heat from the body; or by indirect calorimetry, which measures oxygen utilization and carbon dioxide production (Ndahimana and Kim, 2017). Because indirect calorimetry is a standard procedure in exercise physiology laboratories, we focus on this method in this review.

Indirect calorimetry measures the volume of oxygen (O_2) and carbon dioxide (CO_2) inspired and expired. The synthesis of adenosine triphosphate (ATP) in mitochondria requires oxygen to capture electrons from the electron transport chain during the oxidation of carbohydrates and fat. The coupling between oxygen uptake (VO_2) and CO_2 production (VCO_2) has known stoichiometry, which allows the calculation of substrate oxidation and energy expenditure from VO_2 and VCO_2 measurements obtained *via* indirect calorimetry. Carbohydrate and fat metabolism is also well characterized (Frayn, 1983). The oxidation of 1 g of glucose requires 0.747 L of O_2 and provides 17 kJ (4 kcal), that of 1 g of fat requires 2.03 L of O_2 and provides 37 kJ (9 kcal), and that of 1 g of protein 0.966 L of O_2 and provides 17 kJ (4 kcal). Knowing these values allows for the calculation of energy expenditure from oxygen consumption and the respiratory exchange ratio (RER). The RER further provides information regarding whether the source of the energy utilized originates from carbohydrates or fat. The oxygen consumption determines the amount of substrate oxidized and, therefore, the energy released within the body.

The REE is the amount of energy a person uses at rest and is related to body size and composition (Henry, 2005). On a population basis, the REE accounts for ∼60% of the total energy expenditure. The average oxygen consumption for an adult human sitting at rest is approximately 3.5 mL/(kg·min^{-1}) (Henry, 2005), which corresponds to approximately 1,750 kcal for a person weighing 70 kg sitting still all day (Jetté et al., 1990). On a population basis, physical activity rarely accounts for more than 40% of the total energy expenditure (Westerterp, 2000). Unless a person is very active or a professional athlete, it is very difficult for anyone to exceed their REE during activity.

ENERGY EXPENDITURE DURING EXERCISE

The AEE during physical activity depends on both daily activities and exercise. Energy expenditure during a session of endurance exercise depends on the duration, type, and intensity of the exercise, as well as cardiorespiratory fitness. The maximal oxygen uptake (VO_{2max}) is a reflection of cardiorespiratory fitness and is indicative of how much energy a person can utilize during 1 min. VO_{2max} is usually related to body weight. In some instances, however, relating VO_{2max} to fat-free mass is of more value as body composition can differ substantially within the same body mass index (BMI) range.

Members of our group often examine well-trained, lean young males with a VO_{2max} of 50–75 mL/(kg·min^{-1}) (Rustad et al., 2016; Sollie et al., 2018); in contrast, the VO_{2max} for untrained middle-aged males is approximately 45 mL/(kg·min^{-1}) (Langleite et al., 2016; Jelstad et al., 2019). Obese people have reduced cardiorespiratory fitness and their VO_{2max} may be reduced to 20 mL/(kg·min^{-1}) or less (Christ-Roberts et al., 2004; Vind et al., 2011); however, this is not necessarily solely due to low cardiorespiratory fitness but may also result from an increase in body weight. Furthermore, obese people may experience decreased mobility. A combination of low cardiorespiratory fitness and reduced mobility helps explain why exercise becomes an overwhelming task for an obese person, especially knowing that they must run 100 km to get rid of 1 kg of fat (see **BOX 1**).

Oxygen uptake increases gradually during high-intensity exercise, leading to an O_2 deficit (Krogh and Lindhard, 1920). Moreover, a large O_2 deficit develops during exercise performed at above the anaerobic threshold (Medbø and Tabata, 1989). This makes it difficult to use indirect calorimetry to calculate energy expenditure during HIIT and, especially, SIT. Generally, the oxygen debt acquired during anaerobic activity will be an underestimate of the energy expenditure during the anaerobic work. Combining this O_2 deficit with the excess post-exercise oxygen consumption (EPOC) effect, continuous measurement with indirect calorimetry in the recovery period is required for the estimation of extra oxygen consumption used to pay for the oxygen debt and EPOC-related processes (**Figure 1**).

Total work in healthy young males during cycling SIT with four intervals of 30 s corresponds to an energy use of ∼75 kcal (McCartney et al., 1986; Spriet et al., 1989), whereas during a

BOX 1 | Energy expenditure calculation during running.
Energy expenditure during treadmill running is often estimated by oxygen uptake. External work (the force causing displacement) is difficult to measure in a person running. Instead, running economy (RE) is calculated according to an oxygen consumption of ∼0.2 L/(kg·km^{-1}), equal to ∼1 kcal/(kg·km^{-1}) (Litleskare et al., 2020). Therefore, it costs a person weighing 77 kg approximately 7,700 kcal to run 100 km, corresponding to the energy in 1 kg of fat. (Formula: RE = VO_2·kg^{-1}·km^{-1}).

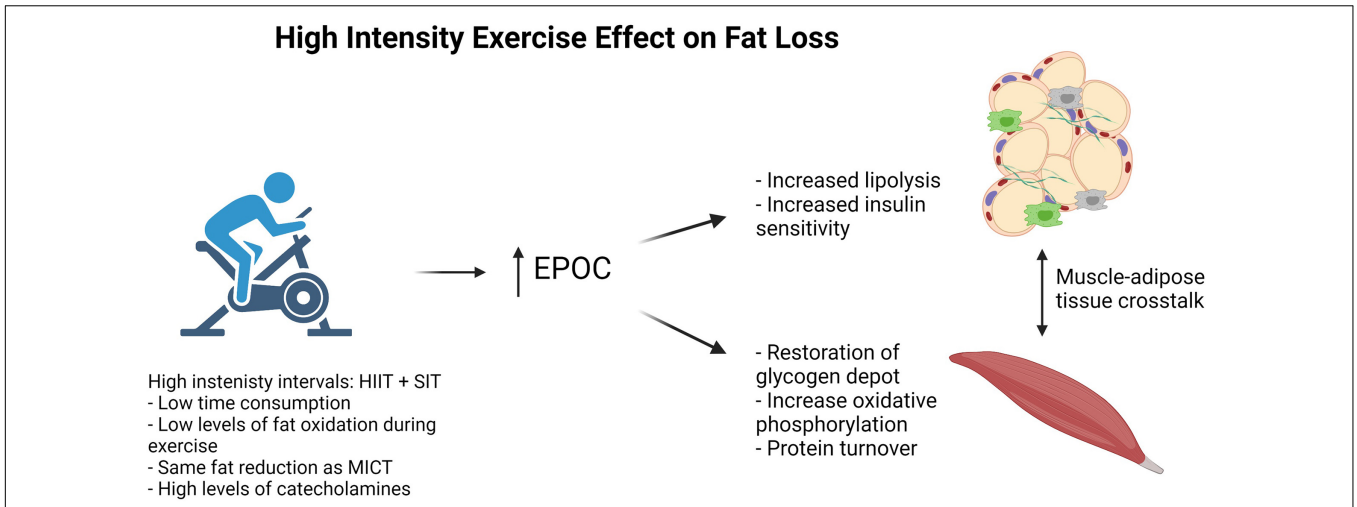

FIGURE 1 | High intensity exercise effect on fat loss.

session of MICT, such as running for 45 min at 75% MHR, a young healthy person weighing 70 kg uses 700 kcal. This clearly indicates that SIT requires only a fraction of the energy expended in MICT. As previously mentioned, HIIT is often performed as 4–7 bouts of work at 85–95% of MHR with 2–3 min of rest between bouts (Bækkerud et al., 2016; Granata et al., 2016; Meinild Lundby et al., 2018). Depending on the length and number of bouts, energy expenditure during HIIT is often the same, or similar to, that for MICT of the same duration (MacInnis and Gibala, 2017).

ENERGY AFTER EXERCISE

Although fat loss is the same, the workload in MICT is much greater than that in HIIT and SIT, indicating that fat loss during HIIT and SIT is greater than expected. Several mechanisms can help explain this surprising outcome. First, as has been suggested, REE may increase in response to high-intensity training; however, no direct evidence exists for such an effect (Karstoft et al., 2017). Secondly, fat loss could result from participants reducing their relative food intake in response to HIIT during a particular study. However, there is evidence to suggest this is not the case (Rosenkilde et al., 2013). Thirdly, although exercise-induced VO_2 utilization drops immediately after cessation of exercise, it remains elevated for up to 24 h after an exercise bout compared with that in the resting state (Tucker et al., 2016). This increase in VO_2 utilization is known as EPOC. There is evidence that the duration of an exercise bout shows a linear relationship with EPOC, whereas an exponential relationship exists between increasing exercise intensity and EPOC (Moniz et al., 2020). Despite these findings, results regarding whether EPOC is responsible for the fat loss associated with high-intensity exercise are conflicting (Tucker et al., 2016).

Biological processes responsible for an increase in EPOC include enhanced protein synthesis related to muscle remodeling and the restoration of intracellular lipid and glycogen deposits in both the liver and skeletal muscle (Moniz et al., 2020) (**Figure 1**). As energy stores are limited in skeletal muscle after exercise, energy must be supplemented from other tissues, including AT. Thus, AT must adapt to the increased energy demands of the skeletal muscle. We hypothesize that, among these adaptations, there is an improvement in AT function that leads to a more favorable shift between carbohydrate and fat oxidation, called metabolic flexibility (Goodpaster and Sparks, 2017). Metabolic flexibility is the ability to shift from fat to carbohydrate oxidation with, for instance, increasing exercise intensity, and then back from carbohydrate to fat oxidation during rest. Improved metabolic flexibility results in a larger fraction of total energy expenditure coming from β-oxidation in the resting state, thereby increasing total fat oxidation. Furthermore, an increase in mitochondrial oxidative phosphorylation in adipocytes could contribute to an increase in EPOC. However, evidence that this occurs in adult humans in response to exercise is scarce, and will not be further discussed (Cannon and Nedergaard, 2011).

FAT AND EXCESS ADIPOSITY

In the healthy state, most fat is stored in AT. There are two types of AT, namely, white and brown AT. In infants, brown AT is involved in thermogenesis, while its role in adult humans is still debated and has been reviewed elsewhere (Rosen and Spiegelman, 2014). White AT is specialized for fat storage and is distributed around different depots in the body. Apart from storing energy, white AT is also a multifunctional organ with endocrine functions that affect whole-body metabolism (Scheja and Heeren, 2019). The energy stored in AT allows humans to survive for long periods without food. Young healthy people with ∼20% body fat can survive for 1–2 months without food (Cahill, 1970). The longest documented period of starvation in a human is 382 days (Stewart and Fleming, 1973). During this period, the weight of the individual declined from 207 to 82 kg, corresponding to a weight loss of 125 kg. Assuming

(although fictive, as fat loss only is unrealistic in such a long fast) that all this weight loss was related to fat, with an energy content of 7,700 kcal/kg, this equates to ∼1,000,000 kcal, i.e., an average of ∼2,500 kcal/day during the 382 days of fasting (Stewart and Fleming, 1973).

High body fat content not only causes major health problems but also prevents people from exercising effectively. Total fat mass is most accurately measured by magnetic resonance imaging (MRI) or computed tomography (CT) (Wells and Fewtrell, 2006). However, MRI and CT are time-consuming and expensive, and cheaper and faster measurement methods such DXA scan and bioimpedance, or anthropometric measurements such as BMI or waist–hip ratio, are often used instead to evaluate body composition and metabolic health. BMI only reflects weight in relation to height and is, therefore, a suboptimal measurement as humans can attain similar BMIs with different body compositions. However, on a population level, an increase either in BMI or total fat mass can increase the risk of obesity-related diseases (Van Pelt et al., 2002; Bays et al., 2007).

METABOLIC DIFFERENCES IN FAT DEPOTS

From a metabolic perspective, it is important to distinguish between different fat depots within the body. White AT is divided into two main types—VAT and subcutaneous white adipose tissue (SAT). VAT is fat located in different depots surrounding internal organs, whereas SAT is located under the skin throughout most of the body. In obesity, most SAT is located on the abdomen or hips. An excess of abdominal fat, termed android obesity, is more common in men, whereas excessive fat storage on the hips and legs (glutofemoral fat mass), often referred to as gynoid obesity, is more common in women. Greater amounts of abdominal (android) fat increase the risk of T2DM, NAFLD, and CVD, whereas peripheral fat centered at the hips and legs (gynoid) has been suggested to exert a protective effect against CVD (Mooney et al., 2013; Karpe and Pinnick, 2015). Studies have shown that increased gynoid obesity is associated with more favorable lipid and glucose metabolism independently of the amount of VAT (Stefan, 2020).

Increasing the amount of VAT alone can increase the risk of the above-mentioned diseases independently of BMI and total fat mass, underlining the importance of fat localization (Tchernof and Després, 2013). The exact mechanism underlying why VAT is more harmful than SAT remains unknown. Features that could make VAT more metabolically harmful include differences in innervation and that VAT has direct access to the portal vein, leading to the released fat finding its way directly to the liver (Nguyen et al., 2014). Furthermore, adrenergic receptors expressed in adipocytes in VAT differ from those expressed in SAT adipocytes (Stefan, 2020). It has also been speculated that fat is stored in the visceral depots when the fat-storing capacity in the subcutaneous depots is exceeded, indicating an overflow of fat and ectopic lipid deposition (Snel et al., 2012). Regardless of the mechanisms, reducing the amount of abdominal visceral fat is of great interest from a health perspective.

ADIPOSE TISSUE AND ITS FUNCTION

Fat has an energy content of 9,000 kcal/kg. However, because AT in humans also contains a small amount of water and other cells, the actual *in vivo* energy content in 1 kg of AT is approximately 7,700 kcal (Flatt, 1995; Heymsfield et al., 2012). Despite accounting for only 50–60% of the total cell number, adipocytes comprise over 85% of the volume in white AT (Lenz et al., 2020). In adipocytes, fat is stored as triacylglycerol in lipid droplets that make up > 95% of the intracellular content (Arner, 2005). That almost all the volume of AT is comprised of fat is indicative of its importance as a storage depot. However, even though the volume of non-adipocytes in AT is limited, these cells exert a significant influence on AT function. Non-adipocytes in AT include immune cells, preadipocytes, endothelial cells, and fibroblasts (Lenz et al., 2020), known collectively as the stromal vascular fraction (Bourin et al., 2013). These cells are responsible for adipogenesis, angiogenesis, extracellular matrix modeling, and regulation of inflammation and, hence, contribute to both the function and dysfunction of AT (Crewe et al., 2017).

Healthy white AT is a dynamic and flexible organ that stores fat when in excess and provides energy when needed. After a meal, insulin is secreted from pancreatic β-cells in response to increased levels of glucose, amino acids, and incretins in the blood (Wilcox, 2005). Insulin signaling in adipocytes through the insulin receptor increases glucose and fatty acid (FA) uptake into the adipocytes (Arner, 2005). Insulin suppresses lipolysis by increasing the activity of phosphodiesterase 3B (PDE3B), leading to decreased cAMP-mediated activation of protein kinase A (PKA), and, consequently, reduced phosphorylation of hormone-sensitive lipase (HSL) and perilipin (PLIN). This ultimately promotes lipogenesis, resulting in triglyceride storage and, therefore, adipocyte expansion (Zechner et al., 2009; Petersen and Shulman, 2018). In contrast, stressors such as exercise and starvation promote catecholamine secretion. Catecholamines act through β-adrenergic receptors in adipocytes, inducing an increase in cAMP levels, which activates PKA and ultimately enhances lipolysis (Arner, 2005). PKA activates lipolysis at several steps, including through PLIN and HSL phosphorylation. PLIN phosphorylates CGI-58, allowing it to bind to and fully activate ATGL, while phosphorylated HSL is directly involved in lipolysis (Zechner et al., 2009). When insulin levels are relatively low, AT releases FFAs into the bloodstream, making them available for muscle and other tissues when needed (Arner, 2005). Beta-oxidation accounts for 80% of the total energy-related oxidation in skeletal muscle at rest after an overnight fast, and this percentage increases further in response to prolonged fasting (Kelley and Simoneau, 1994). In skeletal muscle, FFAs are either stored as triglycerides in lipid droplets or metabolized via β-oxidation, providing energy for exercise and other energy-consuming processes (Kiens, 2006). Importantly, there is a continuous balance between the oxidation of glucose and fat during both rest and activity. Fat oxidation increases the levels of acetyl-CoA and inhibits glucose oxidation via the Randle cycle, whereas excessive glucose uptake increases malonyl-CoA levels, which inhibits fat oxidation (Hue and Taegtmeyer, 2009).

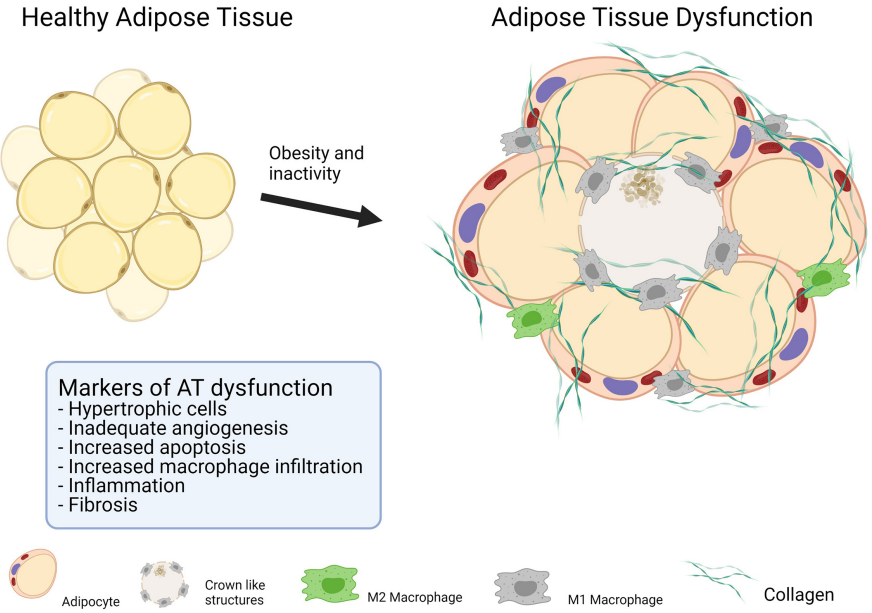

FIGURE 2 | Makers of adipose tissue dysfunction.

Metabolic flexibility, the ability to switch between fat and carbohydrate oxidation with varying energy requirements seems to be important for proper metabolic regulation (Goodpaster and Sparks, 2017). Metabolic flexibility is attenuated in obesity and more so in T2DM (Smith et al., 2018). In the resting state, obese people have a higher RER value when compared with lean individuals, meaning a larger fraction of the energy expended derives from carbohydrate oxidation, an effect that is even more pronounced in patients with T2DM (Goodpaster and Sparks, 2017). Furthermore, during insulin stimulation, obese individuals and patients with T2DM are not able to increase the RER value to the same extent as lean individuals (Kelley and Simoneau, 1994; Goodpaster and Sparks, 2017). However, during high-intensity exercise, obese individuals and patients with T2DM can increase the RER to values comparable to those observed in lean individuals (Kelley and Mandarino, 2000). The exact role of impaired metabolic flexibility remains unknown, and whether it is a consequence of insulin resistance or an early impairment that contributes to insulin resistance remains to be clarified.

Disrupted signaling along intracellular pathways responsible for lipid metabolism is one of the features of impaired metabolic flexibility in obesity (Boucher et al., 2014). As mentioned earlier, insulin primarily inhibits lipolysis while catecholamines primarily promote lipolysis. Insulin resistance in adipocytes is associated with impaired phosphorylation of insulin receptor substrate 1 (IRS-1) in obesity, which is further impaired in T2DM (Copps and White, 2012). The inability of insulin to inhibit lipolysis results in an increased efflux of FFAs into the bloodstream. The counter-regulatory pathway involving catecholamine-related HSL stimulation is also attenuated in obesity, rendering adipocytes insensitive to catecholamines (Arner, 2005). Thus, obesity reduces the flexibility to switch between lipolysis and lipogenesis, exacerbating a vicious cycle whereby an increase in lipolysis results in ectopic lipid deposition and further insulin resistance.

KEY FEATURES OF AT DYSFUNCTION

The localization of fat depots is a strong predictor of metabolic diseases. Regardless of its localization, AT content and function differ markedly between obese and healthy lean people (Rydén et al., 2014), and is likely to be further altered in patients with T2DM compared with obese individuals with the same BMI (Camastra et al., 2017). Collectively, these changes are termed AT dysfunction (Crewe et al., 2017), which is characterized by an unhealthy AT expansion characterized by the presence of hypertrophic adipocytes, excessive accumulation of extracellular matrix (ECM) components, interstitial fibrosis, exaggerated pro-inflammatory macrophage infiltration, and dysregulated angiogenesis (**Figure 2**). Alterations in the ECM and impaired angiogenesis can be difficult to measure in human fat samples; however, the mRNA levels of proteins associated with the ECM and angiogenesis are often used as markers of these processes in AT dysfunction (Åkra et al., 2020). Low-grade inflammation, macrophage infiltration, and adipocyte cell size are frequently used to determine the degree of AT dysfunction (Longo et al., 2019).

AT dysfunction and the resulting impaired AT expandability cause local and systemic low-grade inflammation, fat overflow, and ectopic fat deposition, all of which are associated with insulin resistance (Crewe et al., 2017). Whether AT dysfunction is the cause or consequence of insulin resistance remains unknown. Furthermore, although the exact pathophysiological mechanism underlying NAFLD remains unknown, its development is

associated with the insulin receptor, low-grade inflammation, and ectopic lipid deposition. Consequently, NAFLD is exacerbated with AT dysfunction (Cimini et al., 2017; Tarantino et al., 2019).

Hypertrophic adipocytes show a positive correlation with insulin resistance and the risk of CVD (Muir et al., 2016), while increases in macrophage infiltration and the numbers of apoptotic adipocytes are also seen in dysfunctional AT (Crewe et al., 2017). The exact sequence of events and pathways involved are still under debate. One of the most studied hypotheses postulates that relative hypoxia in AT mediates a cascade of events that lead to the increased apoptosis and low-grade inflammation seen in obesity (Trayhurn and Wood, 2004). Adipocytes increase in size as a result of obesity, with some becoming larger than 100 μm (Yin et al., 2009). At this distance, oxygen diffusion is compromised due to the limitation of the diffusion capacity. Hypoxia *per se* decreases insulin signaling and disrupts lipid metabolism in AT (Regazzetti et al., 2009; Yin et al., 2009). Furthermore, the cytokine hypoxia-inducible factor 1 alpha (HIF-1α) is upregulated in a hypoxic environment. HIF-1α is a multifunctional molecule best known for its function in promoting angiogenesis. However, in dysfunctional AT, HIF-1α fails to promote angiogenesis, inducing instead dysregulated ECM remodeling, resulting in fibrosis that disrupts proper angiogenesis, further exacerbating hypoxia in AT (Sun et al., 2013).

Although adipocytes undergo apoptosis under normal conditions, an increased rate is observed in obesity (Camastra et al., 2017). Adipocytes have a lifetime of approximately 8–10 years, indicating that ∼10% of these cells must be replaced annually (Spalding et al., 2008). Accordingly, a balance between apoptosis and adipogenesis is required for the maintenance of AT. Nevertheless, apoptotic cells attract macrophages. Evidence suggests that most macrophages in AT are in close proximity to apoptotic adipocytes (Murano et al., 2008). The finding that macrophage numbers are increased in AT of obese individuals is not surprising. Macrophages are the main cell type responsible for the increased low-grade inflammation seen in AT dysfunction. However, evidence suggests that it is $CD8^+$ T lymphocytes that initiate the processes of inflammation in AT (Nishimura et al., 2009). Low-grade inflammation drives the secretion of a broad spectrum of cytokines, which further increase low-grade inflammation, resulting in a vicious cycle. Tumor necrosis factor-alpha (TNF-α), interleukin 1 beta (IL-1β), and monocyte chemoattractant protein-1 (MCP-1) are some of the best-characterized cytokines in AT. TNF-α induces an increase in the M1/M2 polarization ratio in macrophages. M1 macrophages enhance the pro-inflammatory response, whereas M2 macrophages have anti-inflammatory properties. Additionally, TNF-α inhibits both the insulin and the catecholamine signaling pathways in adipocytes, resulting in decreased metabolic flexibility (Langin and Arner, 2006; Guilherme et al., 2008). The blood levels of IL-1β are elevated in systemic rheumatic diseases, which is believed to mediate the inflammation seen in these conditions. The role of IL-1β in obesity-related low-grade inflammation is not completely understood, but it has been suggested that it induces β-cell damage in T2DM (Gabay et al., 2010; Eguchi and Manabe, 2013). MCP-1 is secreted from macrophages and is chemotactic for monocytes, inducing further macrophage infiltration into the AT (Sakurai et al., 2017).

Importantly, under these conditions, AT dysfunction disrupts fat metabolism in adipocytes at multiple sites. Notably, an increased resistance to insulin results in elevated lipolysis, resulting in elevated levels of circulating FFAs. In addition to insulin resistance, reduced sensitivity to catecholamines also decreases metabolic flexibility. Fat overflow, together with low-grade systemic inflammation, results in ectopic lipid deposition in the liver, muscle, and pancreatic β-cells. Lipid deposition in the liver and muscle directly increases insulin resistance (Snel et al., 2012), while in pancreatic β-cells it is believed to be lipotoxic, decreasing their ability to secrete insulin (Robertson et al., 2004). A combination of insulin resistance and decreased insulin secretion is expected to increase the risk for T2DM.

EXERCISE AND METABOLIC REGULATION

Physical activity is the cornerstone for improving metabolic health, and a lack of physical activity increases the risk of obesity and obesity-associated diseases such as T2DM, CVD, and NAFLD (Booth et al., 2017). For T2DM and CVD, a broad range of pharmaceuticals drugs are also available that can halt the progression of both diseases. In contrast, the pharmaceutical options for the management of NAFLD are limited (Tarantino et al., 2019). Because NAFLD has become the most common cause of chronic liver disease, it is important that therapeutic options for this condition are identified. Physical activity has proven to be an effective intervention for treating and preventing NAFLD (van der Windt et al., 2018; Wang et al., 2020). Exactly how exercise improves NAFLD is not completely understood (van der Windt et al., 2018); however, complex crosstalk between adipokines, hepatokines, and myokines has been suggested to play a central role in this improvement (de Oliveira Dos Santos et al., 2021).

The effect of exercise training on glucose tolerance and insulin sensitivity has been extensively researched (Sandvei et al., 2012; Langleite et al., 2016; Jelstad et al., 2019). The improved insulin sensitivity in skeletal muscle in response to exercise training can be in part explained by the upregulation of proteins involved in glucose metabolism and the insulin signaling pathway, including the insulin-sensitive glucose transporter 4 (GLUT4), hexokinase II, AKT2, glycogen synthase, and AMP-activated protein kinase (AMPK), as well as mitochondrial proteins (Wojtaszewski and Richter, 2006; Vind et al., 2011; Sylow et al., 2017). Although not as thoroughly investigated as skeletal muscle, it seems that at least some of these changes also happen in AT. In humans, insulin sensitivity in AT is accompanied by an increase in glucose transportation and glycolytic pathway (Riis et al., 2018). Furthermore, it has been shown that fat oxidation at rest increases in response to exercise, suggesting an improvement in AT/skeletal muscle axis (Calles-Escandón et al., 1996).

Sustained adherence to endurance exercise increases oxidative capacity and, consequently, the capacity to utilize energy. In many interventional exercise training studies, no significant reduction in body weight is seen either in obese or lean subjects, despite an improvement in metabolic regulation (Sandvei et al., 2012; Langleite et al., 2016). In exercise interventions, standardized work is normally performed at a specific workload or intensity for a given duration. For instance, in a study performed by our research group, aerobic training sessions involving ∼40 min of cycling at high intensity (75% VO_{2max}) resulted in an energy expenditure of 600 kcal for untrained, lean, middle-aged males (Jelstad et al., 2019). Three weekly sessions each with a 600 kcal expenditure (a total of 1,800 kcal per week) correspond to the energy in 234 g of fat and 12 weeks of training intervention with such energy expenditure corresponds to the energy in 2.8 kg of fat. As previously mentioned, most of the energy used during high-intensity exercise is sourced from carbohydrates. Regardless of the source of the energy expended and the increased energy expenditure during exercise intervention, there must be a total negative energy balance to attain a reduction in body weight.

Exercise training can change body composition without changing total body weight by decreasing fat mass and increasing muscle mass (Langleite et al., 2016). In a 12-week training intervention consisting of 2 weekly sessions of HIIT and two sessions of resistance training, we observed a reduction in both subcutaneous and visceral fat deposits concomitant with an increase in muscle mass, resulting in no significant reduction in body weight (Langleite et al., 2016). From an energy perspective, 1 kg of fat contains 7,700 kcal, whereas muscle tissue is 70% water and the energy content in 1 kg of muscle is ∼1,000 kcal. The small increase in muscle mass has little effect on the REE because the metabolic rate in resting skeletal muscle is only ∼10–15 kcal/(kg·day^{-1}) (Elia and Livesey, 1992). Although higher than the metabolic rate in AT ∼5 kcal/(kg·day^{-1}), the increase accounts for only a small proportion of the whole-body energy expenditure. The brain, heart, kidney, liver, and gastrointestinal tract are the tissues that utilize the most energy at rest, and, for practical reasons, are not under the influence of the training (Elia and Livesey, 1992). Regardless of the small changes in REE, the AEE can increase in response to HIIT and SIT. As previously mentioned, these regimens increase muscle mass and oxidative phosphorylation (and VO_{2max}), and, therefore, the ability to utilize more energy during a given time will be higher (Chrøis et al., 2020). There is evidence that HIIT promotes a greater improvement in mitochondrial oxidative capacity compared with MICT (MacInnis et al., 2017); however, to our knowledge, whether this can explain the increased fat loss has not been investigated.

Although energy expenditure is lower during SIT than during MICT, both regimens seem to undergo similar metabolic adaptations, such as increased VO_{2max} and a reduction in VAT, fasting glucose, and HbA1c (Burgomaster et al., 2008; Sandvei et al., 2012). The reasons for the similar improvements are not fully understood. Nonetheless, there is some evidence that a progressive increase in intensity results in a progressive increase in intracellular signaling in key metabolism-favorable pathways. High-intensity exercise activates both AMPK and peroxisome proliferator-activated receptor co-activator (PGC-1α) to a greater extent than low-intensity exercise in skeletal muscle (Wojtaszewski et al., 2000; Egan et al., 2010). AMPK is a pleiotropic protein with intracellular effects on lipid, glucose, and protein metabolism (Hardie et al., 2012), while PGC-1α is a potent driver of mitochondrial biogenesis and is also involved in glycogen metabolism (Wu et al., 1999; Wende et al., 2007; Kjøbsted et al., 2016). Furthermore, in a study comparing the metabolic effects of 10-fold higher energy expenditure in MICT compared with SIT, oxidative enzyme activity in muscle showed similar increases in both training regimens (Burgomaster et al., 2008). Whether this occurs in AT as well as in skeletal muscle remains to be investigated. Furthermore, the concentrations of catecholamines increase substantially during SIT, and anaerobic processes provide most of the energy during this workout. After 30 s "all-out," the adrenaline concentration increases to high values (4 nM), more than double that in other types of exercise, resulting in an increased metabolic rate (Esbjörnsson-Liljedahl et al., 2002; Zouhal et al., 2008).

Among other functions, adrenaline increases glycogen breakdown. Increased intramuscular glycogen content is seen together with increased insulin sensitivity in response to several weeks of endurance exercise training (Vind et al., 2011). However, the glycogen store in muscles serves as the energy substrate during exercise, and an acute reduction in muscular glycogen content increases insulin sensitivity in the working muscle (Jensen et al., 1997, 2006, 2011). A single MICT session consisting of cycling for 60 min at 70% VO_{2max} reduced the glycogen amount by 50% in muscles (Pedersen et al., 2015). In comparison, a single SIT session stimulates glycogen breakdown and a single 30 s all-out cycling episode decreases the glycogen content in skeletal muscle by approximately 25% (Jacobs et al., 1982; Esbjörnsson-Liljedahl et al., 1999, 2002). Three bouts of 30 s all-out cycling with 20 min rest between sprints decreased glycogen content by approximately 50% (Esbjörnsson-Liljedahl et al., 2002). With only a total of 90 s of work, a SIT session results in the same reduction in glycogen content as 60 min of a MICT session.

THE EFFECT OF EXERCISE ON ABDOMINAL FAT

The effect of exercise training on metabolic parameters, such as insulin sensitivity and fat loss, is unquestionably positive, even in the absence of a reduction in total body weight (Langleite et al., 2016). Exercise training can reduce abdominal visceral and subcutaneous fat mass even when there is no loss of total body weight. There is evidence to support that the relative reduction in VAT is greater than the reduction in SAT in response to exercise training in overweight people (Langleite et al., 2016). If exercise training is accompanied by a loss of total body weight, there is an even bigger relative reduction of visceral fat compared to the subcutaneous fat loss (Ross et al., 2000). If the speculative hypothesis that the amount of VAT increases when the capacity of SAT depots are exceeded, an improvement of AT function in

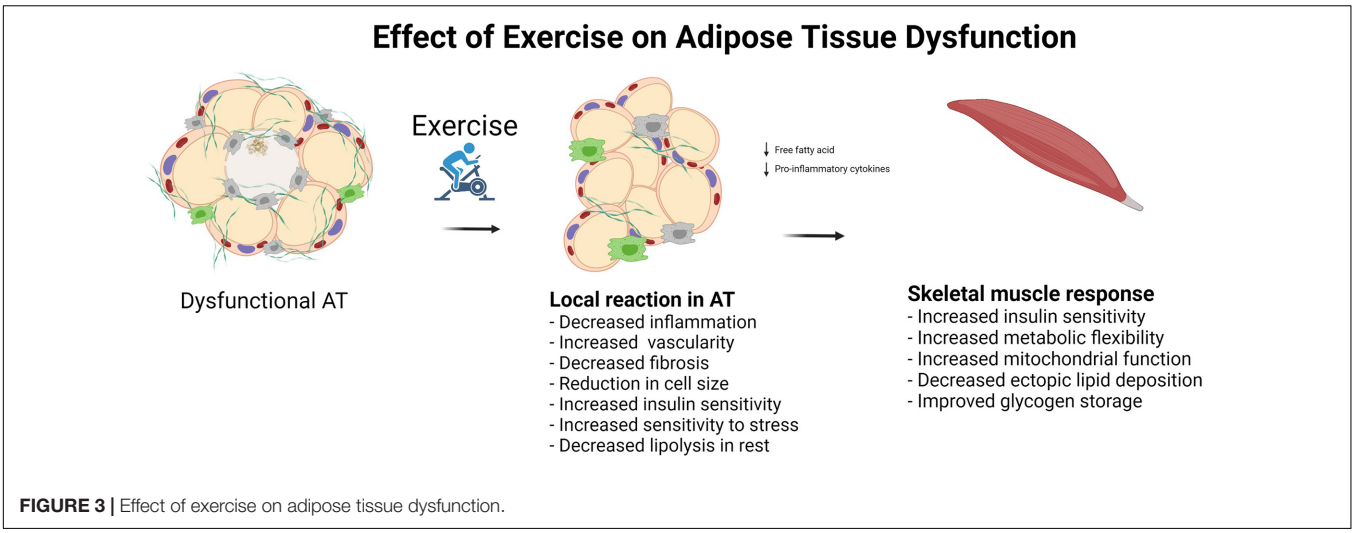

FIGURE 3 | Effect of exercise on adipose tissue dysfunction.

response to exercise could also explain, why there is a relative larger loss of VAT.

While various meta-analyses have shown that MICT and HIIT leads to, at best, modest changes in body fat (Keating et al., 2017; Sultana et al., 2019), exercise intensity seems to play an important role in VAT modulation. A meta-analysis of the effect of exercise on VAT showed that high intensity exercise training induced a larger reduction of VAT compared to moderate and low intensity (Vissers et al., 2013), and moderate intensity exercise training (MICT) reduced VAT more than low intensity exercise training. This indicates that increasing intensity is associated with a greater reduction of VAT. The same study analyzed the effect of training volume (duration of sessions), and found no association between training volume and reduction of VAT or total fat mass. This is somewhat surprising, considering that fat is the predominant source of energy during low-moderate exercise. If the sympathetic nervous activation is greater with increasing exercise intensity, leading to greater VAT innervation, this may explain the increase in lipolysis in VAT and, thereby, also the reduction of VAT. Furthermore, a study has shown that insulin sensitivity, measured as increased glucose uptake using PET–CT combined with hyperinsulinemic clamp, increases in VAT in response to HIIT, but not moderate-intensity training (Honkala et al., 2020). There is evidence that exercise intensity is more important than duration for reducing VAT after a training period. This could in part explain why short-duration high-intensity exercise training can reduce fat, thereby obtaining good metabolic results. However, the associated molecular mechanisms remain unclear.

One of the mechanisms proposed to underlie the exercise-induced reduction of VAT involves signaling through IL-6. This cytokine is produced by a variety of cells, including adipocytes, lymphocytes, and macrophages. The plasma levels of IL-6 correlate well with systemic low-grade inflammation (Pedersen, 2017). Despite its positive correlation with disease, recent studies have suggested that IL-6 can also function as an anti-inflammatory cytokine (Pedersen, 2017)—an interesting duality. When IL-6 is secreted from contracting muscle tissue, it acts as an anti-inflammatory myokine, and is one of the first cytokines to be upregulated after exercise. An acute increase in IL-6 concentrations is believed to enhance whole-body lipolysis and β-oxidation (van Hall et al., 2003). Recently, blocking IL-6 with tocilizumab (an anti-IL-6 receptor antibody) was reported to abolish the reduction of visceral fat promoted by cycling exercise of unreported length and intensity three times a week. However, the average heart rate was 146 during exercise for participants between the ages of 40 and 45, which should be considered moderate intensity (Wedell-Neergaard et al., 2019). This highlights the importance of the crosstalk between muscle and AT, and also underlines the complexity of the effects associated with cytokines originating from different tissues and at different concentrations.

THE EFFECT OF EXERCISE ON AT DYSFUNCTION

In large cross-sectional population studies, moderate-to-high intensity physical activity is associated with reduced levels of circulating markers of low-grade inflammation, namely, IL-6, resistin, and leptin, and an increase in the concentration of the plasma anti-inflammatory marker adiponectin (Vella et al., 2017) (**Figure 3**). However, exercise interventions without weight loss have shown a limited ability to reduce the levels of known markers of low-grade inflammation (Allen et al., 2017; Kelly et al., 2017). In a study by Stinkens et al. (2018), which included obese men with and without T2DM, no changes in adipocyte cell size or the levels of markers of low-grade inflammation were detected after 12 weeks of exercise training consisting of two sessions of endurance exercise at 70% VO_{2max} for 30 min and one session of resistance training per week. The exercise in this study was of moderate intensity and relatively short duration. Furthermore, there was no loss of total body weight, although there was a 0.7-kg reduction in fat mass. Fat reduction was determined by DXA scan, and whether the abdominal fat was visceral or subcutaneous

was not reported. Despite a lack of improvements in the concentrations of these markers of AT dysfunction, the authors found that insulin sensitivity was increased in skeletal muscle, but not in adipocytes or liver, as measured by hyperinsulinemic clamp (Stinkens et al., 2018). The participants had some degree of AT dysfunction related to obesity and T2DM; however, as the study did not include a lean, healthy control group, it is difficult to fully interpret the results.

Riis et al. (2018) investigated the molecular adaptations in AT in lean, healthy young male subjects [age 21 (18–24)] that underwent three sessions per week of endurance exercise of varying intensity for 10 weeks on a bicycle ergometer. They found that insulin sensitivity in AT, as measured using the AT insulin resistance index, was improved in the exercise group compared with that of the control group (Riis et al., 2018), concomitant with an increase in insulin receptor protein abundance as well as that of the downstream proteins involved in glucose oxidation in AT. Although the participants did not lose body weight, they displayed a significant reduction in fat mass. No changes in the levels of markers related to browning or beiging were reported in AT biopsies (Riis et al., 2018). Taken together, these observations suggest that exercise training can improve AT function, thereby contributing to improved metabolic flexibility. This study also emphasizes the positive effect of training on AT, even in young, lean, healthy individuals.

Exercise training alone has not been shown to reduce adipocyte size in obese individuals (Honkala et al., 2020). A meta-analysis of adipocyte cell size in overweight and obese individuals showed a linear relationship between the reduction of adipocyte cell size and the amount of weight lost (Murphy et al., 2017). In the same analysis, the authors compared the reduction of adipocyte cell size among different methods used to lose weight (bariatric surgery, dietary restriction, and exercise) and found no difference among the groups. This suggests that it may be necessary to reduce body weight in order to reduce adipocyte cell size (**Figure 3**).

In summary, regarding the effect of exercise training on AT, exercise *per se* does not reduce body weight. However, even without weight loss, exercise improves body composition by reducing fat mass and increasing muscle mass. Contracting skeletal muscle may play an important role in the reduction of VAT *via* the production and secretion of myokines such as IL-6. Insulin sensitivity in both adipocytes and skeletal muscle improves with exercise training. Despite the exercise-related improvement in AT function, the effect of exercise training on markers of AT dysfunction remains to be clarified. Evidence suggests that exercise *per se* does not reduce adipocyte cell size without weight loss; however, exercise training does, to some extent, reduce low-grade inflammation that is believed to evolve from AT dysfunction. Furthermore, exercise improves insulin sensitivity, glucose metabolism, and oxidative phosphorylation, all of which are impaired in dysfunctional AT.

CONCLUSION/PERSPECTIVES

From an evolutionary perspective, AT stores energy for utilization in periods of low energy availability; however, a shortage of food does not often occur in the Western world, which has led to an increased prevalence of obesity and its related diseases. Obesity is not just an accumulation of fat in adipocytes but is also characterized by unfavorable lipid metabolism, AT dysfunction, ectopic lipid deposition, systemic low-grade inflammation, and insulin resistance. AT dysfunction may contribute to an unhealthy body fat distribution due to impaired fat storage in SAT, leading to an increase in VAT.

Nowadays, exercise training is often performed to lose weight and prevent lifestyle diseases. Losing weight through exercise can be an overwhelming task for untrained obese individuals, requiring a substantial amount of time. Nevertheless, even without inducing weight loss, exercise training reduces both total and visceral fat mass. HIIT and SIT aim to reduce the duration of exercise. Short-duration high-intensity exercise regimens are effective at reducing abdominal fat, indicating that they exert a positive effect on AT, which cannot be fully explained by the fat oxidized during the exercise bouts. A possible physiological mechanism explaining fat loss, in response to exercise at high intensities, is EPOC. The acute effects behind the increased EPOC does most likely not occur in the adipose tissue, but the energy can still be derived from AT. We argue that AT function is improved in response to increased energy utilization. Furthermore, AT dysfunction in obesity or T2DM is likely to be ameliorated in response to high-intensity exercise. However, the effect of exercise training on AT function and dysfunction, particularly with high intensity, remains to be thoroughly investigated.

AUTHOR CONTRIBUTIONS

KJK drafted the manuscript. TL-I, MHP, KH, and JJ commented on and read the final version. All authors contributed to the article and approved the submitted version.

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