PRECISION PHYSICAL ACTIVITY AND EXERCISE PRESCRIPTIONS FOR DISEASE PREVENTION: THE EFFECT OF INTERINDIVIDUAL VARIABILITY UNDER DIFFERENT TRAINING APPROACHES

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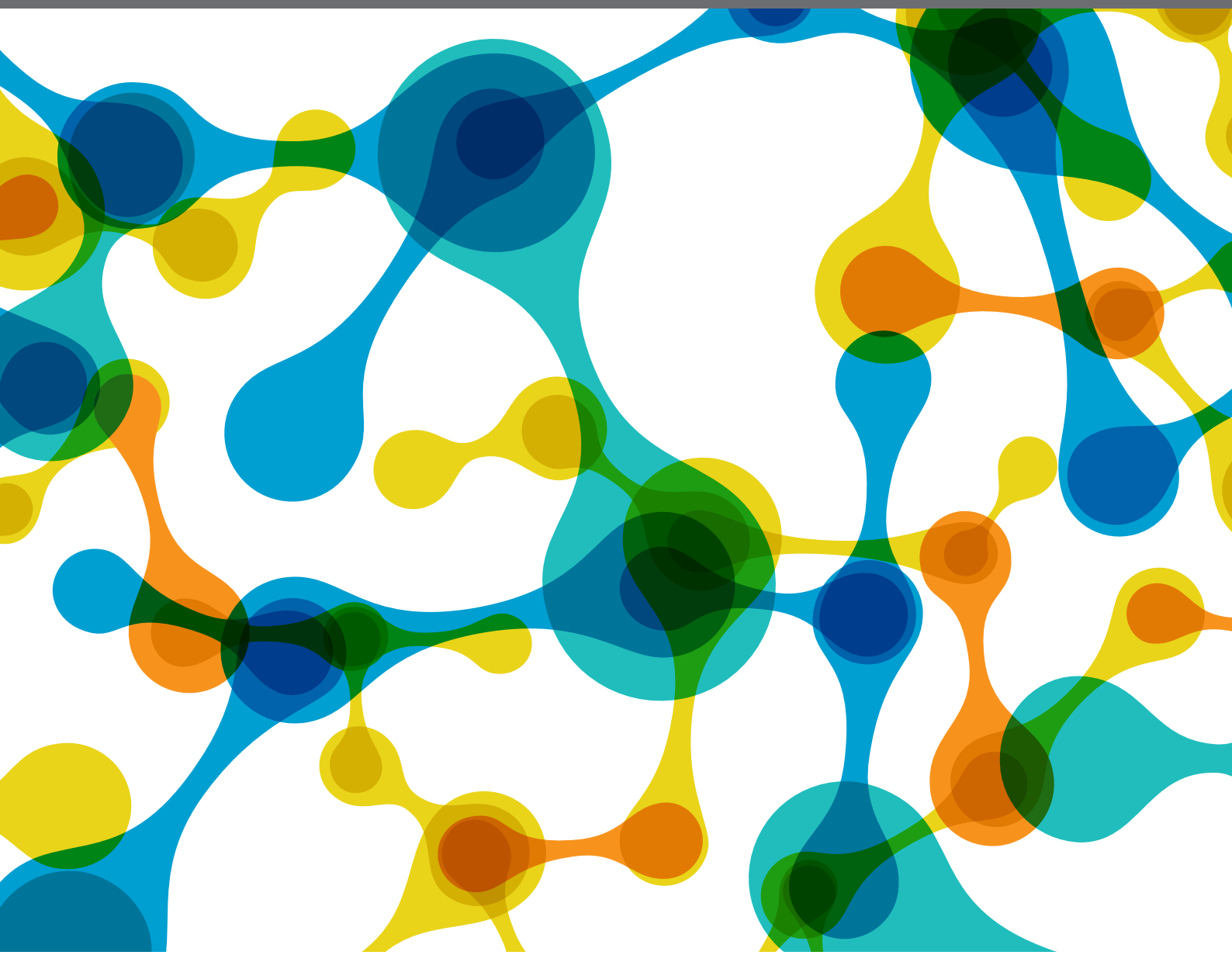

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PRECISION PHYSICAL ACTIVITY AND EXERCISE PRESCRIPTIONS FOR DISEASE PREVENTION: THE EFFECT OF INTERINDIVIDUAL VARIABILITY UNDER DIFFERENT TRAINING APPROACHES

Topic Editors:
Robinson Ramírez-Vélez, Public University of Navarra, Spain
Mikel Izquierdo, Public University of Navarra, Spain

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EDITORIAL
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Editorial: Precision Physical Activity and Exercise Prescriptions for Disease Prevention: The Effect of Interindividual Variability Under Different Training Approaches

Robinson Ramírez-Vélez and Mikel Izquierdo *

Department of Health Sciences, Navarrabiomed, CIBER of Frailty and Healthy Aging (CIBERFES), Instituto de Salud Carlos III, IdiSNA, Public University of Navarra, Pamplona, Spain

Keywords: non-communicable chronic diseases (NCDs), precision physical activity, interindividual variability, responders in clinical trials, high-intensity interval training (HIIT), cardiovascular training, resistance training (RT)

Editorial on the Research Topic

Precision Physical Activity and Exercise Prescriptions for Disease Prevention: The Effect of Interindividual Variability Under Different Training Approaches

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University of Illinois at Urbana-Champaign, United States

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

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INTRODUCTION

Optimizing exercise training to improve health biomarkers and reduce the risk of chronic disease and premature mortality is imperative to long-term health (Ross et al., 2016; Ramírez-Vélez et al., 2017). Indeed, in the era of "precision medicine," it is reasonable to assume that the prescription of exercise as a treatment modality should be individually tailored to the specific characteristics of the patient with respect to program variables. This Research Topic consists of 11 articles, of which nine contain original data, one is a longitudinal study and two are review/opinion articles.

REVIEW/OPINION AND EPIDEMIOLOGY ARTICLES

Solomon's review lays the groundwork of the Research Topic, discussing the evidence and, perhaps more importantly, documenting the origins of inter-individual variability for people with or at risk of diabetes, in relation to responders and non-responders. Solomon underlines the compelling need for prospective trials to identify physiological or molecular signatures that can predict inter-individual variability with regards to exercise-induced changes in blood glucose levels.

Lazarus and Harridge's opinion review claims that the shape of a person's overall performance profile with regards to age is fundamentally independent of discipline, distance, or phenotype. The authors posit that with appropriate training this same profile and trajectory, albeit with a decline in performance times, would be produced by all individuals engaging in sufficient physical activity/exercise. The authors' evidence would indicate that being physically active is by far the best approach for achieving optimal aging.

Whitney and Peterson evaluated the capacity of diverse post-processing methods of handgrip strength to predict mortality and incident cerebrovascular events in 4,143 participants aged ≥65 years from the National Health and Aging Trends Study who were followed for 6 years. Their findings suggest that the variety of post-processing methods might have differing predictive

capacity in the elderly in relation to the outcome of interest. Nevertheless, because absolute handgrip measures correlated with both cerebrovascular events and mortality, they may be considered beneficial for screening in the elderly.

INTERVENTIONAL ARTICLES

Domínguez-Sanchéz et al. compared neurotrophic factor responses after one session of high-intensity interval training (HIIT), resistance training (RT), or both, in a group of overweight and physically inactive adults aged 18–30 years. The main finding was that compared with baseline levels, all three protocols provoked greater changes in neurotrophin levels. Also, the combined (HIIT + RT) and RT regimens elicited greater changes in the levels of brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4/5 than the HIIT regimen alone.

Lewis et al. showed for the first time that genetic variability of the acid ceramidase gene has a significant effect on exercise tolerance and achievement of an exercise program. Given the recognized health benefits of regular exercise, assessing individual exercise potential influenced by genetic variation before starting an intervention may be useful in maximizing exercise adherence and, therefore, the potential health benefits. These results reinforce the idea that individual differences in biological response to exercise exposure are heritable, with estimates ranging from 29 to 70%.

Valenzuela et al. illustrate the beauty and complexity of effects of a 14-week intradialytic combined exercise (aerobic + RT) protocol on patients' mental and health status. Their data suggest that intradialytic combined exercise benefits were observed for 6-min walk test, 10-repetition sit-to-stand performance time and handgrip strength. These results are of major clinical importance, as they suggest the benefits of exercise in dialytic patients, albeit with the assumption that these benefits represent the response of the majority of individuals to potentially reduce morbimortality risk in these patients.

Plyometric jump training (PJT) has received increasing attention in recent years as a modality to increase muscular strength, particularly in the legs. Ramirez-Campillo et al. present the physical fitness responses to work-matched soccer training vs. PJT performed using a novel training regimen, which allows improved individualization of training approaches.

Álvarez et al. reported improvements in obesity markers, metabolic risk factors, and endurance performance in both prehypertensive and normotensive groups. Also, these changes in blood pressure were accompanied by other acknowledged improvements of HIIT for body composition and metabolic and endurance performance, in both study cohorts. These authors suggest that to normalize high blood pressure and improve lipid profiles, an appropriate type of exercise training must be chosen. In addition, other non-pharmacological strategies are required to prevent hypertension.

Myokines and exercise-induced proteins/hormones are known to play important roles in a variety of physiological functions. In this context, He et al. compared the response following one session of HIIT or RT on the levels of several myokines/hormones involved in metabolic function, at baseline and 0, 1, 3, 24, 48, and 72 h post-exercise in 17 healthy, non-athletic men (23 ± 3 years). With the exception of fibroblast-growth factor-21, no overall differences were found in the myokine response to HIIT or RT. However, the authors observed considerable interindividual variability, with some subjects specifically responding to some but not other training session types.

Naves et al. studied and compare the effects of two different 8-week interval-training programs—SIT (23-min) and HIIT (33-min)—on anthropometric parameters and cardiorespiratory fitness in healthy young women, in 49 young active women with a mean age 30.4 ± 6.1 years. Their main findings were that both protocols led to improvements in anthropometric parameters and also cardiorespiratory fitness, even in the absence of changes to dietary intake. Moreover, greater reductions in the sum of skinfolds were seen after the SIT protocol as compared with HIIT.

With personalized medicine becoming increasingly widespread, Williams et al. compared, in a large sample of subjects ($n = 677$) from different laboratories (18 centers), the experiential rates of likely responders in an assortment of aerobic training interventions. Interestingly, the authors found that studies with the shortest duration and high-volume training loads often had the least significant gains and fewer clinically meaningful VO_2peak responders. Because, many of the benefits of exercise are closely associated with improvements in CRF, as determined by peak or maximal oxygen consumption (VO_2peak/VO_2max), the authors suggest that future large, well-controlled studies with comparator groups and cross-over designs may help to identify influential variables and the ideal training load for $\dot{V}O_2$peak trainability.

PERSPECTIVES

Papers in this Research Topic highlight the notion that personalized exercise is a feasible and effective lifestyle modification strategy, for all individuals with, or at risk of, non-communicable chronic diseases (DiMenna and Arad, 2018). Accordingly, more research is needed to compare the training paradigms in defined subgroups, for instance, at-risk subjects, and at different stages of disease join to the point. With many issues unresolved, further research is warranted before exercise can conscientiously be prescribed as "precision medicine" to address cardiometabolic risk factors and their progression to many non-communicable diseases.

AUTHOR CONTRIBUTIONS

RR-V and MI: drafted the manuscript. All authors approved the final version.

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ORIGINAL RESEARCH
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A Multi-Center Comparison of $\dot{V}O_{2peak}$ Trainability Between Interval Training and Moderate Intensity Continuous Training

*Camilla J. Williams[1], Brendon J. Gurd[2], Jacob T. Bonafiglia[2], Sarah Voisin[3], Zhixiu Li[4], Nicholas Harvey[5], Ilaria Croci[1,6], Jenna L. Taylor[1], Trishan Gajanand[1], Joyce S. Ramos[7], Robert G. Fassett[1], Jonathan P. Little[8], Monique E. Francois[8], Christopher M. Hearon Jr[9], Satyam Sarma[9], Sylvan L.J.E. Janssen[9,10], Emeline M. Van Craenenbroeck[11], Paul Beckers[11], Véronique A. Cornelissen[12], Nele Pattyn[12], Erin J. Howden[13], Shelley E. Keating[1], Anja Bye[6,14], Dorthe Stensvold[6], Ulrik Wisloff[1,6], Ioannis Papadimitriou[3], Xu Yan[3,15], David J. Bishop[3,16], Nir Eynon[3] and Jeff S. Coombes[1]**

[1] School of Human Movement and Nutrition Sciences, The University of Queensland, Brisbane, QLD, Australia, [2] School of Kinesiology and Health Studies, Queen's University, Kingston, ON, Canada, [3] Institute for Health and Sport (iHeS), Victoria University, Melbourne, VIC, Australia, [4] Translational Genomics Group, Institute of Health and Biomedical Innovation, Queensland University of Technology at Translational Research Institute, Princess Alexandra Hospital, Brisbane, QLD, Australia, [5] Faculty of Health Sciences and Medicine, Bond University, Robina, QLD, Australia, [6] K.G. Jebsen Center of Exercise in Medicine, Department of Circulation and Medical Imaging, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway, [7] SHAPE Research Centre, Exercise Science and Clinical Exercise Physiology, College of Nursing and Health Sciences, Flinders University, Adelaide, SA, Australia, [8] School of Health and Exercise Sciences, University of British Columbia, Kelowna, BC, Canada, [9] Internal Medicine, Institute for Exercise and Environmental Medicine, University of Texas Southwestern Medical Center, Dallas, TX, United States, [10] Department of Physiology, Radboud University Medical Center, Nijmegen, Netherlands, [11] Cardiology Department, Antwerp University Hospital, Antwerp, Belgium, [12] Department of Rehabilitation Sciences – Research Group for Rehabilitation in Internal Disorders, Catholic University of Leuven, Leuven, Belgium, [13] Baker Heart and Diabetes Institute, Melbourne, VIC, Australia, [14] St. Olavs Hospital, Trondheim, Norway, [15] Australian Institute for Musculoskeletal Science (AIMSS), Melbourne, VIC, Australia, [16] School of Medical and Health Sciences, Edith Cowan University, Joondalup, WA, Australia

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Edited by:
Mikel Izquierdo, Universidad Pública de Navarra, Spain

Reviewed by:
Thierry Busso, Université Jean Monnet, France
Laurent Bosquet, University of Poitiers, France

***Correspondence:**
Jeff S. Coombes
j.coombes@uq.edu.au

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There is heterogeneity in the observed $\dot{V}O_{2peak}$ response to similar exercise training, and different exercise approaches produce variable degrees of exercise response (trainability). The aim of this study was to combine data from different laboratories to compare $\dot{V}O_{2peak}$ trainability between various volumes of interval training and Moderate Intensity Continuous Training (MICT). For interval training, volumes were classified by the duration of total interval time. High-volume High Intensity Interval Training (HIIT) included studies that had participants complete more than 15 min of high intensity efforts per session. Low-volume HIIT/Sprint Interval Training (SIT) included studies using less than 15 min of high intensity efforts per session. In total, 677 participants across 18 aerobic exercise training interventions from eight different universities in five countries were included in the analysis. Participants had completed 3 weeks or more of either high-volume HIIT (n = 299), low-volume HIIT/SIT (n = 116), or MICT (n = 262) and were predominately men (n = 495) with a mix of healthy, elderly and clinical populations. Each training intervention improved mean $\dot{V}O_{2peak}$ at the group level ($P < 0.001$). After adjusting for covariates, high-volume HIIT had a significantly greater ($P < 0.05$) absolute $\dot{V}O_{2peak}$ increase (0.29 L/min) compared to MICT (0.20 L/min) and low-volume HIIT/SIT (0.18 L/min). Adjusted relative $\dot{V}O_{2peak}$ increase was also significantly greater ($P < 0.01$)

in high-volume HIIT (3.3 ml/kg/min) than MICT (2.4 ml/kg/min) and insignificantly greater (P = 0.09) than low-volume HIIT/SIT (2.5 mL/kg/min). Based on a high threshold for a likely response (technical error of measurement plus the minimal clinically important difference), high-volume HIIT had significantly more ($P < 0.01$) likely responders (31%) compared to low-volume HIIT/SIT (16%) and MICT (21%). Covariates such as age, sex, the individual study, population group, sessions per week, study duration and the average between pre and post $\dot{V}O_{2peak}$ explained only 17.3% of the variance in $\dot{V}O_{2peak}$ trainability. In conclusion, high-volume HIIT had more likely responders to improvements in $\dot{V}O_{2peak}$ compared to low-volume HIIT/SIT and MICT.

Keywords: cardiorespiratory fitness, $\dot{V}O_{2max}$, $\dot{V}O_{2peak}$, exercise training, response heterogeneity, trainability

INTRODUCTION

Health guidelines recommend aerobic exercise training for improving cardiorespiratory fitness (CRF) and reducing the risk of chronic disease and premature mortality (WHO, 2015; Ross et al., 2016). An increase of one metabolic equivalent (3.5 mL/kg/min) results in a 10–25% improvement in survival over an approximate 10-year follow-up (Blair et al., 1995; Gulati et al., 2003; Myers, 2003; Myers et al., 2011; Nes et al., 2014). There are various forms of aerobic exercise training that can be differentiated by their intensity and duration. Moderate Intensity Continuous Training (MICT) generally consists of 30–60 min of aerobic exercise at 64–76% peak heart rate (ACOS Medicine, 2017), while interval training involves more intense bouts interspersed by recovery periods (Weston et al., 2014). Interval training can be separated based on intensity into High-Intensity Interval Training (HIIT) or Sprint Interval Training (SIT). HIIT can be further defined by volume. Although classically associated with weekly loads in athletes, volume has gained acceptance to define the total duration of HIIT interval lengths (Boyd et al., 2013; Scribbans et al., 2014; Ramos et al., 2017; Eigendorf et al., 2018; Reljic et al., 2018). High-volume High-Intensity Interval Training (HIIT) typically includes repeated intervals of near maximal aerobic efforts for a specific period (e.g., 4 × 4-minute intervals at 90% peak heart rate), with a rest/recovery period in between (e.g., 3 min at 65% peak heart rate). Low-volume HIIT has fewer or shorter intervals (e.g., 6 × 1-minute intervals at 120% peak work rate) and SIT is defined as supramaximal exertion (e.g., 8 × 20-second intervals at 170% peak work rate) with active recovery/rest between intervals. Interval training has recently become popular because it is more time efficient (Phillips et al., 2017), and sometimes more enjoyable than MICT (Bartlett et al., 2011; Jung et al., 2014).

Meta-analyses have shown that high-volume HIIT is comparable, if not superior, to MICT for improving CRF ($\dot{V}O_{2max}$/$\dot{V}O_{2peak}$) and other health biomarkers (Gist et al., 2014; Weston et al., 2014; Milanovic et al., 2015; Ramos et al., 2015; Batacan et al., 2017). High-volume HIIT produces greater $\dot{V}O_{2max}$/$\dot{V}O_{2peak}$ changes than low-volume HIIT and SIT protocols at the group level (Bacon et al., 2013; Astorino and Schubert, 2014; Gist et al., 2014; Milanovic et al., 2015); where $\dot{V}O_{2max}$ is a maximal effort on graded exercise test with a plateau in oxygen consumption, and $\dot{V}O_{2peak}$ is a maximal effort on a graded exercise test without a plateau in oxygen consumption (Coombes and Skinner, 2014). However, there is heterogeneity in the observed CRF response to an exercise intervention (i.e., the "trainability" of an individual). Some individuals show large improvements in CRF (often described as "responders"), whereas others show little to no-improvements ("low-responders") following the same apparent exercise training stimulus (Bacon et al., 2013; Astorino and Schubert, 2014; Coombes and Skinner, 2014; Mann et al., 2014; Bouchard et al., 2015). Optimizing exercise training to improve CRF is imperative to long-term health; therefore, it is important to understand how factors such as the type of aerobic exercise intervention can influence observed rates of CRF trainability.

In the largest study to date on CRF trainability (The HERITAGE study; n = 742), $\dot{V}O_{2max}$ gain following 20 weeks of endurance training was 400 mL on average, with 7% of participants gaining 100 mL/min or less and 8% gaining 700 mL/min or more (Bouchard et al., 1999). However, this observed heterogeneity in $\dot{V}O_{2max}$ trainability may have resulted from technical error of measurement (TEM); a combination of random within-individual variation and/or measurement error (Atkinson and Batterham, 2015; Hecksteden et al., 2015, 2018; Hopkins, 2015; Williamson et al., 2017). Furthermore, the variability in training response should consider the minimal clinically important difference (MCID). Without considering the TEM and the MCID, identifying the probability of an individual's response is inconclusive and CRF trainability may be misclassified (Atkinson and Batterham, 2015). Despite this, many studies to-date have used zero-change or a proportion of a group to classify "adverse-responders", "non-responders", "low-responders" and "high responders" to CRF training (Scharhag-Rosenberger et al., 2012; Mann et al., 2014; Gurd et al., 2016). Using this terminology based on arbitrary indicators for response is problematic and has created much debate (Montero and Lundby, 2017; Hecksteden et al., 2018). Some investigators have proposed that the concept of "non-responders" is a myth and that simply increasing the training load converts the majority of "non-responders" to "responders" (Bacon et al., 2013; Joyner, 2017; Montero and Lundby, 2017). Training load considers both the intensity and duration of exercise (Banister et al., 1992). Recently, Howden et al. (2015, 2018) found that the number of non-responders is minimal if the intensity and duration of training is high enough. Individuals were able to generate a higher

stroke volume and cardiac output (Howden et al., 2015, 2018), both of which are imperative for increasing the ability of the heart to improve $\dot{V}$O2max (Levine, 2008).

The primary aim of this study was to utilize a large multi-center approach (n = 677 participants across 18 studies) to compare the number of likely responders between different training loads: high-volume HIIT, low-volume HIIT/SIT, and MICT interventions. In this study, we have taken into account the TEM and the MCID to categorize participants as either a "likely responder", "likely non-responder", "likely adverse responder" or "uncertain". The use of these categories provides information on the spread of participant responses relative to the MCID. Based on the literature to date, we hypothesized that high-volume HIIT will have more likely responders compared to MICT and low-volume HIIT/SIT.

MATERIALS AND METHODS

Participant Characteristics and Recruiting

This study includes the initial results of a larger study (PREDICT-HIIT) examining genetic predictors for $\dot{V}O_{2peak}$ trainability from HIIT/SIT and MICT interventions. Studies and potential participants were identified by contacts made through university affiliations (i.e., researchers involved in relevant studies). Studies were included if they met the following criteria: (1) participated in a HIIT, SIT, or MICT training study three or more weeks in duration within the last 15 years, (2) had an objective measure of $\dot{V}O_{2peak}$ (indirect calorimetry from a graded exercise test to volitional fatigue on a cycle ergometer or treadmill) before and after training, and (3) participant DNA collection was possible. Eligibility was open to male and female adults over the age of 18. Participants were included if they had greater than 80% attendance to the supervised protocol. Ethical approval was obtained from the various institutions and by the Bellberry ethical committee at the host institution (#2016-02-062-A-1).

High Intensity Interval Training (HIIT) and SIT were classified according to the intensity thresholds provided by (Weston et al., 2014). High-volume HIIT was further defined as $\geq$ 15 min of high-intensity efforts in total during the session and low-volume HIIT was defined as <15 min of high-intensity efforts in total during the session. SIT was classified as repeats of <1 min above maximal efforts (per bout). MICT was defined as 30 min or more of continuous exercise at 64–76% maximum heart rate (HR_{max}) or equivalent. For analysis purposes, low-volume HIIT and SIT studies were combined because their training loads were similar. Training loads were based on Edwards' training impulse (TRIMP); time in each training heart rate zone multiplied by the relative weighting factor of exercise intensity (Edwards, 1993).

Data Analysis

Normality and homoscedasticity for $\dot{V}O_{2peak}$ response were assessed using the Shapiro–Wilk and Levene's tests. Data are presented as mean ± standard deviation where appropriate. We used a paired sample t-test to calculate the group mean $\dot{V}O_{2peak}$ response for high-volume HIIT, low-volume HIIT/SIT and MICT. Effect sizes were based on Cohen's d. We used an analysis of covariance (ANCOVA) to compare adjusted relative $\dot{V}O_{2peak}$ responses between high-volume HIIT, low-volume HIIT/SIT and MICT. Values were adjusted for age, sex, individual study, duration of individual study, number of sessions each week, population group (e.g., coronary artery disease) and the average between pre and post-test scores (to avoid regression to the mean) (Barnett et al., 2015). The ANCOVA for absolute $\dot{V}O_{2peak}$ also included baseline weight as a covariate. Ethnicity was not a common identifier across studies and therefore was not included as a covariate. *Post hoc* testing used Tukey's least significance difference test. A regression analysis determined the contribution of the covariates to the $\dot{V}O_{2peak}$ response. We used an analysis of variance (ANOVA) to compare men and women group mean $\dot{V}O_{2peak}$ responses within each intervention, and to compare $\dot{V}O_{2peak}$ responses between population groups. Statistical analyses were completed using SPSS (version 23.0, SPSS Inc., Chicago, IL, United States).

The thresholds for response categories used a combination of the TEM and the MCID. Categories included "likely responder", "likely non-responder", "likely adverse responder" and "uncertain". **Table 1** shows the categories illustrated with an example. Combining the MCID and TEM for the threshold improves the confidence in the "likely responder"/"non responder" classifications (e.g., compared to 2 TEM threshold (Hecksteden et al., 2018)). The TEMs for each individual study were first estimated by multiplying the mean baseline $\dot{V}O_{2peak}$ value by a previously published coefficient of variation (CV) for $\dot{V}O_{2peak}$ of 5.6% (Katch et al., 1982). These were then averaged to obtain the TEM for each group that was used in the calculation to categorize individuals. The use of a CV of 5.6% has been suggested by others (Hecksteden et al., 2018) and is more conservative than what has been previously used (3.5%) (Edgett et al., 2018). It has been demonstrated that as little as a 1 mL/kg/min can be clinically important in individuals with coronary artery disease (Keteyian et al., 2008). Despite this, we used 3.5 mL/kg/min as the MCID based on evidence that it is associated with a 10–25% decreased risk of all-cause mortality in studies with an approximate 10-year

TABLE 1 | Criteria for the responder categories with examples.

Category	Criteria	Example if an intervention in a study had a TEM of 5 mL/kg/min and an MCID of 3.5 mL/kg/min
Likely responder	> 1 TEM above the + MCID	> 8.5 mL/kg/min
Likely non-responder	> 1 TEM below + MCID to < 1 TEM below the –MCID	–1.5 mL/kg/min to –8.5 mL/kg/min
Likely adverse responder	> 1 TEM below the -MCID	< 8.5 mL/kg/min
Uncertain	< 1 TEM above to < 1 TEM below + MCID	–1.5 mL/kg/min to 8.5 mL/kg/min

follow-up (Blair et al., 1995; Gulati et al., 2003; Myers et al., 2011; Nes et al., 2014). A likely responder was considered a ṾO2peak response of above one MCID plus the TEM. Individual TEMs were calculated for each study resulting in different thresholds. These individual TEMs were averaged to provide a threshold for each training intervention (high-volume HIIT, low-volume HIIT/SIT and MICT). A likely responder for the high-volume HIIT group was above 5.3 mL/kg/min, low-volume HIIT/SIT group was 5.2 mL/kg/min and MICT group was 5.0 mL/kg/min. A comparison of likely responders between interventions was calculated using Medcalc statistical software, based on the "n-1" Chi-squared test (MedCalc, 2018).

RESULTS

In total, 677 participants across 18 studies from eight different universities provided data for this analysis (**Table 2**). These came from the University of Queensland, Australia (n = 191), Antwerp University and the Catholic University of Leuven, Belgium (n = 180), the Norwegian University of Science and Technology, Norway (n = 126), The Gene SMART cohort (PMID: 29143594) at Victoria University, Australia (n = 59), Queens University, Canada (n = 55), the University of British Columbia, Canada (n = 38), and the University of Texas Southwestern Medical Center, United States (n = 28).

Participants were from various populations including those with coronary artery disease (n = 256), type-2 diabetes (n = 73), the metabolic syndrome (n = 76), as well as individuals who were active and healthy (n = 118) and individuals middle-aged or over 75 years (n = 154). We collated data from females (n = 182) and males (n = 495). The mean age was 56.3 ± 16.0 years.

Relative $\dot{V}O_{2peak}$

Table 3 and **Figure 1** provide the changes in unadjusted relative $\dot{V}O_{2peak}$. Group mean relative $\dot{V}O_{2peak}$ scores significantly increased after all intervention types, with small effect sizes after high-volume HIIT (3.4 mL/kg/min, 95% CI 3.0 to 3.9 mL/kg/min, $P < 0.001$) Cohen's d = 0.3; MICT (2.5 mL/kg/min, 95% CI 2.1 to 3.0 mL/kg/min, $P < 0.001$) Cohen's d = 0.3; and low-volume HIIT/SIT (2.0 mL/kg/min, 95% CI 1.5 to 2.5 mL/kg/min, $P < 0.001$) Cohen's d = 0.2. **Table 4** presents the adjusted group means. A significant group difference (P = 0.01) was found with ANCOVA for relative $\dot{V}O_{2peak}$ response between high-volume HIIT and MICT (0.84 mL/kg/min, 95% CI 0.2 to 1.5 mL/kg/min). There was no significant difference (P = 0.09) between high-volume HIIT and low-volume HIIT/SIT (0.78 mL/kg/min, 95% CI −0.13 to 1.67 mL/kg/min). There was no significant difference (P = 0.9) between MICT and low-volume HIIT/SIT (−0.06 mL/kg/min, 95% CI −0.89 to 1.02).

Absolute $\dot{V}O_{2peak}$

Absolute $\dot{V}O_{2peak}$ values were significantly increased after all intervention types (**Table 4**). There were small effect sizes after high-volume HIIT (0.27 L/min, 95% CI −0.33 to −0.23, $P < 0.001$) Cohen's d = 0.3; MICT (0.19 L/min, 95% CI −0.24 to −0.12, $P < 0.001$) Cohen's d = 0.2; and low-volume HIIT/SIT (0.16 L/min, 95% CI −0.22 to −0.10 to, $P < 0.001$) Cohen's d = 0.2. **Table 4** presents the adjusted group means. Small significant group differences were found with ANCOVA for the change in absolute $\dot{V}O_{2peak}$ between high-volume and low-volume HIIT/SIT (0.12 L/min, 95% CI 0.02 to 0.22 L/min, $P < 0.05$) and between high-volume HIIT and MICT (0.09 mL/kg/min, 95% CI 0.02 to 0.17 L/min, P = 0.01). There was no significant difference (P = 0.61) between MICT and low-volume HIIT/SIT (−0.02 L/min, 95% CI −0.13 to 0.08 L/min).

Different Populations

There was a significant difference between the increases in men and women's absolute $\dot{V}O_{2peak}$ values in high-volume HIIT. Men had a greater ($P < 0.01$) increase (0.49 L/min, 95% CI 0.26 to 0.38) compared to women (0.15 L/min, 95% CI 0.09 to 0.21). There were no other significant differences between men and women's relative and absolute $\dot{V}O_{2peak}$ responses to high-volume HIIT, low-volume HIIT/SIT or MICT.

Table 5 shows that when analyzed according to the population type, middle-aged and elderly participants had a significantly greater ($P < 0.001$) increase in relative $\dot{V}O_{2peak}$ with high-volume HIIT than MICT. Young and healthy participants responded significantly more favorably ($P < 0.05$) to MICT than low-volume HIIT/SIT and high-volume HIIT. Participants with coronary artery disease and middle-aged and elderly participants in the high-volume HIIT group had a $\dot{V}O_{2peak}$ response greater than the MCID. Those with coronary artery disease and young and healthy participants also had a $\dot{V}O_{2peak}$ response greater than the MCID with MICT. All other population groups and training interventions failed to reach the MCID.

Overall, the covariates examined (sex, age, individual study, study duration, sessions per week, population group and the average between pre and post-test scores) contributed to 17.3% of the change in relative $\dot{V}O_{2peak}$ ($P < 0.001$). Individual studies had the largest impact, explaining 13.5% of $\dot{V}O_{2peak}$ response ($P < 0.001$).

Categories of $\dot{V}O_{2peak}$ Responders

Table 3 and **Figure 2** outlines the thresholds and percentages of likely responders, likely non-responders, likely adverse responders and those uncertain (not classified as a likely responder or likely non-responder) for each training intervention, and the individual studies contributing to these training interventions. High-volume HIIT had significantly more likely responders (31%) compared to MICT (21%) and low-volume HIIT/SIT (16%), $P < 0.01$. There were comparable responders classified as uncertain (∼33%) across high-volume HIIT, low-volume HIIT/SIT and MICT. On average, high-volume HIIT had a greater training load (∼100 Arbitrary Units (AU)) compared to low-volume HIIT/SIT (∼33 AU) and MICT (∼75 AU).

Studies with short durations (3–4 weeks) had fewer likely responders (13% average) irrespective of whether the

TABLE 2 | Included studies for each intervention.

	High-volume HIIT	Low-volume HIIT/SIT	MICT
University of Queensland (UQ)	Study (Ramos et al., 2016): *n* = 25 people with metabolic syndrome, 16-wk study, 4 × 4 protocol (38 min total with 16 min high intensity −10 min warm up at 60–70% HR $_{peak}$, followed by 4x4 min at 85–90% HR$_{peak}$, 3-min recovery in between each set at 50–70% HR $_{peak}$), 3x/wk. **Training load per session: ¬97 AU** Study (Taylor et al., 2017): *n* = 37 people with CAD, 12-wk study, 4 × 4 protocol (38 min total with 16 min high intensity −10 min warm up at 11–13, followed by 4 × 4 min at 15–18 RPE,, 3-min recovery in between each set at 11–13), 3x/wk. **Training load per session: ¬97 AU**	Study (Ramos et al., 2016): *n* = 26 people with metabolic syndrome,16-wk study, 1 × 4 protocol (17 min total with 4 min high intensity -10 min warm up at 50–60% HR $_{peak}$, 1 × 4 min at 85–95% HR $_{peak}$, 3 min recovery in between each set at 50–70% HR $_{peak}$), 3x/wk. **Training load per session: ¬23.5 AU** Study (unpublished); *n* = 19 with Type-2 diabetes, 8-wk study, 1x4 protocol (26 min total with 4 min high intensity -3 min warm up and cool down at 50–60% HR $_{peak}$,1x4 min at 85–95% HR $_{peak}$) plus 8 × 1 min resistance exercises with 1min recovery between, RPE 17 + , 3x/wk. **Training load per session: ¬50 AU**	Study (Ramos et al., 2016): *n* = 25 people with metabolic syndrome, 16-wk study, 30 min at 60–70% HR $_{peak}$; 5x/wk. **Training load per session: ¬60 AU** Study (Taylor et al., 2017): *n* = 39 people with CAD, 12-wk study, 40 min at 11–13 RPE, 3x/week. **Training load per session ¬80** Study (unpublished): *n* = 20 people with Type-2 diabetes, 8-wk study, 22 min 30 s at 55–69% HR $_{peak}$, plus 30 min of moderate intensity resistance exercises × 2/wk (8 exercises, RPE 11–13, 2 sets of 10 repetitions). **Training load per session ¬84.5** AU AND 2x/wk of MICT alone (52.5 min). **Training load per session ¬45 AU**
Antwerp University/ Catholic University of Leuven	Study (Pattyn et al., 2016): *n* = 89 people with CAD, 12-wk data; 4 × 4 protocol (38 min total with 16 min high intensity −10 min warm up at 50–70% HR $_{peak}$, followed by 4 × 4 min at 85–95% HR$_{peak}$, 3-min recovery in between each set at 50–70% HR $_{peak}$), 3x/week, 3x/wk. **Training load per session: ¬97**	**N/A**	Study (Pattyn et al., 2016): *n* = 91 people with CAD, 12-week data, 47 min at 60–70% HR $_{peak}$, 3x/week. **Training load per session: ¬94 AU**
Norwegian University of Science and Technology (NTNU)	Study (Stensvold et al., 2015): *n* = 49 seniors (70 +), 12-mth data, 4 × 4 protocol (38 min total with 16 min high intensity −10 min warm up at 60–70% HR $_{peak}$, followed by 4 × 4 min at 85–90% HR$_{peak}$, 3-min recovery in between each set at 50–70% HR $_{peak}$), 3x/week. **Training load per session: ¬97 AU**	**N/A**	Study (Stensvold et al., 2015): *n* = 77 seniors, 12-month data, 50 min at 60–70% HR $_{peak}$, 2x/wk. **Training load per session: ¬100**
Victoria University (VU) the Gene SMART cohort.	Study (Yan et al., 2017): *n* = 59 active and healthy males, 4 wk-study, up to 45 min total with 16–28 min high intensity (5 min warm up at 60 W; 8–14 × 2-min intervals at LT power + 40–70% of change in WR peak and power at LT, 1-min recovery periods at 60 W), 3x/wk. **Training load per session: ¬90- 157 AU**	**N/A**	**N/A**
Queen's University	Study (unpublished): *n* = 12 active and healthy, 4-wk study, 4x4 protocol (38 min total with 16 min high intensity -10 min warm up at 70–75% HR $_{peak}$, followed by 4 × 4 min at 90–95% HR$_{peak}$, 3-min recovery in between each set at 70–75% HR $_{peak}$), 3x/week. **Training load per session: ¬97 AU**	Studies (Boyd et al., 2013; Ma et al., 2013; Scribbans et al., 2014; Bonafiglia et al., 2016, 2017): *n* = 31 healthy participants, 4–6-wk Tabata protocol, 4 min total and up to 2 min 40 s of high intensity = 8 × 20 s sprints at 170% WR$_{peak}$ with 10-s load-less cycling between, 4x/wk. **Training load per session: ¬20 AU** Study (Raleigh et al., 2016): *n* = 2 healthy participants, 3-wk study, up to 24 min total (8–12 × 1-min intervals at 100% WR $_{peak}$), 4x/wk, 1 min warm-up and recovery load-less cycling between repeats. **Training load: ¬59 AU**	Study (Preobrazenski et al., 2018): *n* = 10 active and healthy adults, 4-wk study, 30 min at 65% WR $_{peak}$, 4x/wk. **Training load per session: ¬60 AU**
University of British Columbia (UBC)	**N/A**	Study (Francois et al., 2017): *n* = 34 with Type-2 diabetes, 12-wk study, up to 25 min total exercise with up to 10 min high intensity in total (4–10 × 1 min bursts at 90% HR $_{max}$; 1 min recovery between, 3 min warm up and cool down), 3x/week. **Training load per session: ¬40** AU	**N/A**

(Continued)

TABLE 2 | Continued

	High-volume HIIT	Low-volume HIIT/SIT	MICT
		Study (Forbes et al., 2017): n = 4 healthy females, 3-wk study, 3 sessions each wk, up to 25 min in total, with 10 min high intensity. Session 1 = 30-sec all-out cycling sprints with 4 min recovery (progressing from 4 to 6 repeats). **Training load per session**: ~ **32.5 AU**. Session 2 = 6-s all-out sprints with 24 sec rest (progress from 10 to 20 repeats). **Training load per session: ~10–20 AU.** Session 3 = 1 min sprints and recovery (progressing from 8 to 10 repeats). High intensity intervals = 85% W_{max}. Recovery intervals = 15% W_{max}. **Training load per session: ~45 AU**	
University of Texas Southwestern Medical Center (UTSW)	Study (Howden et al., 2018): n = 28 sedentary middle-aged men and women, 2-y longitudinal study, 4 × 4 protocol* up to 2x/wk in first 6 mth and 1/wk maintenance. **Training load per session: ~97 AU**. Base training, 30–60 min, 2 x/wk. **Training load per session: ~60–120 AU.** MSS lactate training, 30 min, 1x/wk. **Training load per session: ~120–150 AU**. Recovery training, 30 min, 1x/wk. **Training load per session: ~60 AU.** Strength training 1–2 days/wk.	N/A	N/A
Total (n)	299	116	262

Numbers (n), percentage (%), heart rate (HR), work rate (WR), rating of perceived exertion (RPE), maximal steady state (MSS), Watts (W), lactate threshold (LT)coronary artery disease (CAD), second (s), minutes (min), week (wk), months (mth), year, (y), repetitions (reps), 4 × 4 protocol (38 min total with 16 min high intensity – 10 min warm up at 70–75% HR $_{peak}$, followed by 4 × 4 min above 95% HR_{peak}, 3-min recovery in between each set at 60–75% HR $_{peak}$). Tabata protocol = 8 × 20 s sprints at 170% WR_{peak} with 10-s load-less cycling between, 4x/wk. Training load = (time in each training zone multiplied by relative weighting of exercise intensity), arbitrary units (AU).*

intervention was high-volume HIIT, low-volume HIIT/SIT or MICT (Boyd et al., 2013; Ma et al., 2013; Scribbans et al., 2014; Bonafiglia et al., 2016, 2017; Raleigh et al., 2016; Yan et al., 2017). Participants from the shorter duration studies were younger (mean - 23.1 years) and had a higher $\dot{V}O_{2peak}$ (44.4 mL/kg/min) prior to the intervention (Stensvold et al., 2015; Howden et al., 2018). From the individual studies over 4 weeks in duration, the study (Howden et al., 2018) with the most likely responders (53%) had the greatest average training load per session (up to ∼ 150 AU), the longest-running intervention (2 years) and the most training sessions each week (up to five). Most studies had three training sessions per week with 28% of participants classified as a likely responder (Stensvold et al., 2015; Pattyn et al., 2016; Ramos et al., 2016; Forbes et al., 2017; Francois et al., 2017; Yan et al., 2017). One study had two sessions each week, with 13% of participants classified as a likely responder (Stensvold et al., 2015).

Studies with middle-aged (50–60 years) and elderly participants (70+ years) had significantly ($P < 0.001$) more likely responders with high-volume HIIT (44% average) compared to MICT (13% average) (Stensvold et al., 2015; Howden et al., 2018). Studies with coronary artery disease participants had comparable likely responders with high-volume HIIT (38% average) and MICT (27% average), $P = 0.06$ (Pattyn et al., 2016; Taylor et al., 2017). Those with metabolic syndrome and/or type-2 diabetes (Ramos et al., 2016; Francois et al., 2017) had significantly more likely responders with high-volume HIIT (34% average) compared to low-volume HIIT/SIT (17% average) and MICT (8% average), $P < 0.001$. Likely adverse responders were from studies with elderly participants; three participants came from a MICT intervention (Stensvold et al., 2015) and two participants came from a high-volume HIIT intervention (Stensvold et al., 2015). Likely adverse responders also included one participant with coronary artery disease from a high-volume HIIT intervention and two from a MICT intervention (Pattyn et al., 2016), and one participant with metabolic syndrome from a high-volume HIIT intervention and 1 from a MICT intervention (Ramos et al., 2016).

DISCUSSION

Establishing a dose (i.e., intensity, frequency and duration) of exercise training that improves the number of observed responders with a clinically meaningful improvement to exercise training may reduce the prevalence of chronic disease risk and all-cause mortality associated with a low cardiorespiratory fitness. However, much of the research-to-date has focused largely on group-mean changes, with minimal studies comparing interventions and clinically meaningful responses. Furthermore, many of these studies have been small in sample size (between 10 and 20 participants), with potentially large variations in participant baseline physical activity levels, and training responses that are statistically underpowered. With personalized medicine becoming increasingly widespread, the aim of this study was to compare, in a relatively large sample size (n = 677) from different laboratories, the observed rates of likely responders

TABLE 3 | Baseline and relative $\dot{V}O_{2peak}$ response for each individual study, as well as averages for all studies combined.

Training intervention	Total *n* (% F)	Age (years)	Baseline BMI (kg/m^2)	Pre-training $\dot{V}O_{2peak}$ (mL/kg/min)	Change (Δ) mLkg/min	Δ %	*P*-value (Cohen's *d*)	TEM (mL/kg/min)	Likely responder *n* (%)	Uncertain *n* (%)	Likely non-responder *n* (%)	Likely adverse responder *n* (%)
High-volume HIIT total	**299 (22)**	**54.3 ± 16.5**	**27.0 ± 4.4**	**31.4 ± 11.0**	**3.4 ± 4.2**	**10.8**	**< 0.001 (*d* = 0.3)**	**1.8**	**92 (31)**	**98 (33)**	**105 (35)**	**4 (1)**
UQ												
(1) 4 × 4 × 3/wk, 16 wk (Ramos et al., 2016)	25 (52)	57.4 ± 1.8	32.4 ± 7.2	24.8 ± 5.0	2.9 ± 5.8	11.7	0.02 (*d* = 0.5)	1.4	9 (36)	4 (16)	11 (44)	1 (4)
(2) 4 × 4 × 3/wk, 12 wk (Taylor et al., 2017)	37 (14)	66.0 ± 6.7	28.7 ± 3.7	27.3 ± 5.7	2.6 ± 4.0	9.5	< 0.001 (*d* = 0.4)	1.5	12 (32)	8 (22)	17 (46)	0
Antwerp/Leuven 4 × 4 × 3/wk, 12-wk (Pattyn et al., 2016):	89 (8)	58.3 ± 10.0	28.0 ± 4.4	23.3 ± 5.9	4.9 ± 4.0	21.0	< 0.001 (*d* = 0.7)	1.6	38 (43)	28 (32)	21 (24)	1 (1)
NTNU												
(1) 4 × 4 × 3/wk, 1 year (Stensvold et al., 2015)	49 (39)	71.7 ± 1.8	25.3 ± 3.2	31.8 ± 6.7	3.9 ± 4.3	12.3	< 0.001 (*d* = 0.6)	1.8	17 (35)	19 (39)	11 (22)	2 (4)
VU												
(1) 8–14 × 2min × 3/wk, 4 wk (Yan et al., 2017)	59 (0)	31.0 ± 8.2	25.2 ± 3.2	46.7 ± 7.1	0.1 ± 2.7	0.2	0.053	2.6	0	20 (34)	39 (68)	0
Queen's												
(1) 4 × 4 × 3/wk, 3wk	12 (50)	22.0 ± 2.2	25.4 ± 4.9	46.7 ± 8.6	2.5 ± 2.6	5.3	< 0.01 (*d* = 0.3)	2.6	1 (8)	7 (59)	4 (33)	0
UTS												
(1) 4 × 4 × 1/wk, base and recovery × 1–2/wk, MSS × 1/wk, strength × 2/wk, 2 years (Howden et al., 2018)	28 (54)	53.5 ± 4.8	25.6 ± 3.0	28.8 ± 5.0	5.6 ± 2.9	19.4	< 0.001 (*d* = 1.0)	1.6	15 (53)	10 (36)	3 (11)	0
Low-volume HIIT/SIT	**116 (43)**	**48.1 ± 18.1**	**30.3 ± 6.6**	**30.6 ± 12.8**	**2.0 ± 2.9**	**6.5**	**< 0.001 (*d* = 0.2)**	**1.7**	**18 (16)**	**37 (32)**	**61 (52)**	**0**
UQ												
(1) 1 × 4, × 3/wk, 16 wk (Ramos et al., 2016)	26 (35)	57.1 ± 7.4	31.0 ± 5.2	26.5 ± 6.3	2.3 ± 2.7	8.7	< 0.001 (*d* = 1.0)	1.5	3 (12)	10 (38)	13 (50)	0
(2) 1 × 4 + strength × 3/wk, 8 wk	19 (9)	59.5 ± 8.7	34.5 ± 6.1	21.8 ± 4.8	0.7 ± 3.1	1.8	0.4	1.2	3 (16)	1 (5)	15 (79)	0

(Continued)

TABLE 3 | Continued

Training intervention	Total *n* (% F)	Age (years)	Baseline BMI (kg/m^2)	Pre-training $\dot{V}O_{2peak}$ (mL/kg/min)	Change (Δ) mLkg/min	Δ %	*P*-value (Cohen's *d*)	TEM (mL/kg/min)	Likely responder *n* (%)	Uncertain *n* (%)	Likely non-responder *n* (%)	Likely adverse responder *n* (%)
Queens												
(1–5) 8 × 20-second sprints, 4x/wk (Boyd et al., 2013; Ma et al., 2013; Scribbans et al., 2014; Bonafiglia et al., 2016, 2017)	15 (40)	20.9 ± 1.0	24.8 ± 2.8	44.4 ± 7.1	0.6 ± 3.7	1.5	0.6	2.5	2 (13)	3 (20)	10 (67)	0
3wk	12 (0)	21.5 ± 3.7	24.4 ± 4.5	50.9 ± 9.0	3.8 ± 3.3	7.5	< 0.01 (*d* = 0.4)	2.9	3 (25)	7 (58)	2 (17)	0
4wk	4 (0)	22.0 ± 1.2	25.2 ± 1.5	47.3 ± 6.2	3.1 ± 2.7	6.6	0.1	2.8	1 (25)	3 (75)	0	0
6wk	2 (50)	21.5 ± 2.1	34.6 ± 2.5	35.0 ± 1.8	1.6 ± 1.7	4.6	0.4	1.9	0 (0)	1 (50)	1 (50)	0
(6) 8–12 × 1 min intervals × 4/wk, 3 wk (Raleigh et al., 2016)												
UBC												
(1) 4–10 × 1 min × 3/wk, 12 wk (Francois et al., 2017)	34 (69)	55.3 ± 13.6	33.4 ± 6.6	22.1 ± 7.3	2.2 ± 2.0	6.6	< 0.001 (*d* = 0.3)	1.2	4 (12)	8 (24)	22 (64)	0
(2) Up to 1 min intervals × 3/wk, 3 wk (Forbes et al., 2017)	4 (100)	21.5 ± 4.4	NA	40.1 ± 7.7	3.6 ± 3.0	8.9	0.1	2.2	1 (25)	2 (50)	1 (25)	0
MICT	**262 (26)**	**62.0 ± 12.1**	**27.6 ± 5.3**	**27.5 ± 8.1**	**2.5 ± 3.8**	**9.1**	**< 0.001 (*d* = 0.3)**	**1.5**	**55 (21)**	**89 (34)**	**110 (42)**	**8 (3)**
UQ												
(1) 30 min × 5/wk, 16 wk (Ramos et al., 2016)	25 (32)	54.5 ± 9.6	32.5 ± 6.0	27.5 ± 8.0	1.4 ± 5.6	5.1	0.2	1.5	4 (16)	5 (20)	13 (52)	3 (12)
(2) 40 min × 3/wk, 12 wk (Taylor et al., 2017)	39 (18)	65.3 ± 6.8	26.9 ± 2.3	27.4 ± 7.5	1.9 ± 4.0	6.9	< 0.01 (*d* = 0.2)	1.5	7 (18)	11 (28)	19 (49)	2 (5)
(3) 22.5 min + 30 min strength × 2/wk and 52.5 min × 2/wk, 8 wk	20 (8)	60.5 ± 7.0	30.6 ± 10.2	25.4 ± 6.6	0.2 ± 2.0	0.8	0.7	1.4	0	3 (15)	17 (85)	0
Antwerp/Leuven												
(1) 47 min × 3/wk, 12 wk (Pattyn et al., 2016)	91 (10)	57.9 ± 8.7	28.3 ± 4.3	22.7 ± 5.6	4.3 ± 3.25	18.9	< 0.001 (*d* = 0.7)	1.3	33 (36)	34 (38)	24 (27)	0
NTNU												
(1) 50 min × 2/wk, 12 mth (Stensvold et al., 2015)	77 (45)	72.5 ± 2.1	24.7 ± 2.9	31.1 ± 5.9	1.5 ± 3.4	4.8	< 0.001 (*d* = 0.2)	1.7	10 (13)	28 (36)	36 (47)	3 (4)
Queens												
(1) 30 min × 4/wk, 4wk	10 (0)	23.1 ± 5.3	25.9 ± 4.4	47.2 ± 5.7	4.0 ± 2.2	8.5	< 0.001 (*d* = 0.8)	2.6	2 (20)	7 (70)	1 (10)	0
Total	**677 (27)**	**56.3 ± 16.0**	**27.8 ± 5.3**	**29.7 ± 10.5**	**2.8 ± 3.9**	**9.4**	**< 0.001 (*d* = 0.3)**	**1.7**	**162 (24)**	**229 (34)**	**274 (40)**	**12 (2)**

**Values shown are mean ± standard deviation (SD). Number of participants (n), percentage (%), body mass index (BMI), $\dot{V}O_{2peak}$ (peak aerobic fitness), weeks (wk), minutes (min), technical error of measurement (TEM), female (F), maximal steady state (MSS). **The TEM for each individual study was different to the average TEM for each of the three training groups (high-volume HIIT, low-volume HIIT/SIT and MICT).*

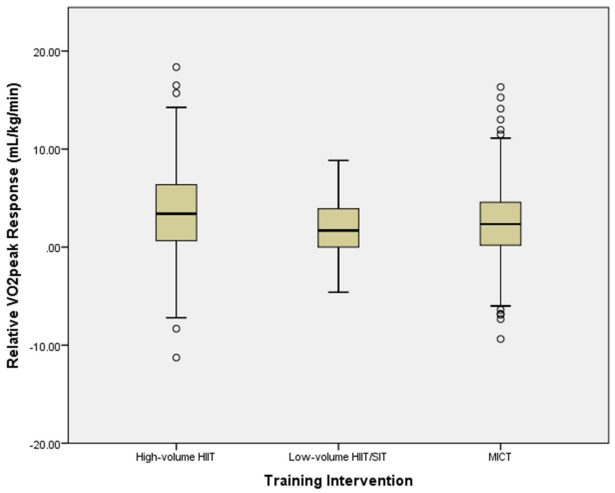

FIGURE 1 | Mean relative $\dot{V}O_{2peak}$ response following each training intervention (raw data). Boxes contain the median (horizontal line), 25th and 75th percentile (bottom and top of box, respectively), the minimum and maximum response (bottom and top of whiskers). Individual "outliers" are dots above and below whiskers.

TABLE 4 | Adjusted means for absolute and relative $\dot{V}O_{2peak}$ response.

Intervention	Relative mean $\dot{V}O_{2peak}$ increase*		Absolute $\dot{V}O_{2peak}$ increase**	
	mL/kg/min ± SD	95% CI	L/min ± SD	95% CI
High-volume HIIT	3.3 ± 3.7	2.9–3.7	0.29 ± 0.40	0.25–0.34
Low-volume HIIT	2.5 ± 4.1	1.7–3.3	0.18 ± 0.44	0.09–0.26
MICT	2.4 ± 3.8	2.0–2.9	0.20 ± 0.42	0.15–0.26

**Adjusted for sex, age, individual study, study duration, sessions per week, population group and the average between pre and post-test scores. **Adjusted for sex, age, individual study, study duration, sessions per week, population group, baseline weight, and the average between pre and post-test scores and the pre weight.*

between a variety of aerobic training interventions. Our study adds to the current literature by showing high-volume HIIT has significantly more likely responders compared to low-volume HIIT/SIT and MICT.

Meta-analyses have shown that high-volume HIIT is comparable, if not superior, to MICT for improving CRF ($\dot{V}O_{2max}$/$\dot{V}O_{2peak}$) and other health biomarkers (Gist et al., 2014; Weston et al., 2014; Milanovic et al., 2015; Ramos et al., 2015; Batacan et al., 2017). The group mean changes from this study were similar to previous research indicating that high-volume HIIT had a larger mean $\dot{V}O_{2peak}$ gain than MICT and low-volume HIIT/SIT and studies with the greatest $\dot{V}O_{2peak}$ gains used longer high intensity intervals/high-volume HIIT (Bacon et al., 2013). Despite these group mean changes, there was considerable heterogeneity in $\dot{V}O_{2peak}$ responses in each intervention (**Figure 3**). An approach to assess whether the inter-individual training response is true that has gained much support involves comparing the adjusted standard deviations between the training group and a control comparator group

TABLE 5 | $\dot{V}O_{2peak}$ response in different population groups.

Population	Relative $\dot{V}O_{2peak}$ increase (mL/kg/min ± SD)		
	High-volume HIIT	Low-volume HIIT/SIT	MICT
Coronary artery disease	4.19 ± 4.12	NA	3.59 ± 3.66
Type II diabetes and/or metabolic syndrome	2.73 ± 4.13	1.86 ± 4.07	0.95 ± 4.01
Middle-aged and elderly	4.50 ± 3.93^	NA	1.50 ± 3.36
Young and healthy	1.10 ± 3.11*	2.28 ± 3.53	4.02 ± 2.23

*Not assessed due to no participants (NA). Significantly different to MICT *P < 0.05, ^P < 0.001.*

(Atkinson and Batterham, 2015; Williamson et al., 2017). If the standard deviation of the training group is clinically significant and larger than the control group, it can be assumed that a true individual response has occurred. Because our data did not include a control comparator group, we felt this approach was not warranted. Nonetheless, when looking at the standard deviation changes of the adjusted group means, they were all greater than the MCID (3.5 mL/kg/min). Overall, the number of "responders" for each intervention was slightly lower in comparison to previous reports (Scharhag-Rosenberger et al., 2012; Gurd et al., 2016; Hecksteden et al., 2018). However, many studies to-date have based thresholds for response on a percentage of change or one TEM away from zero; a lower threshold will produce more "responders" (Scharhag-Rosenberger et al., 2012; Gurd et al., 2016; Hecksteden et al., 2018). Furthermore, these studies have predominantly examined healthy but sedentary populations, whereas our data had a mix of clinical and healthy populations (Timmons et al., 2010; Scharhag-Rosenberger et al., 2012; Gurd et al., 2016; Hecksteden et al., 2018). For example, our data demonstrated that participants with coronary artery disease and middle-aged and elderly participants in the high-volume HIIT group had a $\dot{V}O_{2peak}$ response greater than the MCID. Those with coronary artery disease and young and healthy participants also had a $\dot{V}O_{2peak}$ response greater than the MCID with MICT. All other population groups and training interventions failed to reach the MCID. Middle-aged adults and the elderly, participants with type II diabetes and/or metabolic syndrome had a greater proportion of responders with high-volume HIIT; whereas response rates between exercise training loads were similar in participants with coronary artery disease and those who were young and healthy. There were 4 participants from our data that were classified as "likely adverse responders"; rather than a true adverse response, these participants may have performed poorly on the testing day.

It has been argued that some people are "dose-sensitive" as opposed to a "non-responder" (Montero and Lundby, 2017; Williamson et al., 2017). If physiological systems are maximized, it seems possible that everyone can improve their $\dot{V}O_{2peak}$ (Bacon et al., 2013; Joyner, 2017; Montero and Lundby, 2017). A clinically meaningful $\dot{V}O_{2peak}$ response is unlikely if maximal stroke volume, oxygen transport and oxygen utilization does not improve (Sarzynski et al., 2017). Furthermore, exercise can alter

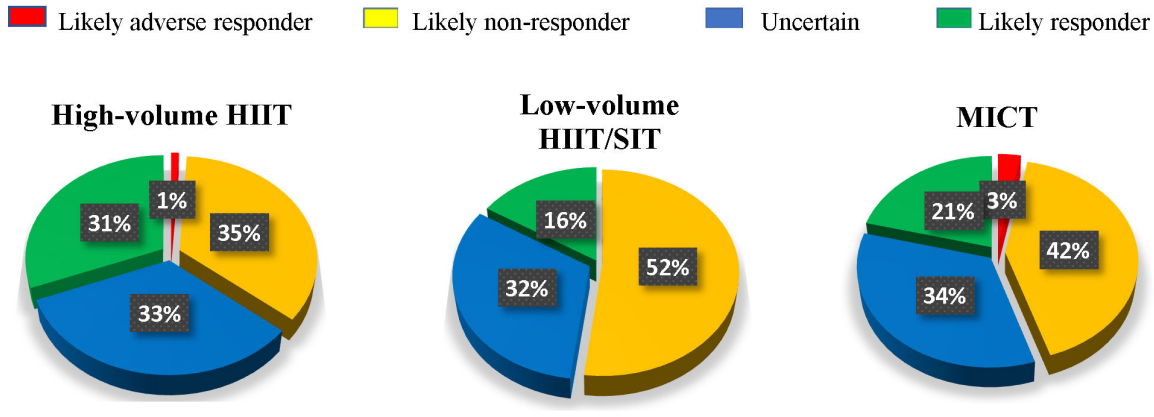

FIGURE 2 | Percentage of likely responders to changes in relative $\dot{V}O_{2peak}$ in each training intervention.

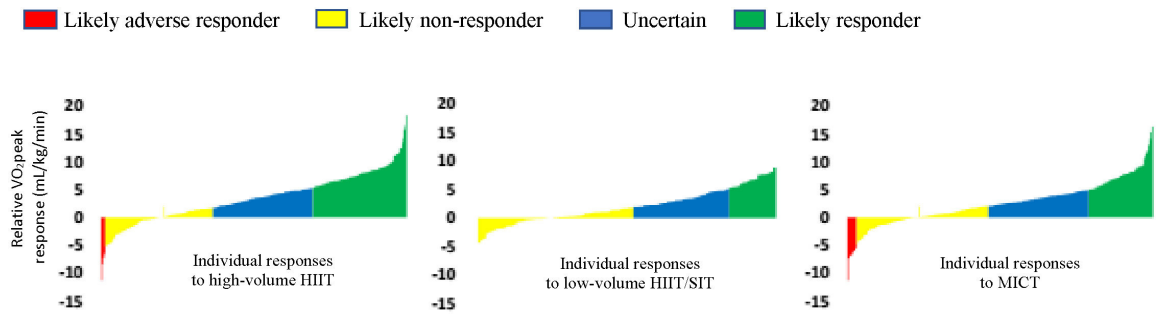

FIGURE 3 | Waterfall plots of the relative $\dot{V}O_{2peak}$ (mL/kg/min) response rates for each intervention (raw data).

the expression of genes related to mitochondrial function and energy use (Barres et al., 2012; Denham et al., 2015). Methylation can increase gene expression and affect metabolic adaptions in skeletal muscle (Barres et al., 2012). In skeletal muscle, most genes related to metabolism are demethylated following long-term exercise training (Voisin et al., 2014). These changes appear to be dose dependent and transient, with higher intensity exercise (80% heart rate maximum) causing greater demethylation and gene expression compared to lower-intensity exercise (40% heart rate maximum) where the total volume of exercise (caloric expenditure) is similar (Barres et al., 2012). To summarize, a higher training load may be more effective in those "dose sensitive" or those considered a "low responder" to training because participants are working at a threshold high enough to activate certain genes and molecular pathways required to induce a clinically meaningful exercise training response (Mann et al., 2014). Our analysis demonstrated that studies with the longest duration intervention and highest overall training loads produced the greatest $\dot{V}O_{2peak}$ gains and more likely responders (Stensvold et al., 2015; Pattyn et al., 2016; Howden et al., 2018). Typically these studies included high-volume HIIT. Our results complement previous research that indicates a greater training load correlates with fewer non-responders. For example, a recent study on 78 young males found that when training was increased from 60 to 180 min to 240–300 min per week, the number of responders (1 × technical error of measurement relative to zero) increased from 30–71 to 100%, respectively, (Montero and Lundby, 2017). It would be interesting to see if those who were deemed a "likely non-responder" from our analysis would "respond" with an increase in training duration, frequency or intensity.

Our analysis showed that age, sex, the individual study, study duration, number of sessions each week, the population group and the average between pre and post-test scores predicted 17.3% of the variance in training response; with the individual study being the highest predictor (13.5%) for $\dot{V}O_{2peak}$ response. This suggests there were other more substantial factors that affected $\dot{V}O_{2peak}$ trainability. In the HERITAGE study, 15% of the variation in the response to MICT was attributed to baseline $\dot{V}O_{2peak}$, age, sex, body mass and ethnicity combined; with approximately 6% attributed to workload fluctuations, 20% to technical error and daily changes, and up to 50% to genetic make-up (Sarzynski et al., 2017). A systematic review from our group identified 97 genetic variants that have been associated with $\dot{V}O_{2peak}$ trainability (Williams et al., 2017). It would be interesting to explore if those classified as a likely non-responder within our study have common genetic variants that may contribute to them being "dose sensitive". This will be investigated in our PREDICT-HIIT Study by combining the $\dot{V}O_{2peak}$ data presented here with genetic analyses.

Although there were several limitations to our study, the heterogeneity of the participants and training approaches should improve external ecological validity. Combining meaningful data from small, individual studies, as we have presented here, is necessary if we seek robust, reproducible, and translational results in exercise science (Eynon et al., 2017). Data was collated from 18 different studies with different protocols and equipment for testing. Participants were predominantly males, training status varied between studies (active vs. sedentary populations), and there was a mixture of clinical (CAD, diabetes, metabolic syndrome) and healthy populations. The age (between groups, 18–81 years), volume of work (60 min to 4 min and 50% heart rate peak to 170% work rate peak) and overall duration (3 to 104 weeks) varied considerably for the individual studies included in the current analysis. These factors are very likely to contribute to training response. Furthermore, some of the individual studies did not control for variables like diet, medication use, smoking status, sleep and recovery time. Lack of sleep or poor nutrition may negatively affect the intensity an individual can train and how fast they can recover between sessions; possibly combining to reduce training response through several interactions, such as genetic and epigenetic changes (Timmons, 2011; Voisin et al., 2014; Hecksteden et al., 2015; Paul et al., 2015; Yan et al., 2016). Our TEM was calculated using one that has been previously published (Katch et al., 1982). A more robust approach is to measure an individual's $\dot{V}O_{2peak}$ response in a test-retest study (Hopkins, 2000) or with a time-matched control group. This information was not collected for each individual intervention from our study. We also acknowledge that our research focuses on several select studies and represents a small portion of MICT, HIIT and SIT related literature. Finally, adherence to the exercise training prescription has been found to impact on studies comparing HIIT to MICT (Pattyn et al., 2016; Ellingsen et al., 2017). In these studies people allocated to the HIIT group did not meet the target exercise intensities and those in the MICT group trained at a higher intensity. In our analysis we have not taken this into account and used an intention to treat analysis approach with the belief that it would be more externally valid.

Future research with cross-over designs will determine if a participant may have a better response to an alternative intervention. Such a design is costly and seldom used but potentially decreases the random variation that may occur from comparing just one pre and post-test score, and measures how an individual will respond to different training interventions (Hecksteden et al., 2015). A recent cross over study (Bonafiglia et al., 2016) compared the number of responders to SIT with MICT. Participants (n = 21) had to complete four sessions a week of SIT or MICT (separated by a 3-month washout period). Both interventions produced similar group mean changes in $\dot{V}O_{2peak}$, and similar rates of response (based on 2 × TEM). Some individuals responded to MICT, but not to SIT and vice versa; whereas others did not improve their $\dot{V}O_{2peak}$ in either intervention (Bonafiglia et al., 2016). Thus, those who fail to have a clinically meaningful $\dot{V}O_{2peak}$ response to an exercise training approach within our study may benefit from another form of training.

In conclusion, high-volume HIIT had a greater average training load and significantly more likely responders compared to low-volume HIIT/SIT and MICT. Individual studies with the smallest duration and training loads generally had the least significant gains and fewer clinically meaningful $\dot{V}O_{2peak}$ responders. Future large, well-controlled studies with comparator groups and cross-over designs may help to identify influential variables and the ideal training load for $\dot{V}O_{2peak}$ trainability.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

CW, JC, and NE contributed to the conception and design of the study. CW organized the database and wrote the first draft of the manuscript. CW, SV, and ZL performed the statistical analysis. NH, IC, NH, JT, TG, JR, RF, JL, MF, BG, JB, CH, SS, SK, SJ, EVC, PB, VC, NP, EH, UW, AB, DS, DB, IP, and XY were investigators involved with the studies used in analysis and assisted with data collation. All authors contributed to manuscript revision, read, and approved the submitted version.

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The Association Between Differing Grip Strength Measures and Mortality and Cerebrovascular Event in Older Adults: National Health and Aging Trends Study

Daniel G. Whitney and Mark D. Peterson**

Department of Physical Medicine and Rehabilitation, University of Michigan, Ann Arbor, MI, United States

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****Correspondence:***
Daniel G. Whitney
dgwhit@med.umich.edu
Mark D. Peterson
mdpeterz@med.umich.edu

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The purpose of this study was to compare the predictive capacity of different post-processing methods of hand grip strength (GS) for mortality and incident cerebrovascular events in older adults. A sample of 4,143 participants aged 65 years and older was included from the National Health and Aging Trends Study (NHATS) and followed for 6 years. GS measures included baseline (i.e., round 1) (1) absolute GS, (2) GS divided by body mass (NGS_{mass}), and (3) GS divided by body mass index (NGS_{BMI}), as well as (4) change in absolute GS from round 1 to round 2 (GS_{1-2}). Cox proportional hazards regression models were used to examine the association between sex- and age group-specific tertiles of GS measures (weak, moderate-strength, strong) with mortality (n = 641) and incident cerebrovascular events (n = 329). Absolute GS (hazard ratio [HR] = 1.83; 95% confidence interval [CI] = 1.51–2.22), NGS_{mass} (HR = 1.46; 95% CI = 1.21–1.76), and NGS_{BMI} (HR = 1.50; 95% CI = 1.24–1.82) were each associated with mortality among weak participants, but not GS_{1-2} (HR = 1.10; 95% CI = 0.99–1.46). NGS_{mass} (HR = 1.54; 95% CI = 1.19–2.01) and NGS_{BMI} (HR = 1.37; 95% CI = 1.06–1.79) were both associated with incident cerebrovascular event among weak participants, but not absolute GS (HR = 1.12; 95% CI = 0.86–1.47) or GS_{1-2} (HR = 1.11; 95% CI = 0.85–1.44). Absolute GS, NGS_{mass}, and NGS_{BMI} were each associated with mortality, whereas only NGS_{mass} and NGS_{BMI} were associated with cerebrovascular event. These findings suggest that different post-processing methods of GS may have differing predictive capacity in the elderly depending on the outcome of interest; however, since NGS measures were associated with both mortality and cerebrovascular events, they may be considered advantageous for screening in older adults.

Keywords: grip strength, normalized grip strength, mortality, cerebrovascular event, elderly, National Health and Aging Trends Study

INTRODUCTION

Muscle strength capacity is a primary determinant of many functional aspects of daily living in older adults, including physical function, cardiometabolic health, and psychosocial wellbeing. The preservation of muscle strength with advancing age, through physical activity and exercise, is an important determinant of disease prevention and longevity (McGrath et al., 2018a). Hand grip strength (GS) is a reliable, inexpensive, and easily utilized surrogate of muscle strength (Peolsson et al., 2001; Savva et al., 2014), and has shown validity and reliability between different devices (Chkeir et al., 2012). Given that GS is highly associated with other measures of muscle strength (e.g., lower extremity strength capacity), it may be considered a valid proxy indicator of overall strength capacity (Cooper et al., 2013). Muscle weakness, as determined by GS assessment, is associated with increased risk of functional disabilities (McGrath et al., 2017b, 2018c), fracture (Dixon et al., 2005), cardiometabolic disease (Peterson et al., 2016a,b,d, 2017; McGrath et al., 2017c), musculoskeletal morbidities (Rikkonen et al., 2012; McGrath et al., 2017a), and early mortality (Leong et al., 2015; Peterson et al., 2016c; Oksuzyan et al., 2017; Celis-Morales et al., 2018). Moreover, statistical modeling of GS significantly improves prediction of morbidity and mortality beyond established office based risk scores (Celis-Morales et al., 2018), and is a stronger predictor of all-cause mortality than even systolic blood pressure (Leong et al., 2015).

Representatives from a variety of institutions participating in the Foundation for the National Institutes of Health (FNIH) Sarcopenia Project concluded that GS should be utilized to assess muscle weakness in the clinical setting (Studenski et al., 2014). There has been debate about the optimal methods for modeling GS in statistical prediction across health outcomes and populations. Most studies investigate absolute GS in an attempt to simplify the interpretation of findings in singular units; whereas we and others have preferred the use of normalizing GS (NGS) by incorporating body composition measures relative to GS (Lawman et al., 2016; Peterson et al., 2016a,b,c, 2017; McGrath et al., 2017a,b,c, 2018c). The FNIH Sarcopenia Project found that muscle weakness defined by GS normalized to body mass index was a stronger predictor of mobility impairment than absolute GS (McLean et al., 2014). Other post-processing techniques that are easily interpretable and computed in a clinical setting include normalizing GS to body mass (Peterson et al., 2016a,b) or assessing change in GS over time (Sirola et al., 2006; Karvonen-Gutierrez et al., 2018). Identifying which post-processing methods of GS is the strongest predictor of clinically important outcomes among older adults will provide clinicians better predictive options for evaluating muscle weakness in the elderly population. This has important implications for longitudinal monitoring or evaluating the efficacy of exercise interventions aimed at mitigating adverse health outcomes in the elderly. Accordingly, the purpose of this study was to determine which of the most common and easily utilized post-processing methods of GS (i.e., absolute GS, normalized GS, change in GS) was the strongest predictor of mortality and incident cerebrovascular events (myocardial infarction or stroke) in a sample of Medicare beneficiaries aged 65 and older.

MATERIALS AND METHODS

Participants

Data were from the National Health and Aging Trends Study (NHATS). NHATS utilized a multistage survey design, sampling >8,000 Medicare beneficiaries aged 65+ with an annual face-to-face interview conducted by trained study personnel. Non-Hispanic Blacks and those aged 90+ were oversampled. NHATS started in 2011 and subjects were assessed each year for a total of six rounds. Response rates were 71% at baseline. Additional information pertaining to NHATS study design, methodology, and survey instruments is available from https://www.nhats.org/. The NHATS study protocol was approved by The Johns Hopkins University Institutional Review Board.

Of the 8,245 participants at baseline, 4,102 participants were excluded from the analyses because they dropped out of the study, were unable to answer survey questions on their own, had dementia, or had incomplete data for baseline GS, baseline body mass, baseline height, round 2 GS, or survival. Survey weights were not applied because the purpose of this study was to compare the different post-processing methods of GS for mortality and incident cerebrovascular events. Therefore, the sample is the same for each of the outcome variables.

Outcome Variables

The participant's death was reported to the study personnel by informants during attempts to contact the participant for their annual interview. Since inclusion criteria required data for round 2 GS, survival time was computed as the annual rate for living and deceased participants from round 1 (alive) to rounds 3–6.

An incident cerebrovascular event was determined if participants reported myocardial infarction or stroke on the basis of an affirmative response to: "Please tell me if a doctor ever told you that you had [a heart attack or myocardial infarction/a stroke]?" Participants were excluded from analyses with cerebrovascular event as the outcome variable if they reported a cerebrovascular event at round 1. Myocardial infarction and stroke were combined into 1 category because of the low number of individuals who experienced either event that also met inclusion criteria.

Grip Strength Variables

The NHATS measured absolute GS (in kg) using a digital, adjustable hand dynamometer (Jamar Plus) in those that did not have surgery or flare up of pain in both hands or wrists, or have surgery in the arms or shoulders within the last 3 months. Participants were asked to squeeze the dynamometer as hard as they could with their arm at their side and elbow bent at 90 degrees. GS was measured twice and the highest value was used for this investigation. Height and body mass were self-reported. Body mass index (BMI) was calculated as follows:

body mass (kg)/height (m)^2. Four GS measures were computed: (1) baseline absolute GS; (2) baseline absolute GS divided by body mass (NGS_{mass}); (3) baseline absolute GS divided by BMI (NGS_{BMI}); and (4) percent change in GS from round 1 to round 2 (GS_{1-2}).

Demographic Variables

Age and sex were available for all participants. Age was categorized into the following groups: 65–74, 75–84, and 85+ years. Weight status was determined by BMI and separated into the following categories: underweight (<18.5 kg/m^2); normal weight (18.5–24.9 kg/m^2); overweight (25.0–29.9 kg/m^2); and obese (≥30.0 kg/m^2).

Statistical Analysis

Descriptive characteristics and GS measures were summarized as means ± SD or frequency (percentage). For each GS measure, sex- and age group-specific tertiles were created to categorize participants into the following muscle strength capacity groups: weak, moderate-strength, and strong. This method allowed for the comparative predictive assessment of GS for each of the outcomes of interest without introducing the confounding effects of age and sex. Unadjusted Cox proportional hazards regression models were used to examine the association between each transformed GS measure and mortality and incident cerebrovascular events. Participants were right censored at round 6 if they were alive (when modeling for mortality) or had no cerebrovascular events (when modeling for incident cerebrovascular events). Since GS measures were standardized using sex and age groups, the hazard ratios (HR) and 95% confidence intervals (CI) were examined to determine which sex- and age-adjusted GS measures were the strongest predictor of each outcome. All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, United States).

RESULTS

Descriptive characteristics and GSs of study participants in the entire sample (n = 4,143) and by sex (45.1% male) are presented in **Table 1**. Over the 6 rounds, 641 participants were reported to be deceased (15.5%). Of those without a reported cerebrovascular event at round 1 (n = 3,309), 329 had acquired a cerebrovascular event (9.9%).

Table 2 shows the results of the Cox regression models for the association between sex- and age-specific GS tertiles (reference: strong participants [highest tertile]) and mortality. For weak participants (lowest tertile), GS had the largest HR with mortality (HR = 1.83; 95% CI = 1.51–2.22), followed by NGS_{BMI} (HR = 1.50; 95% CI = 1.24–1.82) and NGS_{mass} (HR = 1.46; 95% CI = 1.21–1.76). GS_{1-2} was not significantly associated with mortality for weak participants (HR = 1.20; 95% CI = 0.99–1.46). For moderate-strength participants (middle tertile), only GS was significantly associated with mortality (HR = 1.42; 95% CI = 1.16–1.73).

TABLE 1 | Baseline descriptive characteristics and grip strength (GS) measures of the participants.

	Overall (n = 4,143)	Men (n = 1,869)	Women (n = 2,274)
Age, n (%)			
65–74	1,817 (43.9)	872 (46.7)	945 (41.6)
75–84	1,649 (39.8)	727 (38.9)	922 (40.5)
85+	677 (16.3)	270 (14.4)	407 (17.9)
Weight status, n (%)			
Underweight	76 (1.8)	19 (1.0)	57 (2.5)
Normal weight	1,366 (33.0)	553 (29.6)	813 (35.8)
Overweight	1,571 (37.9)	833 (44.6)	738 (32.4)
Obese	1,130 (27.3)	464 (24.8)	666 (29.3)
GS			
Mean ± SD	27.2 ± 10.5	35.0 ± 9.6	20.9 ± 5.9
NGS_{mass}, mean ± SD	0.35 ± 0.12	0.42 ± 0.12	0.30 ± 0.10
NGS_{BMI}, mean ± SD	1.02 ± 0.41	1.30 ± 0.39	0.79 ± 0.26
GS_{1-2}, mean ± SD	−0.51 ± 22.5	−0.81 ± 22.3	−0.26 ± 22.7
Deceased, n	641	330	311
Cerebrovascular event, n	329	156	173

NGS, normalized GS; NGS_{mass}, GS divided by body mass; NGS_{BMI}, GS divided by body mass index; GS_{1-2}, percent change of GS from round 1 to round 2 (1 year).

TABLE 2 | Cox proportional hazards regression for the association between sex- and age group-specific tertiles for grip strength (GS) measures (reference group: strong tertile) with mortality (n = 4,143) and cerebrovascular event (n = 3,309).

	Mortality	Cerebrovascular event
Exposure	**HR (95% CI)**	**HR (95% CI)**
GS		
Weak	**1.83 (1.51, 2.22)**	1.12 (0.86, 1.47)
Moderate-strength	**1.42 (1.16, 1.73)**	1.12 (0.87, 1.44)
NGS_{mass}		
Weak	**1.46 (1.21, 1.76)**	**1.54 (1.19, 2.01)**
Moderate-strength	1.03 (0.84, 1.26)	1.06 (0.81, 1.40)
NGS_{BMI}		
Weak	**1.50 (1.24, 1.82)**	**1.37 (1.06, 1.79)**
Moderate-strength	1.07 (0.87, 1.31)	1.05 (0.80, 1.37)
GS_{1-2}		
Weak	1.20 (0.99, 1.46)	1.11 (0.85, 1.44)
Moderate-strength	1.14 (0.94, 1.38)	1.03 (0.79, 1.34)

HR, hazard ratio; CI, confidence interval; NGS, normalized grip strength; NGS_{mass}, absolute grip strength divided by body mass; NGS_{BMI}, absolute grip strength divided by body mass index; GS_{1-2}, percent change of grip strength from round 1 to round 2. Significant HRs are bolded.

Table 2 shows the results of the Cox regression models for the association between sex- and age-specific GS tertiles (reference: strong participants) and incident cerebrovascular events. For weak participants, NGS_{mass} had the largest HR with cerebrovascular event (HR = 1.54; 95% CI = 1.19–2.01), followed by NGS_{BMI} (HR = 1.37; 95% CI = 1.06–1.79). GS (HR = 1.12; 95% CI = 0.86–1.47) and GS_{1-2} (HR = 1.11; 95% CI = 0.85–1.44) were not significantly associated with a cerebrovascular event. For moderate-strength participants, none of the predictors were

significantly associated with a cerebrovascular event (HR = 1.03–1.12; all $p > 0.05$).

DISCUSSION

The primary findings of this study were that both GS and NGS measures were significant predictors of mortality in older adults, and that NGS measures were significant predictors of incident cerebrovascular events. For predicting mortality, GS had a higher HR than NGS or change in GS; whereas NGS measures were stronger predictors of cerebrovascular events than GS or changes in GS. These findings suggest that different post-processing methods of GS may have differing predictive capacities in the elderly depending on the outcome of interest; however, since NGS was robustly associated with both mortality and cerebrovascular events, it may be considered as a viable standalone tool for screening in older adults. Moreover, considering the well-established role of exercise and physical activity on muscle strength, body composition, and mitigating adverse health outcomes, NGS may serve as a better proxy than absolute GS for determining efficacy of exercise interventions because it encompasses both muscle strength and body composition.

The findings that absolute and NGS measures were associated with mortality and cerebrovascular event are consistent with previous reports (Leong et al., 2015; Peterson et al., 2016c; Oksuzyan et al., 2017; Celis-Morales et al., 2018). The finding that change in GS was not associated with adverse health outcomes is consistent with another report (Karvonen-Gutierrez et al., 2018). While it seems intuitive that a higher rate of strength decline would correspond to a higher rate of acquiring adverse health outcomes, our methodology was limited in adequately addressing this notion. In the current investigation, we used a time interval of 1 year to assess strength change, which may not have been long enough to capture greater strength declines across advancing age. Moreover, our sample included adults 65 years and older. The rate of strength decline from young- or middle-age may be more predictive of later functioning and health outcomes in the elder years.

The difference in associations between absolute GS versus NGS measures with mortality and cerebrovascular events may reflect the influence of body composition or the role of obesity. Myint et al. (2014) found that measures of body composition (BMI, body fat percent, and waist-to-hip ratio) were stronger predictors of incident cardiovascular disease than mortality in middle- and older-age adults. Therefore, by incorporating body mass or BMI, NGS may be a superior predictor for cerebrovascular events than for mortality, as it encompasses important constituents (i.e., body composition) for cerebrovascular function.

Another potential explanation for the unique associations found for absolute GS vs. NGS measures is the so-called "obesity paradox," where there is lower mortality in those with cardiovascular disease who are obese compared to non-obese (Curtis et al., 2005; Angeras et al., 2013; Flegal et al., 2013), but not in those who are morbidly obese (Angeras et al., 2013). In the publically available NHATS dataset, mortality information is denoted as "deceased" or not, thus providing a "catch-all" cause of mortality. Further, in the current investigation, all participants who reported a cerebrovascular event were alive in the same round. Therefore, since absolute GS is a general measure of muscle strength capacity, it may capture the wider and non-specific construct of all-cause mortality and reflect the obesity paradox, i.e., those with greater BMIs may have greater absolute GSs (Lawman et al., 2016). On the other hand, GS normalized to body composition may be more specific to cardiometabolic-related morbidity and mortality, i.e., those with greater body masses or BMIs relative to GS may reflect poor cerebrovascular and metabolic health profiles (Lawman et al., 2016). Unfortunately, we were unable to determine how the different post-processing methods of GS were associated with specific causes of mortality.

The association between muscle weakness and adverse health outcomes is likely driven in part by poor physical functioning (McGrath et al., 2018b). While muscle weakness is inversely associated with physical functioning (Henriksen et al., 2012; Ryder et al., 2013), normalized strength is more strongly associated with physical functioning than absolute strength (Schiller et al., 2000; Henriksen et al., 2012). The caveat in examining absolute GS is that individuals with a high body mass or BMI likely have a higher GS relative to their physical functioning ability. Therefore, normalized strength capacity may provide a better indicator of the ability for that individual to maneuver his/her body through space and perform physical activities. Interestingly, when we examined the strength profiles of the obese participants who were classified as "strong" according to absolute GS (upper GS tertile), nearly 80% were considered "moderate-strength" or "weak" according to NGS measures. These findings highlight the potential benefits of using NGS in evaluating muscle strength capacity in the elderly.

There are other limitations that need to be discussed. First, height and weight were self-reported, which may have influenced measures adjusting for body composition. Stommel and Schoenborn (2009) found that misclassification of weight status by self-report height and weight to determine BMI was more pronounced on the extreme ends, including underweight and obese. However, deviations of BMI values were modest, with the majority (56%) of misclassifications having self-reported BMI values within one-unit interval of their measured BMI. Second, we did not adjust models for sociodemographics, socioeconomics, or morbidities. Whether the difference in associations among the GSs with outcomes are mediated uniquely by confounding variables is unknown and requires future investigation.

CONCLUSION

In conclusion, NGS measures were significantly associated with both mortality and incident cerebrovascular event, whereas absolute GS was only significantly associated with mortality. Changes in GS were not significantly associated with mortality or incident cerebrovascular event; however, the lack of association

may have been due to a short follow up period. These findings are important as they provide evidence of unique associations between clinically important aging outcomes with a variety of commonly used post-processing methods of GS that can be easily utilized in a clinical setting.

AUTHOR CONTRIBUTIONS

DW and MP designed the study and approved the final manuscript. DW contributed to data ascertainment, data analysis, and prepared the manuscript.

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Effects of High-Intensity Interval Training vs. Sprint Interval Training on Anthropometric Measures and Cardiorespiratory Fitness in Healthy Young Women

*João Pedro A. Naves[1], Ricardo B. Viana[1], Ana Cristina S. Rebelo[2], Claudio Andre B. de Lira[1], Gustavo D. Pimentel[3], Patrícia Cristina B. Lobo[3], Jordana C. de Oliveira[2], Rodrigo Ramirez-Campillo[4] and Paulo Gentil[1]**

[1] Department of Physical Education, Faculty of Physical Education and Dance, Federal University of Goiás, Goiânia, Brazil, [2] Department of Morphology, Biological Sciences Institute, Federal University of Goiás, Goiânia, Brazil, [3] Clinical and Sports Nutrition Research Laboratory, Nutrition Faculty, Federal University of Goiás, Goiânia, Brazil, [4] Laboratory of Measurement and Assessment in Sport, Department of Physical Activity Sciences, Research Nucleus in Health, Physical Activity and Sport, Universidad de Los Lagos, Osorno, Chile

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***Correspondence:**
Paulo Gentil
paulogentil@hotmail.com

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Purpose: To compare the effects of 8 weeks of two types of interval training, Sprint Interval Training (SIT) and High-Intensity Interval Training (HIIT), on anthropometric measures and cardiorespiratory fitness in healthy young women.

Methods: A randomized clinical trial in which 49 young active women [age, 30.4 ± 6.1 years; body mass index, 24.8 ± 3.1 $kg.m^{-2}$; peak oxygen consumption (VO_2peak), 34.9±7.5 $mL.kg^{-1}.min^{-1}$] were randomly allocated into a SIT or HIIT group. The SIT group performed four bouts of 30 s *all-out* cycling efforts interspersed with 4 min of recovery (passive or light cycling with no load). The HIIT group performed four bouts of 4-min efforts at 90–95% of peak heart rate (HRpeak) interspersed with 3 min of active recovery at 50–60% of HRpeak. At baseline and after 8 weeks of intervention, waist circumference, skinfolds (triceps, subscapular, suprailiac, abdominal, and thigh), body mass and BMI were measured by standard procedures and cardiorespiratory fitness was assessed by cardiorespiratory graded exertion test on an electromagnetically braked cycle ergometer.

Results: The HIIT and SIT groups improved, respectively, 14.5 ± 22.9% ($P < 0.001$) and 16.9 ± 23.4% ($P < 0.001$) in VO_2peak after intervention, with no significant difference between groups. Sum of skinfolds reduced 15.8 ± 7.9 and 22.2 ± 6.4 from baseline ($P < 0.001$) for HIIT and SIT groups, respectively, with greater reduction for SIT compared to HIIT ($P < 0.05$). There were statistically significant decreases in waist circumference ($P < 0.001$) for the HIIT (−3.1 ± 1.1%) and SIT (−3.3 ± 1.8%) groups, with no significant difference between groups. Only SIT showed significant reductions in body weight and BMI ($p < 0.05$).

Conclusions: Eight weeks of HIIT and SIT resulted in improvements in anthropometric measures and cardiorespiratory fitness, even in the absence of changes in dietary intake.

In addition, the SIT protocol induced greater reductions than the HIIT protocol in the sum of skinfolds. Both protocols appear to be time-efficient interventions, since the HIIT and SIT protocols took 33 and 23 min (16 and 2 min of effective training) per session, respectively.

Keywords: interval training, exercise, physical fitness, weight loss, cardiorespiratory fitness

INTRODUCTION

Interval training (IT) has been used for many decades with different purposes, such as improvements to health parameters (Wisløff et al., 2009; Kemi and Wisløff, 2010; Weston et al., 2013), performance (McMillan et al., 2005; Gibala and McGee, 2008; Gibala and Jones, 2013), and weight loss (Trapp et al., 2008; Boutcher, 2011). Typically, IT implicates alternating periods of relatively intense exercise with periods of lower-intensity effort or complete rest for recovery (Gibala et al., 2014). Two of the most common forms of IT are high-intensity interval training (HIIT) and sprint interval training (SIT) (Gibala et al., 2014). The target intensity during HIIT is usually "near maximal" or between 80 and 100% of maximal heart rate (HRmax) or maximum oxygen consumption (VO_2max), while SIT protocols usually involve "*all-out*" efforts (Buchheit and Laursen, 2013).

Regarding the applications for weight loss, a review found that fat loss after IT was greater than that after moderate-interval continuous training (MICT) (60–80% of HRmax) (Boutcher, 2011). Moreover, studies on the effects of IT on post exercise energy expenditure and fat oxidation (Treuth et al., 1996; Laforgia et al., 1997; Greer et al., 2015) and weight loss (Tremblay and Bouchard, 1994; Trapp et al., 2008; Burgos et al., 2017) suggest that IT is more efficient than continuous models, including MICT (Zhang et al., 2017). In fact, weight loss seems to be higher, even if the caloric expenditure obtained with IT is lower than (Tremblay and Bouchard, 1994) or equal to that of MICT (Trapp et al., 2008). These results can be attributed to the effects of IT on metabolism, promoting increased resting energy expenditure and fat utilization (Kiens and Richter, 1998; Knab et al., 2011; Kelly et al., 2013). Moreover, it seems that fat loss is greater at higher exercise intensities (Tremblay and Bouchard, 1994). However, we were not able to find any study in relation to the effects of SIT vs. HIIT on body composition in healthy young women.

Considering the meaningful differences between IT variations (Viana et al., 2018), it is important to analyze each protocol in detail to get further insight on how variations would be more suitable for a specific purpose in a given population. Two of the most popular types of interval training protocol are those presented by the Wisløff group (Wisløff et al., 2007) and the Gibala group (Gibala et al., 2006), which can be classified as HIIT and SIT, respectively. Such protocols have gained notoriety for inducing cardiovascular and performance adaptations equal to or greater than those induced by MICT despite the lower volume of exercise. However, despite their popularity, the effects of these protocols on markers of body fatness need more clarification, and we are not aware of any comparison between them. Thus, the aim of the present study was to compare the effects of two types of IT (HIIT and SIT) on anthropometric measures and cardiorespiratory fitness in healthy young women.

MATERIALS AND METHODS

Study Design

The participants performed a HIIT or SIT protocol on a mechanically braked cycle ergometer (Evolution SR, Schwinn, USA) three times per week (Monday, Wednesday, and Friday) for 8 weeks. One week before and 1 week after the intervention, anthropometric evaluation, and a cardiorespiratory graded exertion test (GXT) on a cycle ergometer were performed. The volunteers were asked to not perform any other exercise activity apart from the study protocol. The HIIT and SIT sessions lasted 33 and 23 min, respectively. Due to the nature of the interventions, it was not possible to blind the participants and supervisors involved in the study. However, all assessments were completed by blinded technicians. When a participant missed fewer than three training sessions non-consecutively, the sessions were replaced at the end of the period, but when three or more sessions were missed, the participant was excluded from the study.

Participants were advised to maintain their usual diet. Six 24-h dietary recalls were performed at the beginning and end of the intervention.

Participants

Forty-nine healthy women (**Table 1**) were recruited through advertisements on social media and through word of mouth. The following inclusion criteria were adopted: (i) body mass index (BMI) between 18.5 and 29.9 $kg.m^{-2}$, (ii) physically active ($\geq$150 min per week), (iii) pre-menopause, and (iv) not using stimulants (e.g., caffeine, energy drinks, or thermogenic drugs). Exclusion criteria were (i) contraindications to physical activity assessed through the *Physical Activity Readiness Questionnaire*—PAR-Q (Canadian Society for Exercise Physiology, 2002) and (ii) any history of interventions for body mass loss (surgical or hormonal treatment). **Figure 1** shows the flow diagram with all reasons for participants' exclusion and abandonment of the intervention.

All participants were informed of the potential risks and benefits of the study and signed an informed consent form. All experimental procedures were approved by the University Ethics Committee (Approval number: 1.542.353). The study conformed to the principles outlined in the Declaration of Helsinki.

TABLE 1 | Anthropometric and physiological characteristics of participants before and after 8 weeks of exercise training.

	HIIT ($n = 25$)				SIT ($n = 24$)				Between groups (Pre)	Between groups (Pre-Post)
	Pre	Post	ES	*P*	Pre	Post	ES	*P*	*P*	*P*
Age (years)	31.0 ± 6.0	–	–	–	29.8 ± 6.4	–	–	–	0.823	–
Height (m)	1.63 ± 0.05	–	–	–	1.64 ± 0.05	–	–	–	0.814	–
HRpeak (beats/min)	182 ± 10	–	–	–	179 ± 13	–	–	–	0.021	–
Body mass (kg)	66.3 ± 10.2	65.9 ± 9.9	$-0.039_{(trivial)}$	0.280	67.8 ± 8.1	67.0 ± 8.1	$-0.098_{(trivial)}$	0.015	0.156	0.360
Body mass index ($kg.m^{-2}$)	24.5 ± 3.3	24.4 ± 3.2	$-0.030_{(trivial)}$	0.402	25.2 ± 3.2	24.9 ± 3.3	$-0.092_{(trivial)}$	0.019	0.950	0.293
Skinfolds (mm)										
Triceps	22.0 ± 6.3	18.8 ± 5.2	$-0.553_{(medium)}$	< 0.001	27.6 ± 7.7	22.8 ± 5.6	$-0.712_{(medium)}$	< 0.001	0.213	0.909
Subscapular	21.2 ± 9.9	17.3 ± 7.3	$-0.448_{(small)}$	< 0.001	27.4 ± 8.6	20.3 ± 5.8	$-0.967_{(large)}$	< 0.001	0.393	0.074
Suprailiac	21.0 ± 11.2	17.4 ± 8.8	$-0.357_{(small)}$	< 0.001	30.4 ± 8.3	23.2 ± 5.3	$-1.033_{(large)}$	< 0.001	0.084	0.374
Abdominal	25.0 ± 11.2	19.7 ± 8.5	$-0.533_{(medium)}$	< 0.001	35.5 ± 6.9	25.8 ± 4.7	$-1.643_{(large)}$	< 0.001	0.029	0.111
Thigh	32.7 ± 8.6	28.2 ± 7.7	$-0.551_{(medium)}$	< 0.001	38.3 ± 7.9	30.7 ± 6.8	$-1.031_{(large)}$	< 0.001	0.883	0.020
∑skinfolds (mm)	121.9 ± 43.8	101.4 ± 34.6	$-0.519_{(medium)}$	< 0.001	159.1 ± 35.1	122.8 ± 24.8	$-1.194_{(large)}$	< 0.001	0.310	0.045
Waist circumference (cm)	74.6 ± 8.0	72.3 ± 7.8	$-0.291_{(small)}$	< 0.001	77.6 ± 7.0	75.1 ± 6.8	$-0.362_{(small)}$	< 0.001	0.483	0.739
VO_2peak ($mL.kg^{-1}.min^{-1}$)	37.7 ± 7.2	42.1 ± 5.5	$0.686_{(medium)}$	< 0.001	32.0 ± 7.2	36.5 ± 6.7	$0.647_{(medium)}$	< 0.001	0.860	0.097
iVO_2peak (watts)	159 ± 31	167 ± 27	$0.275_{(small)}$	0.028	138 ± 26	149 ± 20	$0.474_{(small)}$	0.003	0.303	0.402

Data are expressed as means ± standard deviation. p (values) for within-group (time) effect and interaction (time × group) effect. ES, effect size; HIIT, high-intensity interval training; SIT, sprint interval training; HRpeak, peak heart rate; ∑Skinfolds, sum of five skinfolds; VO_2peak, peak oxygen uptake; iVO_2peak, intensity associated to peak oxygen uptake.

Interval Training Intervention

During the study period, the participants were requested to avoid any form of physical activity besides the study protocols. The research team constantly monitored and questioned the participants to verify if they complied with the recommendations and to record any adverse event (dizziness, nausea, muscle soreness…). The intervention lasted 8 weeks, with three sessions per week (Monday, Wednesday, and Friday).

The SIT group performed a warm-up of 5 min at light load and self-selected speed, followed by four 30-s all-out bouts interspersed with 4 min of recovery (passive or light cycling with no load). If necessary, the load was adjusted to allow the participant to maintain cycling cadence ≥ 60 rpm.

The HIIT protocol consisted of a warm-up of 5 min at 50% of peak heart rate (HRpeak) (FT1, Polar, Finland), followed by four bouts of 4-min efforts at 90–95% of HRpeak interspersed with 3 min of active recovery at 50–60% of HRpeak. The load was adjusted when the HR deviated from the established zone. During recovery, the cadence was self-selected and the load was reduced to the minimum by one of the researchers. All training sessions for both groups were directly supervised by professionals experienced with the training prescription in a ratio of one supervisor per volunteer. During both protocols, standardized verbal stimuli were offered.

Outcomes Measures

Cardiorespiratory Graded Exertion Test

Participants performed a GXT on an electromagnetically braked cycle ergometer (CG04, Inbramed, Brazil) to determine their peak oxygen consumption (VO_2peak), intensity associated with VO_2peak (iVO_2peak), and HRpeak. Testing was performed 3–7 days before and after the training period. Briefly, following a 2-min warm-up at 50 W, the load was increased by 25 W every minute until volitional exhaustion, defined as the point at which the participant was not able to sustain a cadence ≥50 rpm. Participants wore a mouthpiece and nose clip, and gas was collected breath by breath by a specific pneumotach connected to the analyzer. VO_2 and carbon dioxide production (VCO_2) were analyzed by a metabolic gas collection system (VO2000, MedGraphics, USA) every 10 s. After exhaustion, the load was reduced to 50 W for 2 min of recovery. The highest VO_2 measured at the cessation of exercise was called VO_2peak because no participants reached the criteria for VO_2max (Howley, 2007). To identify iVO_2peak, the highest workload was considered. HR was constantly monitored throughout the test using a HR monitor (Polar RS800, Kempele, Finland). The rating of perceived exertion (RPE) was assessed every minute using the 6–20 Borg scale (Borg, 1982).

Anthropometric Measures

Each participant's height and body mass were measured to the nearest 0.1 cm and 0.1 kg, respectively. All anthropometric measurements (3–7 days before and after the training period) were carried out at the same phase of the menstrual cycle (follicular phase) and by the same examiner (to avoid inter-examiner variability), who was previously trained and experienced in these types of measurements and was blinded to group allocation. BMI was calculated from these data. Waist circumference was measured at the level of the smallest circumference above the umbilicus and below the xiphoid appendix (American College of Sports Medicine, 2011).

Five subcutaneous skinfolds (triceps, subscapular, suprailiac, abdominal, and thigh) were measured on the right side of the body using an adipometer (Premier, Cescorf, Brazil) and

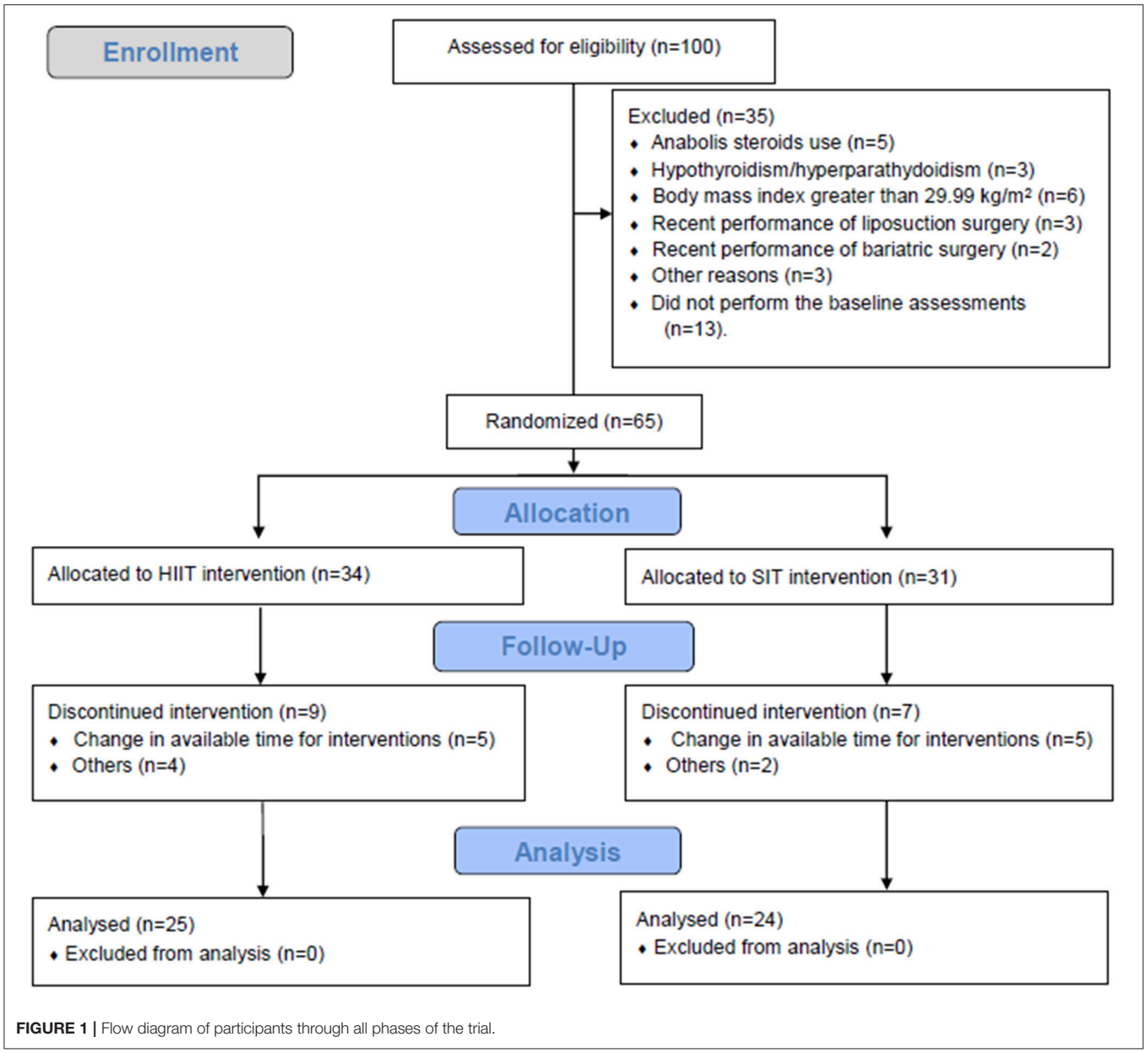

FIGURE 1 | Flow diagram of participants through all phases of the trial.

following the recommendations of the American College of Sports Medicine (2011). The mean of three valid measurements obtained at each skinfold site was used in the analysis. Intraclass correlation coefficient was 0.991 for triceps, 0.993 for subscapular, 0.996 for suprailiac, 0.995 for abdominal, and 0.986 the thigh skinfold. The Typical Error Measurement (TEM) was 0.7 mm for triceps, 0.8 mm for subscapular, 0.7 mm suprailiac, 0.7 mm for abdominal, 1.0 mm for thigh, and 11.2 mm for sum of five skinfolds (Σskinfolds).

Dietary Intake Evaluation

Six dietary recalls were applied by a dietitian, with three 24-h food recalls in the first and three in the eighth week. The quantification of the home measures to their equivalent in grams was made according to values of the Table for Evaluation of Food Consumption in Domestic Measures (Pinheiro et al., 2009).

Food intake was calculated in the *Dietpro® Clinical* software, version 5.8.1 (S. SISTEMAS, Minas Gerais, Brazil), using as reference the values of the Food Composition Table (Philippi, 2015), Brazilian Food Composition Table (TACO) (Núcleo de Estudos e Pesquisas em Alimentação, 2011), and United States Department of Agriculture (USDA). Total energy (kcal), carbohydrates (g), proteins (g), and lipids (g) were obtained. After the quantification, the mean initial and final intakes were compared for the results.

Statistical Analysis

Data were entered into an Excel spreadsheet (Microsoft) and imported into Statistical Package for the Social Sciences (version

20.0; SPSS Inc., Chicago, IL). Based on tests and retests for 49 participants, the standard error of the measurement (SEM) was calculated for triceps, subscapular, suprailiac, abdominal, and thigh skinfolds as previously described (Barbalho et al., 2017). Responsiveness was defined as changes that exceeded two times the SEM in favor of beneficial changes, since this response is supposed to be a true physiological adaptation beyond what might be expected to result from technical and/or biological variability (Barbalho et al., 2017). The responsiveness threshold was set at 0.7 mm for triceps, 0.8 mm for subscapular, 0.7 mm for suprailiac, 0.7 mm for abdominal, 1.0 mm for thigh, and 11.2 mm for sum of skinfolds. Paired *t*-tests were used to compare pre and post values of anthropometry measures, $\dot{V}O_2$peak, and $i\dot{V}O_2$peak within each group. Analysis of covariance (ANCOVA) was used to compare post-intervention values, using baseline values as covariate. Pearson's chi-squared test was used to analyze the distribution of R and NR between groups. Measures of the effect size (ES) for differences were calculated by dividing the mean difference by the standard deviation (*SD*) of the pre-training measurement. The magnitude of the ES was classified according to the following criteria: $d < 0.2$ was considered "trivial," $0.2 < d < 0.5$ was considered "small," $0.5 < d < 0.8$ represented "medium," and $d > 0.8$ constituted "large" (Cohen, 1988). Data are presented as numbers and percentages for categorical variables and are expressed as mean ± *SD*. A significance level of 0.05 was adopted for all statistical tests.

RESULTS

Adherence to training in HIIT and SIT groups was 76.5 and 74.2%, respectively. Only one participant from each group needed to replace one exercise session at the end of the intervention period. Moreover, one participant from the HIIT group reported vomiting and two participants from the SIT group reported dizziness after a training session.

Cardiorespiratory Fitness

The HIIT and SIT groups improved ($P < 0.001$) VO_2peak by 14.5 ± 22.9 and 16.9 ± 23.4%, respectively, as well as iVO_2peak by 6.2 ± 12.2 and 10.6 ± 18.1%, respectively (**Table 1**). The ANCOVA revealed no significant difference between groups for the changes in VO_2peak and iVO_2peak ($P > 0.05$).

Anthropometric Measures

Decreases ($P < 0.05$) in body mass (−1.2 ± 2.6 kg) and BMI (−1.2 ± 2.6 $kg.m^{-2}$) were observed only for the SIT group (**Table 1**).

The sum of the five skinfolds was reduced by 15.8 ± 7.9 and 22.2 ± 6.4% from baseline ($P < 0.001$) for the HIIT and SIT groups, respectively (**Figure 2** and **Table 1**). The results of ANCOVA revealed that the reductions were greater for SIT than for HIIT. The HIIT and SIT groups significantly decreased ($P < 0.001$) triceps (−13.8 ± 9.4 and −16.4 ± 7.8%), subscapular (−15.7 ± 11.8 and −24.0 ± 10.6%), suprailiac (−13.8 ± 14.9 and −22.0 ± 9.5%), abdominal (−19.8 ± 10.4 and −26.8 ± 6.7%), and thigh skinfolds (−13.6 ± 8.0 and −19.6 ± 8.1%) (**Table 1**). There were no significant differences between the groups ($P > 0.05$) in triceps, subscapular, suprailiac, and abdominal skinfold reductions. However, decreases in thigh skinfold were greater for the SIT group ($P = 0.020$). Waist circumference was reduced (P<0.001) for the HIIT (−3.1 ± 1.1%) and the SIT groups (−3.3 ± 1.8%), with no significant difference ($P = 0.739$) between groups (**Table 1**).

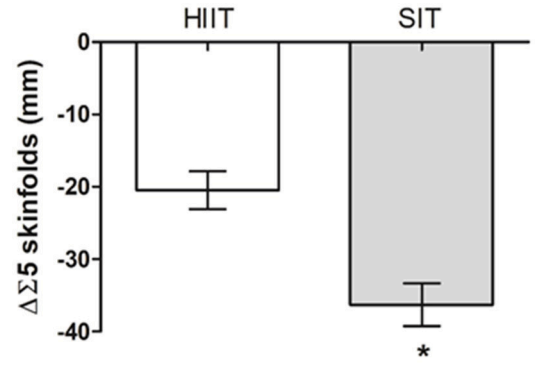

FIGURE 2 | Changes in the sum of skinfolds (ΔΣ5 skinfolds) induced by High intensity interval training (HIIT, $n = 25$) and Sprint interval training (SIT, $n = 24$). Data are expressed as means ± standard deviation*$P < 0.05$.

Dietary Intake

No significant difference ($P > 0.05$) was found in dietary intake between the HIIT and SIT groups at baseline and after 8 weeks of training. In addition, no significant difference was found after the intervention period for both groups (**Table 2**).

Responders and Non-responders

Forty-one participants were classified as responders (R) to triceps skinfold (20 in the HIIT and 21 in the SIT group), 41 to subscapular skinfold (20 in HIIT and 21 in SIT), 44 to suprailiac (21 in HIIT and 23 in SIT), 45 to abdominal (21 in HIIT and 24 in SIT), 47 to thigh (23 in HIIT and six in 24), and 43 to ∑skinfolds (20 in HIIT and 23 in SIT) (**Figure 3**). The SIT group presented more R in abdominal skinfolds when compared with the HIIT group; however, no significant difference was found ($P > 0.05$) in the prevalence of R between HIIT and SIT protocols for triceps, subscapular, suprailiac, thigh, and ∑skinfolds.

DISCUSSION

To the best of our knowledge, this is the first study to compare the effects of HIIT and SIT on anthropometric measures and cardiorespiratory fitness in healthy young females. Our results suggest that 8 weeks of HIIT and SIT improve markers of body fatness and cardiorespiratory fitness, even in the absence of changes in dietary intake. However, our results suggest that the SIT protocol is more efficient than the HIIT protocol for some parameters. In addition, we found greater prevalence of responders for abdominal and suprailiac skinfolds in the SIT group than in the HIIT group.

TABLE 2 | Dietary intake of participants before and after 8 weeks of exercise training.

	HIIT ($n = 25$)				SIT ($n = 24$)				Between groups
	Pre	Post	ES	*P*	Pre	Post	ES	*P*	*P*
Energy intake (kcal)	1594.6 ± 429.9	1577.8 ± 424.9	$-0.039_{(trivial)}$	0.894	1442.8 ± 657.1	1420.6 ± 384.8	$-0.041_{(trivial)}$	0.863	0.243
Carbohydrate (g)	184.7 ± 67.5	186.1 ± 65.8	$0.021_{(trivial)}$	0.930	171.9 ± 79.6	163.5 ± 56.6	$-0.121_{(trivial)}$	0.620	0.258
Protein (g)	75.5 ± 23.8	69.9 ± 20.4	$-0.252_{(small)}$	0.272	64.4 ± 21.6	65.6 ± 21.6	$0.055_{(trivial)}$	0.822	0.893
Lipids (g)	61.4 ± 20.2	61.3 ± 22.9	$-0.004_{(trivial)}$	0.988	53.9 ± 33.0	55.9 ± 16.9	$0.076_{(trivial)}$	0.762	0.420
Monounsaturated fat (g)	17.1 ± 6.8	16.6 ± 8.0	$-0.067_{(trivial)}$	0.824	14.7 ± 10.9	15.5 ± 6.6	$0.088_{(trivial)}$	0.729	0.645
Polyunsaturated fat (g)	9.3 ± 5.0	9.5 ± 4.1	$0.043_{(trivial)}$	0.881	9.9 ± 6.3	10.2 ± 4.8	$0.053_{(trivial)}$	0.876	0.594
Saturated fat (g)	17.0 ± 7.2	16.3 ± 6.8	$0.099_{(trivial)}$	0.726	14.6 ± 10.7	14.8 ± 4.3	$0.024_{(trivial)}$	0.946	0.469
Calcium (g)	501.7 ± 266.5	536.8 ± 235.6	$0.139_{(small)}$	0.616	572.2 ± 392.9	488.3 ± 193	$-0.271_{(small)}$	0.242	0.322
Sodium (g)	43.6 ± 159.1	12.1 ± 4.9	$-0.279_{(small)}$	0.691	11.8 ± 7.1	11.7 ± 6.2	$-0.015_{(trivial)}$	0.571	0.362
Dietary fiber (g)	1539.9 ± 730.9	1628.5 ± 566.1	$0.135_{(trivial)}$	0.192	1575.6 ± 962.9	1446.7 ± 781.9	$-0.146_{(trivial)}$	0.996	0.884

Data are expressed as means ± standard deviation. p (values) for within-group (time) effect and interaction (time × group) effect. ES, effect size; HIIT, high-intensity interval training; SIT, sprint interval training.

In agreement with previous studies showing that different forms of IT significantly increased VO_2max (Gibala et al., 2006; Wisløff et al., 2007; Bacon et al., 2013; Sloth et al., 2013; Gist et al., 2014), the present study found that 8 weeks of HIIT and SIT increased VO_2peak by 14.5 and 16.9%, respectively. Considering that low cardiorespiratory fitness is a strong independent risk factor for cardiovascular disease and all-cause mortality (Kodama et al., 2009; Barry et al., 2014) and that "lack of time" is a common barrier to regular exercise adoption (Weston et al., 2013), IT might help to increase exercise adherence. While the general recommendations suggest a minimum of 150 min of moderate aerobic activity or vigorous exercise for 75 min per week (World Health Organization, 2010), we found that with only 23 min of SIT performed three times per week, it is possible to increase cardiorespiratory fitness. Moreover, the increases for both groups were similar to those found in previous studies involving protocols with longer durations (Scribbans et al., 2016).

The cardiorespiratory fitness increases observed in the present study are similar to those reported in previous IT interventions (Trapp et al., 2008; Macpherson et al., 2011; Bagley et al., 2016; Higgins et al., 2016). Bagley et al. (2016) submitted 17 women and 24 men to a SIT protocol (4 x 20 s sprints on a cycle ergometer at 175% VO_2max followed by 2 min of active recovery, three times per week for 12 weeks) and found VO_2max increased by 18.7 and 6.0% for women and men, respectively. In the study of Higgins et al. (2016), 52 inactive, overweight/obese young women performed one of two experimental interventions: SIT (5–7 x 30 s sprints "*all out*" followed by 4 min of active recovery) and continuous cycling at 60–70% of heart rate reserve. After 6 weeks, the SIT group increased VO_2peak by 14.1%. The study of Macpherson et al. (2011) involved men and women ($n = 10$ per group) training three times per week for 6 weeks with SIT (30 s all-out running sprints on a manually driven treadmill, four to six bouts per session, 4 min of recovery per bout) vs. MICT (65% VO_2max for 30 to 60 min). After the intervention, the SIT and MICT groups showed increases of 11.5 and 12.5% in VO_2peak. These studies indicate that SIT, involving cycling and running, provides an efficient stimulus to improve aerobic metabolism despite its short duration. An important aspect is that the previously mentioned studies (Macpherson et al., 2011; Bagley et al., 2016; Higgins et al., 2016) also used active recovery. Active recovery contributes to increased aerobic metabolic activity and can also influence performance (Buchheit and Laursen, 2013). It appears that active recovery may decrease muscle oxygenation (Buchheit et al., 2009) and impair PCr resynthesis (Spencer et al., 2006). In addition, the active recovery might decrease the performance of the next effort when the intensity is ≥ 45% iVO2max). Therefore, if active recovery is chosen, it should last at least 3–4 min at a low intensity (Belcastro and Bonen, 1975) to allow maintenance of the high intensity of exercise during the following interval.

Both types of IT promoted reductions in body mass, markers of subcutaneous fat (skinfolds) and waist circumference, which is in agreement with the suggestion of Astorino and Schubert (2018) that HIIT and SIT increase whole-body fat oxidation. An important aspect of the present study is that the participants did not present a statistically significant difference between the groups in the pre-intervention period. In addition, our results were similar to those of Hazell et al. (2014), who reported that 6 weeks of a running SIT protocol of similar intensity and duration to the one used in our intervention reduced fat mass by 1.2 kg with 0.5 kg reduction in body mass despite no changes in dietary intake.

Previous studies using different forms of IT with longer periods of training also found fat loss in postmenopausal women with type II diabetes (Maillard et al., 2016), inactive young women (Trapp et al., 2008; Panissa et al., 2016), overweight men and women (Heydari et al., 2012; Higgins et al., 2016), and mixed samples of young men and women (Tremblay and Bouchard, 1994; Macpherson et al., 2011). One of the few studies comparing different IT protocols (Tong et al., 2018) compared the effects of SIT and HIIT in reducing abdominal visceral fat in 46 obese women. The participants were assigned to one of three experimental groups: SIT (6 s all-out sprint followed by a 9 s

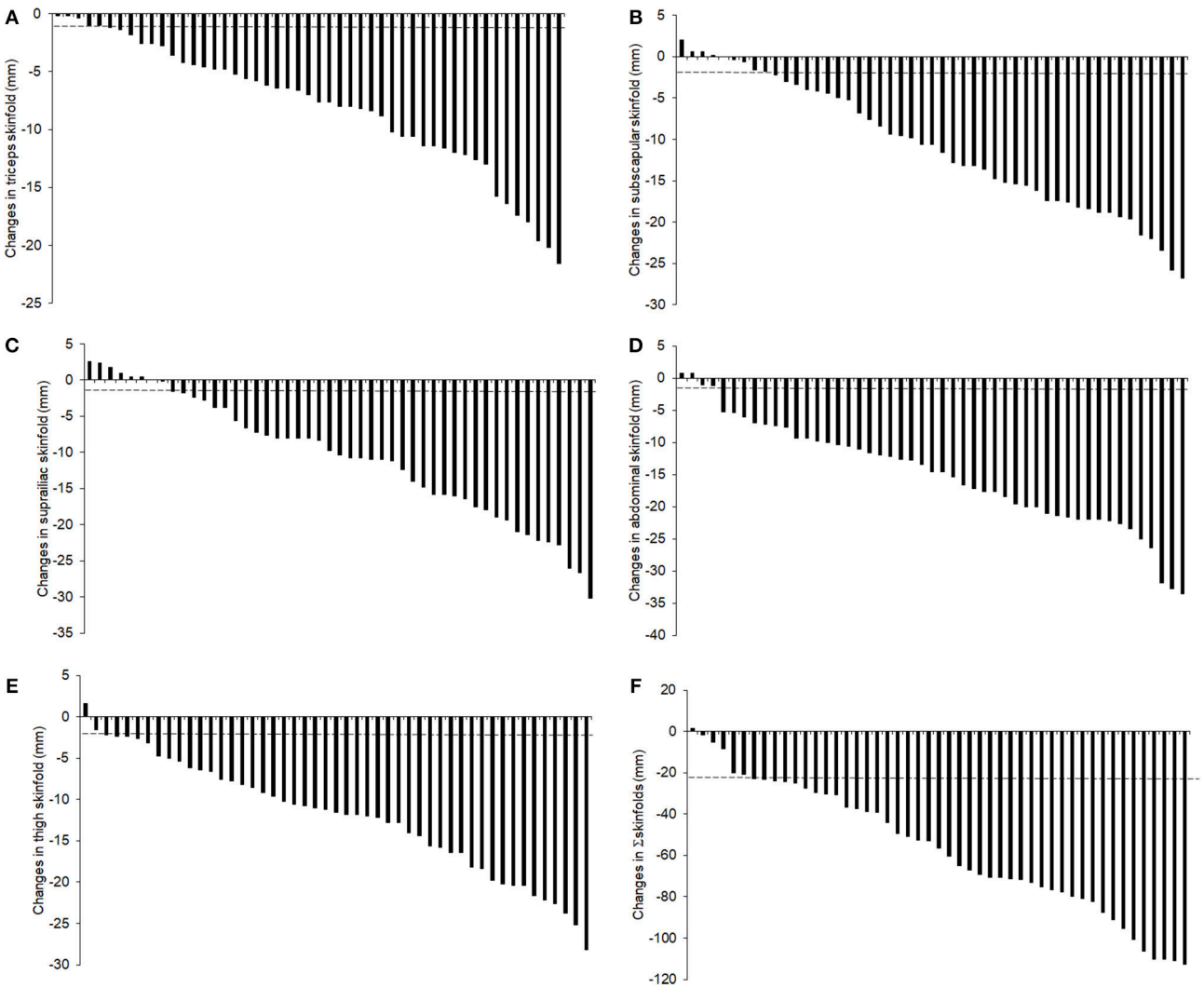

FIGURE 3 | Histogram of the relative changes in triceps **(A)**, subscapular **(B)**, suprailiac **(C)**, abdominal **(D)** thigh **(E),** and Σ5 skinfolds **(F)** for each individual after 8 weeks interval training in young women.

passive recovery for 80 cycles), HIIT (4 min exercise bouts at an intensity of 90% VO_2max, followed by a 3-min passive recovery). and Control group (no exercise). After 12 weeks, there was a reduction in abdominal visceral and subcutaneous fat. However, SIT group had lower reduction in subcutaneous abdominal fat (−17.4 vs. 40.7 cm^2) and trunk fat mass (1.2 vs. 2.0 kg) than HIIT group. No difference was found between SIT and HIIT for visceral abdominal fat, total fat mass, gynoid, and android fat mass. Probably, the difference between the results present by Tong et al. (2018) and our study was related to training protocol, since the short duration of SIT in the study by Tong et al. (6 vs. 30 s) might have lead to a less pronounced effect on fat metabolism and post exercise energy expenditure (Islam et al., 2017).

It is important to note that, according to our results, women reduced the Σskinfolds without changes in dietary intake, which is similar to results previously reported by Zhang et al. (2017) and Trapp et al. (2008), who observed changes in body composition after intervention with IT without changes in dietary intake. Several studies have suggested that the increases in post-exercise fat oxidation seem to be influenced by glycogen depletion (Withers et al., 1991; Kiens and Richter, 1998), and protocols that rely more on the glycolytic system might be more advantageous in this aspect (Whyte et al., 2013; Tucker et al., 2016). The higher reduction in the sum of skinfolds promoted by SIT might be due an increased oxidation of fat during the rest period, as previously reported (Withers et al., 1991). In agreement with this, previous studies have shown that IT protocols that lead to glycogen depletion result in increased fat oxidation (Withers et al., 1991; Kiens and Richter, 1998; Whyte et al., 2013; Tucker et al., 2016). Therefore, it appears that restoration of glycogen has a metabolic priority during recovery, leading to an increase in fat oxidation (Kiens and Richter, 1998).

One important aspect of the present study is that training was performed in a standard fitness facility using commercially available stationary bicycles, which is important to its practical application. However, one important aspect that limits the generalization of our results is that our training sessions were closely supervised at a 1:1 ratio. Considering that previous studies show that the results of an exercise intervention depend on supervision (Gentil and Bottaro, 2010; Knab et al., 2011; Ramírez-Campillo et al., 2017), the current findings might not be reproducible in unsupervised situations. Another apparent limitation is that our study did not identify the responders and non-responders to VO_2peak. In addition, lacks a control group that did not perform any type of exercise and the lack of a more accurate instrument for measuring body composition. However, since the participants did not change their nutritional habits and were evaluated at the same phase of the menstrual cycle, seasonal variations are unlikely to have been able to alter the results. As for the skinfolds measures, whilst we recognize that it might be a limited method to estimated body composition, it has been shown to be a highly reproduceable and widely used method (Jackson et al., 2009; Silva et al., 2009; Alves et al., 2017; Astorino et al., 2018); therefore, it is our opinion that it might be suitable to access the changes induced by an intervention on markers of body fatness.

CONCLUSION

Both HIIT and SIT protocols increased cardiorespiratory fitness and promoted reductions in adiposity indicators in healthy young women, even in the absence of dietary changes. Moreover, the SIT protocol induced greater improvements in some markers of body fatness than the HIIT protocol.

Considering the low physical activity levels in the population, the high prevalence of excessive body fat, and the fact that lack of time is a common barrier to exercise adoption (Weston et al., 2013; Vella et al., 2017), both protocols appear to be viable alternatives, since HIIT and SIT protocols lasted 33 and 23 min, respectively. In addition, one advantage of SIT is that it does not need complex tests to define the intensity of the exercise, which might contribute to its widespread use in cases where no clinical contraindications exist.

AUTHOR CONTRIBUTIONS

JN and PG conceived and designed the research. JN, PL, and JdO performed experiments. JN, RV, and PG analyzed data. JN, RV, AR, CdL, GP, RR-C, and PG interpreted results of experiments. JN and PG drafted manuscript. AR, CdL, GP, RR-C, and PG edited and revised manuscript. All authors approved final version of manuscript.

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frontiers in Physiology

ORIGINAL RESEARCH
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Myokine Response to High-Intensity Interval vs. Resistance Exercise: An Individual Approach

Zihong He[1], Ye Tian[2], Pedro L. Valenzuela[3], Chuanye Huang[4], Jiexiu Zhao[1], Ping Hong[5], Zilin He[6], Shuhui Yin[7] and Alejandro Lucia[8,9]*

[1] Biology Center, China Institute of Sport Science, Beijing, China, [2] Culture Development Center, General Administration of Sport of China, Beijing, China, [3] Physiology Unit, Systems Biology Department, University of Alcalá, Alcalá de Henares, Spain, [4] Graduate School, Shandong Sport University, Jinan, China, [5] Winter Sports Administrative Center, General Administration of Sport of China, Beijing, China, [6] Cardiovascular Department, Beijing Jian Gong Hospital, Beijing, China, [7] Institute of Hepatobiliary Gastrointestinal Disease, The Rocket Force General Hospital of People's Liberation Army (PLA), Beijing, China, [8] Faculty of Sport Sciences, European University of Madrid, Villaviciosa de Odón, Spain, [9] Instituto de Investigación Hospital 12 de Octubre ('i+12'), Córdoba, Spain

Purpose: This study aimed to compare the response to acute exercise of several myokines/hormones involved in metabolic function between two types of training sessions that are growing in popularity for their purported cardiometabolic benefits, high-intensity interval (HIIT) and resistance training (RT).

Methods: Seventeen healthy, non-athletic men (23 ± 3 years) participated in this cross-over study. They randomly performed a HIIT [with short (HIIT1) or long (HIIT2) intervals] or a RT session. The concentration of fibroblast-growth factor (FGF) 21, follistatin, ghrelin, interleukin-15, irisin, myostatin, and peptide YY was measured at baseline and 0, 1, 3, 24, 48, and 72 h post-exercise. An individual approach was adopted to determine the rate of responsiveness to each specific cytokine and training mode.

Results: A significant condition (session type) by time interaction ($p = 0.004$) effect was observed for FGF21, with RT eliciting a greater area under the curve (AUC) concentration than HIIT1 ($p = 0.02$). The AUC for follistatin was significantly greater after HIIT2 compared with RT ($p = 0.02$). Individual responsiveness to all session types ranged between 19 and 93% depending on the cytokine. However, most subjects (71–100%) responded positively for all cytokines (except for irisin, with only 53% of responders) after 1+ session type.

Conclusion: Except for FGF21, our results show no overall differences in the myokine response to HIIT or RT. A considerable individual variability was observed, with some subjects responding to some but not other training session types. Notwithstanding, most responded to at least one training session. Thus, it is mostly the individual response of each subject rather than general recommendations on type of training session (i.e., RT vs. HIIT or HIIT subtypes) that must be taken into consideration for maximizing cardiometabolic benefits in the context of personalized exercise prescription.

Keywords: cytokines, metabolism, physical activity, training, responders

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***Correspondence:**
Ye Tian
tianye@ciss.cn

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INTRODUCTION

Regular physical exercise is an effective lifestyle intervention for the prevention and treatment of some of the most common non-communicable diseases, notably cardiometabolic conditions and many types of cancer (Fiuza-Luces et al., 2013, 2018; Ruiz-Casado et al., 2017). The numerous exercise benefits for cardiometabolic health and weight management are partly mediated by the production of cytokines or peptides in contracting muscles, the so-called myokines; which are released to the blood and exert endocrine or paracrine effects in other cells, tissues or organs (Pedersen and Febbraio, 2012; Fiuza-Luces et al., 2018).

Myokines and exercise-induced proteins/hormones in general play a role in a variety of physiological functions, including mainly muscle growth and metabolic homeostasis. Myostatin, the first described secreted muscle factor to fulfil the criteria of a myokine (Fiuza-Luces et al., 2013), is a negative regulator of muscle growth (Huang et al., 2011), whereas follistatin is a myostatin-binding protein that promotes skeletal muscle development through the activation of the mammalian target of rapamycin pathway (Winbanks et al., 2012). Besides their main function related to muscle plasticity, myostatin and follistatin also play a role in metabolism [i.e., reduction of body fat, improvement of glucose homeostasis and browning of white adipose tissue (WAT)] (Huang et al., 2011; Braga et al., 2014). Another important contraction-induced myokine is interleukin (IL)-15 (Fiuza-Luces et al., 2018), owing to its potential effects on metabolic homeostasis, through a decrease in WAT mass (Nielsen et al., 2008), and an enhancement of glucose tolerance (Kim H.J. et al., 2013) and glucose uptake by muscle tissue (Busquets et al., 2006). Other myokines that are also involved in metabolic homeostasis have been proposed as therapeutic targets for the management of obesity and its related complications. Notably, fibroblast growth factor (FGF) 21 is involved in glucose regulation and lipid utilization, and promotes weight loss and WAT browning (Woo et al., 2013; Fisher and Maratos-Flier, 2016). Irisin is also involved in the promotion of WAT browning with subsequent increases in thermogenesis (Elbelt et al., 2013; Perakakis et al., 2017). In turn, some hormones such as ghrelin (also known as the "hunger hormone") and the peptide YY (PYY) provide metabolic benefits and promote weight management mainly through their influence on appetite, although they also exert a role on glucose and fatty acid homeostasis (Karra et al., 2009; Pradhan et al., 2013; Pinkney, 2014).

Although it is known that regular exercise might benefit cardiometabolic health through the cumulative effects of repeated episodes of exercise-induced increases in myokines or proteins/hormones (Ruiz-Casado et al., 2017), scarce evidence is available regarding which type of exercise session elicits a more robust effect on the release of these molecules. Further, although the existence of a wide inter-individual variability in the biological responses to a given exercise session and its importance for personalized exercise prescription is increasingly recognized, with some subjects achieving meaningful benefits (known as "responders") and others showing no changes ("non-responders") (Mann et al., 2014), most studies still report biological responses to exercise as group average.

Two training modes are gaining increasing popularity for health promotion and weight management. High intensity interval training (HIIT), which involves short bursts of high-intensity exercise (i.e., from less than 1 min to a maximum of 2–4 min) interspersed with short recovery periods is receiving considerable attention partly owing to the low time commitment it requires (<20 min per session) (Gibala and McGee, 2008). This training method has proven effective for the improvement of important health indicators such as cardiorespiratory fitness, metabolic biomarkers (e.g., of glucose control/insulin resistance) and body composition in both healthy and clinical populations (Gibala et al., 2012; Weston K.S. et al., 2014; Weston M. et al., 2014; Milanović et al., 2015; Wewege et al., 2017). Attending to the most recent annual survey of the American College of Sports Medicine, resistance training (RT) is also rapidly becoming one of the largest fitness trends (Thompson, 2017). RT has proven effective not only for the promotion of muscle mass/strength gains as traditionally thought (Borde et al., 2015), but also for reducing cardiometabolic risk factors such as obesity, insulin resistance or hypertension (Ibanez et al., 2005; Strasser et al., 2010).

The main purpose of this study was to compare the response of several myokines/hormones involved in cardiometabolic health to HIIT (two session types) vs. RT, with an analysis of both average and individual responses. Moreover, the effect of these training session types on resting metabolic rate (RMR) was also analyzed as a secondary endpoint given the influence of RMR on total daily energy expenditure and consequently on weight management and cardiometabolic health in general.

MATERIALS AND METHODS

Participants

Seventeen male subjects participated in this study [(mean ± SD) age, 23 ± 2 years; body mass index, 22 ± 2 kg · m^2]. Inclusion criteria were being healthy (i.e., free of any cardiovascular disease, diabetes or abnormal glucose tolerance, or any other acute/chronic disease) and performing no regular physical exercise (i.e., less than 20 min twice a week, or less than a total of 75 min during the week). Participants were required to maintain the same dietary habits during the study length, as well as to refrain from doing exercise, smoking, or drinking coffee or alcohol.

The experimental protocol was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Chinese Institute of Sport Science. Before their inclusion in the study, subjects were informed of the objects and procedures and provided both verbal and written informed consent.

Experimental Design

The study followed a cross-over, counterbalanced design. Each participant was assigned to perform a HIIT session with short (HIIT1) or long (HIIT2) intervals, or a RT session, in a randomized order, with a 7 days period between sessions.

One week before the first training session participants performed an incremental exercise test for VO_{2max} determination (see below). The day of the exercise sessions participants attended to the laboratory in the morning under fasting conditions, and we obtained blood samples and measured their RMR (baseline measures). Thereafter participants had the same standardized breakfast [one cup of soy milk, one egg, and two ∼50 g steamed stuffed buns (filled with pork)] and 2 h later they completed the prescribed training session. RMR was analyzed before (baseline) and 24, 48, and 72 h after each training session. All blood variables were analyzed before (baseline) and immediately after each training session, as well as 1, 3, 24, 48, and 72 h after each session.

Training Sessions

Two common types of HIIT sessions were designed. HIIT1 consisted of two sets of six 30 s treadmill running bouts at 100% of the speed (V_{max}) eliciting the VO_{2max} in the previous incremental text (see below), with 90 s of active recovery (50% of V_{max}) between bouts and 4 min of passive recovery between sets. HIIT2 consisted of five 4 min bouts at 90% V_{max}, with 4 min of active recovery (50% of V_{max}) between bouts. Both sessions lasted approximately 45–50 min and were conducted on the same treadmill that was used for VO_{2max} determination (pulsar4.0; H/P/cosmos, Traunstein, Germany).

The RT session was based on the recommendations of the American College of Sports Medicine (American College of Sports Medicine [ACSM], 2009). Seven types of exercises targeting all the main muscle groups (back squat, bench press, barbell deadlift, barbell row, barbell military press, standing biceps curl, and sit-ups) were prescribed. Participants performed four sets of 8–10 repetitions at 70–75% of their one repetition maximum (1RM, which had been determined during a previous familiarization session) for all exercises except for sit-ups, for which they performed four sets of 20 repetitions without external weight (i.e., just their body weight). Subjects rested for 60–90 s between exercises and for 4 min between sets. Each session lasted ∼50 min.

Measurements

Body Composition

Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively, using a calibrated stadiometer and platform scale (JianminII, Beijing Xin Dong Hua Teng, Beijing, China). Body composition (fat and muscle mass, expressed in relative values) was determined by whole body dual energy X-ray absorptiometry scan (GE LUNAR DPX system, Madison, WI, United States).

Maximal Oxygen Uptake

The treadmill speed was initially set at 7 km · h^{-1} and thereafter was increased by 1 km · h^{-1} every 2 min until volitional exhaustion, while treadmill inclination was kept constant (at 0%). The test was deemed valid if at least three of the following criteria were met: (*i*) a plateau in VO_2 was observed despite increasing exercise intensity; (ii) the subject was no longer able to maintain the required speed; (iii) the respiratory exchange ratio exceeded 1.10; and (iv) the age-predicted maximum heart rate (HR_{max}, 220 *minus* age, in years) was achieved. Gas-exchange data were collected breath-by-breath during the tests with a metabolic cart (MetaMax 3B, Cortex, Biophysik, Germany).

Energy Expenditure During HIIT

Oxygen uptake (VO_2) was analyzed with the aforementioned metabolic cart during HIIT sessions but not during RT sessions due to technical/logistic reasons. We also measured the heart rate (HR) response during HIIT sessions with a HR monitor (Polar RS400, Polar Electro, Kempele, Finland).

Resting Metabolic Rate

During measurements participants lied supine in a bed for 20 min (the first 3 min were discarded from the analyzes) in a room that had minimal light and noise and with ambient temperature maintained at 22 ± 1°C. VO_2 was analyzed with the aforementioned metabolic cart and a variation < 25 ml · min^{-1} was used to determine that the collection was acceptable.

Blood Variables

Blood samples (10 ml each) were drawn from the antecubital vein and centrifuged (3000 × *g*) for 10 min. The serum was then kept at −80°C. Enzyme-linked immunosorbent assay (ELISA) was used for the analysis of the concentration of FGF21 [R&D Systems (Minneapolis, MN, United States), number: DF2100], follistatin (R&D Systems, number: DFN00), myostatin (R&D Systems, number: DGDF80), IL-15 (R&D Systems, number: 0707170149), irisin (Phoenix Pharmaceuticals (Burlingame, CA, United States), number: EK-067-29), acyl ghrelin (Phoenix Pharmaceuticals, number: EK-031-30), and PYY [Millipore (Burlington, MA, United States), number: EZHPYYT66K]. The standard curves were analyzed by double parallel tube. All the changes were analyzed as a percentage of baseline values. The peak concentration and the area under the curve (AUC) displayed by the concentration-time data (trapezoid rule) were analyzed for each molecule.

Statistical Analysis

All data analyzed are available as **Supplementary Material**. Data are presented as mean ± SD. The normal distribution (Shapiro–Wilk test) and homoscedasticity (Levene's test) of the data were checked before any statistical treatment. Student's paired *t*-tests were conducted to analyze the differences in energy expenditure during HIIT1 and HIIT2. A two-factor [condition (HIIT1, HIIT2, RT) and time] repeated-measures ANOVA was used to compare the response over time of the blood variables and RMR between the three types of training sessions (HIIT1, HIIT2, RT). A Greenhouse–Geisser correction was applied when Mauchly's Test of Sphericity was violated. One-way repeated measures ANOVA was used to analyze differences between training sessions (HIIT1, HIIT2, RT) in peak levels and AUC for each blood variable. All statistical analyses were conducted using a statistical software package (SPSS 23.0, United States) setting the significance level at $\alpha = 0.05$.

The rate of responders was calculated for each blood variable and for RMR. Responsiveness was defined as positive changes

that exceeded two times the typical error of measurement (TE) (Hopkins, 2000). The TE was calculated for each variable as the standard error of within-subjects standard deviation for all baseline measures (three measurements for each variable) (Hopkins, 2015). This value was multiplied by 2 and expressed as a percentage of the condition's mean. The responsiveness threshold (i.e., 2 × TE expressed as a percentage of the three measures' mean) for FGF21, follistatin, myostatin, IL-15, irisin, ghrelin and PYY was 95.5, 72.7, 36.9, 139.5, 37.7, 77.2, and 47.9%, respectively, whereas it equaled 15.9% for RMR. Student's unpaired *t*-tests were performed to analyze differences in body composition and VO_2max between responders and non-responders. We used Fishers' exact test to compare the rate of responders for each blood variable between training session types (3 [HIIT1, HIIT2, RT] × 2 [responder vs. non-responder] contingency table). When a significant *p*-value ($p < 0.05$) was observed, we performed the test using 2 × 2 contingency tables to determine differences between specific training session types.

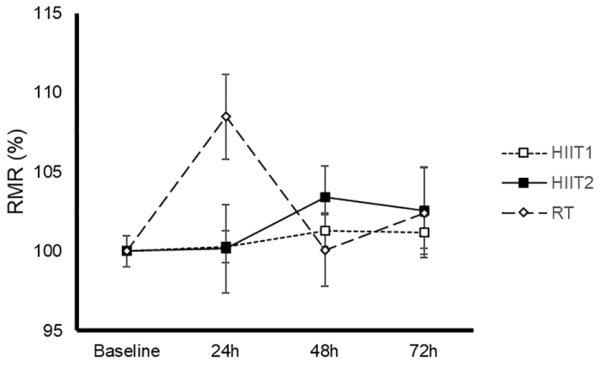

FIGURE 1 | Time-course of resting metabolic rate (RMR) after a session of high-intensity interval training with short (HIIT1) or long intervals (HIIT2), or resistance training (RT). Data are mean ± standard error. No significant time ($p = 0.164$) or condition (HIIT1, HHT2 or RT) by time interaction effect ($p = 0.058$) was found. Abbreviations: HIIT1, high intensity interval training session with short intervals; HIIT2, high intensity interval training session with long intervals; RT, resistance training.

RESULTS

Subjects' VO_{2max} averaged 49 ± 5 ml · kg · min^{-1}. Their total fat and muscle mass averaged 10 ± 4 and 57 ± 5 kg, respectively. All participants completed the prescribed training sessions at the required intensities.

Energy Expenditure During HIIT Sessions

HIIT1 induced a higher mean heart rate than HIIT2 (88 ± 4 vs. 83 ± 5% of HR_{max}, respectively; $p < 0.001$), as well as a higher mean energy aerobic expenditure (82 ± 6 vs. 64 ± 5% of VO_{2max}, respectively; $p < 0.001$ or 826 ± 110 vs. 641 ± 72 kcal, respectively; $p < 0.001$). The differences in caloric expenditure were mostly due to a higher contribution of carbohydrate metabolism in HIIT1 (154 ± 31 vs. 119 ± 16 g, respectively; $p < 0.001$), with no significant differences being observed for fat (11 ± 4 vs. and 10 ± 4 g, $p = 0.540$) or protein oxidation (22 ± 6 vs. 14 ± 2g, $p = 0.062$).

Resting Metabolic Rate

We found no significant time ($p = 0.164$) or condition by time interaction effect ($p = 0.058$) (**Figure 1**). Only 6, 12, and 24% of the subjects could be considered responders attending to their RMR after HIIT1, HIIT2, and RT, respectively.

Blood Variables

The time course and peak and AUC concentrations of each myokine/hormone in response to exercise are presented in **Figures 2**–**4**, respectively. The individual response of each subject to the different training sessions is presented in **Table 1**. The rate of responsiveness for each molecule attending to the type of training session performed and independently of the training session mode is presented in **Figures 5**, **6**, respectively.

Fibroblast Growth Factor 21

A significant time ($p = 0.000$) and condition by time interaction ($p = 0.004$) effect was observed for FGF21 (**Figure 2A**). Both HIIT1 and HIIT2 induced a significant increase in FGF21 levels at 3 ($p = 0.002$) and 0 h ($p < 0.001$) post-exercise, whereas the values of this protein increased above baseline values 48 h after RT ($p = 0.025$). A significantly higher AUC concentration was observed for RT vs. HIIT1 ($p = 0.020$), but not vs. HIIT 2 ($p = 0.122$) (**Figure 4A**).

Most subjects (>∼50%) could be considered non-responders for the FGF21 response to all types of training sessions, with no significant differences between sessions ($p = 0.115$) (**Figure 5A**). However, only 5 subjects (29%) did not increase their FGF21 levels in response to at least one of the three different training session types (**Table 1** and **Figure 6**). No differences in fat mass ($p = 0.765$), muscle mass ($p = 0.353$) or VO_{2max} ($p = 0.182$) were observed between responders and non-responders for FGF21 (data not shown).

Follistatin

A significant time ($p < 0.001$) but not condition by time interaction ($p = 0.176$) was observed for follistatin (**Figure 2B**). RT yielded an almost significantly lower peak follistatin concentration than HIIT1 ($p = 0.056$) and HIIT2 ($p = 0.085$) (**Figure 3B**), and a significantly lower AUC than HIIT 2 ($p = 0.016$) (**Figure 4B**).

There was a significant relationship between training session type and responsiveness to follistatin ($p = 0.019$). Most subjects could be considered responders to follistatin after the HIIT sessions (**Figure 5B**). In contrast, 59% of participants did not show an increase in follistatin levels after RT, being this rate significantly lower than that observed with HIIT2 ($p = 0.010$) but not HIIT1 ($p = 0.303$) (**Figure 5B**). Only one subject (6%) did not show an increase in follistatin levels in response to any of the three training session types (**Table 1** and **Figure 6**).

Myostatin

A significant time ($p < 0.001$) but not condition by time interaction effect ($p = 0.280$) was observed

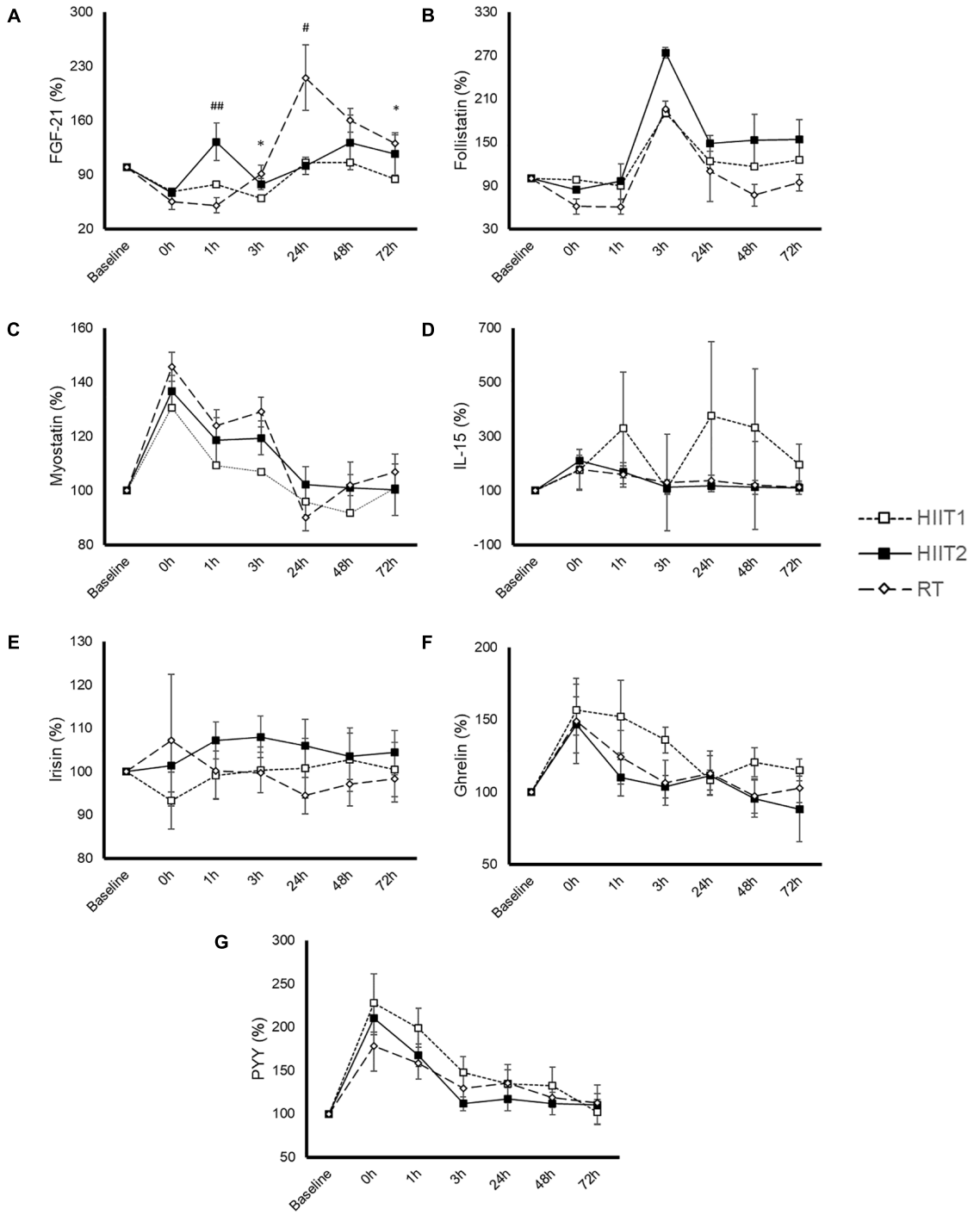

FIGURE 2 | Time-course response of fibroblast growth factor 21 (FGF21) **(A)**, follistatin **(B)**, myostatin **(C)**, interleukin (IL)-15 **(D)**, irisin **(E)**, ghrelin **(F),** and peptide YY (PYY) **(G)** to a session of high-intensity interval training with short (HIIT1) or long intervals (HIIT2), or resistance training (RT). All molecules were measured in 17 subjects except for IL-15 and PYY, which were measured in 9 and 14 subjects, respectively. Data are mean ± standard error. A significant ($p < 0.05$) time effect was observed for all variables except for irisin. A significant condition by time interaction was just observed for FGF21 ($p = 0.004$).

for myostatin (**Figure 2C**). No differences were observed between training session types in the peak (**Figure 3C**) or AUC concentration (**Figure 4C**) of this myokine.

Similar rates of responders were observed between conditions ($p = 0.260$) (**Figure 5C**), and although the rate of responders was overall low attending to each specific type of session (40–70%), all participants showed an increase in myostatin

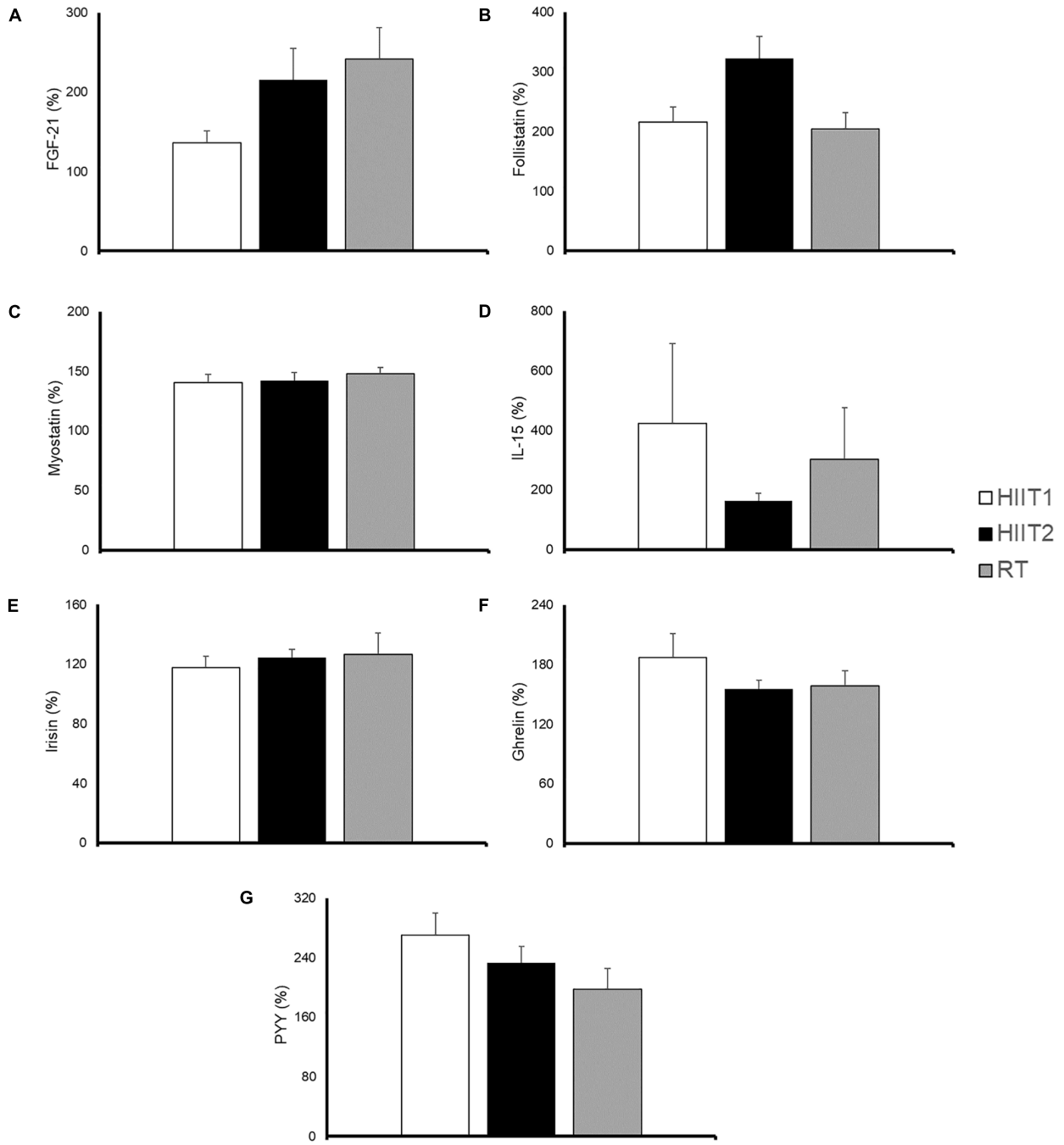

FIGURE 3 | Peak concentration of fibroblast growth factor-21 (FGF-21) **(A)**, follistatin **(B)**, myostatin **(C)**, interleukin (IL)-15 **(D)**, irisin **(E)**, ghrelin **(F),** and peptide YY (PYY) **(G)** in response to a session of high-intensity interval training with short (HIIT1) or long intervals (HIIT2), or resistance training (RT). All molecules were measured in 17 subjects except for IL-15 and PYY, which were measured in 9 and 14 subjects, respectively. Data are mean ± standard error. There were no significant differences between conditions.

levels after at least one training session type (**Table 1** and **Figure 6**).

Interleukin-15

Eight participants presented IL-15 concentrations below the minimum detection levels and thus their results could not be analyzed (total n for analyses = 9). No significant time ($p = 0.373$) or condition by time interaction ($p = 0.324$) was observed for this myokine (**Figure 2D**), with no differences between conditions in peak (**Figure 3D**) or AUC concentration (**Figure 4D**).

Approximately half of the participants (44%) could be considered non-responders for IL-15 attending to each specific

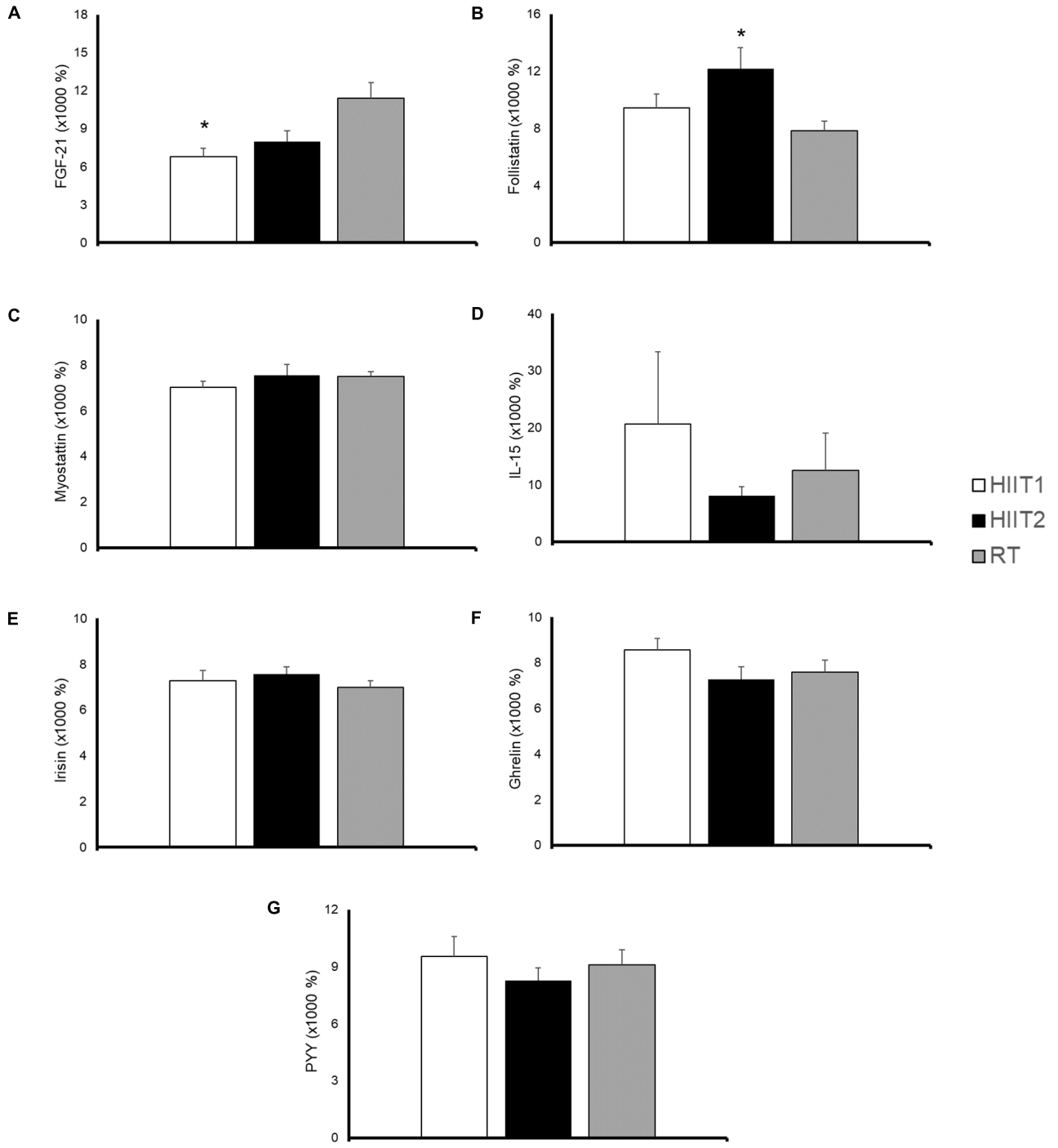

FIGURE 4 | Area under the curve (calculated from concentration-time data from 0 to 72 h post-exercise) of fibroblast growth factor-21 (FGF-21) **(A)**, follistatin **(B)**, myostatin **(C)**, interleukin (IL)-15 **(D)**, irisin **(E)**, ghrelin **(F)**, and peptide YY **(G)** in response to a session of high-intensity interval training with short (HIIT1) or long intervals (HIIT2), or a resistance training (RT) session. All molecules were measured in 17 subjects except for IL-15 and PYY, which were measured in 9 and 14 subjects, respectively. Data are mean ± standard error. Significantly different from RT: $*p < 0.05$.

training mode, with no differences between them ($p = 1.0$) (**Figure 5D**). However, all but two subjects (78%) responded positively after at least one type of training session (**Table 1** and **Figure 6**).

Irisin

No significant time ($p = 0.892$) or condition by time interaction effect ($p = 0.543$) was observed for irisin levels (**Figure 2E**), with no differences between

TABLE 1 | Individual biological response to the different training session types.

	FGF21			Follistatin			Myostatin			IL-15			Irisin			Ghrelin			PYY		
Subject	HIIT1	HIIT2	RT	HIIT1	HIIT2	RT	HIIT1	HIIT2	RT	HIIT1	HIIT2	RT	HIIT1	HIIT2	RT	HIIT1	HIIT2	RT	HIIT1	HIIT2	RT
1		+	+	+	+	+	+	+	+	+	+		+			+			+	+	+
2				+	+	+		+	+	NA	NA	NA	+						+	+	
3			+	+	+	+		+	+	NA	NA	NA						+	+	+	+
4				+	+	+			+	NA	NA	NA				+			+	+	+
5							+			NA	NA	NA							+	+	+
6		+	+	+	+				+	NA	NA	NA				+		+	+	+	+
7		+		+			+	+		+				+		+			+	+	
8					+	+		+	+		+		+				+		+	+	
9					+	+	+	+	+			+					+		+		+
10						+		+		+		+	+		+				+	+	
11						+			+		+	+								+	+
12						+			+			+				+			+	+	+
13		+			+	+		+	+	+	+	+		+	+			+	+	+	+
14						+	+	+	+			+		+		+			NA	NA	NA
15							+	+	+	NA	NA	NA					+	+	NA	NA	NA
16					+	+	+			NA	NA	NA			+		+	+	NA	NA	NA
17					+	+		+		NA	NA	NA		+	+	+			+	+	+

Responsiveness (indicated as "+") was determined as a positive change greater than the smallest worthwhile change (calculated for each cytokine as twice the typical error of measurement and expressed as a percentage). Abbreviations: FGF21, fibroblast growth factor; HIIT1, high intensity interval training session with short intervals; HIIT2, high intensity interval training session with long intervals; IL-15, interleukin-15; N/A, not available; PYY, peptide YY; RT, resistance training.

conditions in peak (**Figure 3E**) or AUC concentration (**Figure 4E**).

The rate of responders to irisin for all training session types was overall low (>75%), with no significant differences between them ($p = 1.00$) (**Figure 5E**). Eight subjects (47%) did not exhibit an increase in irisin levels in response to any of the three types of training session (**Table 1** and **Figure 6**). No differences in fat mass ($p = 0.721$), muscle mass ($p = 0.250$) or VO_{2max} ($p = 0.156$) were observed between responders and non-responders for irisin (data not shown).

Ghrelin

A significant time ($p < 0.001$) but not condition by time interaction ($p = 0.286$) was observed for ghrelin (**Figure 2F**). No significant differences ($p > 0.05$) were observed between conditions for ghrelin peak (**Figure 3F**) or AUC concentration (**Figure 4F**).

The rate of responders for all three training modes was low (<50%), with no differences between conditions ($p = 0.808$) (**Figure 5F**). However, only four subjects (24%) did not show an increase in ghrelin levels in response to any type of session (**Table 1** and **Figure 6**). No differences in fat mass ($p = 0.702$), muscle mass ($p = 0.911$) or VO_{2max} ($p = 0.478$) were observed between responders and non-responders for ghrelin (data not shown).

Peptide YY

Three participants presented a PYY concentration below the minimum detection levels and thus their results could not be analyzed (total n for analyses = 14). A significant time ($p < 0.001$) but not condition by time interaction ($p = 0.452$) was observed for PYY (**Figure 2G**). There were no differences between conditions in peak (**Figure 3G**) or AUC (**Figure 4G**) PYY concentration.

Almost all the subjects could be considered responders to PYY after the HIIT sessions, with no significant differences between conditions ($p = 0.326$) (**Figure 5G**). All the subjects increased their PYY levels in response to at least two training modes (**Table 1** and **Figure 6**).

DISCUSSION

We have measured the exercise response of several myokines/hormones that are involved in metabolic regulation and weight management after a session of RT or HIIT, both of which are gaining popularity for their purported cardiometabolic benefits. In addition, we assessed inter-individual variability. Thus, our study is of potential relevance in the context of personalized exercise prescription for maximizing the cardiometabolic benefits of this crucial lifestyle intervention. In this context, the main finding of this study was the great inter-individual variability observed in the acute cytokine response to different types of training sessions, with the rate of responsiveness to each session ranging between 19 and 93% depending on the analyzed molecule. However, our individual approach shows that most subjects (71–100%) showed a positive response of all blood variables to at least one session type (except

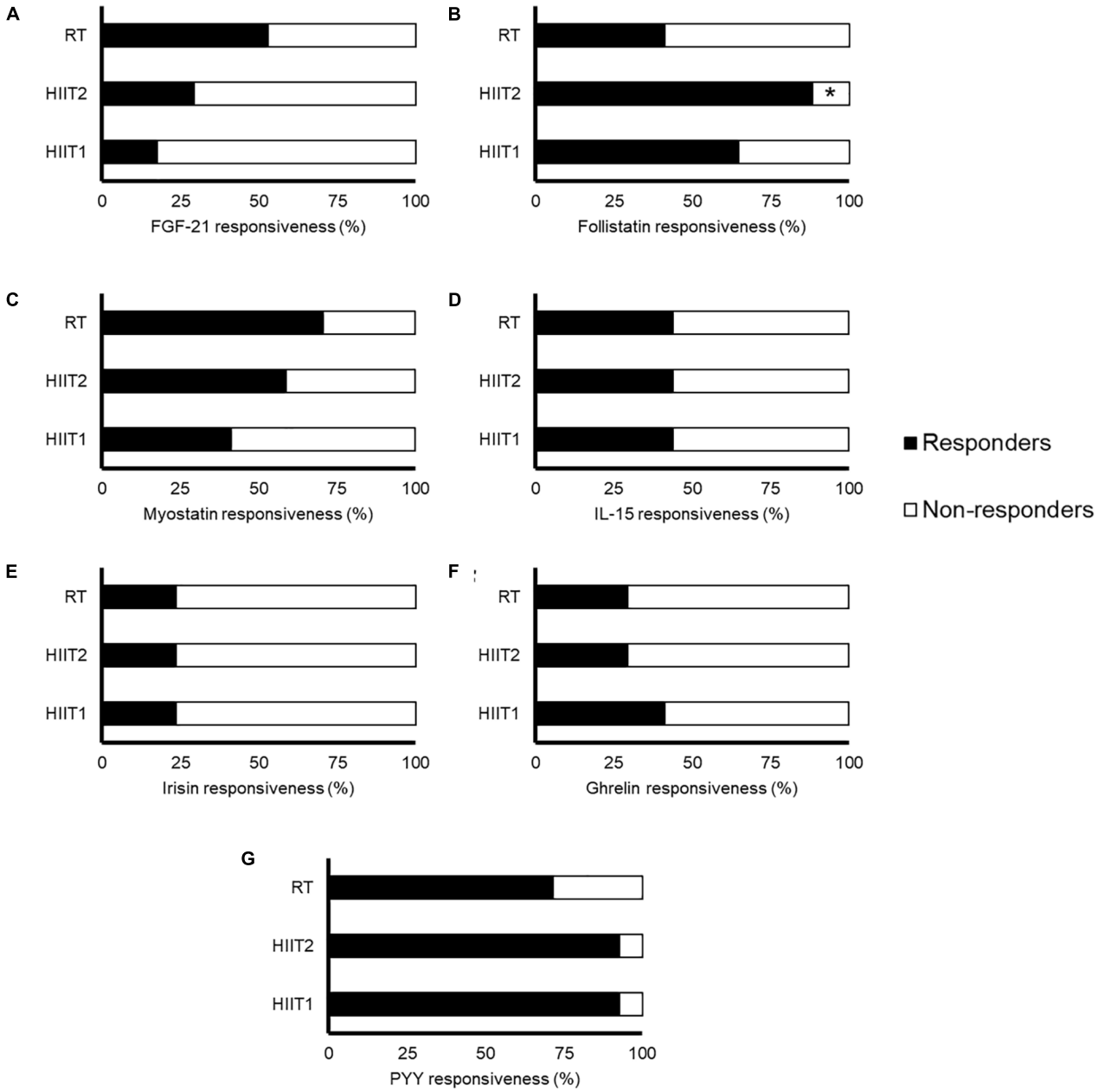

FIGURE 5 | Rate of responsiveness of fibroblast growth factor-21 (FGF-21) **(A)**, follistatin **(B)**, myostatin **(C)**, interleukin (IL)-15 **(D)**, irisin **(E)**, ghrelin **(F)**, and peptide YY **(G)** in response to a session of high-intensity interval training with short (HIIT1) or long intervals (HIIT2), or a resistance training (RT) session. Responsiveness was determined as a positive change greater than the smallest worthwhile change (calculated for each molecule as twice the typical error of measurement and expressed as a percentage). All molecules were measured in 17 subjects except for IL-15 and PYY, which were measured in 9 and 14 subjects, respectively. Significantly different from RT: $*p < 0.05$.

for irisin, with only 53% of responders). By contrast, the majority of subjects were non-responders for changes in RMR in the days following the exercise sessions, and no differences in the time course of RMR post-exercise were found between the three conditions. Given the beneficial role of exercise-induced factors (notably, myokines) for cardiometabolic health (Pedersen, 2011; Fiuza-Luces et al., 2018), our results suggest that training prescription should include a variety of stimuli including both RT and HIIT sessions in order to obtain the greatest benefits from exercise.

The myokines/hormones analyzed here play a major role in muscle growth and/or metabolism. Interestingly, all three session types resulted in a transient (up to 3 h after exercise) increase in myostatin, a transforming growth factor (TGF) β family member that acts as a negative regulator of muscle growth, followed by a subsequent gradual decrease, reaching again approximately

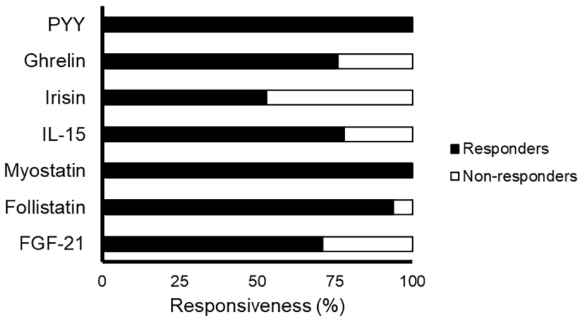

FIGURE 6 | Rate of responsiveness of the blood variables to exercise independently of the training mode. Responsiveness was determined as a positive change greater than the smallest worthwhile change (calculated for each molecule as twice the typical error of measurement and expressed as a percentage). All molecules were measured in 17 subjects except for IL-15 and PYY, which were measured in 9 and 14 subjects, respectively. Abbreviations: FGF21, fibroblast growth factor 21; HIIT1, High intensity interval training session with short intervals; HIIT2, High intensity interval training session with long intervals; IL-15, interleukin-15; PYY, peptide YY; RT, resistance training.

baseline levels 72 h post-exercise. The effects of acute exercise on myostatin remain unclear, with some studies finding a down-regulation of myostatin mRNA expression after different types of exercise (Louis et al., 2007; Hittel et al., 2010; Lundberg et al., 2012) but others failing to find such changes (Jensky et al., 2010; MacKenzie et al., 2013). Although MacKenzie et al. (2013) found a decrease in myostatin transcriptional activity after resistance exercise, the exercise stimulus also activated Notch, an TGFβ inhibitor. The authors concluded that despite the acute increase in myostatin expression, the inhibition of its transcriptional activity might contribute to exercise-induced skeletal muscle hypertrophy (MacKenzie et al., 2013).

In the present study the increase in myostatin concentration upon exercise termination occurred concomitantly with an increase 3 h post-exercise in follistatin, a myostatin-binding protein involved in skeletal muscle development, energy metabolism and WAT browning (Winbanks et al., 2012; Braga et al., 2014). Previous research has demonstrated that this protein is released into the circulation in response to exercise, which could have potential effects on muscle hypertrophy and metabolism (Hansen et al., 2011). Our results show that the increase in follistatin was more marked after HIIT1 and especially HIIT2 compared to RT, with only one subject (6%) not responding positively to any of the HIIT sessions. Therefore, HIIT, especially if including long intervals, appears as the most effective strategy to increase follistatin concentration.

FGF21 has been proposed as a myokine induced by the PI3K–AKT pathway that plays important metabolic roles (Lee et al., 2012). Thus, FGF21 protects muscle tissue against insulin resistance (Lee et al., 2012), augments brown fat thermogenesis in concert with irisin (Lee et al., 2014), and is related to weight loss and WAT browning (Woo et al., 2013; Fisher and Maratos-Flier, 2016). We found an overall increase in the levels of FGF21 after exercise, which is in agreement with previous research (Kim K.H. et al., 2013; Tanimura et al., 2016). However, although all exercise types induced increases in FGF21, an interesting finding was that the increase tended to be greater after RT than after the HIIT sessions, remaining elevated above baseline values even 48 h after the former. Given the potential of FGF21 as a therapeutic target against metabolic disorders such as obesity and diabetes (Giralt et al., 2015; Strowski, 2017), these results could have promising clinical implications, being RT the most recommended training mode for the stimulation of FGF21.

In contrast, no consistent increases in IL-15 levels were observed after any of the training session types. Increased circulating levels of this myokine have been previously observed in young subjects immediately and up to 24 h after a RT session (Riechman, 2004; Pérez-López et al., 2018). A strong inverse relationship has been reported between the serum IL-15 response to exercise and the training volume and time under tension during a session, suggesting that prolonged muscle activation (such as that possibly elicited here by the 50 min session, which included a total of 28 sets of different whole-body exercises) might attenuate the release of this myokine (Pérez-López et al., 2018). Thus, although the present study does not support a stimulating role of the applied exercise training sessions on IL-15 secretion, other training types (i.e., brief but intense RT sessions) might increase the levels of this anti-catabolic/anti-obesogenic myokine. More research is, however, needed to elucidate how circulating IL-15 might influence skeletal muscle and adipose tissue mass in humans.

Another interesting finding of our study was the low rate (<25%) of responders for irisin, with no significant time or condition by time effect been found for this protein. Great attention has been given in recent years to the potential of irisin for the prevention and treatment of obesity and its related complications due to its purported role in WAT browning and energy expenditure (Pedersen and Febbraio, 2012; Elbelt et al., 2013). Previous research has shown an overall increase in circulating irisin levels after acute exercise, with the effect being independent of the type of exercise training session (resistance vs. aerobic training) but fitness level being the best response predictor (i.e., being fit is associated with a ∼twofold increase in post-exercise irisin) (see Fox et al., 2018 for a review). In this regard, we found no differences in VO_{2max} between responders and non-responders, but no data were available regarding participants' muscle strength or specific training background, which might have also conditioned the irisin response. Notwithstanding, controversy now exists around irisin: concerns have been raised regarding inconsistencies between animal and human data (Raschke et al., 2013) and methodological issues (such as potential cross-reactivity of the commercially available anti-irisin antibodies with other proteins) (Albrecht et al., 2015). Our results add further controversy to this topic, as a very low rate of responders for all three different training modes was observed. Therefore, further research should address the actual effect of exercise on irisin concentrations and the physiological consequences of these increases.

Lastly, we observed an acute increase in the appetite-regulating hormone PYY in all the subjects upon exercise termination in response to at least one type of session, with

most subjects (70%) increasing their PYY levels in response to each specific training mode. In line with these results, meta-analytical evidence concluded that exercise influences appetite by increasing the levels of hormones such as PYY, pancreatic polypeptide or glucagon-like peptide 1, which suppress food intake (Schubert et al., 2014). The influence of acute exercise on appetite might also be mediated, at least partly, by a reduction of acylated ghrelin levels. However, whereas some studies observed increased ghrelin levels in response to exercise (Broom et al., 2008; King et al., 2017), others reported opposite findings (Jürimäe et al., 2007; Mackelvie et al., 2007). Our results show that only a few participants (30–40%) presented acute increases in ghrelin levels in response to each of the three training sessions. However, a great percentage of the subjects (76%) showed an acute increase in response in ghrelin to at least one type of training session. The individual variability observed in our and other studies (King et al., 2017) might contribute to the existing controversy on the effects of exercise on ghrelin levels.

Some methodological limitations must be noted. Although all sessions had approximately the same duration (∼45–50 min), they were not matched for external or internal load (as reflected by the different energy expenditures). In addition, due to budget constraints we did not measure some important myokines such as interleukin-6 or brain-derived neurotrophic factor. Moreover, the time points at which blood samples were taken were chosen attending to practical/feasibility reasons rather than to an *a priori* analysis of the time-course of each myokine in response to exercise. Thus, we cannot rule out the existence of potential differences between sessions in the myokine response at time points others than those chosen for the present study. Lastly, the responsiveness threshold (2 × TE) takes into account the random error, that is, the variability provoked by the technical error of measurement and the biological day-to-day changes of each variable. Notwithstanding, we cannot discern if acute changes of a magnitude greater than this threshold actually translate into clinically meaningful benefits/adaptations. It is also important to highlight that the results obtained here might not be generalized to other populations such as overweight or elderly subjects, in whom the production of exercise-induced myokines would be maybe expected to induce greater health benefits than in healthy young adults as those assessed here. Therefore, future research should address the specific response among different population segments. More research is also warranted analyzing the myokine response to different training modes or loads for a given type of training (e.g., RT with varying training intensities or volumes). Finally, it must be emphasized that acute myokine responses as those studied here are not necessarily linked with actual chronic adaptations to training (Barros et al., 2017).

CONCLUSION

The present study shows an overall higher FGF21 response to RT than to HIIT in healthy young subjects. In turn, a higher follistatin response was observed with HIIT (at least with long intervals, i.e., HIIIT2) than with RT. Most important, there was a considerable inter-individual variability in the response of the different cytokines irrespective of the type of exercise session. Notwithstanding, most subjects responded positively to at least one training mode except irisin, for which half of the participants showed no response. Given the involvement of the studied myokines and hormones on cardiometabolic health, our results suggest that, to obtain the greatest benefits, training prescription should be individualized in order to provide the necessary stimulus to each subject.

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ZihH, YT, CH, JZ, PH, ZilH, and SY conceived the study and performed the experiments. PV and AL analyzed the data and drafted the manuscript. All authors significantly contributed to the final version of the manuscript.

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frontiers in Physiology

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Prevalence of Non-responders for Blood Pressure and Cardiometabolic Risk Factors Among Prehypertensive Women After Long-Term High-Intensity Interval Training

Cristian Álvarez[1†], Rodrigo Ramírez-Campillo[1†], Carlos Cristi-Montero[2†], Robinson Ramírez-Vélez[3†] and Mikel Izquierdo[4†]*

[1] Laboratory of Human Performance, Quality of Life and Wellness Research Group, Department of Physical Activity Sciences, Universidad de Los Lagos, Osorno, Chile, [2] IRyS Group, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile, [3] Centro de Estudios en Medición de la Actividad Física (CEMA), Escuela de Medicina y Ciencias de la Salud, Universidad del Rosario, Bogotá, Colombia, [4] Department of Health Sciences, Public University of Navarra, Navarrabiomed, CIBER of Frailty and Healthy Aging (CIBERFES), Instituto de Salud Carlos III, Pamplona, Spain

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Billy Sperlich,
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Ivan Bautmans,
Vrije Universiteit Brussel, Belgium

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

[†]All authors have contributed equally to this work

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Background: Exercise is known to improve cardiometabolic outcomes; however, results are typically reported as mean values, and there is wide interindividual variability in terms of response that has not been explored in populations at risk for hypertension. Our aim was to investigate both the effects on and the prevalence of non-responders (NRs) for decreasing blood pressure (BP) and other risk factors among prehypertensive women after long-term high-intensity interval training (HIIT). A secondary aim was to report potential variables that can predict decreases in BP after HIIT.

Methods: Sedentary overweight/obese women (age 35.9 ± 5.4 year; body mass index [BMI] 30.9 ± 6.2 kg/m^2) were assigned to a prehypertensive (PreHTN; N = 44) or normotensive (NT; N = 40) group according to their ambulatory BP at baseline. Subjects underwent a thrice-weekly 16-week HIIT program (7–10 × 1 min exercise with 2 min of rest). Training-induced changes in body composition and cardiovascular, metabolic, strength, and endurance performance markers were measured, and the prevalence of NRs was reported as a percentage. All outcomes were analyzed by multivariable regression.

Results: Statistically significant ($P < 0.05$) decreases in systolic BP (SBP) were detected in the PreHTN group (Δ −8 mmHg) compared with baseline, whereas the NT group (Δ + 3 mmHg) showed a non-significant increase in SBP. Diastolic BP (DBP) was significantly decreased in the PreHTN group (Δ −5.8 mmHg) and non-significantly decreased (Δ −2 mmHg) in the NT group. Also, there were significant differences ($P < 0.0001$) in the prevalence of NRs based on SBP between the PreHTN and NT groups (11.4 vs. 68.8%), but similar prevalence of NRs based on DBP. SBP alone was a powerful predictive factor for a beneficial SBP reduction, explaining 51.2% of the results, which was similar to other more complex models tested.

Conclusion: The prevalence of NRs based on SBP and DBP was different between prehypertensive and normotensive subjects after 16 weeks of HIIT. Other comorbidities such as body composition and metabolic outcomes showed almost similar modifications between prehypertensive and normotensive subjects, being the most basic predictive factor for BP reduction baseline SBP, which we refer to as 'BP health status' (51.2%). This improvement in BP was accompanied by other known improvements of HIIT on body composition, metabolic and endurance performance in both study cohorts.

Trial Registration: ClinicalTrials.gov ID: NCT03000140 (Register 20 December, 2016).

Keywords: high-intensity interval training, prehypertension, responders, non-responders, women, risk factors, systolic blood pressure

INTRODUCTION

The prehypertensive state is generally associated with physical inactivity (not engaged in physical activity according to international physical activity guidelines) (O'Donovan et al., 2010) and other risk factors including diet, sodium intake, and smoking, which is the most important contributor to hypertension (HTN) development (Díaz-Martínez et al., 2017). HTN is also the most common primary diagnosis in Chile, and has increased in adult women from 25.0% in 2009–2010 to 27.7% in 2017 (Hawley, 2009). Because a single session of endurance exercise decreases 24-h blood pressure (BP) (Karoline de Morais et al., 2015), chronic exercise (i.e., regular exposure to exercise) provides powerful benefits for prehypertensive subjects (Park et al., 2006), where the changes (i.e., decreases) of blood pressure in normotensive subjects have been regularly reported. These effects have been corroborated by meta-analyses and, accordingly, prolonged endurance training has been recognized as a strategy to decrease BP and other comorbidities in prehypertensive and hypertensive populations (Montero et al., 2014).

Unfortunately, while there is ample evidence to support that exercise training (e.g., endurance exercise) decreases BP (Pescatello et al., 2015), 'lack of time' is frequently cited by the sedentary population as the main barrier to adherence to exercise guidelines (i.e., less than 150 min of low-moderate-intensity exercise/week or 75 min of vigorous-intensity exercise/week) (Trost et al., 2002). Therefore, more research in exercise science is needed for the prevention of HTN and the management of both early prehypertension and common comorbidities including obesity (e.g., waist circumference or fat mass) and dyslipidemia (i.e., to decrease low-density lipoprotein or triglycerides) (Russo et al., 2018). In this regard, high-intensity interval training (HIIT, defined as repeated bouts of high-intensity exercise interspersed with rest periods) has been reported to be a powerful regimen for improving body composition and cardiovascular, metabolic, and performance variables (Gibala et al., 2012). HIIT also reduces both arterial stiffness and microvascular dysfunction in hypertensive individuals (Shrout et al., 2017), and increases the expression of key proteins such as peroxisome proliferator-activated receptor-γ coactivator 1α and vascular endothelial growth factor, that play an important role in arterial remodeling, in trained/healthy subjects (Lim et al., 2012). Moreover, when compared with endurance exercise, HIIT has been shown to improve endothelial function in subjects at risk for HTN (Guimaraes et al., 2010) and also in patients with metabolic syndrome (Tjønna et al., 2008). Despite these beneficial effects from HIIT, it remains unknown whether HIIT impacts BP and comorbidities associated with the prehypertensive state more than normotensive subjects.

Most exercise intervention studies report mean data; however, there is usually wide interindividual variability (Bouchard and Rankinen, 2001). This implies that under the same stimulus, some subjects, referred to as responders (Rs), may achieve benefits, while others, referred to as non-responders (NRs), may exhibit a worsened or unchanged response (Bonafiglia et al., 2016). Although genetic [i.e., some polymorphisms (Yako et al., 2018)] and environmental (regular exercise, diet, and sleep) factors have been described to predictive Rs and NRs (Mann et al., 2014), not all of these factors have been explored in detail, such as the effects of a different 'BP health status' (e.g., prehypertension vs. HTN) on the prevalence of NRs after training. For example, it has been widely known that individuals with HTN show power and clinical decreases in blood pressure after traditional endurance exercise training (Cade et al., 1984). A recent report showed that after 6 weeks of HIIT ∼60% of participants were NRs for a decrease in diastolic BP (DBP) (Higgins et al., 2015). By contrast, after 20 weeks of endurance training a minority of subjects were NRs (12.2%), without any decreases in systolic BP (SBP) (Bouchard et al., 2012). Additionally, when prehypertensive individuals underwent 6 months of different training regimens [i.e., endurance, resistant, or concurrent training (i.e., endurance plus resistant training)], ∼60% were NRs for decreases in SBP or DBP (Moker et al., 2014).

Determining both the effects on and prevalence of NRs after HIIT is important for choosing the appropriate exercise regimen and optimizing responses in different cohorts (e.g., athletes or

Abbreviations: $1RM_{LE}$, one-maximum repetition test of leg extension; 2KMWT, 2 kilometer walking test; BMI, body mass index; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein; HIIT, high-intensity interval training; LDL-C, low-density lipoproteins; NRs, non-responders; NTG, normotensive group; OR, odds ratios; PreHTN, prehypertensive group; Rs, responders; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides.

individuals with risk factors for HTN). Furthermore, knowing which variables can predict more changes after training could be useful to more efficiently determine the time to invest in a particular exercise modality. Along this line, some authors have shown that the magnitude of acute changes from chronic exercise is a good predictor of improvements in metabolism (Hecksteden et al., 2013a) and decreases in BP (Hecksteden et al., 2013b). However, neither of these studies simultaneously reported the effects on and the prevalence of NRs, nor determined the variables that can predict responses in populations at risk for HTN. Several studies have reported that certain factors such as exercise modality (Álvarez et al., 2017), acute exercise (Hecksteden et al., 2013b), baseline differences in aerobic fitness (Bouchard and Rankinen, 2001), or metabolic health status (i.e., healthy or diagnosed with a metabolic disease) (Alvarez et al., 2017) can influence the prevalence of NRs, but all have been conducted mainly with normotensive cohorts. Thus, due to the lack of evidence on the effects on and prevalence of NRs after HIIT among prehypertensive than normotensive subjects, and on the potential predictive factors for lowering BP, the aim of the present study was to investigate both the effects on and the prevalence of NRs for decreased BP and comorbidities among prehypertensive women after HIIT. A secondary aim was to report potential variables that predict decreases in BP. Based on previous genetic studies, where the training variation can be attributed to multiple factors, and considering a non-epidemiological sample size (Mann et al., 2014), we hypothesized that independent of training-induced changes there would be a similar effect from HIIT and a similar prevalence of NRs between prehypertensive and normotensive subjects after a 16-week HIIT intervention.

MATERIALS AND METHODS

Subjects

We studied overweight or obese sedentary women [body mass index (BMI) between 25 and 30 kg/m^2; aged 30–40 years] who had been diagnosed with prehypertension for at least 1 month but no more than 3 months by our research team. The study inclusion criteria were as follows: (a) ambulatory SBP >120 and <140 mmHg and/or DBP ≥80 and <90 mmHg, according to standard classifications of blood pressure (Pescatello et al., 2015); (b) lack of drug therapy in the previous 3 months; (c) BMI >24 and <35 kg/m^2; (d) physical inactivity (i.e., <150 min of low/moderate or <75 min of moderate/vigorous physical activity/week (O'Donovan et al., 2010), as assessed by the International Physical Activity Questionnaire previously validated in the Chilean population) (Seron et al., 2010); and (e) independent of commonly altered metabolic variables (normocholesterolemic/or slightly hypercholesterolemic) [total cholesterol (TC) <200/≥200 mg/dL, low-density lipoprotein cholesterol (LDL-C) <140/≥140 mg/dL, high-density lipoprotein cholesterol (HDL-C) ≤30/>30 mg/dL, and triglycerides (TG) ≤150/>150 mg/dL] following standard classifications (Turnbull et al., 2005). All subjects were not under pharmacological therapy. Subjects with (a) cardiovascular contraindications to exercise; (b) histories of stroke; (c) asthma or chronic obstructive pulmonary disease; (d) musculoskeletal disorders such as muscle or back pain; and (e) a history of smoking in the last 3 months were excluded. A minimum compliance to the exercise program of 70% was required for patients in the intervention group to be included in the final statistical analysis. The trial is registered on ClinicalTrials.gov; ID: NCT03000140 (registered 20 December 2016).

One hundred and ninety-nine healthy and prehypertensive subjects (aged 25 to 40 years) from the Family Healthcare Center Tomas Rojas of Los Lagos (Chile) were invited to participate by phone; these participants were provided with explanations about the study aims (first stage), informed about the study and invited to be formally screened. Subsequently, one hundred and eighty-five (N = 185) subjects agreed to participate in the second stage of screening, the first baseline measurements and the third stage of education regarding the experimental procedures for exercise training. The subjects underwent a structured medical history, medical record review, and physical examination by a physician to assess eligibility based on the criteria. All participants underwent 16 weeks of HIIT by cycling on ergometers and were statistically analyzed as two non-randomized groups: the prehypertensive (PreHTN) and normotensive (NTG) groups. After the intervention period, forty-four subjects were included in the final sample (PreHTN, aged 35.2 ± 5.1 years; BMI 31.9 ± 5.8 kg/m^2; N = 44), and forty subjects were included in the normotensive group (NTG, aged 36.6 ± 5.8 years; BMI 30.0 ± 6.6 kg/m^2; N = 40). The study design is shown in **Figure 1**, and the study protocol is shown in **Figure 2**.

Classification of Responders (Rs) and Non-responders (NRs)

To classify the participants as Rs and NRs based on a decrease in SBP/DBP or the other dependent covariables, the typical error (TE) was calculated, similar to recent studies (Bonafiglia et al., 2016), using the following equation:

$$\mathrm{TE} = SD_{diff} \Big/ \sqrt{2}$$

where SD_{diff} is the variance (standard deviation) of the different scores observed between the two repeats of each test. NRs were defined as an individual who failed to demonstrate a decrease or increase (in favor of beneficial changes) that was greater than two times the TE away from zero. A change beyond two times the TE indicates that there is a high probability (i.e., 12 to 1 odds) that this response is a true physiological adaptation beyond what might be expected to result from technical and/or biological variability (Hopkins, 2000). Thus, the cutoff values were the following for body mass, 2 × TE = 0.500 kg; BMI, 2 × TE = 0.21 kg/m^2; waist circumference, 2 × TE = 1.1 cm; tricipital skinfold, 2 × TE = 1.3 mm; suprailiac skinfold, 2 × TE = 2.6 mm; abdominal skinfold, 2 × TE = 2.9 mm; fat mass, 2 × TE = 3.8%; muscle mass, 2 × TE = 0.3 kg; systolic BP, 2 × TE = 8.0 mmHg; diastolic BP, 4.9 mmHg; heart rate at rest, 2 × TE = 5.4 beats/min; fasting glucose, 2 × TE = 4.5 mg/dL;

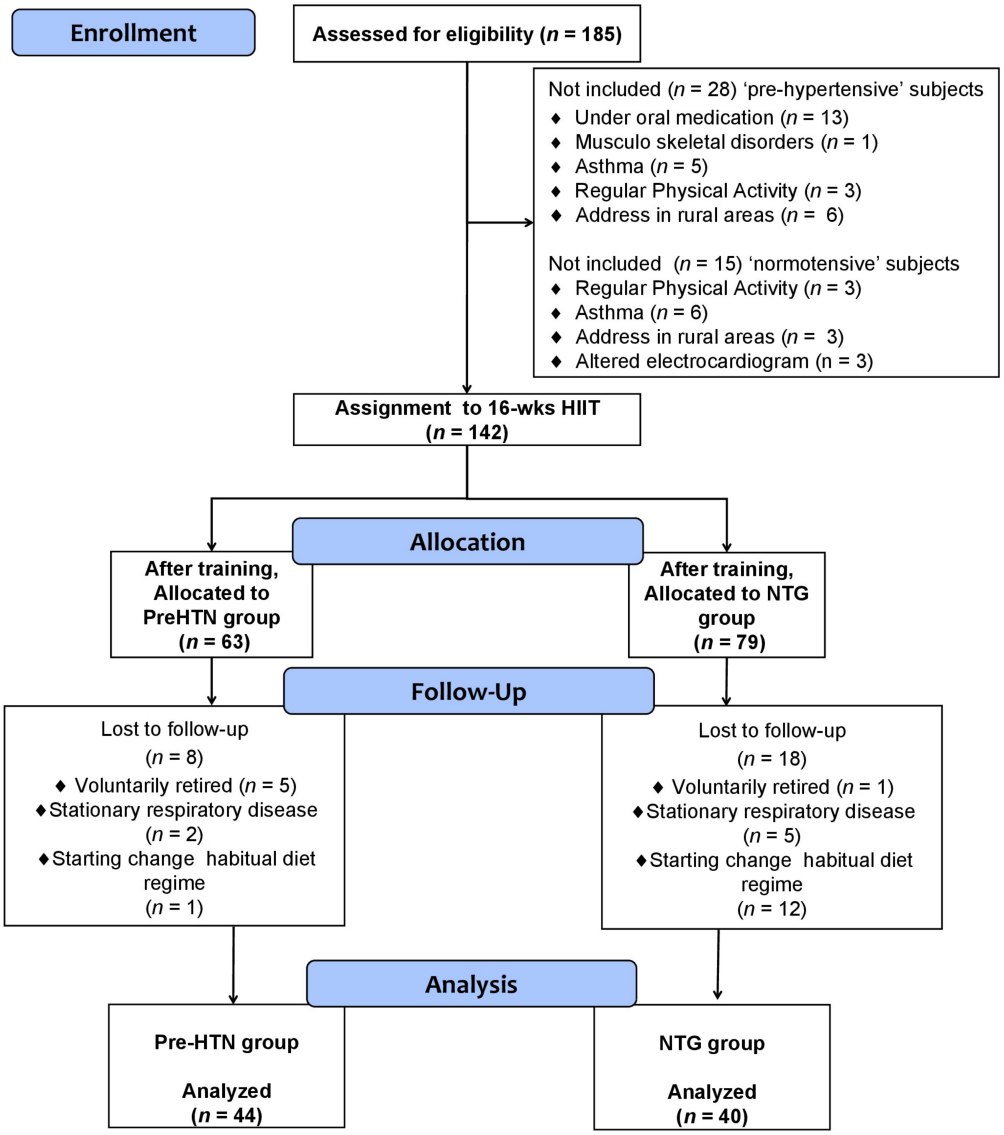

FIGURE 1 | Study design.

TC, 2 × TE = 7.1 mg/dL; LDL-C, 2 × TE = 5.2 mg/dL; HDL-C, 2 × TE = 3.9 mg/dL; TG, 2 × TE = 14.6 mg/dL; $1RM_{LE}$, 2 × TE = 5.0 kg; and 2KMWT, 2 × TE = 1.5 min/s.

HIIT Program

Before the intervention, all subjects were familiarized with the HIIT program over three sessions. The participants underwent a thrice-weekly progressive program for 16 weeks. All exercise sessions were performed on cycle ergometers (OXFORD^{TM}, model BE2601, OXFORD Inc., Santiago, Chile) and were supervised by an exercise physiologist. The HIIT program consisted of high-intensity intervals of work (cycling) for 1 min at a subjective intensity of 8–10 points on the modified Borg scale, which ranges from 1–10 points, similar to other reports (Álvarez et al., 2017) separated by an inactive (no movement on the bicycle) recovery period of 2 min. The training sessions were structured according to the following progression (presented in time cycling/rest/repetitions): weeks 1–4: 1/2/7; weeks 5–8: 1/2/8; weeks 9–12: 1/2/9; and weeks 13–16: 1/2/10. The total range of time investment was as follows: weeks 1–4, 21 min; and weeks 13–16, 30 min/session). Heart rate was continuously monitored among the subjects (ProTrainer 5, Polar Electro, Inc., Kempele, Finland), and their efforts were adjusted to maintain cycling at the subjective effort proposed. Thus, when a subject reported starting the first interval of cycling at 8–10 points on the Borg scale (corresponding to a 100-watt load, for example), this level was rechecked at each of the 3 sessions, and it was usually necessary to increase the load (watts) of cycling to maintain an initial intensity of cycling at 8–10 points, according to the normal exercise adaptations to a new threshold. This subjective intensity

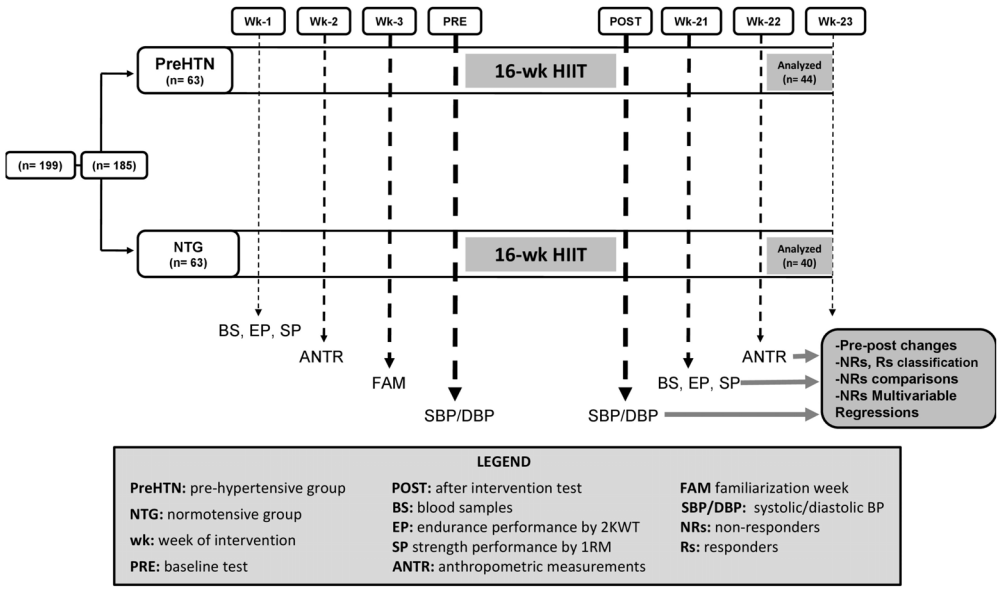

FIGURE 2 | Study protocol.

corresponded with all intervals of work to a range from 70–100% of the maximum heart rate based on age.

Exercise Training Compliance

Compliance indices for attendance were calculated for the groups by dividing the total number of sessions attended by the number of training sessions prescribed in percentages. Intensity adherence was calculated from the mean 8 ± 2 points on the Borg modified scale (1–10) based on the cycling ratings across all exercises and sessions for weeks 2–16. The results showed that exercise compliance was 85.1% (41 sessions) in the PreHTN group and 93.7% (45 sessions) in the NTG, and there were no significant differences between groups.

Assessments

Body Composition Assessment

For three sessions prior to the start of the pediment, participants were familiarized with the tests, and 1 week before and after the 16-week follow-up, anthropometric, cardiovascular, metabolic, and performance measurements were obtained. Body mass (kg) was measured (to the nearest 0.1 kg) using a professional scale (Health o Meter^{TM} Professional, Sunbeam Products, Inc., Chicago, IL, United States). Height (m) was assessed using the same machine to the nearest 0.1 m of accuracy, similar to previous studies (Jebb et al., 2007). BMI was calculated as body mass divided by height squared (kg/m^2). Waist circumference (cm) was measured to the nearest 0.1 cm using a flexible and unextendible measuring tape (Hoechstmass^{TM}, Sulzbach, Germany). Four skinfold thickness measurements were obtained (tricipital, suprailiac, subscapular, and abdominal) using a Langue^{TM} caliper (Beta Technology, Inc., Santa Cruz, CA, United States) by a professional, and the same evaluator made the measurements in both the pre- and posttest stages following the standard protocols (Marfell-Jones et al., 2006). The percent (%) fat mass and % muscle mass were assessed by bioimpedance using a digital scale (Omron HBF-INT^{TM}, Omron Healthcare, Inc., Lake Forest, IL, United States). This procedure was conducted without metal or watches on the body to increase precision, and the average of three measurements was used.

Cardiovascular Health Assessment

Ambulatory SBP and DBP were measured using an automatic monitor (Omron^{TM} HEM 7114^{TM}, Omron Healthcare, Inc., Lake Forest, IL, United States) in triplicate (2-min interval between measurements) with subjects in a seated position after they had rested for 15 min. The average data were recorded for each participant based on standard classification procedures (Mancia et al., 2013). Heart rate at rest was measured using a monitor (ProTrainer 5^{TM}, Polar Electro Inc., Kempele, Finland) after at least 15 min of rest.

Strength Performance Assessment

Strength performance was assessed 1 week before and after the intervention using the one-repetition maximum leg extension ($1RM_{LE}$) test, which was implemented according to similar procedures previously described (Álvarez et al., 2017). The $1RM_{LE}$ was performed using an exercise machine (OXFORD^{TM}, model EE4002, Santiago, Chile) in the morning between 9 and

11 o'clock. The highest load of three attempts was reported as an average value.

Endurance Performance Assessment

On the third day, after 2 days of familiarization, the endurance performance test was conducted. The test involved a 2-km walking test (2KMWT) (Álvarez et al., 2017) in an indoor sports court (100-m track) after a 10-min warm-up of walking at low intensity and performing slow movements involving the knee and ankle joints. The subjects were instructed to walk as fast as possible with a steady pace and were warned not to run. Heart rate was continuously monitored (ProTrainer 5^{TM}, Polar Electro Inc., Kempele, Finland) during the test. To ensure an accurate test, participants were encouraged to walk faster if their heart rate was lower than 70% of the maximum heart rate based on age. The time spent on the 2KMWT and the average heart rate during the 2KMWT were registered and used for the analysis.

Blood Analyses

Blood samples (4 ml) were collected before and after the 16-week follow-up in the morning and after a 10-h overnight fast. Posttraining blood sampling of the subjects was performed at least 48 h after the last exercise session to avoid any acute effects of exercise. The samples were placed on ice and centrifuged at 4000 rpm for 5 min at 4°C. Plasma samples were immediately transferred to prechilled microtubes and stored at −20°C for subsequent analysis. Plasma glucose was analyzed by enzymatic methods using standard kits (Wiener Lab, Inc., Rosario, Argentina) and an automatic analyzer (Metrolab 2300 Plus^{TM}, Metrolab Biomed, Inc., Buenos Aires, Argentina). TC, HDL-C and TG were analyzed using an enzymatic calorimetric method (Diagnostica mbH, Alemania). LDL-C was calculated using the Friedewald equation (Friedewald et al., 1972).

Statistical Analyses

Data are presented as the mean ± standard deviation (SD). Normality and homoscedasticity assumptions for all data were checked using the Shapiro-Wilk and Levene tests. Wilcoxon's test was used for non-parametric data. Student's *t*-test was performed to test for differences between groups at baseline and for each delta between groups. In addition, the non-parametric Wilcoxon test was applied for variables with non-normal distribution (TC and 2KMWT). To reduce within-group variability, a univariate test (ANCOVA) was performed for SBP and DBP as main variables using anthropometric covariables. Repeated-measures ANOVA was used to make comparisons based on group and time. After the intervention, delta values (Δ) were calculated in each dependent variable. Subjects were categorized as Rs and NRs according to the previously mentioned criteria (Hopkins, 2000). Bonferroni *post hoc* test was applied to determine the differences between groups. To test for differences between the prevalence of NRs in the PreHTN group and NTG, the Chi-Square test (X^2) was used for categorical variables. The odds ratios (ORs) of NRs to HIIT were applied for both NR variables between groups, with an OR ≥2 indicating a high risk of being an NR. Finally, five different models (Model 1, based on only SBP at baseline; Model 2, based on SBP at baseline plus body composition changes; Model 3, based on SBP at baseline plus body composition and cardiovascular changes; Model 4, based on SBP at baseline plus body composition, cardiovascular, and metabolic changes; and Model 5, based on SBP at baseline plus body composition, cardiovascular, metabolic, and performance changes) were applied in order to predict the SBP changes. All statistical analyses were performed with SPSS software version 18 (SPSS^{TM} Inc., Chicago, IL, United States). The alpha level was fixed at $P < 0.05$ for all tests of statistical significance.

RESULTS

Baseline Measurements

As expected based on the study design, there were significant differences in SBP (128 ± 6 vs. 108 ± 5 mmHg, $P = 0.003$) and DBP (85 ± 8 vs. 75 ± 10 mmHg, $P = 0.007$) at baseline between the PreHTN group and NTG (**Table 1**). There were no baseline differences in the other dependent anthropometric, cardiovascular, metabolic and performance covariables between groups (**Table 1**).

Training-Induced Changes in Body Composition Outcomes

After training, in the PreHTN group, there was a significant decrease in body mass (Δ −3.3 kg, $P < 0.0001$), BMI (Δ −1.4 kg/m^2, $P < 0.0001$), and waist circumference (Δ −2.9 cm, $P < 0.0001$); the NTG also showed similar decreases in body mass (Δ −2.0 kg, $P < 0.0001$), BMI (Δ −0.5 kg/m^2, $P < 0.05$), and waist circumference (Δ −4.2 cm, $P < 0.0001$). Significant differences in waist circumference (Δ −1.3 cm, $P = 0.037$) were observed between groups (**Table 1**). Subcutaneous skinfold measurements, including tricipital (Δ −4.6 mm, $P < 0.05$ and Δ −4.8 mm, $P < 0.05$), suprailiac (Δ −7.3 mm, $P < 0.001$ and Δ −5.6 mm, $P < 0.001$), and abdominal (Δ −11.5 mm, $P < 0.0001$ and Δ −7.6 mm, $P < 0.0001$) skinfold measurements, were similarly decreased in the PreHTN group and NTG. Significant differences between groups were detected only in abdominal skinfold thickness (Δ −3.9 mm, $P < 0.001$) (**Table 1**). The % fat mass was reduced in both the PreHTN group and NTG (Δ −5.8%, $P < 0.0001$; and Δ −3.8%, $P < 0.001$) (**Table 1**). The % muscle mass was not different between groups (**Table 1**).

Training-Induced Changes in Cardiovascular Outcomes

SBP was significantly decreased in the PreHTN group (Δ −8 mmHg, $P < 0.0001$) (**Table 1**); in contrast, the NTG showed no changes in SBP (**Table 1**). There was a significant difference between groups in the change in SBP (Δ −8 vs. + 3.0 mmHg, $P < 0.003$) (**Table 1**). DBP was significantly decreased in the PreHTN group (Δ −5.8 mmHg, $P < 0.0001$) compared to the NTG, and there was a significant difference in the change in DBP (−5.8 vs. −2.0 mmHg, $P = 0.007$) between

TABLE 1 | Characteristics of the sample before and after the 16-weeks follow-up.

	PreHTN Pre	PreHTN Post	Δ	NTG Pre	NTG Post	Δ	PreHTN vs. NTG Baseline†	ΔPreHTN vs. ΔNTG Pre-post†
n	44			40				
Age (y)	35.2 ± 5.1			36.6 ± 5.8			0.572	
Height (m)	1.58 ± 5.6			1.59 ± 6.1				
Time elapsed from diagnosis (y)	1.5			1.2				
Anthropometry								
Body mass (kg)	79.8 ± 14.8	76.4 ± 14.1***	−3.3 ± 2.9[&]	75.2 ± 12.3	73.2 ± 11.8***	−2.0 ± 2.5	$P = 0.131$	$P = 0.091$
Body mass index (kg/m^2)	31.9 ± 5.8	30.5 ± 5.7***	−1.4 ± 1.0	30.0 ± 6.6	29.5 ± 6.4*	−0.5 ± 1.9	$P = 0.182$	$P = 0.167$
Waist circumference (cm)	101.5 ± 11	98.5 ± 12***	−2.9 ± 5.1[&]	98.0 ± 10.7	93.8 ± 10.3***	−4.2 ± 2.9	$P = 0.145$	**$P = 0.037$**
Tricipital skinfold (mm)	26.7 ± 8.2	22.1 ± 6.6*	−4.6 ± 5.5	24.9 ± 7.2	20.1 ± 8.5*	−4.8 ± 5.1	$P = 0.234$	$P = 0.233$
Supra-iliac skinfold (mm)	34.5 ± 9.1	27.2 ± 8.8**	−7.3 ± 5.4	32.5 ± 6.2	26.9 ± 7.3**	−5.6 ± 5.0	$P = 0.189$	$P = 0.246$
Abdominal skinfold (mm)	44.9 ± 8.1	33.4 ± 9.8***	−11.5 ± 6.3[¥]	43.1 ± 7.2	35.5 ± 4.1***	−7.6 ± 5.9	$P = 0.156$	**$P < 0.001$**
Fat mass (%)	42.3 ± 6.8	36.5 ± 8.1***	−5.8 ± 6.2	40.6 ± 7.1	36.8 ± 8.4**	−3.8 ± 5.5	$P = 0.138$	$P = 0.110$
Muscle mass (%)	21.3 ± 7.5	21.5 ± 9.9	+0.2 ± 1.5	22.4 ± 5.9	22.7 ± 9.4	+0.3 ± 1.7	$P = 0.119$	$P = 0.188$
Cardiovascular								
Systolic blood pressure (mmHg)	128.0 ± 6.0	120.0 ± 6.0***	−8.0 ± 7.0[$]	108.0 ± 5.0	111.0 ± 8.0	+3.0 ± 9.8	**$P = 0.003$**	**$P < 0.0001$**
Diastolic blood pressure (mmHg)	85.0 ± 8.0	80.0 ± 10.0***	−5.8 ± 11.7[¥]	75.0 ± 10.0	73.0 ± 10.0	−2.0 ± 4.9	**$P = 0.007$**	**$P < 0.001$**
Heart rate rest (beats/min)	84.0 ± 6.0	80.0 ± 8.0***	−4.0 ± 6.0	82.0 ± 6.0	80.0 ± 8.0	−2.0 ± 5.0	$P = 0.078$	$P = 0.079$
Metabolic								
Fasting glucose (mg/dL)	98.0 ± 9.0	95.0 ± 8.0*	−3.3 ± 9.0[¥]	95.0 ± 9.0	90.0 ± 7.0**	−5.0 ± 5.8	$P = 0.078$	**$P < 0.01$**
Total cholesterol (mg/dL)	190.0 ± 43.0	182.0 ± 33.0	−8.0 ± 25.2	194.0 ± 32.0	180.0 ± 20.0**	−14.0 ± 29.6	$P = 0.652$[‡]	$P = 0.062$[‡]
Low-density lipids (mg/dL)	109.0 ± 37.0	106.0 ± 32.0	−2.6 ± 18.7[¥]	120.0 ± 27.0	110.0 ± 20.0**	−10.0 ± 21.7	$P = 0.114$	**$P < 0.01$**
High-density lipids (mg/dL)	48.0 ± 11.0	53.0 ± 10.0**	5.0 ± 6.6	53.0 ± 10.0	54.0 ± 9.0	+1.0 ± 10.1	$P = 0.076$	$P = 0.221$
Triglycerides (mg/dL)	130.0 ± 61.0	117.0 ± 45.0*	−13.9 ± 30.9[&]	133.0 ± 50.0	111.0 ± 32.0***	−22.0 ± 36.4	$P = 0.132$	**$P = 0.045$**
Strength performance								
$1RM_{LE}$ (kg)	36.0 ± 7.0	39.0 ± 9.0	+3.0 ± 4.0	33.0 ± 7.0	35.0 ± 8.0	+2.0 ± 6.0	$P = 0.088$	$P = 0.153$
Endurance performance								
2KMWT (min.s)	23.47 ± 3.2	19.33 ± 3.3***	−3.14 ± 3.44	23.11 ± 4.4	20.18 ± 4.5***	−3.34 ± 3.41	$P = 0.783$[‡]	$P = 0.328$[‡]

*Results presented as mean ± SD. PreHTN, pre hypertensive group; NTG, normotensive group; Δ, delta pre-post changes according to each biological unit of assessment; $1RM_{LE}$, one maximum repetition of leg-extension strength test; 2KMWT, 2 kilometers walking test. *$P < 0.05$, **$P < 0.001$, ***$P < 0.0001$ post vs. pre intervention within-group. †Compared by the Student t-test. ‡Compared by the Wilcoxon non-parametric test. [&]$P < 0.05$, [¥]$P < 0.001$, $P < 0.0001$ and [$]compared delta changes by student t-test. Bold values denotes significant differences at baseline or at pre-post changes at each specific value $P < 0.05$.*

groups (**Table 1**). A significant reduction in heart rate at rest was detected in the PreHTN group (Δ −4 beats/min, $P < 0.0001$) but not in the NTG (**Table 1**).

Training-Induced Changes in Metabolic Outcomes

Fasting glucose was significantly decreased in both the PreHTN group and NTG (Δ −3.3 mg/dL, $P < 0.05$ and Δ −5 mg/dL, $P < 0.001$), with significant differences between groups (Δ 1.7 mg/dL, $P < 0.01$) (**Table 1**). TC and LDL-C were significantly decreased in the NTG (Δ −14 mg/dL and Δ −10 mg/dL, $P < 0.001$) (**Table 1**). There were significant differences in the changes in LDL-C (Δ + 7.4 mg/dL $P = 0.01$) between groups (**Table 1**). HDL-C was significantly increased (Δ + 5.0 mg/dL, $P < 0.001$) in the PreHTN group, while the NTG showed no significant changes in HDL-C (**Table 1**). TG levels were significantly reduced in both the PreHTN group and NTG (Δ −13.9 mg/dL, $P < 0.05$ and Δ −22 mg/dL, $P < 0.0001$), with significant differences between groups (Δ 8.1 mg/dL, $P = 0.045$) (**Table 1**).

Training-Induced Changes in Performance Outcomes

There were no changes in the $1RM_{LE}$ strength test in either group (**Table 1**). However, there were significant improvements in endurance performance, with decreases in the time spent on the 2KMWT in both the PreHTN group and NTG (Δ −3.14 min and Δ −3.34 min, $P < 0.0001$) (**Table 1**).

Prevalence of NRs After HIIT Exercise Training

There were significant differences in the prevalence of NRs in the PreHTN group vs. the NTG based on the following variables: BMI (13.6 vs. 40.0%, $P = 0.006$), abdominal skinfold (6.8 vs. 15.0%, $P < 0.0001$), LDL-C (72.7 vs. 50.0%, $P = 0.032$), HDL-C (56.8 vs. 77.5%, $P = 0.045$), and TG (70.5 vs. 35.0%, $P < 0.001$) (**Table 2**). The risk (based on OR: 95% CI) of no response was high (≥2-fold) in the PreHTN group for LDL-C (OR 2.6: 1.0 to 6.6) and TG (OR 4.4: 1.7 to 11) (**Table 2**).

TABLE 2 | Prevalence of non-responders by health status (i.e., prehypertensive and normotensive subjects) after intervention.

	Response	PreHTN	NTG	OR (95% IC)	PreHTN vs. NTG X^2
Anthropometry					
	Rs	88.6 (39)	77.5 (31)		
Body mass, %/(*n*=)	NRs	11.4 (5)	22.5 (9)	0.4 (0.1 to 1.4)	$P = 0.171$
Body mass index, %/(*n*=)	NRs	13.6 (6)	40.0 (16)	0.2 (0.08 to 0.6)	**$P = 0.006$**
	Rs	86.4 (38)	60.0 (24)		
Waist circumference, %/(*n*=)	NRs	4.5 (2)	15.0 (6)	0.2 (0.05 to 1.4).	$P = 0.103$
	Rs	95.5 (42)	85.0 (34)		
Tricipital skinfold, %/(*n*=)	NRs	9.0 (4)	0 (0)	0.8 (0.2 to 3.2)	$P = 0.255$
	Rs	90.9 (40)	100 (40)		
Supra-iliac skinfold, %/(*n*=)	NRs	11.3 (5)	12.5 (5)	0.5 (0.2 to 1.8)	$P = 0.331$
	Rs	88.6 (39)	87.5 (35)		
Abdominal skinfold, %/(*n*=)	NRs	6.8 (3)	15.0 (6)	1.5 (0.9 to 3.2)	**$P < 0.0001$**
	Rs	93.1 (41)	85.0 (34)		
Fat mass, %/(*n*=)	NRs	20.4 (9)	20.0 (8)	0.6 (0.1 to 1.1)	$P = 0.651$
	Rs	79.5 (35)	80.0 (32)		
Muscle mass, %/(*n*=)	NRs	97.7 (43)	100 (40)	0.1 (0.1 to 0.9)	$P = 0.288$
	Rs	2.2 (1)	0 (0)		
Cardiovascular					
Heart rate rest, %/(*n*=)	NRs	29.5 (13)	37.5 (15)	0.7 (0.2, 1.7)	$P = 0.322$
	Rs	70.4 (31)	62.5 (25)		
Metabolic					
Fasting glucose, %/(*n*=)	NRs	65.9 (29)	70.0 (28)	0.8 (0.3 to 2.0)	$P = 0.668$
	Rs	34.1 (15)	30.0 (12)		
Total cholesterol, %/(*n*=)	NRs	61.4 (27)	52.5 (21)	1.4 (0.6 to 3.4)	$P = 0.412$
	Rs	38.6 (17)	47.5 (19)		
Low-density lipids, %/(*n*=)	NRs	72.7 (32)	50.0 (20)	2.6 (1.0 to 6.6)[#]	**$P = 0.032$**
	Rs	27.3 (12)	50.0 (20)		
High-density lipids, %/(*n*=)	NRs	56.8 (25)	77.5 (31)	0.3 (0.1 to 0.9)	**$P = 0.045$**
	Rs	43.2 (19)	22.5 (9)		
Triglycerides, %/(*n*=)	NRs	70.5 (31)	35.0 (14)	4.4 (1.7 to 11.0)[#]	**$P = 0.001$**
	Rs	29.5 (13)	65.0 (26)		
Strength performance					
$1RM_{LE}$, %/(*n*=)	NRs	54.5 (24)	67.5 (27)	0.3 (0.1 to 1.8)	$P = 0.466$
	Rs	45.4 (20)	32.5 (13)		
Strength performance					
2KMWT, %/(*n*=)	NRs	15.9 (7)	25.0 (10)	0.9 (0.5, 2.5)	$P = 0.121$
	Rs	84.0 (37)	75.0 (30)		

PreHTN, pre hypertensive group; NTG, healthy group; $1RM_{LE}$, one maximum repetition strength test; 2KMWT, 2 kilometers walking test; OR, odds ratios (95% IC). [#]High risk (≥2 fold) to suffer a non-response. Bold values denote significant difference in the NRs prevalence between groups at level $P < 0.05$.

Figure 3A shows the delta values for individual changes in SBP (ΔSBP in mmHg) in the PreHTN group, in which the prevalence of NRs was 54.5% (24 patients). **Figure 3B** shows the delta values for individual changes (ΔSBP in mmHg) in the NTG, in which the prevalence of NRs was 92.5% (37 patients). There was a significant difference in the prevalence of NRs based on SBP between the PreHTN group and NTG (54.5 vs. 92.5%, $P < 0.0001$) (**Figures 3A,B**).

Figure 3C shows the delta of individual changes in DBP (ΔDBP in mmHg) in the PreHTN group, in which the prevalence of NRs was 54.5% (24 patients). **Figure 3D** shows the delta of individual changes in DBP (ΔDBP in mmHg) in the NTG, in which the prevalence of NRs was 85.0% (34 patients). There was a significant difference in the prevalence of NRs based on DBP between the PreHTN group and NTG (54.5 vs. 85.0%, $P < 0.0001$) (**Figures 3A,D**).

Table 3 shows the five models used to predict a response in SBP, where model 3 (based on baseline SBP + body composition and cardiovascular measurements), model 2 (based on baseline SBP + body composition measurements), and model 1 (based on only baseline SBP), which we named previously as 'health status,' could explain, respectively, 53.8, 52.3, and 51.2% of the total variance in SBP changes after training. Model 5, in which performance variables were added, explained a significant 25.5% of the total variance in SBP changes (**Table 3**).

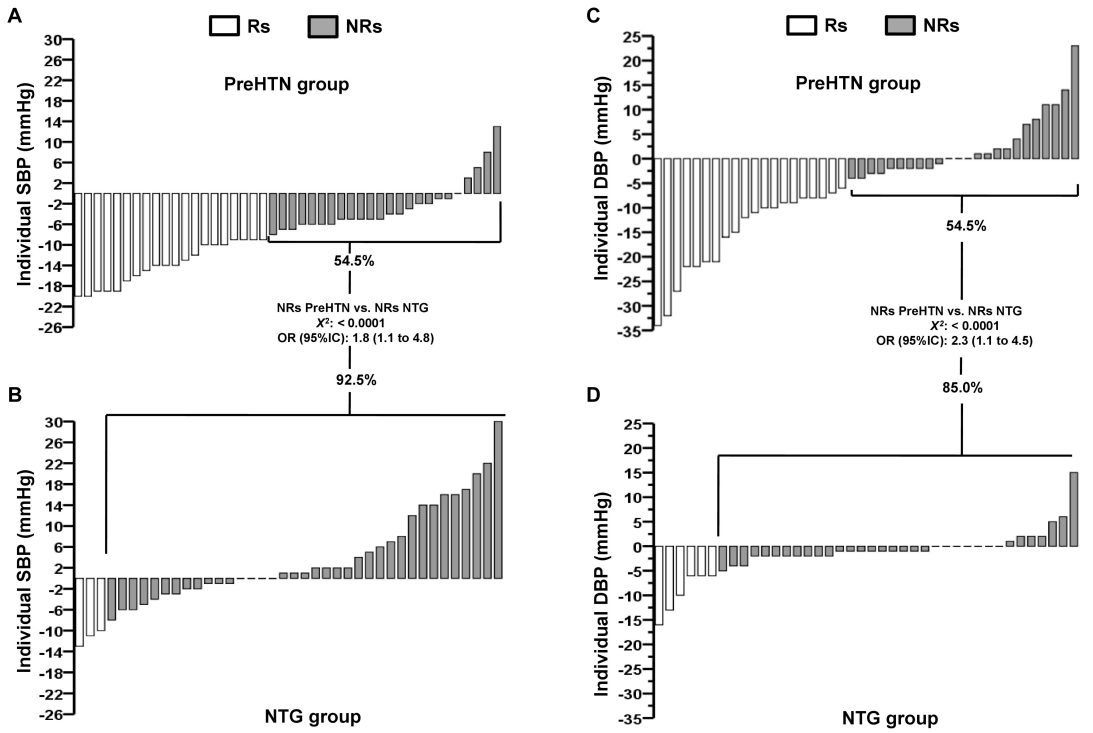

FIGURE 3 | Individual changes for systolic blood pressure in **(A)** prehypertensive and **(B)** normotensive, and individual changes for diastolic blood pressure in **(C)** prehypertensive and **(D)** normotensive subjects after 16-weeks of HIIT. PreHTN, prehypertensive group; NTG, normotensive subjects; SBP, systolic blood pressure; DBP, diastolic blood pressure; Rs, responders; NRs, non-responders; OR, odds ratios.

DISCUSSION

This study has two main findings: (*i*) the prevalence of NRs based on SBP, DBP, and other comorbidities is different between groups; and (*ii*) between the 5 tested models (from the most basic to the most complex) for BP reduction, baseline SBP alone (model 1), referred to as 'BP-health status' by us, is the simplest model with similar (e.g., model 2; 52.3%, model 3; 53.8%, both $P < 0.0001$) efficacy for predicting Rs with a predictive capacity in SBP (51.2%) after 16 weeks of HIIT, when compared with other more complex models that included added outcomes. Decreases in SBP were accompanied by other known effects of exercise, but not in both cohorts. Accordingly, HIIT decreased both SBP (Δ −8 mmHg) and DBP (Δ −5.8 mmHg) only in prehypertensive subjects, whereas improvements in anthropometric/body composition, metabolic and endurance performance were observed in both groups.

Contrary to our hypothesis, the prevalence of NRs between prehypertensive and normotensive subjects after HIIT was different, whereas changes in anthropometric, metabolic, and endurance performance were almost similar. However, there is little evidence at present regarding the prevalence of NRs in terms of changes in SBP/DBP after HIIT (Higgins et al., 2015; Alvarez et al., 2017). Including other training methods, such as endurance or resistance training (Bouchard et al., 2012; Moker et al., 2014), would help confirm which training modality is more appropriate for decreasing BP with fewer NRs. For example, in ∼1600 subjects after 20 weeks of endurance training (30–50 min/session, 3 days/week, 55–75% of the maximum oxygen uptake), 12.2% of subjects were considered NRs based on decreased SBP (Bouchard et al., 2012). After 6 months of endurance (65–80% peak of oxygen uptake, walking/jogging), resistance (8–12 repetitions, 8 exercises, 70–85% of one-maximum repetition, 3 days/week) or combined training, ∼60.9% of subjects were NRs based on decreased SBP and ∼59.1% of subjects were NRs based on decreased DBP (Moker et al., 2014). Furthermore, 6 weeks of HIIT in adults (3 × 1 min maximum intensity with 2 min recovery, 3 days/week) resulted in ∼61.5% NRs in terms of decreased DBP (Higgins et al., 2015). We found that among prehypertensive subjects, 11.4% were NRs for decreased SBP and 31.8% were NRs for decreased DBP. By contrast, the prevalence of NRs based on decreased SBP and DBP in the NT group was greater at 68.8 and 35.0%, respectively. Additionally, among 23 healthy adults who underwent 6 weeks of HIIT (Higgins et al., 2015) a wide interindividual variability was reported, in which Rs showed a decrease in SBP of ∼10 mmHg, and NRs showed an increase in SBP of 10 mmHg.

Interestingly, in terms of BP effects, 30 min of moderate-intensity exercise/day in combination with resistance training for a cumulative 150 min/week decreased BP by 5–7 mmHg in individuals with HTN (Pescatello et al., 2015). Here we report that 16 weeks of HIIT at a low volume of exercise/week (∼21–30 min/week) is able to decrease BP (SBP −8 mmHg and DBP −5.8 mmHg) in a prehypertensive cohort to similar values as for

TABLE 3 | Percentage of total variance explained by 5 models of factors predictive SBP decreases.

	Variables included in the model	R	Total model R^2	% Variance explained by the model	*P*-value
Model 1	Baseline SBP (mmHg)	0.715	0.512	51.2%¶	***P* < 0.0001**
Model 2	Baseline SBP (mmHg)	0.723	0.523	52.3%¶	***P* < 0.0001**
	+*Body composition*; Δbody mass (kg), Δwaist circumference (cm), Δtricipital skinfold (mm), Δsupra-iliac skinfold (mm), ΔAbdominal skinfold (mm), Δ % fat mass (%), and Δ % muscle mass (%)				
Model 3	Baseline SBP (mmHg)	0.734	0.538	53.8%¶	***P* < 0.0001**
	+*Body composition*; Δbody mass (kg), Δwaist circumference (cm), Δtricipital skinfold (mm), Δsupra-iliac skinfold (mm), ΔAbdominal skinfold (mm), Δ % fat mass (%), Δ % muscle mass (%), +*Cardiovascular;* Δ heart rate at rest (beats/min)				
Model 4	Baseline SBP (mmHg)	0.435	0.189	18.9%	$P = 0.072$
	+ *Body composition*; Δbody mass (kg), Δwaist circumference (cm), Δtricipital skinfold (mm), Δsupra-iliac skinfold (mm), ΔAbdominal skinfold (mm), Δ % fat mass (%), Δ % muscle mass (%), + *Cardiovascular;* Δ heart rate at rest (beats/min). + *metabolic;* Δ TC, Δ LDL-C, ΔHDL-C, and ΔTG.				
Model 5	Baseline SBP (mmHg)	0.523	0.255	25.5%	***P* < 0.05**
	+ *Body composition*; Δbody mass (kg), Δwaist circumference (cm), Δtricipital skinfold (mm), Δsupra-iliac skinfold (mm), ΔAbdominal skinfold (mm), Δ % fat mass (%), Δ % muscle mass (%), + *Cardiovascular;* Δ heart rate at rest (beats/min) + *metabolic;* Δ TC, Δ LDL-C, ΔHDL-C, ΔTG + *strength and endurance performance;* $\Delta 1RM_{LE}$ (kg), Δ2KMWT (min.s)				

SBP, systolic blood pressure; Δ, delta changes to each body composition, cardiovascular, metabolic outcome; TC, total cholesterol, LDL-C, low-density lipids, HDL-C, high-density lipids, TG, triglycerides. Bold values denote significant correlation. ¶ denotes the most strongest (>50% variance explained) models predictors of SBP changes after 16-weeks of HIIT.

endurance or resistance training, but with less time investment. The evidence supporting endurance exercise for normalizing BP is not novel (Cade et al., 1984); however, to the best of our knowledge, the evidence for HIIT in decreasing BP has been little explored at all in prehypertensive or hypertensive populations. After 12 weeks of HIIT and endurance training, HIIT decreased the average 24-h ambulatory SBP of hypertensive subjects by 12 mmHg, whereas endurance training decreased it by only 4.5 mmHg (Molmen-Hansen et al., 2012). Similarly, the ambulatory 24-h DBP was decreased by 8 mmHg after HIIT and by 3.5 mmHg after endurance training, leading the authors to conclude that the decrease in BP among patients with HTN is intensity-dependent. In another study, after 16 weeks of HIIT, SBP and DBP were decreased in hypertensive patients by 6 and 4 mmHg, respectively, but these measures were not markedly changed in normotensive patients, with decreases of ∼1 mmHg for both variables (Guimaraes et al., 2010), which is similar to our findings. Moreover, after 12 weeks of HIIT in healthy men, SBP decreased by 18 mmHg (Nybo et al., 2010). Thus, it is not surprising that short HIIT programs (Whyte et al., 2010) lasting only 2 weeks showed decreases in SBP of 6 mmHg and in DBP of 9 mmHg among prehypertensive individuals. The mechanisms by which HIIT leads to decreases in BP have not been fully elucidated, but it has been reported that a combination of factors could be involved, including increasing shear stress, decreasing sympathetic nervous activity, reducing vascular peripheral resistance, and increasing nitric oxide-mediated vasodilatation (Halliwill, 2001). In our study, although the BP increases at the individual level were high in both groups, they were higher in the NT group than in the PreHTN group (**Figure 2**). A report from an epidemiological study has shown increases in SBP of ∼10 mmHg as an adverse response to exercise (Bouchard et al., 2012). Here, we report, using an experimental approach, increases in SBP by ∼30 to 15 mmHg in the normotensive group and by 14 to 20 mmHg in the HTN group, indicating the relevance of reporting data not only as the 'mean,' but also at the interindividual level using a typical sample size (∼20–40 subjects) for experimental studies.

In our study, other anthropometric/body composition effects from HIIT included body mass decreases (−3.3 and −2.0 kg), BMI (−1.4 and −0.5% kg/m^2), tricipital (−4.6 and −4.8 mm), and suprailiac (−7.3 and −5.6 mm) skinfold thickness, and fat mass (−5.8 and −3.8%), which were similar in both the prehypertensive and hypertensive groups. Other authors have reported similar results after 2 weeks of HIIT (Whyte et al., 2010; Boutcher, 2011), and these findings were corroborated with molecular changes after HIIT (Little et al., 2011). For example, in the study of Whyte et al. (2010), 6 sessions of 30 s of 'all out' exercise were shown to decrease body mass by 1 kg and waist circumference by 2.4 cm. In our previous study using 16 weeks of HIIT, we found a 1.6 kg decrease in body mass, 4.1 cm decrease in waist circumference, and ∼20% decrease in subcutaneous fat in patients with type 2 diabetes (T2DM) (Alvarez et al., 2016). Other authors, however, have not observed decreases in body mass after 12 months of HIIT in adolescents (Tjønna et al., 2009). Additionally, in the present study, for some variables we found significant differences between groups in the magnitude of change, including waist circumference (∼1 cm, $P = 0.037$) and abdominal skinfolds (∼4 mm, $P < 0.0001$). We presume that these effects would be in addition to a similar general

effect of HIIT. Fat mass, for example, was decreased similarly between groups; thus, we speculate that the same specific (not measured) molecular mechanisms accrued as a result of the HIIT protocol, and that any small differences in the magnitude of changes between groups may have been influenced by the anthropometric and/or the BP differences between groups at baseline (pre-HIIT). The weight loss, and other fat markers such as waist circumference and subcutaneous skinfold thickness, as well as postexercise adrenergic mechanisms, are relatively well known and described (Boutcher, 2011).

Fasting glucose was decreased after HIIT in both groups, with a more pronounced decrease in the NT group (∼5 mg/dL) over the PreHTN group (∼3.3 mg/dL). Our previous study reported that 16 weeks of HIIT resulted in a decrease in fasting glucose of ∼15% in T2DM patients (Alvarez et al., 2016), showing more pronounced benefits in the same time period than in our present non-diabetic sample. However, we also reported in this study that there were decreases in TG in both groups, as well as TC and LDL-C in the NT group, and increases in HDL-C in the prehypertensive cohort. Interestingly, TC, LDL-C, and TG were decreased by a greater magnitude in the NT group than in the PreHTN group. The additional metabolic benefits of HIIT, including improving dyslipidemia, alongside the benefits on BP are considered relevant for decreasing/preventing comorbidities in prehypertensive populations. Reduced risk of HTN in populations with dyslipidemia is related to higher physical activity levels than the minimal recommended activity level in current guidelines (Williams and Franklin, 2015). In the present study, the HIIT program had a weekly time commitment of ∼60 to ∼90 min divided into three exercise sessions (∼21–30 min/session), which was lower than the minimum 150 min/week of activity recommended in current guidelines (O'Donovan et al., 2010). For example, in T2DM patients, decreases of 2.1/0.9 mmHg in BP reduced the risk of major cardiovascular events by 10% (Turnbull et al., 2005), whereas the risk of developing coronary artery disease was reduced by 2–3% for each 1 mg/dL increase in HDL-C (Maron, 2000). Thus, the 8 mmHg reduction in systolic BP and the ∼5 mg/dL increase in HDL-C observed in prehypertensive women in the present study may have clinical implications. The mechanisms by which HIIT decreases plasmatic lipoprotein levels are unclear, but we presume that a decrease in the intramyocellular fat in the liver could play a role (Heijden et al., 2010).

A similar 16-week HIIT program was shown to improve endurance performance, similarly decreasing the time needed to complete the 2KMWT in T2DM patients by 2 min (Alvarez et al., 2016). There is strong evidence that HIIT increases endurance performance (Gibala et al., 2012), which is corroborated by the findings in our prehypertensive cohort. Twelve weeks of HIIT have been reported to increase the maximum oxygen uptake ∼13%, similar/or more than traditional endurance training of ∼7%, with this outcome considered as a performance marker frequently related with health and disease (Nybo et al., 2010).

Multiple regressions analyses of baseline SBP (model 1) and baseline SBP associated with one (anthropometric, model 2), two (cardiovascular, model 3), three (metabolic, model 4), and four (performance, model 5) additional parameters showed that baseline SBP (model 1) explained a similar percentage of variance (51.2%) to that of the more complex models tested (model 2: 52.3%; model 3: 53.8%; and model 5: 25.5%). Thus, our findings confirm that baseline measurements can be useful for predicting responses to HIIT. More recently, the 'magnitude' of the hypotensive effect has been reported as a predictive factor for decreasing BP after chronic exercise (Hecksteden et al., 2013b). Unfortunately, the authors referred to 4 weeks of training as 'chronic' exercise, and these results are limited to the specific endurance protocol used, showing the importance of effects, the prevalence of NRs, and predictive factors of a response to long-term HIIT.

The strengths of the present study include our data on the effects, prevalence of NRs and predictive factors for decreasing BP in prehypertensive subjects. One limitation was the lack of a true no-exercise control group. Another limitation was that we used BIA to assess body composition variables; however, BIA is not considered the 'gold standard' method. We also did not implement dietary control during the intervention. Nonetheless, we continually reminded subjects to maintain their baseline dietary habits. Finally, as heart rate at rest, fasting glucose, HDL-C, and $1RM_{LE}$ were almost significantly different at baseline, the significant differences in each group pre- and post-intervention (**Table 1**) must be interpreted with caution.

CONCLUSION

In conclusion, the prevalence of NRs based on SBP and DBP was different between prehypertensive and normotensive subjects after 16 weeks of HIIT. Other comorbidities such as body composition and metabolic outcomes showed almost similar modifications between prehypertensive and normotensive subjects, being the most basic predictive factor for BP reduction baseline SBP, which we refer to as 'BP health status' (51.2%). This improvement in BP was accompanied by other known improvements of HIIT on body composition, metabolic, and endurance performance in both study cohorts.

NOVELTY AND SIGNIFICANCE

What Is New?

Although both prehypertensive and normotensive groups showed improvements in fat markers, metabolic risk factors (fasting glucose, lipid profile), and endurance performance, and thus a decrease in comorbidities, there was a different prevalence of non-responders based on decreased systolic and diastolic BP among prehypertensive individuals compared to normotensive individuals.

What Is Relevant?

To normalize high blood pressure and improve lipid profiles, an appropriate type of exercise training must be chosen. In addition, other non-pharmacological strategies are required to prevent hypertension.

Summary

Among participants in a prehypertensive state, altered blood pressure alone (health status) is a powerful predictive factor for the normalization of blood pressure, an increase in endurance performance and improvements in other metabolic risk factors after exercise.

AVAILABILITY OF DATA AND MATERIAL

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

ETHICS STATEMENT

Prior to providing written informed consent, the clinical records of all participants were reviewed to ensure that they met all of the inclusion criteria. Additionally, all subjects were personally informed about the study procedures that would occur before, during and after the intervention. All procedures were approved by the local Ethics Committee of the Family Healthcare Center Tomás Rojas in the city of Los Lagos, Chile.

AUTHOR CONTRIBUTIONS

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

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REVIEW
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The Inherent Human Aging Process and the Facilitating Role of Exercise

*Norman R. Lazarus and Stephen D. R. Harridge**

Centre for Human & Applied Physiological Sciences, School of Basic & Medical Biosciences, Faculty of Life Sciences & Medicine, King's College London, London, United Kingdom

Arguably the best available depictions of the global physiological changes produced by age are the profiles of world record performance times in swimming, athletics, and cycling, depicting the trajectory of decline in maximal integrated physiological performance capability. The curves suggest that the aging process produces a synchronized, controlled decrease in physiological performance over the human lifespan. The shape of the performance profile by age is essentially independent of discipline, distance, or phenotype. Importantly, the specific times of performance are not the driving force in the production of the shape of the declining performance profile. We suggest that in these highly trained individuals the shape of the curve is generated by the aging process operating on a physiology optimized for any given age. We hypothesize that with adequate training this same profile and trajectory, but with lower performance times, would be generated by all individuals who engage in sufficient physical activity/exercise. Unlike performance, data obtained from examining individual physiological systems or tissues do not give information on the unceasing and changing global integrating functions of the aging process. However, these data do give valuable information about the integrity of physiological systems at a particular age and allow a direct comparison to be made between the effects of inactivity and physical activity/exercise. Being physically active has been shown to have global protective effects on physiological systems and thus facilitates the aging process by maintaining physiological integrity. There is emerging evidence which suggests that physiological regulation of aging may be multi-compartmentalized. We do not advocate exercise as a panacea, but all the evidence indicates that being physically active and exercising is far superior to any other alternative for achieving optimal aging.

Keywords: aging, exercise, performance, healthspan, physiology

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Mikel Izquierdo, Universidad Pública de Navarra, Spain

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Gladys Leopoldine Onambele-Pearson, Manchester Metropolitan University, United Kingdom
Beat Knechtle, University Hospital Zurich, Switzerland

***Correspondence:**
Stephen D. R. Harridge
s.harridge@kcl.ac.uk

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INTRODUCTION

The aging process is generally described as being closely associated with the onset of disease. Yet there is an extensive literature documenting that many of these diseases of aging are heavily influenced by lifestyle factors, namely physical inactivity, exercise and diet. Readers are referred elsewhere for more expansive definitions of physical activity and exercise (Centers for Disease Control and Prevention, 2014). It is also becoming clear that not only is the expending of energy (through physical activity or exercise) *per se* important, but that the time spent being sedentary, and especially time spent sitting, is also highly deleterious to physiological function and health across the lifespan (Bouchard et al., 2015). In this review the majority of the discussion and

evidence accessed from the literature is based on the influence of exercise on aging, i.e., those that have undertaken planned, structured, repetitive physical activity that is purposeful in the sense that the objective is an improvement or maintenance of one or more components of physical fitness (Centers for Disease Control and Prevention, 2014). The diseases which relate to a lack of exercise, low levels of physical activity and sedentary behavior include; include sarcopenia, metabolic syndrome, obesity, insulin resistance, type 2 diabetes, non-alcoholic fatty liver disease, coronary heart disease, peripheral artery disease, hypertension, congestive heart failure, endothelial dysfunction, deep vein thrombosis, depression and anxiety, osteoporosis, osteoarthritis, rheumatoid arthritis, as well as a number of different types of cancer including colon, breast, and endometrial cancer (Booth et al., 2014; Pedersen and Saltin, 2015).

The descriptions of the interactions of exercise, diet and health have been common place in textbooks of exercise physiology for many years (e.g., Åstrand and Rodahl, 1986; Blair, 2001; McArdle et al., 2006). Yet whilst the responses and adaptations to exercise on physiological systems are well documented, the aging process itself remains an enigma. Healthy aging appears in the context of exercise physiology, but still is largely omitted as a core part of the medical curriculum, as is evidenced by a perusal of many medical textbooks. Surprisingly, there is also relatively little reference to the effects of exercise on the etiology of diseases of aging and more of a focus on the therapeutic effects of exercise on a wide range of aging related diseases (ACSM: Guidelines for Exercise testing et al., 2014; Pedersen and Saltin, 2015). In recent years there has been a movement to include exercise in therapeutic regimens (Hallal and Lee, 2013), even though the effectiveness of exercise in the treatment of heart diseases has essentially been implied since the seminal work of Morris et al. (1953). The current majority approach to the treatment of the preventable diseases associated with inactivity (e.g., cardiovascular disease, obesity, type 2 diabetes) is to rely on pharmaceutical therapy after the onset of the disease (Partridge, 2014). Exercise may only then be prescribed to correct the physiological deficit caused by the inactivity and energy imbalance. One of the unfortunate outcomes arising from ignoring prevention is demonstrated in a large cohort of individuals in the United Kingdom followed for 70 years, where 75% were on pharmaceutical therapy (Pierce et al., 2012). It has long been argued that this pharmaceutical approach to aging results in multi-pharmaceutical medication as one system failure follows another (Estes and Binney, 1989; Gems, 2011).

The aim of this short review and commentary is to try and present a different perspective of the aging process, emphasizing the global nature of its effects on physiological function and how these effects are modified over time by lifestyle factors and by exercise in particular.

THE NATURE OF THE INHERENT AGING PROCESS

Because aging probably effects every system and cell in the body, the obvious way of measuring these effects would be by having an indicator that accurately reflects the relationship between aging and the changes in physiological function that are occurring over a period of at least eight decades. Indicators of all-cause mortality are generally associated with changes in physiological function that can be modified by exercise. The indicators are therefore malleable. The result of this malleability raises a number of important points. The first is that when, for example, the maximal rate of oxygen consumption (VO_{2max}) of exercisers and non-exercisers of the same age are compared, the exercisers will have a higher VO_{2max} than non-exercisers (Wilson and Tanaka, 2000; Lazarus and Harridge, 2010; Harridge and Lazarus, 2017). The link between a specific age and a specific value for that age will have been broken in a widely accepted marker of all-cause mortality (Blair et al., 1989). Secondly, both in exercisers and non-exercisers it is found that the same VO_{2max} value occurs in subjects differing by two or three decades (Harridge and Lazarus, 2017). The exercisers will have higher values than their non – exercising counterparts. Again, the link between a specific age and a specific VO_{2max} value will have been broken. These facts demonstrate not only malleability, but that exercisers and non – exercisers have different phenotypes. The concept of a physiological marker that appears to be independent of the malleability of the function it is measuring is interesting. The recent paper by Levine et al. (2018) on epigenetic markers suggests such a marker. This needs to be rigorously and widely tested, particularly against defined populations of exercisers and non – exercisers.

It appears that aging affects nearly all physiological systems. In order to follow these changes a measure of global physiological function is necessary. Such a measure is maximal athletic performance where many systems must be integrated to be able to perform at their limits. Thus, a change in performance over time (years) reflects the change in global physiological status with aging. Such performances are depicted in the records of Master Athletes (Baker and Tang, 2010; Lazarus and Harridge, 2017) and in these highly trained individuals, the negative effects of inactivity are completely removed. We suggest that these performance profiles (Gold curves depicted in **Figure 1**) are generated by the effects of the aging process because the following important variables are controlled: (1) all competitors are at maximal physical effort in order to produce best times, (2) the intensity, type and frequency of training will be similar, (3) the type of exercise is the same for all competitors, (4) lifestyle and dietary habits are geared to maximum performance, (5) the ages of the entrants in any championship event cover the human lifespan, (6) performance times are objectively measured and (7) in any single discipline phenotype is probably going to be as homogeneous as can be expected in cross-sectional studies. Thus, confounding variables such as healthcare, nutrition, socio-economic conditions between generations, as well as genetic differences between individuals are ameliorated.

The profiles produced by aging runners, swimmers and cyclists all follow the same age- related decrease in performance (Donato et al., 2003; Baker and Tang, 2010). The decline in performance is curvilinear tending to accelerate after the

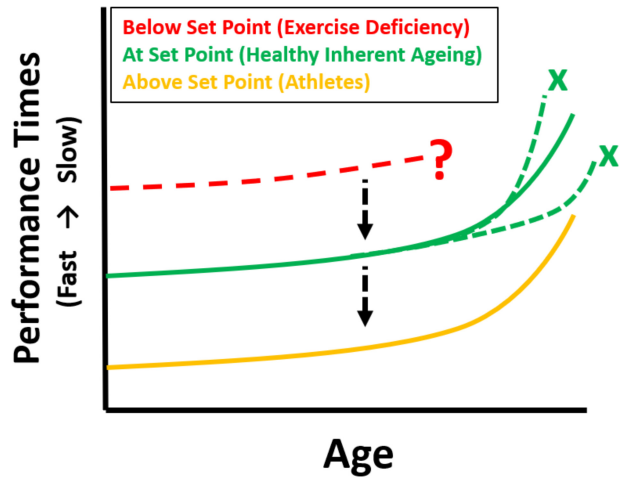

FIGURE 1 | Theoretical longitudinal maximum performance curves (e.g., swimming, running, cycling) for athletes as they age [based on cross-sectional data from World Record performances, Baker and Tang (2010), gold]. Inherently aging individuals exercising at Set Point (green) are predicted to have the same trajectory of decline if they were to undertake a similar athletic event at regular intervals as they age (depicting the change in integrative whole-body physiology due to aging), but with inferior performances. Maintaining the same profile, but improving athletic performance is possible by increasing exercise towards the gold zone. Those below set point show an unpredictable trajectory (red). The black arrows indicate the change to different performance curves with increased levels of physical exercise, the green crosses indicate that if exercising at set point there can be no acceleration or deceleration (i.e., deviation from the predicted curve which represents inherent aging) – assuming no disease. The red dotted curve and question mark indicate both an uncertain trajectory, or indeed the very possibility, of being able to undertake a maximal performance.

seventh or eighth decade. Even more surprising is that the time course is similar in both sprint and endurance events, in other words independent of time of performance and phenotype (Lazarus and Harridge, 2017). The mechanisms whereby the aging process produces superimposable performance profiles from differing phenotypes are unknown. In addition, these data need to be interpreted in the context of factors which include: (i) there is a shrinking pool of competitors in the older age groups, (ii) the fact that older world record holders are unlikely to been the champion athletes in their youth and (iii) master sports is not well developed in all countries.

It has been postulated that the exceptional performance times are partly due to the presence of "athletic genes" (Tucker and Collins, 2012) and therefore non-reflective of the ability of the general population who regularly engage in exercise. That may well be, but the main interest of this review is not in the performance times themselves. The more pertinent question is whether the shape of the performance profile generated by the aging process is a universal aging profile or is it only applicable to champion athletes? This question cannot be directly answered because, not surprisingly, there are no championships for average performers in any discipline. However, examination of swimming performance in Class six as defined by the Paralympic Committee (2015) may give some insight. This class shows that the competitors, although not conforming to the stereotypical phenotype of master swimmers, are capable of producing extraordinary performances. We hypothesize that with the same facilities, coaching and training advice, average swimmers, for example, would produce maximum times appropriate to their abilities and that the profile of decreasing performance as a result of age would be the same as that produced by better physically endowed athletes. This concept is demonstrated as the solid green curve in **Figure 2**.

Aside from hypothetical elite genes there are other related issues that need to be addressed. Firstly, do people engage in exercise because they have a genetic predisposition to respond to exercise, and secondly, do about 80% of people choose inactivity because they cannot respond to exercise? Unsurprisingly, in a cross-sectional study of non-competitive men and women cyclists (albeit again self-selected) it was found that all the physiological systems showed the expected effects of high levels of physical activity (Pollock et al., 2015). The Canadian Heritage studies demonstrate that there are about 20% of the population who will not respond appropriately to exercise (Bouchard, 2012). How the aging process operates in these individuals has yet to be investigated. Another population, the frail, represent a group whose symptomatology can be ascribed to an exercise deficiency (Lazarus et al., 2018). These patients, which represent the very antithesis of exercisers, are also able to exhibit a positive response to an exercise intervention program (Cadore et al., 2014; Maddocks et al., 2016). Sometimes personal choice is as important as genetics.

THE SET POINT HYPOTHESIS: PREDICTIONS AND CONUNDRUMS

We (Lazarus and Harridge, 2017) have recently put forward the hypothesis that for optimal aging to occur, exercise must be at a level that equates to an individual's "set point." At set point physiological function is free of the effects of inactivity (**Figure 2**). The level of exercise necessary to reach set point will vary and is determined by individual differences in physiological ability. Operating on this platform of protected physiological function, the aging process is optimal. Thus, all subjects engaging in exercise at their set point will follow a pathway that results in optimal aging and decreased morbidity in later life. The hypothesis is now extended and suggests that if individuals, irrespective of whether they were at set point or above, could be tested for performance over a lifetime, then the effect of age would generate the same curvilinear-shaped decline in performance irrespective of times (**Figure 1**). Any increase in exercise above the built-in set point does not impinge on the aging process, but it does allow better performance times by expanding physiological capability (**Figure 2**). The inherent aging process generates the same age-related decline in performance for all who engage in exercise, provided other lifestyle factors such as smoking, alcohol intake and diet are controlled.

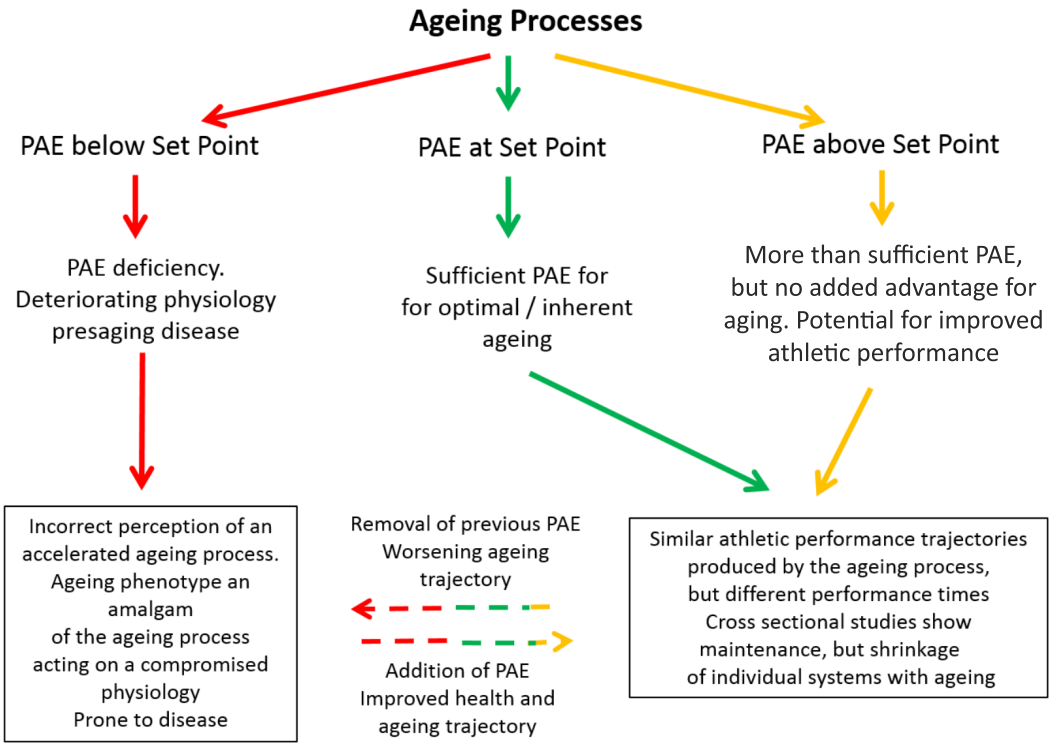

FIGURE 2 | Schematic showing the effects of exercising below (red), at (green), or above (gold) the Set Point. Any protective effects of moving from gold/green to red are unclear (but overall effects are negative). The progression from red through to green will be an overall positive effect, whilst the progression from red through to gold and being able to fulfill athletic potential in later life is unclear. PAE refers to Physical Activity/Exercise.

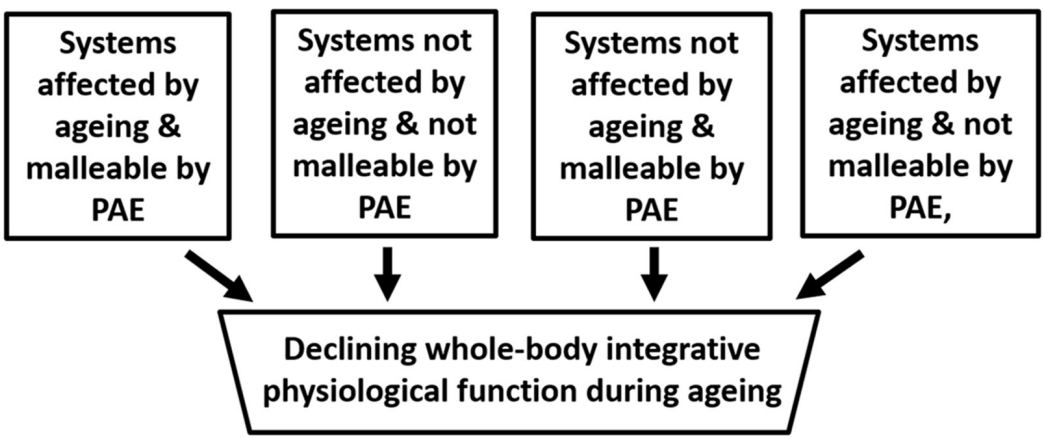

FIGURE 3 | Schematic depicting the compartmentalisation of factors that impact on aging. The assumption here is that no pathologies are present. PAE refers to Physical Activity/Exercise.

Some researchers use terms such as accelerated (Simon et al., 2006) or decelerated (Olshansky et al., 2007) aging. These terms can refer to changes in telomere length with shortened telomeres supposedly indicating accelerated aging. The terms are generally unfortunate because, whatever their basis, they give the impression that the inherent aging process can be manipulated. Instead, we suggest that aging be considered as having two modes of presentation. Either an optimal outcome that results from the inherent aging process operating on a diminishing, but fully primed physiology, or an uncertain outcome resulting from the aging process operating on a diminished and compromised physiology which is the result of inactivity and other lifestyle factors. The shape of each performance curve is independent of ability

and is probably driven by the inherent ageing process. Thus, in theory no deviations in the shape of a curve are allowable (dotted green lines in **Figure 1**) for the disciplines mentioned (e.g., running, swimming, cycling). It remains to be seen if in future years and with more competitive athletes whether the trajectory of change predicted by world records will alter. If it is representative of the aging process then this shape will remain essentially the same.

If the age-dependent change in performance time follows the same curve for all exercisers, irrespective of ability, discipline, distance, or phenotype then do these curves all intersect baseline at the same age? In other words, would all exercisers die at round about the same age? There is some evidence that elite athletes have greater longevity than the general population (Teramoto and Bungum, 2010; Garatachea et al., 2014). This is unsurprising because most of the general population do not engage in sufficient physical activity or exercise and so are prone to all the diseases of inactivity and their consequences (Booth et al., 2014). We are unaware of any longevity data comparing the age of death of athletes from different disciplines. An initial study showed that male athletic champions had a significantly lower mortality than the general population under the age of 50 years; after 50 years of age the mortality was the same (Schnohr, 1971). A more recent systemic review of 54 studies reported superior lifespan longevity outcomes for elite athletes compared to age- and sex-matched controls from the general population and to other athletes (Lemez and Baker, 2015). In a study of 393 former male Finnish elite level athletes (∼72 years), a lower body fat percentage, lower risk for the metabolic syndrome and non-alcoholic fatty liver disease was reported (Laine et al., 2016). Having had a career as an elite-class athlete during young adulthood seemed to offer some protection, but current exercise levels and volume of leisure time physical activity were suggested to be equally important factors. This is predicted by the set point theory. For example, if exercise is markedly reduced after 50 years of age then the athletes would likely revert to the same inactivity-compromised physiology as the general population. Perhaps the most relevant question is whether elite athletes live longer than those subjects who engage in exercise at their set point? There is no information, but our hypothesis states that exercise above set point confers no additional health benefit; therefore, the longevity of these latter subjects should be equivalent to elite athletes. However, longevity is arguably not the most appropriate index to follow. For the elderly, being free from disease and being independent is probably more important than longevity. This independence is reflected by the "healthspan" (Fries, 1980; Seals et al., 2016). Healthspan is optimal at set point and above assuming no over-training pathologies.

Whilst conferring health benefits, exercise above the set point and engaging in very high levels of exercise can carry its own risks. These relate to musculoskeletal wear and injury (Kujala et al., 2005) as well as overtraining suppression of the immune system (Gleeson et al., 1995).

CROSS-SECTIONAL STUDIES: AGING, PHYSIOLOGICAL FUNCTION, AND EXERCISE

Cross-sectional studies, that do not measure performance, allow for a limited but important understanding of the effect of exercise on the aging process. These studies allow comparison of the effects of exercise versus inactivity on physiological systems across the human age spectrum but give no information about the integrating functions of the aging process. This rarely acknowledged deficiency in the interpretation of cross-sectional data has had a profound influence on the perception of aging especially if inactive subjects with compromised physiologies have been used as examples of healthy aging in aging research. For example, it has been reported that mitochondrial function declines with age (Gonzalez-Freire et al., 2018), yet there was no objective assessment of the individuals exercise behavior. The heterogeneity in the results also makes it impossible to ascribe any one level of mitochondrial function to a specific age. Will the same physiological index value suffice for a 70-year-old exerciser and a 70-year-old inactive person? The available data indicate probably not (Wilson and Tanaka, 2000; Pollock et al., 2018). We have previously discussed the issue regarding the relationship (or lack of) between age and function across multiple physiological indices (Lazarus and Harridge, 2010, 2011; Harridge and Lazarus, 2017). Researchers, whatever their field, can thus be prone to incorrectly ascribing changes in physiological indices as being due to age instead of the result of the aging process operating on an inactivity compromised physiology. This lack of appreciation of the interactions between aging, disease and exercise medicalises aging and often wrongly favors pharmaceutical and other treatments over preventive measures (Lazarus et al., 2018).

EFFECTS OF EXERCISE ON PHYSIOLOGICAL SYSTEMS AND LAYERS OF REGULATION DURING AGING

Cross-sectional studies have shown exercise to have a positive effect on many physiological functions and there is an extensive literature on the subject that need not be re-iterated here (Booth et al., 2014). However, as is depicted **Figure 3** physiological systems can be divided into four categories, which in the absence of pathology could be described as those that are highly sensitive to and interact with exercise (e.g., skeletal muscle), those that are independent of both exercise and aging (e.g., absorptive function of the small bowel), those that are not malleable by exercise are age dependent (e.g., maximum heart rate) and those that are not affected by age, but are malleable by exercise and physical activity (e.g., resting heart rate).

In an extensive study, the immune cellular profile and affiliated hormones of a cohort of cyclists aged 55–79 years were compared with both a healthy young group and a group of

clinically heathy older people who did not undertake regular physical exercise (Duggal et al., 2018). The prevailing view is that the thymic microenvironment undergoes architectural and phenotypic changes with age including: loss of stromal epithelial niches, reduced numbers of thymic epithelial cells, replacement of lymphoid tissue with adipose tissue reducing active areas of thymopoiesis (Dixit, 2010; Chinn et al., 2012). However, in this study thymic function and output were maintained in the cyclists with high levels of exercise. The frequency of naïve T cells and recent thymic emigrants were both higher in cyclists compared with inactive elders. The cyclists also had significantly higher serum levels of the thymoprotective cytokine IL7 and lower IL6, which promotes thymic atrophy. Interestingly, the cyclists also showed evidence of reduced immune senescence, namely lower Th17 polarization and higher B regulatory cell. Markers of chronic low-grade inflammation ("inflamaging"), IL-6 and TNFα, were also markedly lower in the cyclists compared to the older non-exercisers and only slight higher than the young. However, the study also showed that $CD28^{-ve}$ $CD57^{+ve}$ senescent CD8 T cells did not differ between cyclists and the older non-exercisers (Duggal et al., 2018). Thus, these cell types may be added to the exercise-independent, but age-dependent category in **Figure 3**.

This study demonstrates that not only between different systems, but within a single system, the regulation of physiological function during aging may be compartmentalized and multi-layered (Harridge and Lazarus, 2017). Furthermore, there is an intriguing relationship between skeletal muscle, the most metabolically sensitive tissue to exercise and the thymus. In culture thymic cells express contractile proteins and can form myofibers and animal data suggest that changes in the density of thymic myoid cells may accompany acute and chronic demands for muscle precursors (Wong et al., 1999). Thymic cells have also been suggested for myoblast transfer (Pagel et al., 2000). Could there be other links in active humans? Certainly, the maintenance of thymus function in the presence of continued skeletal muscle activity suggests molecular or hormonal links that need to be explored.

Undoubtedly, a global function like maximum athletic performance requires an intact immune system (Fitzgerald, 1998). However, performance unfortunately does not inform on the whole gamut of functions of the immune system. It may be that an intervention directed specifically at testing the immune system may be more appropriate. For example, a challenge by a specific antigen such as a flu antigen may be a better option. In this regard an exercise intervention in which participants were examined for improvements of the immune response to influenza vaccination showed that exercising participants had a greater increase in antibody and IFNγ production compared to less active controls (Kohut et al., 2005). However, unless the physical activity and exercise levels of the participants are reported, the results will provide little useful information for determining the global differences between the immune systems of those who are physically active and those that are not (Prezemska-Kosicka et al., 2018). We caution against the use of inactive subjects to confirm some of these associations.

SOME FINAL THOUGHTS ON REGULATION IN COMPLEX BIOLOGICAL SYSTEMS

The mutual interaction of the aging process and exercise ensures that the functional ability of the diminishing physiological base is kept as efficient as possible. The aging process accomplishes this effect by, as yet, unknown integrating and synchronizing mechanisms. We hypothesize a central location/s for the control of these integrating functions, but more data is necessary and at present our hypothesis is heuristic. There are other hypotheses concerning the control of complex systems. Many of these concern non-biological systems and will not be discussed. Kitano (2002) in his review of systems biology makes the point that "many breakthroughs in experimental devices, advanced software, and analytical methods are required before the achievements of systems biology can live up to their much-touted potential." In the 16 years since this statement, there has been an exponential rise in the tools available in the field of systems biology, making advances in this field all the more possible. We would add other caveats; an understanding of the mechanisms whereby the aging process integrates and controls the declining *milieu interieur* over many decades is in its infancy. We hypothesize that as people, engaging in exercise, age-alternate pathways of regulation may be brought into play in order to keep shrinking physiological functions as optimal as possible. There is some evidence that in inactive, aging individuals there are shifts in control mechanisms; however, these shifts are related to failing systems rather than toward the maintenance of physiological integrity (Seidler et al., 2010). These crucial differences in regulation between failing systems and co-ordinate healthy aging should in no way be equated. As this brief review strongly suggests the physiology of aging is unfinished business.

AUTHOR CONTRIBUTIONS

Both authors contributed equally to the work and approved it for publication.

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Inter-individual Variability in Responses to 7 Weeks of Plyometric Jump Training in Male Youth Soccer Players

Rodrigo Ramirez-Campillo[1], Cristian Alvarez[1], Paulo Gentil[2], Jason Moran[3], Felipe García-Pinillos[4], Alicia M. Alonso-Martínez[5†] and Mikel Izquierdo[5]*

[1] Department of Physical Activity Sciences, Research Nucleus in Health, Physical Activity and Sport, Laboratory of Measurement and Assessment in Sport, Universidad de Los Lagos, Osorno, Chile, [2] Faculdade de Educacao Fisica e Danca, Federal University of Goiás, Goiania, Brazil, [3] Department of Sport, University Centre Hartpury, University of the West of England, Bristol, United Kingdom, [4] Department of Physical Education, Sports and Recreation, Universidad de La Frontera, Temuco, Chile, [5] Department of Health Sciences, Navarrabiomed, CIBER of Frailty and Healthy Aging (CIBERFES), Instituto de Salud Carlos III, Pamplona, Public University of Navarra, Navarra, Spain

The purpose of this study was to compare the inter-individual variability in the effects of plyometric jump training (PJT) on measures of physical fitness (sprint time, change of direction speed, countermovement jump, 20- and 40-cm drop jump reactive strength index, multiple five bounds distance, maximal kicking distance, and 2.4-km time trial) in youth soccer players who completed a PJT program versus players who completed soccer training only. In a single-blinded study, participants aged between 10 and 16 years were randomly divided into a PJT group (n = 38) and a control group (n = 38). The experimental group participated in a PJT program twice weekly for 7 weeks, whereas the control group continued with their regular soccer training sessions. Between-group differences were examined using a Mann–Whitney U test. Nonresponders where defined as individuals who failed to demonstrate any beneficial change that was greater than two times the typical error of measurement from zero. The results indicated that the mean group improvement for all physical fitness measures was greater ($p < 0.05$) in the PJT group (Δ = 0.4 to 23.3%; ES = 0.04 to 0.58) than in the control group (Δ = 0.1 to 3.8%; ES = 0.02 to 0.35). In addition, a significantly greater ($p < 0.05$) number of responders across all dependent variables was observed in the PJT group (from 4 up to 33 responders) than in the control group (from 0 up to 9 responders). In conclusion, compared to soccer training only, PJT induced greater physical fitness improvements in youth soccer players, with a greater number of responders for all the physical fitness tests related to jumping, speed, change of direction speed, endurance, and kicking technical ability.

Keywords: football, force-velocity curve, jump training, stretch-shortening cycle, maturation, strength

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

†orcid.org/0000-0002-7204-696X

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INTRODUCTION

The habitual development of "athleticism" to improve health, enhance physical fitness, reduce the relative risk of injury, and develop the confidence and competence of youths is particularly relevant in programs for children and adolescents (Lloyd et al., 2014) into the holistic long-term athletic development process in youths. Of particular relevance among training programes for youth soccer

players is resistance training, a specialized method of conditioning that involves the progressive use of a wide range of resistive loads, including body mass, and a variety of training modalities (e.g., machine-based training, free weight training, plyometric training, complex training, and functional training) to enhance muscular fitness and athletic performance (Behm et al., 2008; Granacher et al., 2016; Barbalho et al., 2018). Plyometric jump training (PJT) is a common resistance training modality that incorporates the stretch-shortening cycle of muscles to acutely improve the rate of force development, with the long-term aim to induce neuromuscular adaptations (Markovic and Mikulic, 2010; Lloyd et al., 2014). The PJT programs have the added advantages of not requiring expensive equipment or large spaces and have been shown to be an enjoyable (Ward et al., 2007) and effective form of training for youth soccer players (Bedoya et al., 2015), inducing physical fitness improvements such as jumping, sprinting, kicking, and change of direction, key traits for soccer (Barnes et al., 2014). These actions might precede most of the goals scored in competitive leagues (Faude et al., 2012) and may correlate with competition success (Arnason et al., 2004). Repeating these maximal-intensity actions across a game is also important (Carling et al., 2012) and might be associated with endurance (Helgerud et al., 2001), which also may be enhanced with PJT in youth soccer players (Ramirez-Campillo et al., 2014b, 2015a,b). On this basis, PJT programs have received extensive attention from researchers in recent years (Ramirez-Campillo et al., 2018a).

However, despite extensive investigation, research articles usually report the group response (i.e., the mean change within a training group) from youth soccer players to PJT without considering the wider inter-individual variability in the response to exercise training (IVRET), in which participants can be broadly classified into two types: responders and nonresponders (NR) (Alvarez et al., 2017a,b,c, 2018a). Additionally, among the few studies of IVRET, most have focused on cardiorespiratory fitness and metabolic measures without considering other measures more relevant to soccer players (i.e., jumping) (Arnason et al., 2004), which not only may exhibit an IVRET but also may show a different response to training in each individual over time (Piirainen et al., 2014). Thus, it is important to not only study the IVRET phenomenon in a single variable but also study a cluster of dependent variables relevant to each study population (Barbalho et al., 2017).

In addition to the above, most of the cited studies applied endurance training stimulus only or a combination of endurance and resistance training. Moreover, most investigations were carried out in adult populations. As the effects of resistance training and PJT may differ between individuals according to their development (Asadi et al., 2017; Moran et al., 2017a,b,c), the IVRET phenomenon typically observed in adults may be different from that in youth populations. Therefore, individual responsiveness to PJT alone remains a phenomenon that warrants further exploration. To our knowledge, only one study has analyzed the IVRET phenomenon after plyometric training (Radnor et al., 2017). However, the study was not on youth soccer players, and only sprint and jumping variables were analyzed.

The purpose of this study was to compare the IVRET of physical fitness measures (jumping, reactive strength index, speed, change of direction ability, kicking performance, and endurance) in youth soccer players who completed a PJT program or soccer training only. According to relevant literature (Radnor et al., 2017), it was hypothesized that a higher number of responders – based on measures of physical fitness – would be observed among youth soccer players after PJT than among those who underwent soccer training only.

MATERIALS AND METHODS

Participants

Seventy-six male soccer players aged between 10 and 16 years (control group: Tanner stage 3.7 ± 1.1; body mass index, 19.9 ± 2.3 $kg{\cdot}m^{-2}$; PJT group: Tanner stage, 3.7 ± 1.1; body mass index, 19.9 ± 1.7 $kg{\cdot}m^{-2}$) volunteered to participate in the study. All participants had previously been engaged in soccer, with (i) more than 2 years of systematic soccer training and competition experience and (ii) continuous soccer training in the last 6 months. Although the participants regularly performed sporadic jumps during training and competition, they had not systematically performed PJT in the 6 months prior to this study and had no history of regular strength training. **Figure 1** depicts the CONSORT diagram of the full recruitment and randomization process. Participants were divided into a PJT group (n = 38) or a control group (n = 38). The experimental group participated in a PJT program twice weekly for 7 weeks, whereas the control group carried out their regular soccer training sessions only. Participants were reminded during each training session to maintain their usual physical activity habits during the experiment. Exclusion criteria included subjects with (a) potential medical problems or a history of ankle, knee, or back pathology in the 3 months before the study, (b) medical or orthopedic problems that compromised their participation or performance in the study, (c) any lower extremity reconstructive surgery in the past 2 years or unresolved musculoskeletal disorders, and (d) subjects who were taking or had previously taken anabolic steroids, growth hormone, or related performance-enhancement drugs of any kind. However, individuals were not excluded if they had been taking vitamins, minerals, or related natural supplements (other than creatine monohydrate).

The following dependent variables were tested in all participants before and after the 7-week intervention: 20-m sprint time, change of direction speed test time (CODS), countermovement jump (CMJ) height, 20- (RSI20) and 40-cm (RSI40) drop jump reactive strength index, multiple 5 bounds distance (MB5), maximal kicking test for distance (MKD), and 2.4-km time trial. Following previous criteria (Alvarez et al., 2018b), NR to each of the dependent variables were defined as individuals who failed to demonstrate an increase or decrease (in favor of beneficial changes) that was greater than two times the typical error of measurement (TE) away from zero. Parental informed consent and participant assent were obtained in advance of the study. The Department Research Ethics

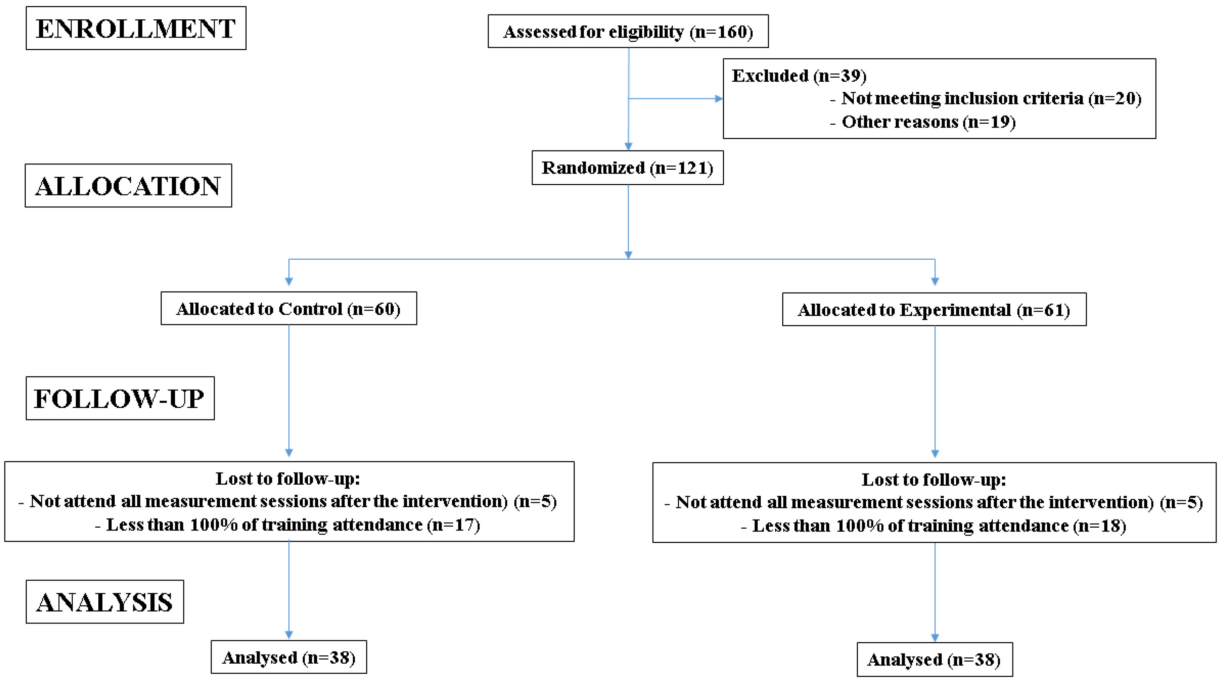

FIGURE 1 | CONSORT diagram of the full recruitment and randomization process.

Committee, in accordance with the Declaration of Helsinki, approved the study.

Sample size was computed according to the changes observed in plyometric (i.e., reactive strength index) performance (d = 0.3 mm·ms^{-1}; SD = 0.35) in a group of young adolescents submitted to the same training program (Ramirez-Campillo et al., 2013, 2014b). Eight participants per group would yield a power of 80% and $\alpha = 0.05$.

Experimental Design

Subjects followed a 90-min familiarization session before testing to reduce any learning effects, and a warm-up was completed at the beginning of each testing session (Andrade et al., 2015). Standardized tests were scheduled >48 h after competition or high-intensity physical training to minimize the influence of fatigue. All tests were performed over 2 days under similar weather, time, and field conditions before and immediately after the 7-week period. On day one, the players' physical characteristics (height, body mass, and self-assessed pubic hair and genital stage) were assessed, and physical fitness tests were conducted in the following order: CMJ, RSI20 and RSI40, MB5, 20-m, and CODS test. On day two, the MKD and a 2.4-km time trial were performed. All tests were administered in the same order before and after training, with players wearing the same sporting attire. Data were recorded by the same investigators who were blinded to the group allocation of the participants. In addition, all participants (and their parents or guardians) were instructed to have a good night's sleep (≥9 h) before each testing day and to be well hydrated. A standardized meal rich in carbohydrates was provided to the participants 2–3 h before measurements. All participants were motivated to give their maximum effort during physical fitness measurements. At least 2 min of rest were allowed between each trial to reduce effects from fatigue. While waiting, the participants performed low-intensity activity to maintain physiological readiness for the next test. The best score of three trials was recorded for all physical fitness tests, apart from the single 2.4-km time trial, which was performed just once. As in previous studies that used similar procedures (Ramirez-Campillo et al., 2013, 2014b), high intra-class correlation coefficients were obtained for the different physical fitness tests, varying between 0.81 and 0.98.

Somatic and Maturity Measures

Height was measured using a wall-mounted stadiometer (Butterfly, Shanghai, China) recorded to the nearest 0.5 cm. Body mass was measured to the nearest 0.1 kg using a digital scale (BC-554 Ironman Body Composition Monitor; Tanita, IL, United States). Body mass index was then calculated (kg·m^{-2}). Maturity was determined by self-assessment of Tanner stage as previously outlined (Ramirez-Campillo et al., 2014b).

Vertical Jump Tests

Testing included the execution of maximal CMJ, RSI20, and RSI40. All jumps were performed on a mobile contact mat (Ergojump; Globus, Codogne, Italy) with arms akimbo. Take-off and landing was standardized to full knee and ankle extension on the same ground position. The participants were instructed to maximize jump height and minimize ground contact time during the RSI20 and RSI40 after descending from 20- and 40-cm boxes, respectively. The RSI was calculated as previously reported

(Ramirez-Campillo et al., 2014b), dividing jumping height (mm) by time contact (ms), thus expressed in $mm \cdot ms^{-1}$.

Multiple Five Bounds Test

The MB5 was started from a standing position from which participants performed a set of five forward jumps with alternative left- and right-leg contacts to cover the longest distance possible. The distance of the MB5 was measured to the nearest 0.5 cm using a tape measure (Ramirez-Campillo et al., 2014b). Participants were motivated to give their maximum effort during three trials, with ∼2 min of rest between trials. Considering its specificity in soccer players, the test is an adequate alternative to vertical jumps as a measure of explosive strength and coordination (Diallo et al., 2001; Meylan and Malatesta, 2009; Ramirez-Campillo et al., 2014b).

Twenty-Meter Sprint and Change of Direction Speed Test

The sprint time was measured to the nearest 0.01 s using single beam infrared reds photoelectric cells (Globus Italia, Codogne, Italy). The starting position was standardized to a still split standing position with the toe of the preferred foot forward and behind the starting line. The sprint start was given by a random sound, which triggered timing. The photoelectric signal was positioned at 20 m and set ∼0.7 m above the floor (i.e., hip level) to capture the trunk movement rather than a false trigger from a limb. The CODS test has been described previously, and its reliability addressed elsewhere (Ramirez-Campillo et al., 2014b). The timing system and procedures were the same as the 20-m sprint with the exception that subjects started lying on their stomach on the floor with their face down.

Maximal Kicking Distance Test

After a standard warm-up, each player kicked a new size 5 soccer ball (Nike Seitiro, FIFA certified) for maximal distance on a soccer field. Two markers were placed on the ground side by side to define the kick line. Participants performed a maximal instep kick with their dominant leg after a run up of two strides. A 75-m metric tape was placed between the kicking line and across the soccer field. An assessor was placed near the region where the ball landed after the kick to mark the point of contact and to measure the distance kicked. The distance was measured to the nearest 0.2 m. All measurements were completed with a wind velocity <20 $km \cdot h^{-1}$ (local Meteorological Service). Previous studies have reported a high level of reliability for similar soccer kicking tests (Ramirez-Campillo et al., 2014b).

Time Trial 2.4-km Test

As previously recommended (Ramirez-Campillo et al., 2014b; Assuncao et al., 2017), the time-trial 2.4 km test was used considering its multiple facet requirement (maximal oxygen consumption, lactate threshold, running economy, muscle power) (Coyle, 1995), likely to affect aerobic-related performance in soccer. After a warm-up run of 800-m and 4 min of rest, players performed six laps of a 400-m outdoor dirt track, timed to the nearest second, with a stopwatch. The wind velocity at all times was ≤ 8.9 $km \cdot h^{-1}$, the relative humidity was between 50 and 70%, and the temperature was between 15 and 20°C (local Meteorological Service). Motivation was considered maximal as the test was conducted as part of the team selection process.

Training Intervention

This study was completed during the mid-portion of the players' competition period. Before this period, participants completed 2 months of summer pre-season training, were three 90 min training sessions were completed per week. The control group did not perform PJT but did perform their usual soccer training, which included 20 min of technical drills, 20 min of tactical drills, 20 min of small-sided games, and 30 min of simulated competitive games per session. In addition, once a week, injury prevention drills were incorporated. To ensure that training loads were similar between groups, the session rating of perceived exertion (RPE) was determined by multiplying the soccer training duration (in minutes) by session RPE, as previously described in studies of young soccer players (Ramirez-Campillo et al., 2014b). Before the initiation of the training period, participants from the PJT group were instructed on proper execution of all the exercises included in the program. During the intervention, the PJT group replaced some technical drills (e.g., ball heading exercises) with plyometric drills within the usual 90-min practice period, twice per week for 7 weeks. This training program has been shown to induce significant physical fitness adaptations in youth soccer players during the in-season period as part of a replacement for some low-intensity technical drills (Ramirez-Campillo et al., 2014b). All plyometric sessions lasted ∼21 min and were performed just after the warm-up to ensure that the players were in a rested state and that they gained optimal benefits from the specific program (Ramirez-Campillo et al., 2018).

Briefly, the PJT included 60 drop jump repetitions per session and was performed on a grass soccer field. The athletes completed three sets of 10 repetitions from 20-, 40-, and 60-cm height boxes, in a random schedule, in order to maximize adaptations (Hernández et al., 2018), for a total of 840 foot contacts after 7 weeks of training. The participants were instructed to jump as high and fast as they could, with maximal voluntary effort (Ramirez-Campillo et al., 2018b), for each repetition. We did not increase the training volume during the 7-week period, as we used high-intensity plyometric exercises performed with maximal effort; however, an adequate training stimulus was applied during each plyometric session, as previously demonstrated in youth soccer players (Ramirez-Campillo et al., 2014a,b). The rest period between repetitions and sets was ∼15 and ∼90 s (Ramirez-Campillo et al., 2013, 2014b), respectively. Previous research has demonstrated that this is an adequate rest interval for this type of training (Ramirez-Campillo et al., 2014b). As players did not have any history of formal PJT, all exercises were supervised with an investigator-to-participant ratio of 1:6. A high investigator-to-participant ratio have demonstrated greater benefits during explosive resistance training interventions (Ramirez-Campillo et al., 2017). Particular attention was paid to exercise demonstration and execution, providing maximal motivation to athletes during each jump. Training sessions were

separated by a minimum period of 48 hours (including games). Aside from the formal training intervention, all participants attended their regular physical education classes.

The reliability of jump heights and contact times for the PJT drills was verified in a randomly assigned subsample of participants (i.e., $n = 2$) during two randomly selected training sessions. During these sessions, ground contact-times and jump heights were tested using the same procedures and equipment as described above. Briefly, the maximal intensity for drop jumps was verified by measuring height and contact-time of the respective drill.

Statistical Analysis

The between group differences in percentage change for all physical fitness variables was examined using a Mann–Whitney U test. Percentage change from baseline testing was calculated for all individuals in each of the physical fitness variables. The NR were identified and defined as individuals who failed to demonstrate an increase or decrease (in favor of beneficial changes) in physical fitness that was greater than two-times the TE away from zero, calculated using a previously stablished equation (Bonafiglia et al., 2016). For the current study, three repeats of each physical fitness test were used in order to calculate the TE. A change beyond two times the TE was representative of a high probability (i.e. 12 to 1 odds) that the observed response was a true physiological adaptation beyond what might be expected to result from technical and/or biological variability. Thus, the TE were the following [CMJ, 0.074 (cm) × 2; RSI20, 0.00054 (mm/ms) × 2; RSI40, 0.00052 (mm/ms) × 2; MB5, 0.017 (m) × 2; 20 m, 0.0074 (s) × 2; CODS, 0.038 (s) × 2; MKD, 0.106 (m) × 2]. For the 2.4 km time trial test, considering that only one maximal attempt was employed during testing, the criteria to determine NR were those athletes that did not reduce the total time in the test. Additionally, the Chi-Square test (X^2) was used for comparisons between groups of subjects who were into the 2 × TE calculated in each outcome (NR), or beyond two times the TE [responders (R)]. Cohen's d effect sizes (ES) were calculated for within groups changes in physical fitness and interpreted using previously outlined ranges (<0.2 = trivial; 0.2–0.6 = small; 0.6–1.2 = moderate; 1.2–2.0 = large; 2.0–4.0 = very large; >4.0 = extremely large).

RESULTS

No differences were observed between the PJT and the control groups in the somatic and maturity measures, nor before nor after the intervention.

At baseline, no differences were observed between the groups for all the dependent variables, with values for the whole group of players being 26.8 ± 5.2 cm for CMJ, 0.102 ± 0.04 mm·ms^{-1} for RSI20, 0.103 ± 0.04 mm·ms^{-1} for RSI40, 8.9 ± 1.2 m for MB5, 4.35 ± 0.5 s for 20 m, 20.2 ± 2.8 s for CODS, 10.6 ± 0.8 min for 2.4-km time trial, and 31.8 ± 7.6 m for the MKD test.

Table 1 shows the mean group response to each intervention and the significant between-group differences in percentage change for all physical fitness variables. For CMJ, RSI20, RSI40, MB5, 20-m, CODS, 2.4-km time trial, and MKD physical fitness variables, a significantly greater ($p < 0.05$) improvement was observed in the PJT group (ES = 0.21, 0.58, 0.37, 0.28, 0.04, 0.27, 0.28, 0.53, respectively) when compared to the control group (ES = 0.13, 0.08, 0.06, 0.01, 0.35, 0.25, 0.04, 0.06, respectively). The individual change in absolute units per each physical fitness test is shown in **Figures 2**, **3**.

When responders in the PJT group were compared to those in the control group, the chi-squared analysis revealed a significantly greater number of responders after PJT for CMJ, RSI20, RSI40, MB5, 20-m, CODS, 2.4-km time trial, and MKD (**Table 1**).

Specifically, for the CMJ test, 47% of the players were identified as responders in the PJT group, compared to only 24% in the control group. For the RSI20 test, 87% of the players were identified as responders in the PJT group, compared to only 13% in the control group. For the RSI40 test, 76% of the players were identified as responders in the PJT group, compared to 18% in the control group. In the MB5 test, 63% of the players were identified as responders in the PJT group, compared to 8% in the control group. For the 20-m test, 11% of the players were identified as responders in the PJT group, compared to 5% in the control

TABLE 1 | Effects of 7 weeks of plyometric jump training plus soccer (Experimental) and only soccer training (Control) on mean group pre-post change (group % change) and number of responders (R) for performance variables.

	Experimental (n = 38)		Control (n = 38)	
	Group % change	R, n	Group % change	R, n
Countermovement jump (cm)	4.4 ± 3.8*	18†	2.4 ± 7.1	9
20-cm reactive strength index (mm·ms^{-1})	23.3 ± 17.3†	33†	−1.7 ± 13.2	5
40-cm reactive strength index (mm·ms^{-1})	16.7 ± 13.2†	29†	−1.0 ± 17.3	7
5 multiple bounds (m)	4.2 ± 4.8†	24†	0.1 ± 2.0	3
20-m sprint time (s)	−0.4 ± 2.7†	4†	3.8 ± 5.3	2
Change of direction speed test (s)	−3.5 ± 2.5†	19†	3.6 ± 2.5	0
2.4-km time trial (min)	−1.9 ± 2.4†	19†	−0.3 ± 1.9	6
Maximal kicking distance test (m)	14.0 ± 10.7†	29†	−1.4 ± 5.2	3

†*Significantly greater than Control ($p < 0.01$); *significantly greater than Control ($p < 0.05$).*

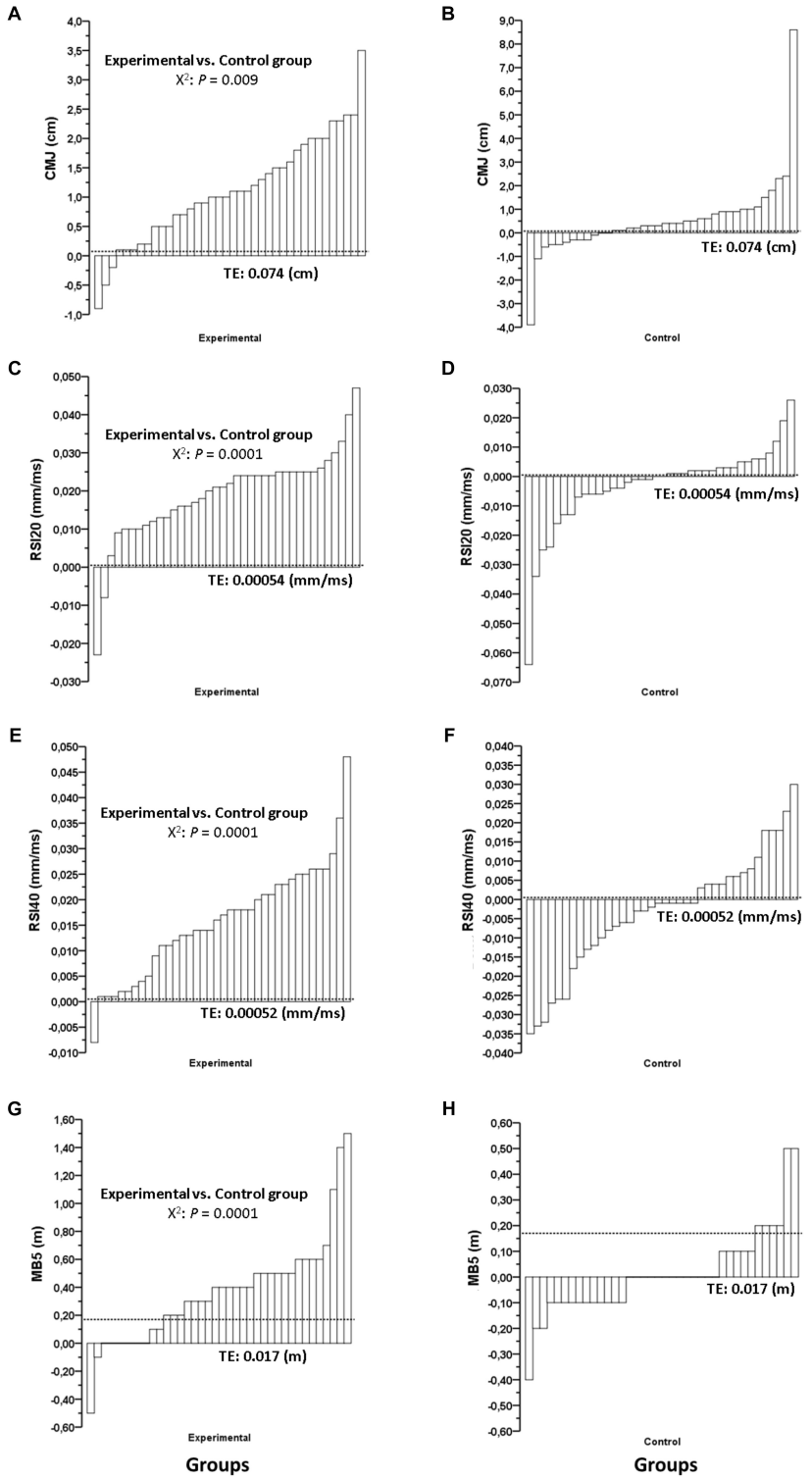

FIGURE 2 | Effects of 7 weeks of plyometric jump training plus soccer (Experimental) and only soccer training (Control) on individual pre-post change for **(A,B)** countermovement jump, **(C,D)** 20-cm reactive strength index, **(E,F)** 40-cm reactive strength index, and **(G,H)** multiple 5 bounds test. Note: all significant *p*-values (<0.05) denote a greater number of responders in the Experimental group compared to the Control group. Responders were identified on an individual basis according to the typical error of measurement (TE), represented by a dotted line. X^2, chi-squared test.

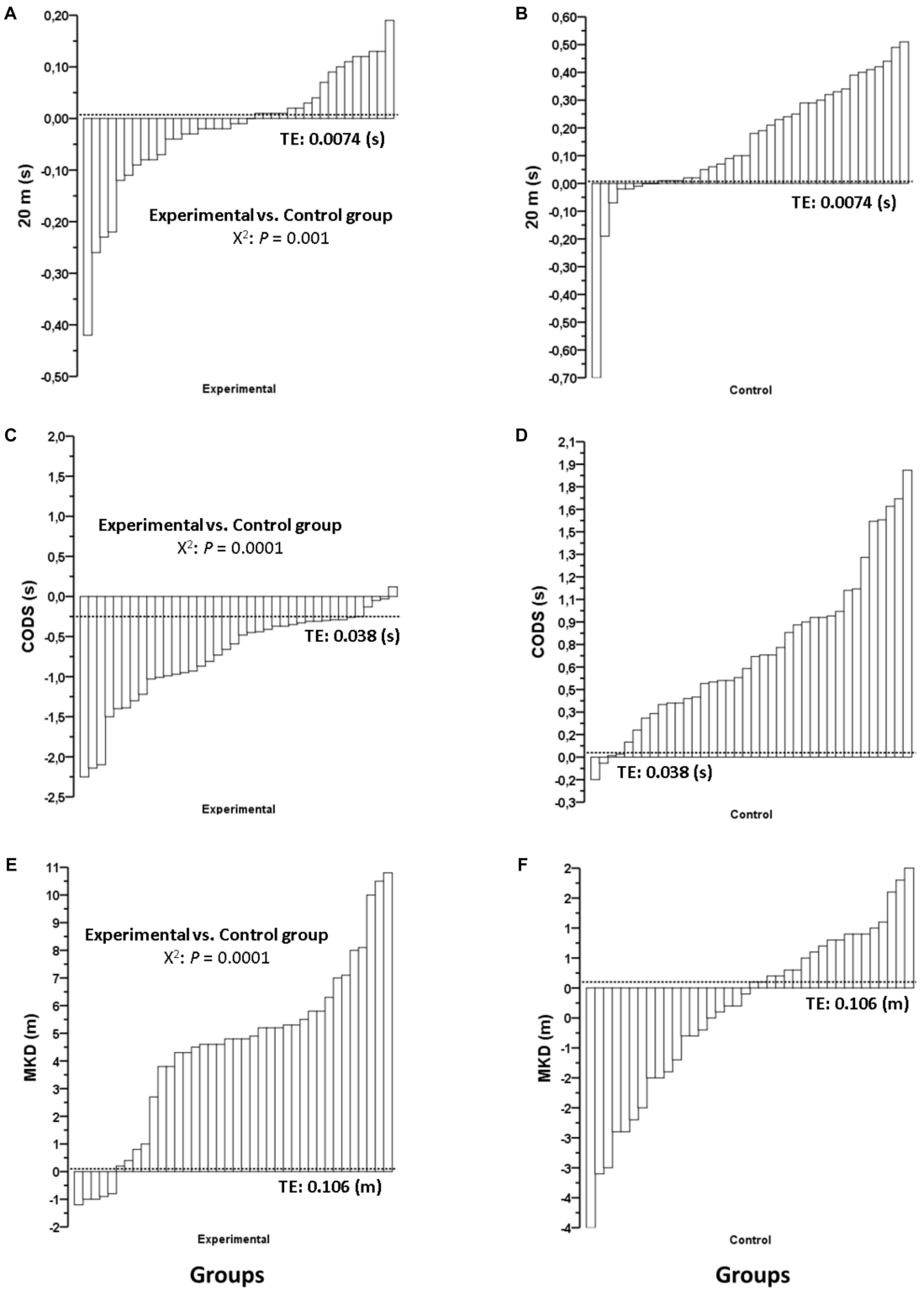

FIGURE 3 | Effects of 7 weeks of plyometric jump training plus soccer (Experimental) and only soccer training (Control) on individual pre-post change for **(A,B)** 20-m sprint time (20 m), **(C,D)** change of direction speed (CODS), and **(E,F)** maximal kicking test for distance (MKD). Note: all significant *p*-values (<0.05) denote a greater number of responders in the Experimental group compared to the Control group. Responders were identified on an individual basis according to the typical error of measurement (TE), represented by a dotted line. X^2, chi-squared test.

group. In the CODS test, 50% of the players were identified as responders in the PJT group, while none of the players from the control group demonstrated a response. Regarding the 2.4-km time trial test, 50% of players were identified as responders in the PJT group, compared to only 16% in the control group. In the MKD test, the percentage of responders for the PJT and the control groups were 76% vs. 8%, respectively.

DISCUSSION

The purpose of this study was to compare the IVRET on measures of physical fitness in youth soccer players who completed PJT versus players who completed soccer training only. The main findings indicate that there was a greater number of responders in the PJT group than in the control group across all variables measured. Thus, the combination of PJT with soccer training induced a greater number of responders than did soccer training only in measures of jumping, speed, change of direction, endurance, and technical abilities in youth soccer players. Current results contribute novel findings regarding the IVRET phenomenon in youth soccer players after a PJT program. The IVRET analysis carried out in the current study may help better assess results from PJT interventions for improved individualization of training approaches.

The current findings show larger improvements in jumping performance in the PJT group than in the control group. These results corroborate previous findings showing that PJT was effective in improving jumping performance in youth soccer players (Bedoya et al., 2015). Improvements in jumping height ability may be a relevant aim for soccer players, since a greater jumping ability may be related to a better position in a competitive league (Arnason et al., 2004), and therefore, the integration of PJT in the regular training schedules of youth players may be an effective method of enhancing competitiveness. The improvement observed in the PJT group may have been induced by increased neural drive to the agonist muscles, improved intermuscular coordination, changes in musculotendinous mechanical stiffness characteristics, changes in muscle size or architecture, and changes in single-fiber mechanics (Markovic and Mikulic, 2010). However, without specific physiological measurements, only speculative conclusions are possible. In a previous study (Radnor et al., 2017), although several resistance training methods were applied in youths, only PJT was associated with a greater number of responders in jumping performance (i.e., RSI) than that in the control group. Our results also showed a greater number of responders in jumping performance in the PJT group (n = 18–33, depending on the jump test) than in the control group (n = 3–9). In addition, our findings expanded previous knowledge, showing that PJT induced a greater number of responders than soccer training only for variables related to vertical jumping, horizontal jumping, acyclical jumping (i.e., CMJ; RSI), and cyclical jumping (i.e., five multiple bounds). Of note, a greater number of responders was observed for jumping actions that involved fast stretch-shortening cycle (SSC) measures (i.e., RSI) than for those involving slow SSC measures. This result may reflect the specific effect of the training program, as only drop jumps were implemented in the current study, similar to previous studies (Ramirez-Campillo et al., 2015a,b). Given the relevance of the rate of force development for youth soccer long-term athletic development (Meylan et al., 2014), the observed improvement in RSI could enhance physical qualities related to game performance.

Regarding the 20-m sprint test, our results indicate that the change in sprinting time was greater in the PJT group than in the control group after 7 weeks. Previous findings confirm that PJT may increase sprint performance (Saez de Villarreal et al., 2012; Assuncao et al., 2017). Moreover, a larger number of responders was observed in the PJT group (n = 4) than in the control group (n = 2). However, it must be noted that the improvement in the 20-m test was rather small (−0.4%) compared to the improvements in other physical fitness variables, possibly owing to the lack of motor pattern similarity between the training stimulus (i.e., vertical) and the sprinting performance test. Therefore, PJT may be the best complement to other methods for inducing positive adaptations that improve sprint performance. Previous studies have also called for more specific training methods to improve sprint performance in youth soccer players (Ramirez-Campillo et al., 2014b, 2015b), especially when the PJT stimulus was of a vertical nature, given the importance of horizontal force production and its relevance to sprint performance (Morin et al., 2012).

An improvement in the CODS test was observed in the PJT group compared to the control group. Performance in this test is commonly improved after PJT programs in youths (Asadi et al., 2017), including youth soccer players (Ramirez-Campillo et al., 2014b; Bedoya et al., 2015). Several underlying factors may help explain the improvements in CODS performance, such as improved muscle power and concentric and eccentric muscle strength (Young et al., 2015). To our knowledge, this was the first study to report and compare responders and NR to PJT in CODS performance among youth soccer players. Our results indicate that the PJT program induced 19 responders; meanwhile, no responders were detected in the control group. These results suggest the advantage of including some specific jump drills in the regular training schedule of youth soccer players to help them perform the CODS movements that commonly occur during a competitive soccer match (Stolen et al., 2005). Moreover, CODS is an important determinant of high performance throughout the course of a soccer player's career and, therefore, must be developed from a young age (le Gall et al., 2010).

Regarding the 2.4-km time trial test, the PJT induced an improvement in this test in the youth soccer players. Improvements in similar tests have been previously reported in youth soccer players after PJT (Ramirez-Campillo et al., 2014b; Assuncao et al., 2017). In addition to the improvements in endurance performance in the PJT group, the number of responders in the PJT group reached 50%, which is considerably higher than the 16% in the control group. The positive effects of PJT on performance in the 2.4-km time trial test might be due to the running economy associated with PT (Barnes and Kilding, 2015), which may have offset fatigue and allowed the athletes to maintain a higher velocity during the test.

In the kicking performance test, the PJT group experienced greater improvement than the control group. This is a particularly interesting observation considering that the PJT group replaced some technical low-intensity soccer drills with high-intensity jumping actions. Moreover, a greater number of responders (n = 29) was observed after PJT than after soccer training only (n = 3). These results are similar to those previously reported (Michailidis et al., 2013). Considering that players had 2 or more years of soccer experience, improvements in kicking performance in the PJT group were probably not related to changes in technical ability and were more likely due to improvements in neuromuscular (Markovic and Mikulic, 2010) and biomechanical adaptations induced by PJT (Lees et al., 2010).

Although with several strengths, some potential limitations should be acknowledge. First, we did not obtain physiological assessments to better understand the underlying mechanisms of PJT induced adaptations in responders and NR athletes. However, physical fitness tests (i.e., jumping) are significantly and highly associated with physiological [i.e., type of muscle fiber (Bosco and Komi, 1979)] and biomechanical parameters (Ham et al., 2007; Chamari et al., 2008; Meylan et al., 2010) as well as with sporting success. The latter is most important for athletic cohorts (Arnason et al., 2004; Wisloff et al., 2004). Second, although the replacement of technical drills during the in-season period in youth soccer players is uncommon, our approach did not induced a negative impact on the player's technical abilities. In fact, our results proved that athletes in the PJT group improved their ability to kick a soccer ball. However, from an ecological valid point of view, although PJT can improve physical fitness in youth male soccer players, to optimize training adaptations, this training strategy should be adequately applied in a more complex training plan that incorporates other explosive (e.g., sprints), endurance, technical, and tactical-oriented training methods. Future studies should aim to obtain physiological assessments to better understand the underlying mechanisms of PJT induced adaptations in responders and NR athletes. Moreover, future studies should aim to analyze the IVRET according to the maturity of soccer players, including both female and males athletes.

In conclusion, compared to soccer training only, PJT induced greater physical fitness improvements in youth soccer players, with a greater number of responders in the PJT group in all the physical fitness tests related to jumping, speed, change of direction, endurance, and kicking technical ability. The current results contribute novel findings regarding the IVRET phenomenon in youth soccer players after a PJT program. The IVRET analysis carried out in the current study may help to better assess results from PJT interventions for improved individualization of training approaches.

AUTHOR CONTRIBUTIONS

RR-C, CA, and MI designed the work. RR-C and CA acquired the data. PG, JM, AMA-M, and CA analyzed and interpreted the data. RR-C, CA, and FG-P drafted the work. All authors critically revised the work, approved the final version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

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REVIEW
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Sources of Inter-individual Variability in the Therapeutic Response of Blood Glucose Control to Exercise in Type 2 Diabetes: Going Beyond Exercise Dose

Thomas P. J. Solomon[1,2]*

[1] School of Sport, Exercise, and Rehabilitation Sciences, University of Birmingham, Birmingham, United Kingdom, [2] Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, United Kingdom

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***Correspondence:**
Thomas P. J. Solomon
t.solomon@bham.ac.uk

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In the context of type 2 diabetes, inter-individual variability in the therapeutic response of blood glucose control to exercise exists to the extent that some individuals, occasionally referred to as "non-responders," may not experience therapeutic benefit to their blood glucose control. This narrative review examines the evidence and, more importantly, identifies the sources of such inter-individual variability. In doing so, this review highlights that no randomized controlled trial of exercise has yet prospectively measured inter-individual variability in blood glucose control in individuals with prediabetes or type 2 diabetes. Of the identified sources of inter-individual variability, neither has a prospective randomized controlled trial yet quantified the impact of exercise dose, exercise frequency, exercise type, behavioral/environmental barriers, exercise-meal timing, or anti-hyperglycemic drugs on changes in blood glucose control, in individuals with prediabetes or type 2 diabetes. In addition, there is also an urgent need for prospective trials to identify molecular or physiological predictors of inter-individual variability in the changes in blood glucose control following exercise. Therefore, the narrative identifies critical science gaps that must be filled if exercise scientists are to succeed in optimizing health care policy recommendations for type 2 diabetes, so that the therapeutic benefit of exercise may be maximized for all individuals with, or at risk of, diabetes.

Keywords: exercise, training, type 2 diabetes, non-responder, variability, blood glucose control, HbA1c, heterogeneity

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is characterized by persistent hyperglycemia (**Table 1**) that increases the risk of retinopathy, nephropathy, neuropathy, and cardiovascular-related mortality. Because diabetes affects 5–10% of the population, healthcare costs create a major socioeconomic burden. For example, in 2012 the United Kingdom, the National Health Service spent ∼10% of its annual budget (∼£24billion) on diabetes management (Hex et al., 2012). Although T2DM is considered a preventable disease, since its incidence is mostly associated with lifestyle factors, patient numbers continue to escalate.

Blood glucose levels are governed by rates of glucose appearance and disappearance that are controlled by a complex interplay between metabolic, endocrine, and neurological systems. This involves direct action of neuronal, gastrointestinal, hepatic, pancreatic, renal, adipose, endothelial, and muscular tissues. Muscle contraction-mediated increases in basal glucose disposal were first documented in the 1960s (Holloszy and Narahara, 1965). In the 1970s and 1980s, it emerged that exercise also increases insulin sensitivity in rodents (Richter et al., 1982) and humans (Soman et al., 1979). An abundance of studies has now confirmed that robust increases in insulin sensitivity occur following exercise in individuals with prediabetes or patients with T2DM. Consequently, exercise is a key part of diabetes therapy included in the American Diabetes Association's (ADA) diabetes prevention and treatment guidelines. Skeletal muscle indeed plays a large role in blood glucose uptake during/following exercise, but at rest and following a meal blood glucose levels are controlled by several tissues. Skeletal muscle insulin sensitivity is not measured in the clinic since it is impractical and because it is the exposure to persistent hyperglycemia (in additional to elevated lipids and inflammatory cytokines) that elicits diabetic complications and cardiovascular mortality. For this important reason, this review will principally focus on evidence from exercise intervention studies where blood glucose control is the primary outcome. Glycated hemoglobin (HbA1c) levels, fasting plasma glucose, and the 2-h plasma glucose value during a 75-g oral glucose tolerance test (OGTT) are the three variables used by clinicians to measure blood glucose control, and to diagnose and monitor treatment in those at risk of developing diabetes and in patients with T2DM (American Diabetes Association, 2018a) (**Table 1**).

TABLE 1 | Diagnostic references range values for high risk for diabetes (prediabetes) and type 2 diabetes (American Diabetes Association, 2018a).

	Prediabetes	Type 2 diabetes
Fasting glucose	≥5.6 to 6.9 mM	≥7 mM
Two-hour OGTT glucose	≥7.8 to 11 mM	≥11.1 mM
HbA1c	≥5.7 to 6.4% (39–47 mmol/mol)	≥6.5% (48 mmol/mol)
Random blood glucose	–	≥11.1 mM

Although there is a robust effect of exercise on insulin sensitivity, the effect of exercise training on blood glucose control is less consistent. Several reports suggest that large inter-individual variability may exist in the therapeutic effect of exercise on blood glucose control. This narrative review will explore such variability and then identify the sources of this variability. By doing so, the narrative will highlight key science gaps that must be filled in order to inform and improve the current clinical guidelines.

EVIDENCE FOR INTER-INDIVIDUAL VARIABILITY IN THE THERAPEUTIC EFFECT OF EXERCISE ON BLOOD GLUCOSE CONTROL, IN INDIVIDUALS WITH (PRE)DIABETES

Clues that inter-individual variability exists have emerged from clinicians' anecdotal observations and alarmist media headlines. However, we can venture beyond subjective assessments and objectively examine such variability. The first evidence comes from the HERITAGE family study in which Boulé and colleagues followed 596 healthy sedentary individuals through a 20-week training intervention (Boulé et al., 2005). Participants exercised on cycle ergometers for 30–50-min on 3 days/week, at 55–75% VO_2max. Glucose tolerance and its determinants (insulin sensitivity and insulin secretion) was determined via intravenous glucose tolerance tests (IVGTT) before and after the intervention. Although there were statistically significant training-induced increases in glucose disappearance rate (K_g), insulin sensitivity (S_i), and disposition index (DI, a marker of insulin secretory function relative to insulin sensitivity), approximately 40% of the participants showed no change or an adverse direction of change (a decrease) in these variables (Boulé et al., 2005). Fasting glucose also significantly improved but its inter-individual variability was not reported. This work made an important advance by presenting the variability of training-induced changes in diabetes-relevant variables; however, participants with prediabetes or T2DM were not included, and neither HbA1c nor 2-h OGTT glucose were measured (Boulé et al., 2005). We followed up this work in 2013 to examine the inter-individual variability in the therapeutic response of blood glucose control in 105 older obese individuals with prediabetes or T2DM, excluding those treated with insulin (Solomon et al., 2013b). All participants underwent a 12–16-week aerobic exercise training intervention consisting of up to 60 min/day supervised walking or cycling on 4–5 days/week at up to 75% of VO_2max. Blood glucose control (HbA1c, fasting glucose, 2-h OGTT glucose) and its determinants, insulin sensitivity (measured by hyperinsulinemic euglycemic clamp) and insulin secretion (plasma C-peptide response to OGTT), were assessed before and following the intervention. Exercise training was well adhered to and there was a small statistically significant reduction in both fasting glucose and 2-h OGTT glucose, along with an statistically significant increase in insulin-sensitivity and disposition index (Solomon et al., 2013b). Following training, HbA1c, fasting glucose, and 2-h OGTT glucose were reduced in only 69, 62, and 68% of the study participants, respectively (Solomon et al., 2013b). This work indicated that approximately 1/3 of this cohort of individuals with prediabetes or T2DM had no improvement or even a deterioration in blood glucose control following exercise training (**Figure 1**). We confirmed that the observations made by Boulé et al. (2005) in healthy individuals are also evident in individuals with pathological blood glucose control. Similar findings were published in Álvarez et al. (2017) who examined the effect of 10-weeks of high-intensity interval training in two groups of women, 20 with normal fasting glucose and 20 with impaired fasting glucose and elevated HOMA-IR values. The authors found a statistically significantly reduction in HOMA-IR but an increase in HOMA-IR in 5 of the 20 participants (Álvarez et al., 2017). In the same year, work from Phillips and

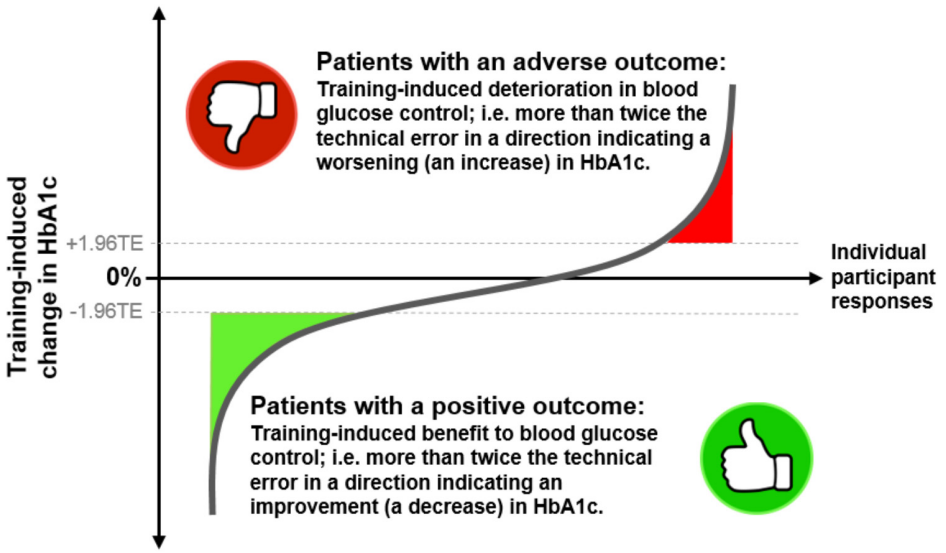

FIGURE 1 | A cartoon depicting the inter-individual variability of changes in HbA1c levels following exercise training in individuals with prediabetes or type 2 diabetes. The *y*-axis represents the training-induced change in HbA1c (post- minus pre-intervention value). The *x*-axis represents the individual participants taking part in a study. Adverse outcomes are illustrated as participants' responses showing a training-induced increase in HbA1c that is more than 1.96 times the technical error (TE). Therapeutic outcomes are shown as training-induced decreases in HbA1c that are greater than 1.96 times the technical error.

colleagues also highlighted variability in changes in HOMA-IR following training in obese or prediabetic individuals (Phillips et al., 2017). It must be highlighted, however, that neither our work (Solomon et al., 2013b) or the work of Boulé (Boulé et al., 2005), or Álvarez (Álvarez et al., 2017), included a non-exercise control group. Therefore, the direct effects of training *per se* are uncertain, and the natural variability (i.e., intra-subject variability) in the measured variables over the time course of the interventions are not known. The work from Phillips did include a non-exercise control group but did not report variability from diabetes-related clinical diagnostics (HbA1c, or blood glucose) (Phillips et al., 2017). More importantly, as will be discussed in the next section, despite concluding that inter-individual variability in the therapeutic effect of exercise on blood glucose control exists, it can be debated whether these studies employed an adequate study design in order to detect such variability and thus accurately identify adverse outcomes.

HOW SHOULD WE QUANTIFY INTER-INDIVIDUAL VARIABILITY AND THEREBY IDENTIFY A NON-RESPONDER TO EXERCISE?

As the previous section alludes, inter-individual training-induced changes in blood glucose control are heterogeneous across the population. The treatment goal for diabetes is to manage blood glucose control by reducing HbA1c, fasting glucose, and 2-h OGTT glucose toward specific target levels. A "non-responder" is a patient displaying a lack of therapeutic benefit (no reduction in HbA1c, in the context of this review) following treatment, while a deterioration in blood glucose control (i.e., an increase in HbA1c) is an "adverse outcome" since this confers an elevated risk of diabetic complications and cardiovascular-related mortality. Previously, this phenomenon has also been referred to as "exercise resistance" (De Filippis et al., 2008; Stephens and Sparks, 2015). Several randomized controlled trials have found no significant improvement in blood glucose control in patients with T2DM following training (Dela et al., 2004; Burns et al., 2007; Karstoft et al., 2013; Terada et al., 2013b). However, these studies present the changes in sample mean (±SD, or ±SEM) and do not provide information regarding the inter-individual responses.

To obtain an accurate assessment of the inter-individual variability in an intervention-induced change in a primary outcome variable, one must be able to quantify the two components of change: random change (induced by technical and/or biological error) and systematic change (induced by the intervention). To separate random from systematic changes, scientists may calculate the typical error of measurement (i.e., the within-subject standard deviation), which reflects the measurement-to-measurement variation in a patient's value. Typical error is equal to the standard deviation of the sum of the observed differences between repeated measurements within each individual. Since variance is equal to standard deviation squared (s^2), the variance of the differences between within-subject repeated-measurements (represented by $s_{diff}{}^2$) is equal to the sum of the variances representing the typical error. This can be written as $s_{diff}{}^2 = s^2 + s^2$ which rearranges to $s = s_{diff}/\sqrt{2}$, therefore the technical error of measurement is equal to the standard deviation of the difference scores divided by the square root of 2. For normally distributed data, 95% of the observations fall within 1.96 standard deviations of the mean. Therefore, to have 95% confidence that an intervention (either

treatment or control) has no effect on a variable of interest in a particular individual and thereby identify a non-responder, the intervention-induced change in that subject should be less than 1.96 times the technical error of measurement. In the context of this review, any diabetic patient not exhibiting an exercise-induced decrease in HbA1c (or fasting glucose or 2-h OGTT glucose) more than 1.96 times the technical error would be a true non-responder. These principles have been discussed in detail by Hopkins (2000) and Senn (2004). Technical error, however, may display heteroscedasticity. For example, it could be greater when the value of the variable is larger, or it may differ across sub-groups (e.g., male vs. female, young vs. old, diabetic vs. non-diabetic, etc.). If so, applying an average typical error to all groups may overestimate some individuals and underestimate others. To account for heteroscedasticity, the typical error of measurement would need to be calculated individually for all such sub-groups or, more simply, the data could be normalized; for example, by log transforming to remove the heteroscedasticity or by expressing the technical error as a percentage of the respective mean (i.e., a coefficient of variation for the technical error). But, how should we use technical error to quantify inter-individual variability?

In a repeated-measures study, if data are analyzed using a linear mixed model with the patient ID number as a random effect and the intervention assigned as a fixed effect, a significant patient-by-treatment interaction would indicate true inter-individual variability. However, this would only be correct if the training effect on an individual is reproducible. Naturally, the blood glucose lowering response to exercise training in T2DM may not only exhibit inter-individual variability but also intra-individual variability of the measurement if the intervention was repeated within an individual. Consequently, the optimal approach for quantifying inter-individual variability in repeated-measures studies is to use a randomized replicated crossover design where the control (no training) and treatment (training) conditions are administered to each participant at least twice (Senn et al., 2011; Hecksteden et al., 2015; Atkinson et al., 2018; Goltz et al., 2018) (**Figure 2**). This design would allow accurate interpretation of a significant patient-by-treatment interaction, thereby revealing the true individual differences in response to exercise. A limitation of this approach, however, is that an adequate wash-out of the training effects would be required which, for exercise studies, creates logistical difficulty. It is unlikely that long-term training studies with a double crossover to determine the patient-by-treatment interaction effect on blood glucose control will ever be conducted in patients with T2DM (**Figure 2**), because blood glucose control would deteriorate while patients were not exercising, and several confounding variables would likely change (e.g., their drug regimen, body weight, etc.). Hecksteden et al. (2015) proposed an indirect approach whereby a separate validity study would be conducted to determine the within-subject variability in their response to repeated training interventions and using a linear mixed model to apply that to the main study. Although not in the context of blood glucose control or in individuals with (pre)diabetes, some training studies have used this approach (Bonafiglia et al., 2016; Gurd et al., 2016) but it is confounded by assumed generalizability, which contradicts the initial reason to accurately determine whether true inter-individual variability actually exists. Fortunately, a more practical alternative solution exists which is to repeatedly test the primary outcome variable within an intervention. Bouchard et al. (2012) explored inter-individual variability in the response of metabolic syndrome related variables to exercise training in order to identify adverse outcomes. They used an approach whereby resting systolic blood pressure, fasting triglycerides, and fasting HDL-cholesterol were measured three times over a 3-week period in sixty subjects from six independent randomized controlled long-term exercise training studies (including HERITAGE, DREW, INFLAME, STRRIDE, and others). Subsequently, they calculated the technical error of measurement for these variables to determine the frequency of exercise-induced adverse outcomes, reporting that 12, 10, and 13% of their sample population had an "adverse response" in systolic blood pressure, triglycerides, and HDL-cholesterol, respectively, following exercise training (Bouchard et al., 2012). While this elegant approach, which was also used by Phillips et al. (2017), provides evidence that non-responders to exercise indeed exist in the context

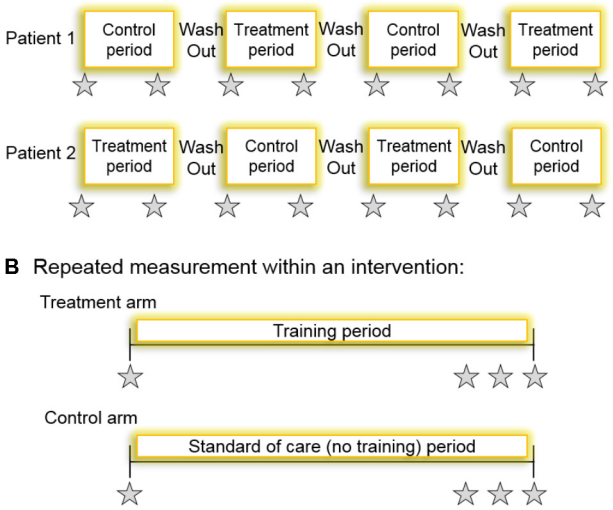

FIGURE 2 | (A) A schematic for a repeated-measures double-crossover study design which, if the patient-by-treatment interaction term were statistically significant, would accurately indicate the presence of inter-individual variability. All patients undergo all interventions twice. The type of trial each participant first undergoes should be randomized and the primary outcome is measured at the beginning and the end of each intervention period. The time between interventions would have to be sufficient such that training effect was washed out. **(B)** Perhaps a more practical and logistically feasible method for determining the technical error of measurement, and thereby identifying non-responders, is a randomized controlled intervention where the primary outcome variable is measured repeatedly within an intervention. Participants are randomized to either the control arm (no training) or the treatment arm (exercise training). Ideally, the variable would be repeatedly measured within a time frame where intervention-induced changes are unlikely (e.g., measuring HbA1c three times within a 2-week period towards the end of a long-term training intervention). Gray stars indicate repeated measurement of the variable, HbA1c, for example.

of cardiometabolic risk factors, surprisingly the authors of neither study presented inter-individual changes in blood glucose control. Blood glucose control (2-h OGTT glucose) was measured, however, by De Lannoy et al. (2017) who found that the number of non-responders ranged from 86 to 98% following different types of training in 171 obese non-diabetic adults.

The above-mentioned studies provide evidence for inter-individual variability in the therapeutic effect of exercise on blood glucose control, in individuals with (pre)diabetes. However, as of 2018, there is an urgent need for a large-scale randomized controlled trial aimed specifically at investigating the variability of long-term training adaptations in blood glucose control in individuals with T2DM. Such a trial should employ a study design allowing analysis of a patient-by-treatment interaction. This would be possible with a repeated-measures crossover where the control and treatment conditions are administered to each participant at least twice, or where the primary outcome variable (HbA1c, fasting glucose, and 2-h OGTT glucose) is repeatedly tested within the control and treatment intervention arms (**Figure 2**). As described above, both approaches have their limitations that investigators need to be aware of. But such approaches would generate technical errors of measurement, enabling training-induced effects on blood glucose control to be reliably compared between independent studies, and allowing interpretations to be made in the context of clinically meaningful responses to interventions in individual subjects. The eternal endeavor of achieving statistical significance between means is useless when trying to identify whether one particular person has responded or not to a treatment. Therefore, measuring technical error, quantifying inter-individual variability, and thereby detecting true adverse outcomes in exercise science will advance the field. However, this will only be achieved if investigators also attempt to control for as many sources of variance as possible.

SOURCES OF INTER-INDIVIDUAL VARIABILITY IN THE THERAPEUTIC EFFECT OF EXERCISE ON BLOOD GLUCOSE CONTROL IN INDIVIDUALS WITH (PRE)DIABETES

The above-described evidence suggests it is very likely that inter-individual variability in the therapeutic effects of exercise on blood glucose control truly exists in patients with T2DM, and that adverse outcomes do occur. However, we do not precisely understand what causes adverse outcomes and, more importantly therefore, we do not currently know how adverse outcomes can be prevented. The standard-of-care guidelines for diabetes which are published annually by the American Diabetes Association, provide excellent, clear and effective evidence-based exercise recommendations that are summarized in **Table 2** (American Diabetes Association, 2018b). However, heterogeneity in the therapeutic effect of exercise between patients inevitably prompts us to explore how adverse outcomes can be avoided. Exercise scientists, clinicians, and fitness trainers often comment that non-responders should simply do more exercise. It is indeed enticing to believe such a sentiment, particularly when it may be true in the context of improving cardiorespiratory fitness (VO_2max) in healthy adults (Montero and Lundby, 2017). Nonetheless, in individuals with (pre)diabetes, several lines of published evidence have identified sources of inter-individual variability in the therapeutic effect of exercise on blood glucose that go beyond exercise dose. The following points discuss these sources in the context of current guidelines which, with future experimental evidence, will be improved by providing more information on exercise dose, exercise type, exercise-meal timing, and anti-hyperglycemic drug-exercise timing, etc.

A. Exercise Dose (Frequency, Intensity, and Time)

The first evidence that exercise dose might contribute to the inter-individual variability of training-induced changes in blood glucose control came from the elegant series of STRRIDE studies. In 2004, over 200 middle-older aged, sedentary, overweight or obese individuals were randomized to one of four 8-month training interventions: (i) low amount/moderate intensity (1200 kcal/week), (ii) low amount/vigorous intensity (1200 kcal/week), (iii) high amount/vigorous intensity (2000 kcal/week), or (iv) non-exercise control. Fasting glucose, and insulin sensitivity, insulin secretion, and disposition index (modeled from an IVGTT), were measured before and after the interventions. Fasting glucose increased in the control group but was unaffected by any of the training interventions, whereas insulin sensitivity was most increased by the low amount/moderate intensity and high amount/vigorous intensity interventions compared to the low amount/vigorous intensity intervention (Houmard et al., 2004; Slentz et al., 2009). Of importance, the expected training-induced compensatory decrease in the insulin secretory response to IVGTT (relative to the increased insulin sensitivity) was smallest following the

TABLE 2 | Exercise recommendations for adults with prediabetes or type 2 diabetes, issued in American Diabetes Association (2018b) standard of care update.

	Exercise recommendations for adults with prediabetes or type 2 diabetes
1	≥150 min of moderate to vigorous intensity aerobic activity per week, spread over at least 3 days/week, with no more than 2 consecutive days without activity.
2	Shorter duration (≥75 min per week) of vigorous-intensity or interval training may be sufficient for younger and more physically fit individuals.
3	Two to three sessions per week of resistance exercise on non-consecutive days.
4	Decrease the amount of time spent in daily sedentary behavior. Prolonged sitting should be interrupted every 30 min.
5	Flexibility training and balance training are recommended 2–3 times per week for older adults with diabetes.

low amount/moderate intensity intervention, leading to the largest increase in glucose disposition in that group (Slentz et al., 2009). The STRRIDE investigators also followed up these investigations in ∼150 middle- to older-aged, sedentary participants with prediabetes randomized to one of four 6-month interventions: (i) low amount/moderate intensity exercise, (ii) high amount/moderate-intensity exercise, (iii) high amount/vigorous-intensity exercise, or (iv) control group (low amount/moderate intensity plus 7% weight loss to mimic the DPP study). This STRRIDE-Prediabetes study (Slentz et al., 2016) found that only the control group improved fasting glucose but that high amount/moderate-intensity exercise was most effective for lowering the blood glucose during OGTT, when compared to either low amount/moderate intensity or high amount/vigorous-intensity exercise. A further study (Malin et al., 2013) found that exercise dose was positively associated with increased glucose disposition index following a 3-month aerobic training intervention in 35 older, obese, individuals with prediabetes. However, no statistically significant associations between exercise dose and changes in blood glucose control were found (Malin et al., 2013). Furthermore, exercise dose was simply estimated from assumed energy expenditure during exercise sessions in a supervised training intervention and participants were not randomized to different exercise dosing groups (Malin et al., 2013). Finally, Terada and colleagues conducted a retrospective analysis of outcomes in 15 middle- to older-aged individuals with T2DM randomly assigned to a 12-week energy expenditure-matched high-intensity interval exercise or moderate-intensity steady-state exercise intervention (Terada et al., 2013a). Capillary blood glucose was measured immediately before and after each exercise bout and multiple regression analyses demonstrated that greater reductions in blood glucose were found in individuals working at higher exercise intensities or engaged in longer exercise bouts (45- or 60- vs. 30-min). Hence, Terada et al. (2013a) showed that a larger exercise dose was more effective for reducing hyperglycemia in diabetes patients.

Exercise frequency has seldom been studied during training interventions in the context of diabetes. Dubé et al. (2012), who studied the effects of 16-weeks of aerobic exercise training on changes in insulin sensitivity in middle-aged overweight and obese non-diabetic individuals, found that although exercise dose was positively correlated with increased insulin sensitivity, exercise frequency did not contribute to the magnitude of the change. Unfortunately, Dubé and colleagues did not report blood glucose control variables (Dubé et al., 2012). Exercise frequency has, however, been studied in detail in people with (pre)diabetes during a 24–48-h period. In van Dijk et al. (2012) found that a 30-min cycle at 50% peak power on two consecutive days elicited the same improvement in continuous glucose monitoring (CGM)-derived blood glucose control compared to a single 60-min ride in thirty older obese patients with T2DM. DiPietro et al. (2013) found that three 15-min post-meal walking bouts (moderate-intensity; 3 METs) was more effective at lowering CGM-derived blood glucose profiles than a single 45-min bout performed in the morning or evening, in older adults with prediabetes. In a similar demographic of prediabetic adults, in Francois et al. (2014) also found that walking bouts (6 × 1 min incline walking at 90% HRmax) performed 30 min before the three meals of the day reduced CGM-derived glucose compared to no exercise or a single 30-min bout of moderate-intensity (60% of maximal heart rate) walking. However, these studies (DiPietro et al., 2013; Francois et al., 2014), along with the work of Dubé et al. (2012) did not present the inter-individual responses. While some studies indicate that exercise dose may indeed influence variability in the changes in blood glucose control following training in people with (pre)diabetes, no study has yet specifically analyzed the inter-individual variability caused by different exercise doses or frequency. While current guidelines (**Table 2**) for preventing and treating T2DM clearly state how many minutes of exercise should be accumulated each week and how frequent exercise sessions should be (American Diabetes Association, 2018b), precise guidance on what a moderate to vigorous intensity equates to is lacking.

B. Exercise Type

Evidence that the type of exercise might play a role in the inter-individual variability in outcomes also originates from the STRIDE team. In 2011, the STRRIDE-AT/RT study randomized ∼200 volunteers to (i) resistance training (3 days/week, 3 sets/day of 8–12 repetitions of 8 different exercises targeting all major muscle groups), (ii) aerobic training (∼120 min/week at 75% of the VO_2max), or (iii) combined resistance plus aerobic training, for 8-months (Bateman et al., 2011). Although the post-minus pre-intervention change in blood glucose increased in the combined resistance-aerobic group and decreased in the aerobic-only and resistance-only group, the large standard error of the change scores indicates probable heterogeneity between subjects. Consequently, the authors found no statistically significant effects of exercise on fasting blood glucose nor statistical differences between groups (Bateman et al., 2011). The HART-D and DARE studies advanced this work by conducting randomized controlled trials comparing the effects of resistance, aerobic, vs. combined resistance-aerobic training on HbA1c in patients with T2DM (Sigal et al., 2007; Church et al., 2010). Both studies employed similar designs, training sessions were fully supervised, but HART-D was longer in duration than DARE (9- vs. 6-months) and made more thorough recording and monitoring of the exercise dose and energy expenditure. HART-D found that only combined resistance-aerobic training significantly reduced HbA1c (Church et al., 2010), while HbA1c was significantly reduced in all 3 exercise groups the DARE trial (Sigal et al., 2007). The between-study differences may be attributable to the greater weight loss (fat mass) seen in DARE vs. HART-D. Further to such work, a randomized controlled trial from my group compared the effects of 4-months of moderate-intensity steady-state walking training (4–5 days/week, ∼60 min/day) vs. energy expenditure matched moderate-intensity interval walking training (ten cycles of 3-min fast, 3- min slow walking) in patients with T2DM (Karstoft et al., 2013). We found that only interval walking training improved CGM-derived glucose control but that this was in the presence of greater weight loss than the continuous walking group. We also found that varying interval length (1-min fast,

1 min slow vs. 3-min fast, 3- min slow walking) had no influence on the improvement in blood glucose control (Jakobsen et al., 2016).

The current guidelines (**Table 2**) do not provide specific guidance on which types of exercise may be used (American Diabetes Association, 2018b). Neither are they explicit on what aerobic or resistance exercise means. Some studies indeed indicate that either aerobic or resistance exercise alone may be sufficient to improve blood glucose control, while other work shows that different modalities of walking can also have diverging outcomes. In an age of interval training popularity, since 2017 the ADA guidelines have included a useful statement concerning vigorous exercise and interval training (American Diabetes Association, 2018b), stating that "≥75 min/week of vigorous-intensity or interval training may be sufficient for younger and more physically fit individuals." However, specific details on what vigorous-intensity or interval exercise may entail is lacking. We also await outcomes from large scale randomized controlled trials with HbA1c as a primary endpoint to further understand the role of exercise type (particularly high intensity interval training) in order to further optimize existing guidelines. Additionally, no current study has yet prospectively examined inter-individual variability in changes in blood glucose control following different types of training in patients with prediabetes or T2DM.

C. Exercise Adherence

In this context, adherence refers to the longevity of maintaining regular exercise following the initial inclusion of exercise to an individual's lifestyle. With the exception of a few studies (Houmard et al., 2004; Sigal et al., 2007; Slentz et al., 2009; Church et al., 2010; Karstoft et al., 2013; Solomon et al., 2013b), exercise adherence is seldom measured or reported in training studies whose primary outcome was blood glucose lowering in (pre)diabetes patients. This is unfortunate since adherence is of utmost importance for assessing the intended stimulus provided by the intervention. That said, many trials exclude patients participating in <80% of the treatment intervention. This creates widespread homogeneity and bias. Nonetheless, a lack of adherence will clearly influence the total exercise dose (daily dose, weekly frequency), which as described above contributes toward inter-individual outcomes. However, exercise adherence goes beyond metabolism and involves health psychology (desire, motivation), behavioral barriers (e.g., self-esteem, self-image), and environmental barriers (e.g., local access to trails/parks/gyms, weather, climate). Such factors are beyond the scope of this review but have been reviewed elsewhere (Wing et al., 2001). Nonetheless, in the interest of implementing and sustaining population-wide physical activity lifestyles changes, a brief description of the influence of such factors on exercise adherence should be included in the current ADA guidelines. This may help clinicians provide more individualized advice. Furthermore, the role of adherence in the inter-individual variability in glycemic outcomes following training in (pre)diabetes patients must be studied in greater depth. This would help inform and further enhance the guidelines.

D. Exercise-Meal Timing

Training in the fed vs. fasted state has long been an intense area of investigation for optimizing athletes' training methods to maximize their performance. This is also true for patients with type 1 diabetes, where several studies have investigated exercise timing in relation to insulin dosing and carbohydrate intake. However, in the context of T2DM, little data exists. In the early 2000s, a series of elegant studies by Poirier and colleagues (Poirier et al., 2000, 2001; Gaudet-Savard et al., 2007) examined fed vs. fasted exercise in men with T2DM. One-hour of moderate-intensity cycling (60% VO_2max) completed 2-h after breakfast reduced blood glucose, whereas fasted exercise did not (Poirier et al., 2001). Further, following a 3-month training intervention (1-h of cycling at 60% VO_2max, three times per week) no change in blood glucose was found when exercise was performed in the fasted state, whereas 20–40% decreases in blood glucose arose when exercise was initiated postprandially (Poirier et al., 2000). However, the authors also documented that the effect of exercise on blood glucose in the fasted state was dependent on the ambient glucose level: blood glucose increased when pre-exercise glucose levels were ≤6 mM but decreased when pre-exercise levels were >8 mM (Gaudet-Savard et al., 2007). In support of these findings, Colberg et al. (2009) found that 20 min of self-paced treadmill walking 15–20 min after eating dinner lowered blood glucose in patients with T2DM, whereas pre-dinner exercise had no effect on blood glucose levels. Data somewhat related to these observations were generated from a retrospective analysis of outcomes from a 12-week training study in patients with T2DM conducted by Terada et al. (2013a). They demonstrated that greater reductions in blood glucose were found when pre-exercise meals were ingested less than 2-h prior to the beginning of exercise sessions rather than more than 2-h. These above-described data led to a series of view-point papers published by Chacko (2014, 2016) who presented an idea that the mid-postprandial period (30–120 min post-ingestion) would be the best time to implement exercise in order to optimize blood glucose control. While Chacko's viewpoints are mostly anecdotal and informed by educated opinion rather than evidence, they nicely highlighted the necessity for research data in this field from a clinician's perspective. That said, Chacko's points are specific to the acute response to exercise. In healthy individuals, ingestion of carbohydrate prior to exercise has been shown to blunt adaptations to training (Van Proeyen et al., 2010) as well as acute bouts of exercise (Gonzalez et al., 2013; Taylor et al., 2018). Thus optimal benefit may be conferred from fasted exercise. However, in people with diabetes, fasted exercise promotes post-exercise hypoglycemia which should be avoided at all costs.

Exercise-meal timing is rarely considered in the design of training studies. As of 2018, no long-term, randomized, controlled, physical activity or exercise intervention trial with a primary focus on blood glucose control has reported exercise-meal timing. It is therefore possible that inappropriate exercise-meal timing partly explains the lack of improvement in blood glucose control in some long-term training studies in individuals with (pre)diabetes (Dela et al., 2004; Burns et al., 2007; Karstoft et al., 2013; Terada et al., 2013b). Despite efforts to elucidate the optimal exercise-meal timing, full

knowledge in this area is lacking. Consequently, as of 2018, no information regarding exercise-meal timing is provided in the ADA guidelines for preventing and treating T2DM (American Diabetes Association, 2018b). Furthermore, no study has yet determined the influence of exercise-meal timing on inter-individual variability in glycemic outcomes. Given recent knowledge that skeletal muscle metabolism in humans follows a diurnal pattern under the control of clock genes (Loizides-Mangold et al., 2017), it is plausible that circadian rhythm is an additional (albeit complicated) factor to additionally consider in future work aimed at optimizing exercise-meal timing for maximal postprandial glucose control.

E. Exercise-Drug Interactions

Anti-hyperglycemic pharmacologic therapy is administered in conjunction with lifestyle management for the treatment of, and to an extent the prevention of, T2DM. At first, metformin monotherapy is initiated to lower hepatic glucose output. If this is not successful at achieving HbA1c targets, dual or triple therapy with various insulin sensitizers (e.g., TZDs like pioglitazone), insulin secretagogues (e.g., sulfonylureas like glimepiride, or GLP-1 receptor agonists like liraglutide), DPPIV inhibitors (e.g., sitagliptin), or sodium-glucose cotransport inhibitors (e.g., canagliflozin) is initiated, with additional insulin injection therapy if HbA1c targets are still not achieved (American Diabetes Association, 2018c). Because pharmacologic therapy is always administered in conjunction with lifestyle management (diet and exercise), it is highly likely that a patient with T2DM who initiates exercise will also be using some anti-hyperglycemic medication. Since exercise affects most of the molecular pathways these compounds target, it is a clinical necessity that researchers understand exercise-drug interactions. Fortunately, several studies have examined this topic.

Sharoff et al. (2010) found that metformin (2000 mg twice/day for 2–3 weeks) did not augment the insulin sensitizing effect of exercise (40-min cycling at 65% VO_2peak) in non-diabetic individuals and may even blunt the beneficial effects of exercise. They followed up by examining the effects of 12-weeks of exercise training (45-min of cycling at 75% HRmax 3-times/week and 2-sets of 12-rep max lifts for all major muscle groups 2-times/week) ± metformin treatment (2000 mg/day) in prediabetic individuals (Malin et al., 2012). They found that the largest increase in insulin sensitivity was present in the exercise only group, compared to the exercise plus metformin and metformin-only groups (Malin et al., 2012). Neither study found an effect on fasting glucose, while HbA1c and OGTT data were not reported. The notion that an interaction between exercise and metformin may blunt therapeutic benefits was supported by a small study in patients with T2DM from Boulé et al. (2011) which showed that exercise reduced the metformin-induced lowering of blood glucose responses to a meal. However, a larger-scale but retrospective analyses of the DARE trial in patients with T2DM from the same authors (Boulé et al., 2013) showed that improvements in HbA1c following 22-weeks of either aerobic, resistance, or combined aerobic plus resistance training, were not different between metformin users ($N = 143$) and non-users ($N = 82$). Erickson et al. (2017a) examined T2DM patients treated with either metformin monotherapy or metformin combined with additional antidiabetic drugs (sulfonylureas, GLP-1 receptor agonists, or DPPiv inhibitors) (Erickson et al., 2017b). They found that post-meal treadmill walking (five 10-min bouts at 60% VO_2max, or three 10-min bouts at 50% VO_2max) reduced postprandial glucose responses in habitual metformin users but that benefits were blunted in those on additional therapy (Erickson et al., 2017a,b). Such studies support the use exercise for managing blood glucose in drug-treated diabetes patients, but had a very small sample size ($N = 8$–10) and were not specifically designed to test exercise-drug interactions.

Besides metformin, several groups have examined exercise-drug interactions for other anti-diabetic drugs. In a longitudinal study by Mensberg et al. (2014), 33 T2DM patients were randomly allocated to 16-weeks of exercise (combined aerobic and resistance) and the GLP-1 receptor agonist, liraglutide (1.8 mg/day), or exercise and placebo (Mensberg et al., 2014). The authors found that HbA1c and fasting glucose were more greatly reduced in the liraglutide-treated group, indicating a beneficial interaction between exercise and GLP-1 receptor agonist therapy for blood glucose management (Mensberg et al., 2014). However, the liraglutide-treated patients also lost more weight, negating the specificity of an exercise-liraglutide interaction directly optimizing glucose control (Mensberg et al., 2014). With a focus on skeletal muscle, using fluorine-18–labeled fluoro-deoxy-glucose and positron emission tomography (PET), Hällsten and colleagues studied the effects of 26-weeks of rosiglitazone (4 mg twice daily) or metformin (1000 mg twice daily) treatment in 45 newly diagnosed patients with T2DM (Hällsten et al., 2002). Despite equal improvements in HbA1c, insulin stimulated muscle glucose uptake and exercise-induced glucose uptake (65-min of single-leg knee-extension at 10% of maximal isometric force) was augmented in the rosiglitazone group but not in the metformin-treated patients (Hällsten et al., 2002). These findings are, however, in keeping with the mechanisms of actions of these drugs. Besides insulin sensitizers, insulin secretagogues have also been studied. Larsen et al. (1999) found that the blood glucose lowering actions of the sulfonylurea glibenclamide (7 mg) and exercise (60-min at 57% of VO_2max) are additive in patients with T2DM. Similarly, Massi-Benedetti et al. (1996) studied the glucose and insulin responses to a single exercise bout in 167 patients with T2DM treated for 14–28 days with glimepiride (3 mg/day) or glibenclamide (10 mg/day). They found that 1-h of cycling at 120 bpm reduced blood glucose in both groups, but lowered endogenous insulin secretion (as shown by reduced C-peptide levels) in the glimepiride group only (Massi-Benedetti et al., 1996). This likely indicates that glimepiride, but not glibenclamide, treatment also increases insulin- and/or- exercise-induced glucose uptake. This idea is supported by *in vitro* observations from Haupt and colleagues who showed that glimepiride activates PI3 kinase and increases insulin-stimulated glycogen synthesis in human primary skeletal muscle cells, where glibenclamide has no effect (Haupt et al., 2002).

While the majority of T2DM patients will use metformin and other anti-hyperglycemic drugs, insulin is used as a final

approach and has been seldom studied in such patients in the context of exercise. van Dijk et al. (2012) found that 24-h CGM-derived hyperglycemia (time above 7.8 mM) and glycemic variability was reduced in sixty patients who had completed a 45–60-min cycling bout at 30–50%Wmax, and that this improvement was not different between insulin-treated or insulin-naïve patients (Van dijk et al., 2013). The same study also reported inter-individual differences in changes in mean 24-h glucose, finding poorer glycemic variability in nine (15%) of the sixty patients (Van dijk et al., 2013). Furthermore, while the study found that the prevalence of hypoglycemia was greater in insulin-treated patients compared with non-insulin-treated patients, this was not influenced by exercise. While hypoglycemia is a common fear and consequence of the use of exercise in insulin-treated diabetes patients, the risk of hypoglycemia can be dramatically reduced through proper instruction, advice, and guidance on carbohydrate intake and insulin-meal-exercise timing [some guidance is given ADA guidelines (American Diabetes Association, 2018b)]. This is a very important consideration that is beyond the intended aim of this review but studies addressing the effect of exercise on the prevalence and inter-individual variability of hypoglycemia in T2DM are lacking.

Besides anti-hyperglycemia drugs, lipid-lowering HMG-CoA reductase inhibitors (e.g., statins) are commonplace in the management of (pre)diabetes. In the 1990/2000s, it was shown that statin use may lead to mitochondrial dysfunction and muscle damage (Thompson et al., 1997; Päivä et al., 2005; Draeger et al., 2006; Schick et al., 2007), prompting a hypothesis that statin treatment may blunt the beneficial effect of exercise. Meex et al. (2010) found that statin treatment (between 5 and 40 mg/day of atorvastatin, simvastatin, rosuvastatin, or pravastatin) combined with regular aerobic plus resistance training more robustly increased insulin sensitivity than training alone. They observed equal weight loss and improved fitness and muscle mitochondrial function between groups but no improvements in fasting glucose or HbA1c (Meex et al., 2010). While this work showed that statins unlikely impair exercise adaptations to glucose metabolism and mitochondrial function, with different dosing regimens and the inclusion of individuals with/without diabetes treated with a mix of hyperglycemia-lowering drugs (metformin and/or sulfonylureas), is it difficult to ascertain the precise exercise-statin interaction from this work. In 2013, two studies examined exercise-statin interactions. Larsen and colleagues compared ten simvastatin-treated (10–40 mg/day for ∼5-years) hypercholesterolemic patients with untreated controls matched by age, weight, body mass index, fat percentage and VO_2max. They found lower insulin sensitivity, lower muscle mitochondrial function, and higher HbA1c levels in statin-treated patients (Larsen et al., 2013). Meanwhile, Mikus et al. (2013) randomized thirty-seven obese individuals with metabolic syndrome to 12-weeks of aerobic exercise with or without simvastatin treatment (40 mg/day). They found that exercise-induced improvements in VO_2max and muscle mitochondrial content were absent in the statin-treated group. Again, these studies indicate potential for statins to influence exercise-related factors but they do not allow one to conclude whether statin use directly influences changes in blood glucose control following exercise.

Other retrospective studies have also examined exercise-drug interactions. For example, in 2014, a small study ($N = 14$) by my group found that the increase in GLP-1- or arginine-stimulated insulin secretion following a single exercise bout was absent in T2DM patients who were drug-treated (Knudsen et al., 2015). This is similar to the above-described findings from Erickson et al. (2017b) who found blunted acute exercise-induced improvements in postprandial glucose in patients treated with multiple anti-hyperglycemics. However, Knudsen et al. (2015) did not examine specific drugs and was not designed to prospectively examine exercise-drug interactions. In their retrospective analysis of outcomes from a 12-week training study in patients with T2DM, Terada et al. (2013a) demonstrated that greater reductions in blood glucose were found when diabetic medications were taken less than 6-h prior to exercise compared to more than 6-h. These observations suggest that drug-exercise timing is important; however, outcomes were not derived from controlled drug administration but from retrospective analyses of patients' drug diaries.

Unfortunately, published work that documents exercise-drug interactions vary in their study designs and target populations, and have mixed outcomes. Consequently, such work has not informed current clinical guidelines. With the exception of a brief comment on insulin-activity timing (American Diabetes Association, 2018c), as of 2018 no information regarding exercise-drug timing is currently provided in the ADA guidelines for preventing and treating T2DM. Although there is an urgent need for a larg-scale prospective trial specifically examining the interactions between anti-hyperglycemic medications and exercise to optimize blood glucose control for patients with T2DM, based on the equivocal evidence available it seems likely that bespoke and carefully monitored exercise-drug timing and dosing is required for patients on an individual basis.

F. Weight Loss

The independent effects of exercise and weight loss on blood glucose control in (pre)diabetes have been well studied, showing that either approach may improve blood glucose control or insulin sensitivity (Goodpaster et al., 2003; Solomon et al., 2008, 2009; Dubé et al., 2011). However, their interactive effects are less understood. Furthermore, a lack of energy balance and prevention of consequent weight loss confounds the interpretation of many exercise studies. For example, we found that interval walking training improved blood glucose control in T2DM patients whereas continuous walking did not; however, interval walkers also displayed greater reduction in body fat mass, precluding a firm conclusion that the benefit was induced by interval walking *per se* (Karstoft et al., 2013).

Although data from Goodpaster et al. (2003) demonstrated the additive effects of diet-induced weight loss (10% of body mass via a 500–1000 kcal/day deficit) and exercise training (40 min at 75% HRmax, 4–6 times/week) on insulin sensitivity, their work did not reveal additive effects on blood glucose control (fasting glucose, or 2-h OGTT glucose). Our work also previously found that improvements in fasting glucose,

2-h OGTT glucose, or insulin sensitivity were equal in obese individuals with prediabetes randomized to 12-weeks of exercise training (60 min/day at 65% VO_2max, 5 days/wk) either with or without 500 kcal/day deficit-induced weight loss (Solomon et al., 2008, 2009). A following study from Goodpaster et al. (2010) found that delaying initiation of physical activity during a weight loss intervention in severely obese individuals had no influence on metabolic outcomes, including improved fasting glucose at 6-months. That said, other studies have found that exercise is critical for maintaining improved glucose control. For example, Thomas and colleagues carefully examined the effects of weight regain on cardiometabolic risk factors following 6-months weight loss (10% of body weight via deficit of 600 kcal/day) with supervised exercise (walking at 60% of VO_2max, 400 kcal/session, 5 days/week) in 100 metabolic syndrome patients (Thomas et al., 2010). They found that individuals randomized to continue exercise during controlled weight regain following initial weight loss, maintained improved blood glucose control while most metabolic variables deteriorated in those who ceased exercise (Thomas et al., 2010). Bouchonville et al. (2014) reported that fasting glucose, and glucose AUC and insulin sensitivity measured during OGTT were more robustly improved following 12-months of diet-induced weight loss (10% of body weight) plus exercise (90-min of combined aerobic and resistance, thrice weekly) when compared to either weight loss or exercise alone, in 100 obese individuals. The additional benefit conveyed by exercise plus diet-induced weight loss is supported by other recent work (Weiss et al., 2015; Francois et al., 2018). But the importance of exercise alone is also underpinned by outcomes from the IDES trial: Balducci et al. (2012) found that the magnitude of increase in fitness following exercise training (twice weekly supervised aerobic and resistance training plus exercise counseling) predicts improvement of cardiometabolic risk factors including HbA1c, independent of weight loss.

We currently lack precise information regarding the interaction between exercise and weight loss from large-scale randomized controlled trials in order to update clinical guidelines. Nonetheless, the above-described observations underpin the necessity of regular exercise to maintain and/or maximize the benefits of weight loss on blood glucose control in individuals with (pre)diabetes. Current clinical guidelines do not convey this sentiment.

G. Inactivity/Sitting Time

From the early 2000s, data has emerged that physical inactivity (daily time spent being sedentary, i.e., sitting or lying while awake) is strongly associated with T2DM risk and interacts with the level of physical activity (Dunstan et al., 2004; Wilmot et al., 2012). This provides evidence that the amount of daily inactivity may influence the inter-individual variability in changes in blood glucose control following exercise training. Further evidence has also emerged demonstrating that interrupting sitting time can be a useful intervention for preventing and managing blood glucose control (reviewed in Dempsey et al., 2016). Additionally, in the 45 and up study published in van der Ploeg et al. (2012), association analyses showed that patients with T2DM must increase their physical activity level and reduce their sedentary time in order to reduce mortality. Such evidence has prompted the ADA to include a statement in their standards of medical care stating that, in addition to increased physical activity and regular exercise, individuals should reduce their sedentary time by breaking up prolonged bouts of sitting with light activity for a few minutes at least every 30 min (**Table 2**) (American Diabetes Association, 2018b). However, since large scale randomized controlled trials examining such phenomena in patients with T2DM are lacking, this guideline is not wholly evidence-based. Furthermore, it is not known when an interruption to sitting time would be best initiated, i.e., in the postabsorptive or postprandial state (see *Exercise-Meal Timing*). Additionally, training studies in diabetes patients seldom report objectively measured physical activity/inactivity levels; consequently, the influence of inactivity on the variability in glycemic outcomes following training is unknown. With advances in tri-axial accelerometry, methods for objectively quantifying sitting time and the transition to standing and activity can be easily implemented with increasing accuracy and low cost. As new data emerges over the coming years, clinical guidelines related to the interruption of sedentary time will be further optimized. In relation to this, since an exercise bout may influence total daily activity levels (Thompson et al., 2014), prospective trials are required to understand the impact of this on glucose control in diabetes.

H. (Epi)genetics

Genome wide association studies have identified several genes associated with increased risk of developing T2DM (reviewed in Prasad and Groop, 2015). Some of these genes are also associated with glycemic outcomes from weight loss lifestyle interventions, such as the Diabetes Prevention Program and the Diabetes Prevention Study (reviewed in Weyrich et al., 2007). With specific reference to the effect of exercise training on blood glucose control, far fewer genetic studies have been published. As described earlier in this paper, the HERITAGE family study demonstrated large heterogeneity in glycemic outcomes following a 20-week exercise training intervention, where approximately 40% of the participants showed no change or an adverse direction of change in IVGTT-derived parameters of blood glucose control (Boulé et al., 2005). Findings from the HERITAGE family study have shown that leptin and leptin receptor gene polymorphisms and a leptin gene trait locus on 7q31 are associated with training-induced changes in the insulin response to IVGTT and fasting insulin, respectively (Lakka et al., 2003, 2004). Rate of glucose disappearance, insulin sensitivity, and disposition index during IVGTT are also improved more following training in C allele carriers at rs2180062 in the *FHL1* gene than in the T allele carriers (Teran-Garcia et al., 2007). Although the HERITAGE family study did not examine individuals with prediabetes or T2DM, in 2010 the study investigators examined whether 8 T2DM susceptibility variants (single nucleotide polymorphisms, SNPs, previously identified through genome-wide linkage analyses) could modulate changes in IVGTT-derived measures of glycemic control following 20 weeks of regular exercise training (Ruchat et al., 2010). After adjustment for multiple comparisons and adjusting for weight loss (change in waist circumference), the authors identified that

a Pro12Ala SNP in the *PPARγ* gene accounted for statistically significant variance in exercise-induced changes in the glucose disappearance rate (ΔK_g, 2.81% of variance explained) glucose effectiveness (ΔS_g, 1.83%), the acute insulin secretory response to glucose (ΔAIR_g, 0.94%), and the disposition index (ΔDI, 2.15%) (Ruchat et al., 2010). The authors also found that carriers of the Ala allele had greater exercise-induced improvements in these IVGTT-derived variables (Ruchat et al., 2010). The findings from this work advanced our knowledge; however, only 8 SNPs were selected and HbA1c or 2-h OGTT glucose were not measured. Furthermore, since the publication date of that study in 2010, several more SNPs associated with diabetes risk have been identified. As such, there is a great need for similar studies in individuals with prediabetes or T2DM. That said, in Klimentidis et al. (2014) examined the influence of 65 T2DM-associated SNPs on the relationship between physical activity level and genetic risk score for T2DM. They found that the protective effect of physical activity was weakest among individuals with high genetic risk for T2DM. Their findings suggest that the role of physical activity in the prevention of diabetes may be blunted in those with high susceptibility for the disease. However, the causality of such correlative findings must be confirmed.

Besides genetics, epigenetics have received little attention in the context of the exercise and blood glucose control in pre(diabetes). Barrès et al. (2012) measured whole genome methylation as well as the methylation status of exercise responsive genes (PGC-1α, PDK4, and PPAR-δ) in skeletal muscle biopsies from healthy adults at rest and following a single exercise bout. Exercise induced a dose-dependent expression of PGC-1α, PDK4, and PPAR-δ, together with a marked hypomethylation on their respective promoters. The authors further showed that acute exercise caused a transient changes in the pattern of DNA methylation in adult skeletal muscle tissue (differentiated non-dividing somatic cells), and that DNA methylation was unaltered following 3-weeks of training despite increased RNA expression of PGC-1α and TFAM promoters (Barrès et al., 2012). This was a seminal observation in exercise biology since it demonstrated that DNA hypomethylation is a likely a transient mechanism involved in mRNA synthesis and that epigenetic regulation of the genome is dynamic to acute stimuli. However, whether promoter hypomethylation induces a functional influence on blood glucose control from exercise in individuals with (pre)diabetes, remains to be investigated.

In data published in 2015 from the HART-D study, a large-scale randomized controlled trial which determined the effect of 9-months of supervised exercise training on HbA1c in patients with T2DM, Stephens et al. (2015) measured the baseline skeletal muscle transcriptome before the intervention. The authors identified 186 genes with differential mRNA expressions between "responders" (training-induced decrease in HbA1c) and "non-responders" (no change in HbA1c) of which ∼25% of these differentially expressed genes were involved in substrate metabolism and mitochondrial dynamics (Stephens et al., 2015). Targeted qRT-PCR analyses of a selection of genes from their array demonstrated that the lack of training effect on HbA1c was linked to lower baseline expression levels of exercise-responsive genes. These included PPARα and ELOVL1, which play a role in lipid metabolism, and CHKB, CISD2, and FOXO1, which are involved in mitochondrial function. Such findings prove very useful in identifying molecular biomarkers of exercise effectiveness in T2DM, and individualized follow-up studies of the "non-responders" are needed in order to understand how their therapeutic benefit from exercise can be achieved.

From the data we have available in 2018, identified genetic/epigenetic/transcriptomic factors explain only a small amount of the variability in outcomes following training. For example, the HERITAGE family study found that less than 5% of the variance in glycemic outcomes following training was explained by 8 T2DM susceptibility variants (Ruchat et al., 2010). As -omics technologies improve and become more widespread and more accessible in exercise science, there is no doubt that metabolite, protein, and microRNA signatures, as well as DNA methylation loci, which predict the magnitude of the therapeutic effect of exercise on blood glucose control in individuals with (pre)diabetes will be identified. Evidence to support this notion was presented by Rowlands et al. (2014) who found multiple alterations in the transcriptome, the methylome, and microRNA arrays following 16-weeks of resistance or endurance training in obese Polynesian individuals with T2DM. Due to a lack of evidence, as of 2018 the ADA guidelines do not include any information on molecular biomarkers which may be used to inform exercise prescription. This is very likely to change over the next 10-years, particularly as outcomes from studies like the Molecular Transducers of Physical Activity Consortium (MoTrPAC) evolve.

I. Direct Effect of Hyperglycemia and Poor Beta-Cell Function

Chronic exposure to high glucose levels deteriorates cellular function and/or causes apoptosis in tissues that regulate blood glucose control. For example, several groups including my own have found that *in vitro* exposure of differentiated skeletal muscle cells (myotubes) to prolonged (>24-h) hyperglycemia reduces insulin-stimulated glucose uptake (Aas et al., 2011; Green et al., 2012). Solomon et al. (2012) we confirmed these observations in humans showing that elevation of plasma glucose 5 mM above basal for 24-h reduced insulin sensitivity in healthy volunteers. Furthermore, primary myotubes isolated from hyperglycemic donors exhibit blunted muscle cell adaptations to electrical pulse stimulated contractions (Feng et al., 2015). Therefore, *in vitro* observations prompt one to hypothesize that chronic exposure to high blood glucose levels (the phenotype of T2DM) may blunt beneficial exercise adaptations. To test this hypothesis, in 2013 we examined the relationship between pre-intervention blood glucose control (HbA1c, fasting glucose, and 2-h OGTT glucose) and changes in glucose control following 3–4-months of exercise training (∼4–5 days/week, up to 60 min/session at 60–70% HRmax) in 105 individuals with prediabetes or T2DM. Interestingly, we found a U-shaped relationship suggesting that individuals with relativity well controlled hyperglycemia respond well to training while patients with poor blood glucose control have poor improvements or even a deterioration in blood glucose control following training (Solomon et al., 2013a). Another

study found that fasting hyperglycemia was also associated with blunted improvements in 2-h OGTT glucose following 3-months of aerobic training in older obese individuals (Malin and Kirwan, 2012). Furthermore, the STRRIDE study found an inverse correlation between baseline fasting glucose and aerobic training-induced improvement in insulin sensitivity (S_i from IVGTT) in overweight individuals: S_i increased in participants with normal fasting glucose (<5.6 mM) but decreased in those with impaired fasting glucose (≥5.6 mM) (AbouAssi et al., 2015). However, these are correlational observations that do not imply causality and may be confounded by other influential variables. Additionally, some studies dispute a role for hyperglycemia in blunting the therapeutic action of exercise. Terada et al. (2013a) found that higher pre-exercise blood glucose concentrations were associated with greater decreases in blood glucose following a single exercise bout in patients with T2DM. Accordingly, van Dijk and colleagues demonstrated that greater HbA1c levels in T2DM patients correlated with greater decreases in mean glucose over the 24-h period following a single exercise bout (Van dijk et al., 2013). However, since the findings from Terada and van Dijk derive from single exercise bouts, they should not be extrapolated to reflect expected outcomes following chronic training.

To help understand the physiological mechanisms that potentially link hyperglycemia with exercise adaptations, the HERITAGE Family study demonstrated that pre-intervention glucose tolerance (K_g during IVGTT) influences training induced changes in glucose-stimulated insulin secretion (AIR_g during IVGTT) (Boulé et al., 2005). Boulé et al. (2005) found that in healthy non-diabetic subjects, training decreased AIR_g in individuals in the quartile with the highest K_g at baseline while AIR_g increased in those in the quartile with the lowest Kg. We repeated this work in people with prediabetes and T2DM, finding that exercise-induced improvements in blood glucose control were lowest in those with poorer pre-intervention pancreatic beta-cell function (Solomon et al., 2013b). This observation supports earlier work in patients with T2DM from Krotkiewski et al. (1985), and is complemented by a study conducted by Dela et al. (2004) who found that T2DM patients with a low C-peptide response to glucagon infusion had no improvement in glucose- or arginine-stimulated insulin secretion following 3-months of aerobic training. *In vitro* incubation of pancreatic beta-cell lines or primary islets in high glucose-containing medium reduces glucose-stimulated insulin secretory function (Donath et al., 1999; Maedler et al., 2002), findings we have also translated into human observations (Solomon et al., 2012). So it may be speculated that chronic exposure to high glucose levels may directly blunt otherwise beneficial exercise-mediated adaptations in the endocrine pancreas. In a pilot project to test that hypothesis, in 2013 we stratified T2DM patients with respect to their HbA1c value and examined insulin secretory function following a single exercise bout (Knudsen et al., 2015). We found that GLP-1 and arginine-mediated potentiation of glucose-stimulated insulin secretion was augmented by exercise in patients with well controlled glycemia (HbA1c < 6%) but worsened in patients with poor glucose control (HbA1c > 6%) (Knudsen et al., 2015). Although this was a small pilot study, it is the first evidence that causally links chronic exposure to hyperglycemia with blunted exercise adaptations in diabetes patients. In combination with above-described work (Krotkiewski et al., 1985; Dela et al., 2004; Solomon et al., 2013b), such data suggest that T2DM patients with poor beta-cell insulin secretory function may not optimally respond to exercise treatment modalities. Research studies are required to determine whether optimizing insulin secretory function in such patients prior to initiating training may restore beneficial exercise adaptations. Chronic cellular exposure to high glucose levels is typically linked with apoptosis driven by inflammation and/or oxidative stress, a process called glucotoxicity (Poitout and Robertson, 2008). However, it remains to be determined whether inflammatory or oxidative stress mechanisms underpin high glucose-induced prevention of beneficial exercise adaptations.

No study to date has examined whether exposure to experimental hyperglycemia (via infusion) or rapid normalization of hyperglycemia in diabetes patients (via insulin or sodium-glucose cotransport inhibitors drugs) can influence exercise adaptations. It remains to be investigated whether glucotoxicity directly influences exercise training-induced improvements in blood glucose control. Knowledge gained from answering such a research question would further inform ADA guidelines and therefore enable clinicians to enhance the management of their patients' hyperglycemia. Such knowledge would also help individualize lifestyle intervention approaches if indeed glucose lowering and/or beta-cell optimizing therapy is required in some patients prior to initiating an exercise regime.

Since several other descriptive characteristics such as age, sex, race, body weight, or duration of diabetes (years since diagnosis) or specific dietary nutrients, may independently influence the above described contributing factors, one may speculate that these may influence exercise-mediated effects on blood glucose control in patients with (pre)diabetes. The same may also be true of activity compensation where an exercise bout may negatively influence total daily activity levels (Thompson et al., 2014). Family history of diabetes should also be considered in future work since individuals with a diabetic parent exhibit a blunted post-exercise insulin-mediated glycogen storage response (Price et al., 1996). However, following regression analyses in one of my own studies (Solomon et al., 2013b), neither age, BMI, sex, or time since diabetes diagnosis had any influence on the hyperglycemia-lowering effect of exercise training. Regression analyses from van Dijk and colleagues also support that neither age, BMI, diabetes duration, or drug treated influence exercise-induced blood glucose control in patients with T2DM (Van dijk et al., 2013). That said, no study has prospectively examined the role of such variables on blood glucose control following training in individuals with prediabetes or T2DM. One exception may be the Look-AHEAD study, a randomized controlled trial examining the effect of an intensive lifestyle intervention (combined diet and exercise induced weight loss) on T2DM remission in 5145 patients. The investigators found that longer-term remission (after 2 to 4-years follow-up) was more likely in patients not using insulin with less than 2-year duration of diabetes, a lower baseline HbA1c, and a greater first-year weight loss (Gregg et al., 2012). Although Look-AHEAD was not an exercise training study *per se*,

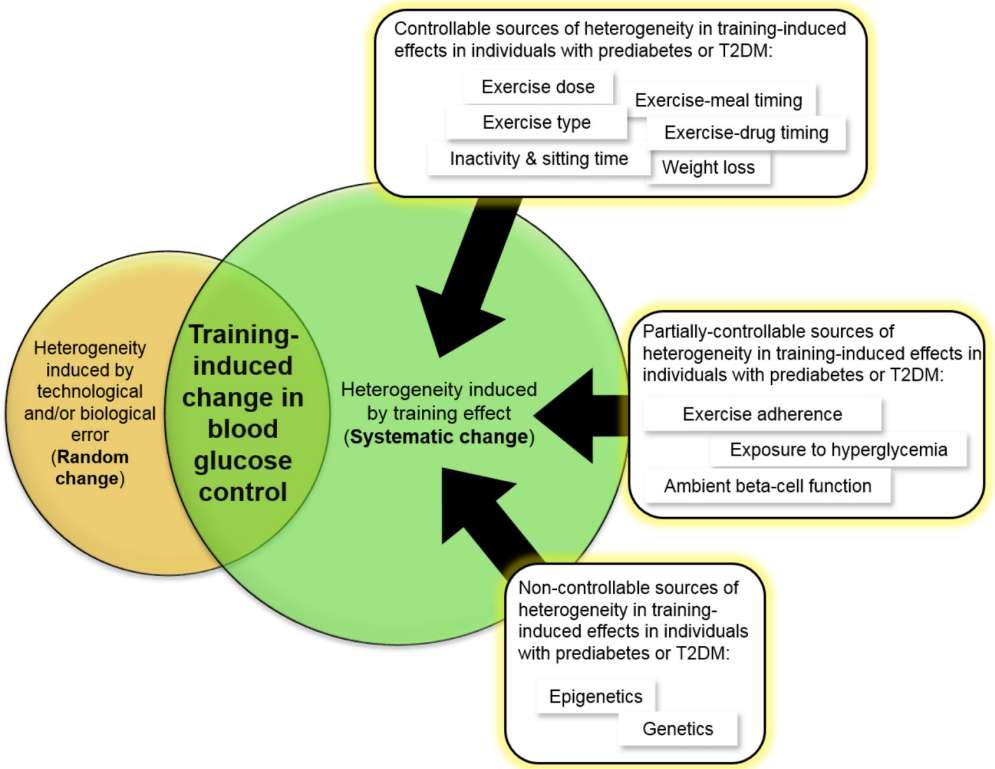

FIGURE 3 | Evidence-based sources of inter-individual variability in the blood glucose lowering effects of exercise in individuals with prediabetes or T2DM. Other sources that have not been adequately studied to conclusively state that they contribute to this variability in individuals with prediabetes or T2DM include age, sex, race, body weight, family history of diabetes, and duration of diabetes.

TABLE 3 | Science gaps which, if filled, will increase our understanding of inter-individual variability in the therapeutic blood glucose lowering effect of exercise for individuals with prediabetes and/or type 2 diabetes.

	Science gaps
1	A randomized controlled trial of exercise training to determine the patient-by-treatment interaction for the change in blood glucose control (HbA1c, fasting glucose, and 2-h OGTT glucose) is needed in people with prediabetes and T2DM. This would help accurately quantify inter-individual variability and identify true non-responders.
2	A study to determine the inter-individual variability in blood glucose control caused by different exercise doses (frequency, intensity, and time) is needed in individuals with prediabetes or T2DM.
3	A study to determine the inter-individual variability in blood glucose control caused by different types of exercise is needed in individuals with prediabetes or T2DM.
4	A description of psychological barriers, behavioral barriers, and environmental barriers to implementing lifestyle changes and incorporating exercise into diabetes treatment should be included in clinical guidelines.
5	A study to determine the optimal exercise-meal timing needed to maximize postprandial glucose control in individuals with prediabetes or T2DM is required.
6	There is an urgent need for a large-scale prospective trial specifically examining the interactions between exercise and anti-hyperglycemic medications to optimize blood glucose control for patients with T2DM.
7	A large scale randomized controlled trial examining the interruption of sitting time with light activity (and its pre-postprandial timing) in patients with T2DM is needed.
8	There is a need for studies to identify metabolite, protein, or microRNA signatures, as well as DNA methylation loci, which predict the magnitude of the therapeutic effect of exercise on blood glucose control in individuals with prediabetes of T2DM.
9	A study determining whether exposure to experimental hyperglycemia (via infusion) or rapid normalization of hyperglycemia in diabetes patients (via insulin or sodium-glucose cotransport inhibitors drugs) can directly influence exercise adaptations is needed.
10	Exercise dose (including frequency, intensity, and time above habitual activity levels), exercise type, exercise adherence, exercise-meal timing, exercise-drug timing, and drug name and dosing, and objectively measured physical activity levels and sedentary time, should always be considered in a study design and be reported in publications.

These future approaches will optimize clinical exercise guidelines, and ultimately help maximize the therapeutic blood glucose lowering effects for all patients. Further detail and references are in the main text.

it does indeed highlight factors to be considered in future exercise studies.

In the free-living "real world" setting, all of the above-described contributing factors play a role in the notable heterogeneity in the therapeutic blood glucose lowering response to exercise in people with (pre)diabetes. In the lab setting, where exercise is supervised and standardized, the influence of several of these above-described sources of variability, particularly exercise adherence, can be controlled and therefore minimized. Yet, in the free-living "real world" setting there are behavioral (desire, self-image, motivation) and environmental (climate, weather, terrain) barriers combined with abundant access to activity reducing transport modalities (cars, busses, trains, elevators, escalators, conveyer belts) which influence the adherence to exercise and thereby encourage an inactive lifestyle. Thus, the true challenge to maximizing the therapeutic potential of exercise is immense.

WHERE DO WE GO FROM HERE?

The purpose of this review was to examine inter-individual variability in the blood glucose lowering effect of exercise in individuals with T2DM, and to identify the sources of such variability. Interpretations should not be extrapolated to other variables (e.g., lipids, blood pressure, etc.), nor should a non-response in blood glucose control following exercise be considered to convey a non-response in other variables. Due to a lack of standardization of study design, differences in methods/assays, variations in timing of post-training measurements, heterogeneity of subject demographics between trials, and probably most importantly, a lack of measurement of clinical diagnostic measures for assessing blood glucose control, a systematic review and meta-analysis on this topic is not possible. However, from the evidence presented above it is highly likely that inter-individual variability in the changes in blood glucose control following exercise exists in the context of T2DM and that true non-responders will be identified. In doing so, one must be aware that "non-responder" does not mean "never responder." Identifying an adverse outcome to a particular intervention should be embraced as a challenge to overcome. By doing so, the knowledge gained will ultimately maximize the therapeutic benefits of exercise for all patients.

Going forward, several sources of variability have been identified (**Figure 3**), and I propose that exercise dose (including frequency, intensity, and time above habitual activity level), exercise type, exercise adherence, exercise-meal timing, exercise-drug timing, and drug name and dosing, and objectively measured physical activity level and sedentary time, should always be considered in a study design and reported in publications. Among many published studies, I admit that I too have been guilty of not always including such details in my papers, either through accidental omission or failure to record such data. Remedying this in future will increase the quality of work in the field and enable comparisons between independent studies. This would facilitate the accurate calculation of technical error of measurement and eventually establish an evidence-based "reference range" indicative of a clinically meaningful exercise-induced improvement in blood glucose control. Such an approach would then enhance the reliability of information used to inform clinical guidelines. That said, **Table 3** highlights the current science gaps that must be urgently filled if we are to understand how to maximize the therapeutic benefit of exercise on blood glucose control for all individuals with prediabetes or T2DM. The new knowledge that will emerge in the next 5–10 years will couple genetic, transcriptomic, epigenetic, and physiological factors with knowledge of exercise dosing, exercise-meal timing, and exercise-drug interactions to help maximize the therapeutic benefit of exercise for all individuals, including those at risk of developing diabetes or those already with T2DM. This creates great confidence that we will soon successfully control the incidence of this preventable disease.

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TS wrote the manuscript and takes responsibility for the integrity of its content.

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Intradialytic Exercise: One Size Doesn't Fit All

Pedro L. Valenzuela[1,2†], Ana de Alba[3†], Raquel Pedrero-Chamizo[4], Javier S. Morales[5], Fernando Cobo[3], Ana Botella[3], Marcela González-Gross[4], Margarita Pérez[5], Alejandro Lucia[5,6] and M. T. Marín-López[3]*

[1] Department of Systems Biology, Universidad de Alcalá, Madrid, Spain, [2] Department of Sport and Health, Spanish Agency for Health Protection in Sport (AEPSAD), Madrid, Spain, [3] Fundación Renal Íñigo Álvarez de Toledo, Madrid, Spain, [4] Faculty of Physical Activity and Sport Sciences, Technical University of Madrid, Madrid, Spain, [5] Faculty of Sport Sciences, European University of Madrid, Madrid, Spain, [6] Research Institute i+12 and CIBER de Envejecimiento y Fragilidad (CIBERFES), Madrid, Spain

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****Correspondence:***
Pedro L. Valenzuela
pedrol.valenzuela@edu.uah.es

† These authors have contributed equally to this work.

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Purpose: Hemodialysis patients commonly have impaired physical performance and mental health. We studied the effects of an intradialytic exercise program on these variables.

Methods: 27 patients (33% women; 68 ± 13 years) were enrolled in a 14-week intradialytic endurance-resistance training program ('exercise' group, 40 programmed sessions per patient); 40 hemodialysis patients (28% women; 68 ± 11 years) performing no exercise during the same time length were used as controls. Endpoints included physical performance (6-min walk test [6MWT], 10-repetition sit to stand [STS-10] and handgrip strength), emotional status (Beck's depression inventory and State-Trait Anxiety Inventory), and mental and physical component scores of the short-from (SF)-12 Health Survey.

Results: There were no differences ($p > 0.05$) between groups at baseline for sex distribution, or mean age, body mass index and time spent on dialysis. Exercise benefits were observed for 6MWT (11 and −3% for the exercise and control groups, respectively; $p < 0.001$), STS-10 performance time (−22 and 6%; $p < 0.001$) and handgrip strength (4 and −4%; $p < 0.02$). No significant benefits ($p > 0.05$) were observed for emotional status endpoints or SF-12 component scores. Despite significant benefits on physical performance, the proportion of clinically meaningful responders was low (<50%). Responsiveness was dependent on baseline physical performance ($p < 0.05$) but not on age or sex ($p > 0.05$).

Conclusion: A 14-week intradialytic training program induced significant improvements on physical performance. However, the rate of clinically meaningful responders observed in the present study was low, being the level of responsiveness dependent on baseline physical status. Efforts to individualize exercise prescription are needed in clinical practice.

Keywords: hemodialysis, end-stage renal disease, chronic kidney disease, physical activity, training, mental health

INTRODUCTION

The prevalence of patients with end-stage renal disease is rapidly growing, especially among the elderly population and patients with comorbidities (particularly diabetes mellitus and hypertension) (Szczech and Lazar, 2004). Consequently, more than two million people are expected to be treated by dialysis for end-stage renal disease by 2030 (Szczech and Lazar, 2004).

Despite important progress in hemodialysis techniques and in the treatment of its associated comorbidities, patients have a much higher morbimortality risk than their healthy counterparts (Foley et al., 1998; Szczech and Lazar, 2004). Dialysis is associated with a deterioration of physical function and mental status. Muscle function (Leal et al., 2011b) and exercise capacity (Painter, 2005) are significantly lower in hemodialysis patients, presenting a peak oxygen consumption that is considerably lower (>50%) compared to their healthy sedentary peers (Painter, 2005). Emotional disorders such as anxiety and depression are prevalent among dialysis patients (Dziubek et al., 2016; King-Wing Ma and Kam-Tao Li, 2016), negatively affecting their social, financial and psychological well-being, as well as their quality of life (QoL) (Christensen and Ehlers, 2002).

Physical fitness is one of the strongest predictors of survival in dialysis patients, with low levels of physical activity and impaired physical performance being associated with increased mortality risk in this population (O'Hare et al., 2003; Sietsema et al., 2004; Stack et al., 2005; Roshanravan et al., 2013; Torino et al., 2014; Morishita et al., 2017). In addition, lower QoL and mental health are also strongly associated with higher risk of death and hospitalization (Knight et al., 2003; Mapes et al., 2003). Therefore, maintaining their physical and mental status closer to their healthy counterparts is of major importance.

Meta-analytical evidence supports the benefits of intradialytic exercise programs for the improvement of several health-related outcomes such as physical performance or mental health (Smart and Steele, 2011; Chung et al., 2017). Yet, exercise benefits in dialytic patients are typically reported under the assumption that the group average represents the response of most individuals. However, a wide interindividual variability can be observed in the human response to a similar training program, which results in subjects being classified as responders (those who achieve clinically meaningful benefits) or non-responders (those who experience a worsening or remain unchanged) (Mann et al., 2014). The aim of this study was to analyze the effects of a 14-week intradialytic combined exercise (endurance + resistance) training program on patients' mental and health status. In addition, we assessed the influence of baseline phenotype on the training response as well as individual variability in training responses to the study endpoints.

MATERIALS AND METHODS

Participants and Study Design

End-stage-renal disease patients undergoing hemodialysis were recruited for the study. Subjects were excluded if they presented one or more of the following conditions: myocardial infarction in the 6 weeks prior to the start of the exercise program, unstable angina, cerebrovascular disease or a high risk for recurrence, musculoskeletal or respiratory (e.g., chronic obstructive pulmonary disease) alterations, uncontrolled hypertension, peripheral vascular disease, active liver disease, osteoporosis, cardiac ejection fraction <45%, blood hemoglobin concentration <10 g/dL, or problematic vascular access (immature arteriovenous fistulas, high risk for extravasation). All participants had the procedures explained and provided written informed consent to participate in the study. The present study was approved by the institutional review board (P141115303, *Fundación Universitaria Hospital de Alcorcón*, Madrid, Spain).

The study took place between January 2015 and May 2016. Patients in the 'exercise' group had to participate in a 14-week intradialytic training program, whereas those of the 'control' group had to maintain their regular lifestyle during this time period without direct intervention from the personnel of this investigation. From a total of 235 patients undergoing hemodialysis in different dialysis centers, two cohorts of 86 and 74 patients met the inclusion criteria to participate as 'exercise' and 'control' group, respectively. From these, 12 and 31 patients, respectively, did not participate. Reasons not to participate were receiving a transplant, leaving the center, not signing the informed consent form after having the study explained to them, and not being interested. For the rest of patients, only those who performed at least two physical tests and two psychological tests at baseline were enrolled in the study. Finally, 27 and 40 patients were included in the exercise and control group, respectively. Participants' descriptive data are presented in **Table 1**.

Exercise Intervention

The intradialytic training intervention consisted of 14 weeks of combined endurance and resistance exercises. Training sessions were conducted at three different dialysis centers but were supervised by the same experienced fitness instructors. Training sessions were performed three times per week and lasted approximately 60 min. A total of 40 training sessions were planned per subject during the intervention portion of the study.

Training sessions started with a warm-up consisting of respiratory and joint mobility exercises. During the main part of the sessions, both resistance and endurance exercises were performed. Resistance exercises included ankle plantarflexion and dorsiflexion, combined knee and hip flexion and extension, hip abduction and adduction, and abdominal exercises. These

TABLE 1 | Descriptive baseline characteristics of the participants.

	Control	Exercise	*p*-value
Women (%)	28	33	0.79
Age (years)	68 ± 11	68 ± 13	0.92
BMI ($kg \cdot m^{-2}$)	27 ± 5	27 ± 6	0.99
Dialysis prescription ($hours \cdot week^{-1}$)	11 ± 1	11 ± 1	0.51
Time on dialysis (years)	5 ± 4	7 ± 5	0.08

Data are Mean ± SD. Abbreviations: BMI, body mass index.

exercises were performed using elastic bands, Styrofoam balls and ankle weights. Endurance exercise consisted of pedaling on a mini bike for 30 min at an intensity corresponding to 12–14 points in the Borg's 6–20 scale (Borg, 1998).

Endpoints

Endpoints were assessed the week before (baseline) and after (post-intervention) the 14-week intervention. Assessment was done on dialysis days, with each participant being tested at the same time of the day (i.e., always in the morning or in the afternoon, before starting dialysis). Before the testing sessions, participants were individually instructed on how to perform all tests with detailed explanations and visual examples. Two testing sessions per patient were required to perform all the tests at each time point, one for all physical performance tests and another one for psychological evaluation. The tests were always performed in the same order.

Physical Performance

We assessed patients' performance in the 10-repetition sit to stand (STS-10), handgrip strength and 6-min walk (6MWT) tests (performed in this order), which are some of the most popular fitness tests in dialysis patients (Koufaki and Kouidi, 2010) and present an excellent test-retest reliability in this population (Segura-Ortí and Martínez-Olmos, 2011).

The STS-10, an index of lower-extremity strength (Csuka and McCarty, 1985), measures the time (in seconds) required to perform 10 consecutive repetitions of sitting down and getting up from a chair. Participants began the test with their arms crossed on their chest and sitting with their back against the chair. They were instructed to perform the task "as fast as possible," starting and finishing at the sitting position. Time was measured with a stopwatch (ONstart 100, Geonaute, France) to the nearest 0.1 s. This test has previously demonstrated a good test-retest reliability in hemodialysis patients (intra-class correlation coefficient [ICC] = 0.88) (Segura-Ortí and Martínez-Olmos, 2011).

Maximal isometric handgrip force has been suggested as a useful tool for the continuous assessment of muscle mass and function in dialysis patients (Leal et al., 2011a). It was measured in both hands using a manual dynamometer (T.K.K.5401, Takei Scientific Instruments, Japan) while participants were in a standing position, with the arm extended and parallel to the body, and without moving the wrist. They performed two maximal repetitions with each hand interspersed with 1-min rest periods between trials, and the mean of all four trials (combined handgrip strength) was analyzed. This test has also proven highly reliable in hemodialysis patients (ICC = 0.95 and 0.96 for the dominant and non-dominant hand, respectively) (Segura-Ortí and Martínez-Olmos, 2011).

The 6MWT was used as a marker of endurance capacity (Rikli and Jones, 1998). It was performed on a 17-meter corridor with marks on every meter, and time was measured with a chronometer (ONstart 100, Geonaute, France). Participants were asked to cover the greatest distance possible during 6 min by walking (not running) continuously and turning around at the final mark. No verbal encouragement was given during the test; however, feedback regarding the remaining time was available. Participants were allowed to rest during the test, and to use any ambulation aid (e.g., crutches) that they used during daily life. A very high test-retest reliability has been previously reported for this test in hemodialysis patients (ICC = 0.94) (Segura-Ortí and Martínez-Olmos, 2011).

Mental and Health Status

Changes in depression symptoms were assessed using the Beck Depression Inventory (BDI) (Beck et al., 1996). In this self-reported questionnaire 21 items are rated on a four-point severity scale and summed to give a total score, with a higher score being suggestive of more severe depression. The BDI has proven a valid depression screening tool in dialysis patients (Preljevic et al., 2012), being one of the most commonly used questionnaires to assess this condition in this patient population (King-Wing Ma and Kam-Tao Li, 2016). This questionnaire has previously yielded high values of internal consistency (Cronbach's $\alpha = 0.89$), sensitivity (0.82) and specificity (0.87–0.89) in dialysis patients (Preljevic et al., 2012). Test–retest coefficients in other populations have been reported to range from 0.62 (7-week interval) to 0.93 (1-week interval) (Julian, 2011).

Health-related QoL (HRQoL) was assessed using the Short-Form 12 (SF-12) health survey, a short version of the SF-36 (Ware and Sherbourne, 1992). A physical (PCS) and a mental component score (MCS) are calculated from this self-reported questionnaire. SF-12 has previously proven reliable in a 6-month longitudinal study performed with dialysis patients (ICC = 0.90 and 0.86 for MCS and PCS, respectively) (Loosman et al., 2015). Moreover, SF-12 scores are associated with short-term and long-term mortality in this population (Loosman et al., 2015).

The level of anxiety was assessed using the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970). We specifically analyzed the anxiety subscale. This test has previously shown a good internal consistency (Cronbach's $\alpha = 0.86$–0.95) and reliability over time ($r = 0.65$–0.75) (Spielberger et al., 1970; Julian, 2011).

Statistical Analysis

All the participants assessed at baseline were considered to be part of the study. Missing individual data at post-intervention were imputed with the 'baseline-observation-carried forward' method, that is, baseline values were used when these data were missing. The normal distribution (Shapiro-Wilk test) and homoscedasticity (Levene's test) of the data were checked before any statistical treatment. Non-normally distributed data (results from STAI and BDI) were log-transformed prior to its analysis. Differences in proportions were evaluated using Pearson's chi-squared test. Differences in baseline characteristics were analyzed using unpaired Student's *t*-tests. Endpoints were analyzed by a two-way mixed ANOVA with time points (baseline and post-intervention) as the within-subject factor and intervention groups (control or exercise) as the between-subject factor. The effect size (partial eta-squared, η_p^2) of the significant group x time interactions was calculated and considered small (>0.01) moderate (>0.06) or large (>0.14) (Cohen, 1988). *Post hoc*

analysis (Bonferroni test) was conducted when a significant interaction (group × time) effect was found.

The rate of clinically meaningful responders was calculated in those endpoints in which a beneficial effect of exercise (i.e., significant group x time interaction) was found. Responsiveness was defined as beneficial changes that exceeded two times the standard error of measurement (SEM) (Hopkins, 2000). The responsiveness threshold for the physical tests was set at 3 kg, 7.2 s, and 56.8 m for handgrip strength, STS-10 and 6MWT, respectively, attending to the SEM values previously reported for these tests in dialysis patients (Segura-Ortí and Martínez-Olmos, 2011). The magnitude of the differences (effect size, ES) in baseline values between responders and non-responders was determined through standardized mean differences (Hedges' g). Pearson's correlation analyses (for physical performance and age) and Pearson's chi-square test (for sex) were used to determine the influence of baseline phenotype on training responsiveness. All analyses were performed using a statistical Package (SPSS, version 23.0).

RESULTS

There were no significant differences between control and exercise groups in baseline characteristics (**Table 1**). All subjects in the exercise group completed at least 80% of the planned training sessions. No major adverse events or health-related issues attributable to exercise were noted.

Four subjects in each group could not complete the baseline 10-STS assessment due to excessive weakness or mobility limitations (i.e., use of crutches), and therefore the sample analyzed for this test was of 36 and 23 for the control and the exercise group, respectively. After the 14-week intervention four subjects in the control group could not perform the 10-STS and one subject in this same group could not perform the psychological tests, and thus we used their baseline values.

No significant changes in physical performance measures were observed in the control group between baseline and post-intervention. By contrast, a significant improvement was observed in the exercise group for 6MWT ($p = 0.006$, ES = 0.31), STS-10 ($p < 0.001$, ES = 0.59) and combined handgrip strength ($p = 0.027$, ES = 0.12). Significant interactions (group × time) with moderate to large effect sizes were found for all physical performance measures (**Table 2**). *Post hoc* analyses revealed significant differences between groups at post-intervention for 6MWT ($p = 0.005$), STS-10 ($p < 0.001$) and combined handgrip strength ($p = 0.017$).

Despite statistically significant benefits, only 30, 46, and 20% of subjects in the exercise group were clinically meaningful responders for 6MWT, STS-10 and handgrip strength test, respectively. Of note, responsiveness was dependent on baseline physical fitness, that is, participants with lower baseline physical fitness showed greater improvements. Indeed, significant differences were found between responders and non-responders for baseline physical performance (**Figure 1**), and a significant inverse relationship was observed between baseline combined handgrip strength and 6MWT, on one hand, and the relative performance improvement in these tests, on the other (**Figure 2**). There were no significant differences between sexes for the rate of responders observed in 6MWT (33 and 22% for men and women, respectively; $p = 0.882$), STS-10 (53 and 33%, respectively; $p = 0.597$) or handgrip strength test (11 and 44%, respectively; $p = 0.141$). No significant relationship ($p > 0.05$) was observed between age and relative improvement on physical performance for any test.

Regarding mental status, BDI scores significantly decreased in the exercise group at post-intervention compared to baseline ($p = 0.006$, ES = 0.29), whereas no significant changes were

TABLE 2 | Effects of an intradialytic exercise program on markers of physical and mental health.

End point	Group	n with baseline data	Baseline	Post-intervention	Change (95% CI)	Group × Time effect	Effect size (η_p^2)[a]
6MWT (m)	Control	40	341 ± 127	330 ± 118	−11 (−27, 5)	**0.001**	0.160
	Exercise	27	380 ± 131	422 ± 136	42 (13, 70)		
STS-10 (s)	Control	36	32 ± 11	34 ± 12	2 (−1, 5)	**<0.001**	0.203
	Exercise	23	26 ± 10	21 ± 8	−6 (−8, −4)		
Handgrip (kg)	Control	40	25 ± 8	24 ± 8	−1 (−2, 0)	**0.02**	0.084
	Exercise	27	28 ± 8	29 ± 8	1 (0, 2)		
STAI-S	Control	40	18 ± 13	18 ± 12	0 (−2, 2)	0.10	–
	Exercise	27	19 ± 9	17 ± 10	−2 (−5, 2)		
BDI	Control	40	15 ± 13	14 ± 10	−1 (−3, 2)	0.32	–
	Exercise	27	10 ± 8	8 ± 7	−2 (−4, −1)		
PCS	Control	40	61 ± 17	66 ± 16	5 (0, 10)	0.36	-
	Exercise	27	62 ± 20	63 ± 23	1 (−5, 8)		
MCS	Control	40	70 ± 20	73 ± 16	3 (−1, 8)	0.54	–
	Exercise	27	75 ± 14	76 ± 15	1 (−3, 5)		

Data are mean ± SD. Significant p-values are highlighted in bold. Handgrip strength corresponds to the mean of the two arms. Abbreviations: 6MWT, six minute-walk test; 95% CI, 95% confidence intervals; BDI, Beck's depression inventory; MCS, mental component summary; PCS, physical component summary; STAI-S, State-Trait Anxiety Inventory; STS-10, sit to stand test. 4 and 1 subjects of the control group could not perform the STS-10 and the psychological tests, respectively, at post-intervention, and their baseline values were used. Data from STAI-S and BDI were log-transformed prior to its analysis, but are shown as raw data for the sake of clarity.

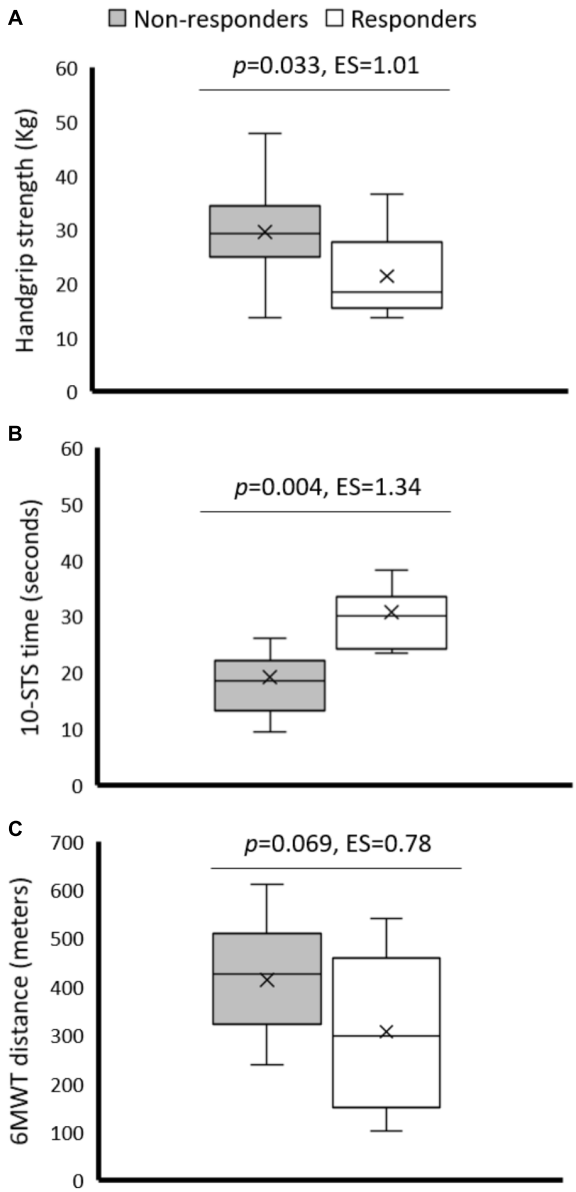

FIGURE 1 | Differences in baseline performance for combined handgrip strength **(A)** 10-repetition sit to stand **(B)** and 6-min walk test **(C)** between responders and non-responders to the intradialytic training program.

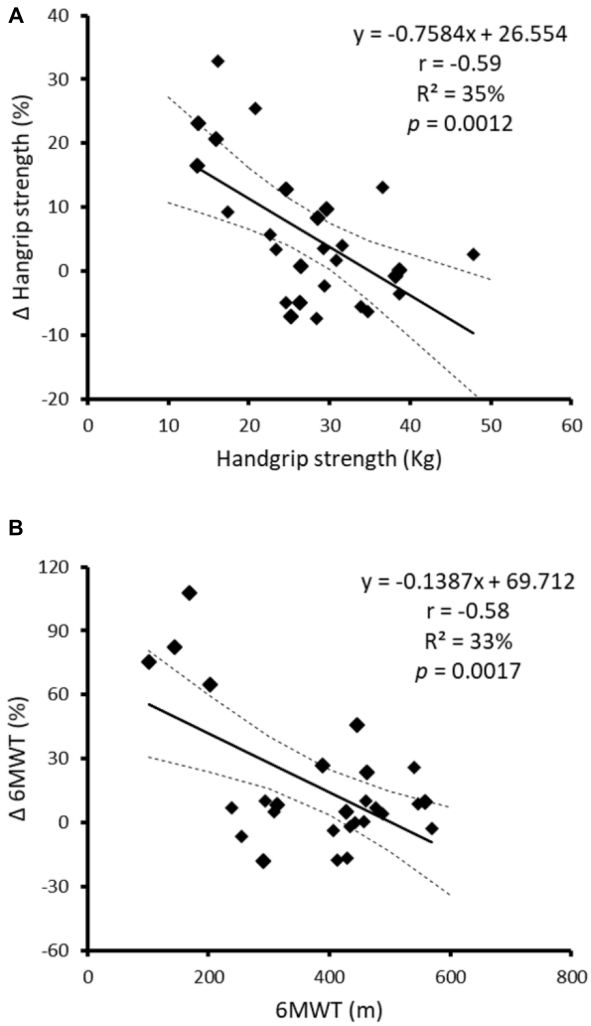

FIGURE 2 | Relationship between baseline performance for combined handgrip strength **(A)** and 6-min walk distance **(B)** with the change observed in these tests after the intradialytic training program. Solid and dashed lines represent regression line and 90% confidence intervals, respectively.

observed in the control group (**Table 2**). No significant differences ($p > 0.05$) between baseline and post-intervention were observed for any of the other mental and health status endpoints in the control or exercise group (**Table 2**). No significant interaction (group x time) was found for any of the mental and health status endpoints (**Table 2**).

DISCUSSION

The present results show that a 14-week intradialytic training program including endurance and resistance exercise induced improvements in mean values of physical performance, which are significantly lower in this population than in their healthy counterparts (Painter, 2005; Leal et al., 2011b). Specifically, significant improvements were observed for the average value of 6MWT, a valid predictor of mortality, cardiovascular events and hospitalization in dialysis patients (Torino et al., 2014). Exercise training also resulted in an increased strength of the lower limb muscles (as reflected by a lower time on average to complete the STS-10 test), which is important because an impaired physical performance of the lower extremities is strongly associated with all-cause mortality in these patients (Roshanravan et al., 2013). We also found an exercise-training induced improvement in handgrip strength, with decreases in this variable being related to a decreased inflammatory status and higher muscle mass and survival expectancy in this population (Leal et al., 2011a). Therefore, these results are of major clinical importance,

as they suggest that an intradialytic exercise program can attenuate dialysis-associated physical impairment and thus might also potentially reduce morbimortality risk in these patients (Morishita et al., 2017).

The effectiveness of intradialytic training programs for the improvement of physical performance has been previously demonstrated (Smart and Steele, 2011; Chung et al., 2017). Although the response to exercise interventions is commonly described in general terms under the assumption that the group average represents the response of most individuals (Mann et al., 2014), it has now been demonstrated that a considerable individual variability can be observed even in tightly controlled studies (Yan et al., 2017). In this context, an interesting finding of the present study is that, despite significant improvements in mean physical performance, the rate of clinically meaningful responders was overall low (<50%). Several hypotheses have been proposed to account for individual variability in response to exercise training (Mann et al., 2014). In our study, baseline physical performance - but not participants' age or sex - partly conditioned the level of responsiveness to the training program, with the less fit patients at baseline being those showing greater benefits. These results suggest that the training stimulus was high enough to induce clinically meaningful improvements in less fit subjects but not in their fitter peers. Notwithstanding, even non-responders presented a lower physical performance at baseline than expected for their age (Casanova et al., 2011; Massy-Westropp et al., 2011). Therefore, efforts to enhance responsiveness in these subjects are needed, which might probably involve applying a higher training stimulus (e.g., higher intensity or volume) (Mann et al., 2014).

Although in agreement with our results some studies have found no changes in variables such as HRQoL or depression after intradialytic training programs (van Vilsteren et al., 2005; Parsons et al., 2006), most studies have reported benefits on these psychological variables (Suh et al., 2002; Levendoğlu et al., 2004; Ouzouni et al., 2009; Dziubek et al., 2016; Frih et al., 2017). Interestingly, the level of anxiety observed in our patients was overall low, with only 4% of subjects in the intervention group presenting a STAI score higher than 40, which is the proposed cut-off for detecting clinically significant symptoms of anxiety) (Knight et al., 1983; Julian, 2011). HRQoL was also surprisingly good, with the observed mean MCS and PCS being higher than those previously reported in other dialysis populations (Mapes et al., 2003; Lacson et al., 2010; Frih et al., 2017). The lack of significant differences in these variables in our study might have been due to the low prevalence of psychological disorders in the analyzed sample, which can be a result of the psychological therapy that all subjects received since they started dialysis. Nevertheless, a significant reduction of 23% in mean BDI scores was observed after the exercise program in the present study, and a reduction of >17.5% has proven to be the threshold above which depressive individuals report feeling better (Button et al., 2015). Therefore, the observed benefits of exercise on depression levels could be of clinical importance despite no statistically significant differences between groups.

Considering the importance of physical activity and performance for dialysis patients (O'Hare et al., 2003; Sietsema et al., 2004; Stack et al., 2005; Matsuzawa et al., 2012; Roshanravan et al., 2013) and their low levels of physical activity (Johansen et al., 2010), promoting physical activity in this population should be a priority. Intradialytic exercise programs have proven safe and effective not only for improving physical performance (Smart and Steele, 2011; Chung et al., 2017) but also dialysis efficacy (Parsons et al., 2006), and therefore these programs should be routinely included in clinical practice. Nevertheless, the present study highlights the need of individualizing training programs so as to achieve an optimal stimulus for every patient.

Our study has some limitations, including mainly the lack of subjects' familiarization sessions with the tests and the fact that we did not perform a randomized controlled trial. In addition, several potential confounders which were not considered here have been proposed to influence inter-individual variability in response to a training stimulus. Particularly a commonly overlooked source of error is within-subject variability, with recent research providing some insights into its importance (Hecksteden et al., 2015, 2018; Lindholm et al., 2016) and another ongoing project, the Gene Smart study, currently embracing this concept (Yan et al., 2017). However, applying the designs that allow to control for confounders like within-subject variability (e.g., performing repeated tests both before and after the intervention, or using a crossover study with repeated training intervention) might not be feasible in patient populations such as the present one. While keeping the aforementioned limitations in mind, a major strength and novelty of our approach was the individualized analysis of training responses, which allowed us to estimate the rate of clinically meaningful responders.

CONCLUSION

A 14-week intradialytic endurance-resistance training program improved patients' physical performance on average. Yet, baseline physical status affected the level of responsiveness to the training program, with only those patients presenting the lowest physical fitness at the beginning of the intervention obtaining clinically meaningful benefits from the training program. Efforts to individualize exercise prescription are needed in clinical practice to enhance responsiveness. Future reach might determine if applying a higher training stimulus (i.e., higher intensity or volume) in the fitter subjects actually results in a clinically meaningful response.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the institutional review board of Fundación Universitaria Hospital de Alcorcón (Madrid, Spain). The protocol was approved by the institutional review board of Fundación Universitaria Hospital de Alcorcón (Madrid, Spain). All subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

AB, RP-C, MG-G, and MP conceived, designed, and supervised the study. AdA, FC, and MM-L supervised the training sessions and performed the evaluations. PV and JM analyzed the data. PV drafted the manuscript. All authors significantly contributed to the final version of the manuscript.

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Genetic Variation in Acid Ceramidase Predicts Non-completion of an Exercise Intervention

*Lauren S. Lewis[1], Kim M. Huffman[2,3], Ira J. Smith[4], Mark P. Donahue[4], Cris A. Slentz[2], Joseph A. Houmard[5], Monica J. Hubal[6], Eric P. Hoffman[6], Elizabeth R. Hauser[2,7], Ilene C. Siegler[8] and William E. Kraus[2,4]**

[1] Department of Obstetrics and Gynecology, Duke University School of Medicine, Durham, NC, United States, [2] Duke Molecular Physiology Institute, Duke University School of Medicine, Durham, NC, United States, [3] Division of Rheumatology and Immunology, Department of Medicine, Duke University School of Medicine, Durham, NC, United States, [4] Division of Cardiology, Department of Medicine, Duke University School of Medicine, Durham, NC, United States, [5] Human Performance Laboratory, East Carolina University, Greenville, NC, United States, [6] Children's Genetic Medical Research Center, Children's National Medical Center, Washington, DC, United States, [7] Cooperative Studies Program-Epidemiology Center Durham, Veterans Administration Medical Center, Durham, NC, United States, [8] Division of Behavioral Medicine, Department of Psychiatry, Duke University School of Medicine, Durham, NC, United States

Genetic variation is associated with a number of lifestyle behaviours; it may be associated with adherence and individual responses to exercise training. We tested single nucleotide polymorphisms (SNPs) in the acid ceramidase gene (*ASAH1*) for association with subject adherence and physiologic benefit with exercise training in two well-characterised randomised, controlled 8-month exercise interventions: STRRIDE I ($n = 239$) and STRRIDE II ($n = 246$). Three *ASAH1* non-coding SNPs in a linkage disequilibrium block were associated with non-completion: rs2898458(G/T), rs7508(A/G), and rs3810(A/G) were associated with non-completion in both additive (OR = 1.8, 1.8, 2.0; $P < 0.05$ all) and dominant (OR = 2.5, 2.6, 3.5; $P < 0.05$ all) models; with less skeletal muscle *ASAH* expression ($p < 0.01$) in a subset ($N = 60$); and poorer training response in cardiorespiratory fitness (peak VO_2 change rs3810 $r^2 = 0.29$, $P = 0.04$; rs2898458 $r^2 = 0.29$, $P = 0.08$; rs7508 $r^2 = 0.28$, $p = 0.09$); and similar in direction and magnitude in both independent exploratory and replication studies. Adherence to exercise may be partly biologically and genetically moderated through metabolic regulatory pathways participating in skeletal muscle adaptation to exercise training.

Keywords: STRRIDE, metabolism, ceramide, exercise adherence, behavioural lifestyle interventions

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University of Missouri, United States

***Correspondence:**
William E. Kraus
william.kraus@duke.edu

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INTRODUCTION

Many of the health benefits of exercise are mediated by metabolic adaptations in skeletal muscle, and increases in cardiorespiratory fitness. As detailed in the Physical Activity Guidelines Advisory Committee Report the benefits of exercise are substantial (DHHS, 2008) however, perhaps the major clinical issue confronting the use of exercise training as a therapeutic option is how to get individuals to initiate an exercise training program and—following that—how to maintain it. Common wisdom holds that adherence issues are primarily related to neurobehavioural and social issues, and that barriers can be identified and addressed using behavioural approaches. Although most attention regarding adherence to lifestyle interventions has traditionally focused on

psychosocial behavioural factors, it is conceivable that there also exist biological and genetic factors determining whether individuals maintain an exercise program once initiated.

An additional issue complicating the use of exercise training as a therapeutic option is the variability in training responses. Conventional wisdom holds that more exercise is better, and that exercise is beneficial for everyone for a myriad of health benefits. Rather, even when controlling for adherence, there is a range of health responses to any given exercise program for any given health parameter (e.g., fitness, blood lipids, insulin sensitivity, blood pressure control, even weight change). In sum, not all individuals respond in a similar—or even in a favourable manner—to an exercise program (Bouchard et al., 2012). Such variation in biological response to a given exercise exposure is heritable; with heritability estimates ranging from 29 to 70%. This suggests a large fraction of the exercise response is moderated by genetic factors. These observations raised the prospect that genetic classifiers might be assembled to predict the variability in responses to exercise.

Genes of metabolic pathways active in skeletal muscle are associated with exercise performance; (Bouchard et al., 2011) and many of the physiologic effects of exercise training result from the adaption of skeletal muscle mitochondria (Duscha et al., 2005; Huffman et al., 2014). Thus, genes involved in skeletal muscle mitochondrial function serve as important candidate genes in studying the heritability of compliance with and response to exercise training. One antagonist of mitochondrial function—ceramide—is a central compound in sphingolipid metabolism. Intracellular ceramide content is increased in obese individuals; and the accumulation of ceramide in non-adipocytes is implicated in many obesity-associated diseases: lipotoxic heart disease, atherosclerosis, and type 2 diabetes (Zhou et al., 2000; Birbes et al., 2002; Hannun and Obeid, 2002; Summers, 2006). Ceramide content is reduced with short exercise bouts and exercise training, although exercise effects on ceramide pathway components are complex and incompletely understood (Dobrzyn and Gorski, 2002a,b; Helge et al., 2004; Bruce et al., 2006; Baranowski et al., 2008; Błachnio-Zabielska et al., 2008, 2011; Bergman et al., 2016). Ceramide impinges on mitochondrial adaptations to exercise by directly inhibiting the respiratory chain, activating apoptotic pathways converging on mitochondria, and promoting programmed cell death and inflammation (Birbes et al., 2002; Hannun and Obeid, 2002; Summers, 2006; Mao and Obeid, 2008); these bioactive effects can be regulated via conversion of ceramide to alternate metabolites, including degradation by acid ceramidase (Mao and Obeid, 2008). Despite the regulatory roles of acid ceramidase on ceramide effects impacting exercise, to the best of our knowledge no one has investigated acid ceramidase genetic variants in exercise responses. We hypothesised that acid ceramidase genetic variants might impact ceramide metabolism and other downstream metabolic responses to exercise; exercise capacity; and thereby influence exercise adherence. Here, using two well-described randomised controlled exercise trials, we capitalised on a 35% non-completion rate to investigate the association of single nucleotide polymorphisms (SNPs) in the acid ceramidase (EC 3.5.1.23) gene (*ASAH1*) with the ability to predict completion of and response to exercise training.

METHODS

Subjects

Two independent exercise trial samples were available: STRRIDE I (NCT00200993) and STRRIDE II (NCT00275145). A complete description of the STRRIDE I design is published elsewhere (Kraus et al., 2001). In summary, subjects were: 40–65 years of age; sedentary (exercised less than once weekly); overweight or obese (BMI 25 to 35 kg/m2); had fasting hyperinsulinemia (>10 IU/mL) with mild to moderate lipid abnormalities (LDL cholesterol between 130 and 190 mg/dL or HDL cholesterol <45 mg/dL for women or 40 mg/dL for men). The study design was similar. STRRIDE II, subjects were: 18–70 years of age; similarly, overweight or obese; sedentary; and with mild lipid abnormalities (**Table 1**). All subjects provided verbal and written informed consent as approved by the of Duke University Investigational Review Board and ECU Investigational Review Board. Subjects meeting inclusion criteria were randomised to one of four exercise groups in each study. Those subjects with DNA available for genetics studies are studied in this report.

Exercise Training

In STRRIDE I, subjects were randomly assigned to one of four groups: (1) non-exercising control; (2) low volume/moderate intensity aerobic exercise, defined as a caloric equivalent of 12 miles/week at 40–55% peak oxygen consumption (peak VO_2); (3) low volume/vigorous intensity, defined as the caloric equivalent of 12 miles/week at 65–80% peak VO_2; and (4) high volume/vigorous intensity exercise, defined as the caloric equivalent of 20 miles/week at 65–85% peak VO_2. Caloric equivalents were determined by the approximate energy expenditure during walking or jogging for a 90 kg person; however, actual exercise modalities included cycle ergometers, treadmills, and elliptical trainers. The subjects underwent a 2-month ramp period in which exercise intensity and duration were gradually increased until the appropriate regimen was reached; this was followed by an additional 6 months of exercise training.

In STRRIDE II, subjects underwent a 3-month control run-in period followed by an 8-month exercise intervention in one of four exercise groups: (1) low volume/vigorous intensity group, identical to the low volume/vigorous intensity group of STRRIDE I; (2) resistance training, in which subjects completed a regimen of three sessions per week during which nine resistance exercises were performed with eight to twelve repetitions at 70–85% of one repetition maximum weight; (3) low volume/vigorous intensity aerobic exercise plus resistance training, during which subjects completed the low volume/vigorous intensity aerobic training protocol in addition to the resistance training protocol; and (4) high volume/vigorous intensity aerobic training, identical to that of STRRIDE I.

For the statistical analyses in which the STRRIDE I and STRRIDE II datasets were combined, the groups were coded as follows: (1) STRRIDE I inactive controls; (2) STRRIDE I low volume/moderate intensity aerobic exercise; (3) STRRIDE I

TABLE 1 | Characteristics of Subjects in STRRIDE I and STRRIDE II (% (N) or mean ± SD).

	STRRIDE I		STRRIDE II		Total		Gene Expression Subset
	Failed to Complete Study	Completed Study	Failed to Complete Study	Completed Study	Failed to Complete Study	Completed Study	
	28 (67)	72 (172)	38 (93)	62 (153)	33 (160)	67 (325)	$n = 60$
Age (y)	51.3 ± 5.8	52.5 ± 4.8	46.5 ± 11.8	48.8 ± 10.2	48.5 ± 10.0	50.8 ± 8.6	51 ± 1.2
Gender**- men	40 (27)	55 (94)	35 (33)	44 (68)	37 (60)	50 (162)	50 (30)
Women	60 (40)	45 (78)	65 (60)	56 (85)	63 (100)	50 (163)	50 (30)
Race**- white	58 (39)	81 (139)	67 (62)	86 (131)	63 (101)	83 (270)	90 (54)
Black	42 (28)	19 (33)	33 (31)	14 (22)	37 (59)	(17) 55	10 (6)
Body mass Index (kg/m2)	30.8 ± 3.6	29.9 ± 2.9	30.6 ± 3.3	30.5 ± 3.3	30.8 ± 3.4	30.2 ± 3.1	30.6 ± 0.4
Minimum Waist Circumference (cm)	96.2 ± 10.7	95.4 ± 9.8	95.7 ± 10.2	96.4 ± 9.6	96.0 ± 10.4	95.9 ± 9.7	96.9 ± 1.2
Pre-intervention peak VO_2 (mL/kg/min)	26.4 ± 6.1	27.9 ± 6.1	26.6 ± 8.8	27.5 ± 6.0	26.5 ± 6.4	27.7 ± 6.0	27.6 ± 0.7

** *p-values for two sample t-test (completers vs. non-completers) significant in STRRIDE I, STRRIDE II, and total.*

plus STRRIDE II low volume/vigorous intensity aerobic exercise groups; (4) STRRIDE I plus STRRIDE II high volume/vigorous intensity aerobic exercise groups; (5) STRRIDE II resistance training group; (6) STRRIDE II low volume/vigorous intensity aerobic exercise plus resistance exercise group. Demographics and physiologic characteristics of the groups are shown in **Table 1**.

Biologic Measures

All phenotypic measures were taken prior to initiation and at completion of the exercise intervention (Month 6 in STRRIDE I and Month 8 in STRRIDE II). Peak VO_2 was measured using a graded treadmill exercise testing protocol with gas exchange analysis (Duscha et al., 2005). Exercise compliance was measured as a percentage of the assigned exercise minutes per week completed by the subject averaged over the intervention period. Non-completion occurred when a subject withdrew from further participation in the study for any of the following reasons: time constraints; injury or illness unrelated to the study; medical problems; family issues; or geographic relocation. No subjects were lost to follow- up.

Genotyping

DNA was isolated from whole blood using a commercial DNA isolation kit and a standard protocol (Qiagen, Inc, Valencia, CA). Acid ceramidase (*ASAH1*) SNPs were selected using the SNPSelector program in which a tagging algorithm prioritised SNPs for low linkage disequilibrium in the HapMap database, allelic frequencies, and regulatory potential (Xu et al., 2005). Six haplotype tagging SNPs in *ASAH1* were identified: rs7844023, rs2898458, rs7508, rs3810, rs2427746, and rs1049874; genotypes were determined using the Taqman assay (Applied Biosystems, Foster City, CA). The Taqman genotyping reaction was then amplified using a GeneAmp PCR system 9700 (95°C for 10 min, then 50 cycles at 92°C for 15 s, 60°C for 1 min). Fluorescence was detected using the 7900HT Taqman sequence detector (Applied Biosystems). Two reference controls were included. All SNPs were successfully genotyped for 95% or more of the individuals in the study; rescreening of 2.4% of subjects gave 100% identical results. Error rate estimates for SNPs meeting the reference control benchmarks were <0.2%.

Gene Expression Profiling

We conducted gene expression analysis on a subset of 60 representative individuals randomly selected for further study from both data sets. The gene expression subgroup included 10 subjects (five men and five women) from each of the six exercise groups described above; paired baseline and post-training samples were always processed in the same assay. Demographics for this cohort were not significantly different from those of the entire cohort (**Table 1**).

Total RNA was extracted using the standard Trizol (Invitrogen, Carlsbad, CA) method and 30 to 50 mg of starting skeletal muscle. Two round amplification of total RNA was performed using a commercially available kit (Affymetrix, Santa Clara, CA). Thirty micrograms of biotinylated cRNA from each sample was hybridised to Affymetrix U133 Plus 2.0 microarrays. More detailed methods associated with microarray gene expression analysis can be found elsewhere (Hittel et al., 2005). Probe set expression levels were generated using the PLIER algorithm (typically 6 iterations) in Expression Console (Affymetrix) and imported directly into Partek Genomics Suite (Partek Inc., St. Louis, MO) for statistical processing.

Statistical Analysis

Haploview (27) was used to assess LD between SNPs using the combined STRRIDE I and STRRIDE II datasets. Genotype association with the non-completion study was analysed using a logistic regression model (SAS software, SAS Institute, Cary, NC). STRRIDE I and STRRIDE II datasets were analysed separately. As independent datasets, they provided an opportunity for validation of significance of individual SNPs in direction

and magnitude of effect: STRRIDE II was considered the testing/exploratory set, and STRRIDE I the validation set. The outcome of non-completion of the exercise intervention was defined as a dichotomous variable with individuals who did not complete the intervention coded as 1; individuals who completed the program were coded as 0. Genotypes were coded using an additive model with 0, 1, or 2 copies of one allele and using a dominant model for the presence vs. absence of the same allele. Race, gender, and exercise group were included in the regression model; these variables differed significantly in those subjects completing the study vs. non-completers (**Table 1**). Due to the very small numbers of individuals reporting Asian or Hispanic ethnicity, only subjects who were either black or white were included in the analysis. To examine the potential for confounding by race, the logistic regression analyses were also performed stratified by race. Odds ratios were estimated for non-completion vs. completion.

Multivariable linear regression models (SAS software, SAS Institute, Cary, NC) were used to model genotype association with peak oxygen consumption (peak VO_2) and exercise compliance in completers. Genotypes were coded for additive and dominant models. As described above, all models included terms for gender, race, and exercise group. Given the consistency of the effects observed for the SNPs associated with non-completion between STRRIDE I and STRRIDE II, the datasets were combined for analysis of change in peak VO_2. Peak VO_2 improvement models were tested with the complete dataset and with a dataset that excluded those subjects in the control and resistance training groups. Results are presented as mean ± SE.

Expression profile statistical analysis used Partek Genomics Suite (Version 6.4). Expression profiles were analysed to test differences in baseline gene expression between genotype groups. Following the gene expression value normalization, we used analysis of variance (ANOVA) with sex and race as covariates to examine genotype influences on mRNA expression also known as an eQTL analysis. $P < 0.01$ were considered statistically significant in this analysis.

RESULTS

Allele Frequencies

For a complete gene analysis of the acid ceramidase gene, six *ASAH1* SNPs were genotyped (rs7844023, rs2898458, rs7508, rs3810, rs2427746, rs1049874) in 239 subjects in STRRIDE I and 246 subjects in STRRIDE II. All allele frequencies were in Hardy Weinberg equilibrium (chi^2 test, $P > 0.05$) except for rs1049874 (**Table 2**). The rs1049874 SNP met all quality control benchmarks for genotyping as evaluated by an independent lab supervisor; it was therefore included in the analysis. Three SNPs, rs2898458, rs7508, and rs3810 were in pair-wise LD in whites ($r^2 > 0.60$). Only SNPs rs2898458 and rs3810 were in strong LD in blacks ($r^2 > 0.70$; **Figure 1**).

Genetic Association With Non-completion: Exploratory Analysis—STRRIDE II

Three *ASAH1* SNPs, rs2898458, rs7508, and rs3810—in LD—were consistently associated with intervention non-completion in both additive and dominant models (**Table 3**). After controlling for group, gender, and race, each additional T allele at rs3810 doubled the odds of failure to complete; in the dominant model, subjects with either one or two copies of the T allele were 3.5 times more likely to fail to complete than those with the GG genotype (additive model $P = 0.01$; dominant model $P = 0.005$; **Figure 2**). Similarly, both rs2898458 allele G and rs7508 allele G significantly increased the odds of non-completion in both additive (OR = 1.8, 1.8; $P = 0.03$, 0.002, respectively) and dominant (OR = 2.5, 2.6; $P = 0.02$, 0.02, respectively) models.

Genetic Association With Study Non-completion: Validation Analysis—STRRIDE I

ASAH1 genotype associations were validated in the STRRIDE I dataset, in which the additive model was significantly associated with non-completion for rs3810 and rs2898458 (OR = 1.8, 1.8; $P = 0.02$, 0.02). When comparing the direction and magnitude of association of each SNP, the two independent datasets showed excellent agreement, providing strong replication for association of these SNPs with failure to complete (**Table 3**, **Figure 3**). The race-stratified analyses supported the combined analysis with the white race group showing results consistent with the analysis of the full datasets for both STRRIDE I and STRRIDE II (supplemental tables).

Genetic Association With Peak Oxygen Consumption in Intervention Completers

To investigate potential mediators of the genetic association with failure-to-complete an exercise intervention, we studied the effects of the *ASAH1* variants on baseline peak VO_2 and change in peak VO_2 with exercise training; we hypothesised that poor exercise capacity or ability to respond to exercise training might explain why persons chose to not complete the protocol. Baseline peak VO_2 was not significantly associated with genotype or study completion (data not shown). However, among completers, improvement in peak VO_2 was correlated with genotype for two of the three *ASAH1* SNPs previously associated with non-completion. When compared to subjects homozygous for the G allele for SNP rs3810 and controlling for race, gender, group, and baseline peak VO_2, each additional T allele decreased the improvement in peak oxygen consumption by 1.4 (mL/kg/min) ($p = 0.0185$, $r^2 = 0.3689$). Similarly, the G allele of SNP rs2898458 showed a smaller improvement in peak VO_2 (0.45 mL/kg/min decrease, $P = 0.0734$, $r^2 = 0.362$).

One would not expect peak VO_2 to change significantly in those subjects assigned to either the control or resistance training groups; we therefore tested the association of improvement in peak VO_2 with genotype in the dataset excluding these two groups. Indeed, the two SNPs mentioned previously remained correlated with improvement in peak VO_2 (rs3810 $r^2 = 0.29$, $P = 0.036$; rs2898458 $r^2 = 0.29$, $P = 0.0834$; **Figure 4**). In this model, rs7508 genotype was also associated with improvement in peak VO_2 ($p = 0.0873$, $r^2 = 0.28$).

TABLE 2 | Genotype Frequency by Race.

SNP	Genotype	STRRIDE I ($N = 239$)		STRRIDE II ($N = 246$)	
		White	Black	White	Black
RS7844023	CC	26.5	25.9	24.5	30.8
	CT	46.5	46.6	53.2	40.4
	CT	27.0	27.6	22.3	28.8
	T Allele frequency	50.3	50.8	48.9	49.0
RS2898458	AA	50.3	7.4	44.4	22.0
	AG	38.2	40.7	47.6	38.0
	GG	11.5	51.9	8.0	40.0
	G Allele frequency	30.6	72.2	31.7	59.0
RS7508	AA	59.4	81.0	53.7	90.4
	AG	32.9	13.8	40.5	9.6
	GG	7.7	5.2	5.8	0.0
	G Allele frequency	24.1	12.1	26.0	4.8
RS3810	GG	45.3	7.1	43.1	11.8
	GT	41.8	39.3	48.4	45.1
	TT	12.9	53.6	8.5	43.1
	T Allele Frequency	33.8	73.2	16.2	65.7
RS2427746	AA	35.5	63.0	27.5	70.6
	AG	41.4	29.6	49.7	27.4
	GG	23.1	7.4	22.8	2.0
	G Allele Frequency	43.8	22.2	47.6	15.6
RS1049874	CC	29.0	12.1	23.0	54.9
	CT	36.7	31.0	51.3	37.2
	TT	34.3	56.9	25.7	7.8
	C Allele Frequency	47.3	27.6	51.3	26.5

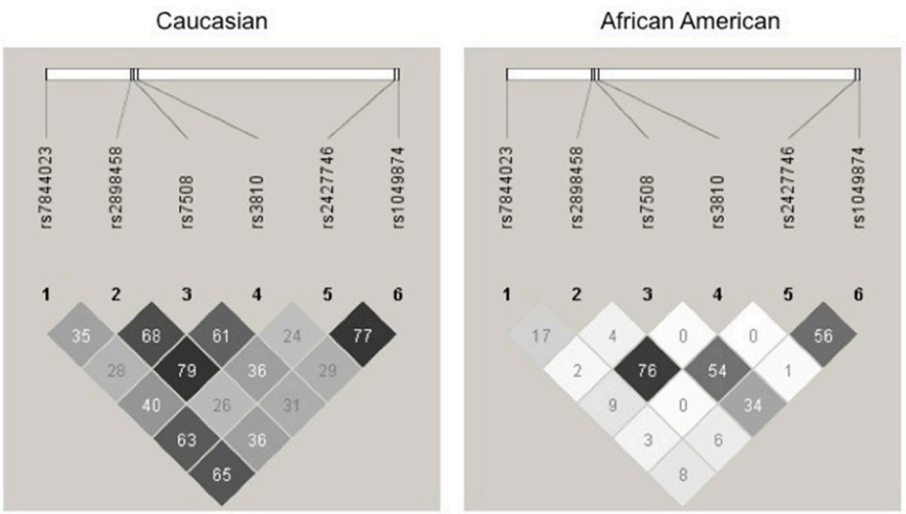

FIGURE 1 | Linkage disequilibrium plot for Acid Ceramidase SNPs**.** SNPs assessed in the current study are shown above the linkage disequilibrium (LD) plots for genotype in white subjects (**left**) and black subjects (**right**) participating in either STRRIDE I or STRRIDE II. Results given are r^2-values for association. The intensity of the block is proportional to the association between two variants. SNPs rs2898458, rs7508, and rs3810 as well as rs2427746 and rs1049874 are shown to be in LD($r^2 > 0.6$) in whites ($n = 371$). Only rs2898458 and rs3810 are in LD ($r^2 > 0.6$) in blacks ($n = 114$).

TABLE 3 | Genotype association with risk of failure to complete study, controlling for race, gender, and intervention group.

		Additive Model		Dominant Model	
		Odds Ratio	***P*-value**	**Odds Ratio**	***P*-value**
rs7844023 (C/**T**)*	STRRIDE I	0.88	0.562	0.88	0.300
	STRRIDE II	0.86	0.513	0.82	0.596
rs2898458 (A/**G**)*	STRRIDE I	**1.80***	**0.022***	1.80	0.384
	STRRIDE II	**1.79***	**0.026***	**2.52***	**0.018***
rs7508 (A/**G**)*	STRRIDE I	1.17	0.546	1.17	0.499
	STRRIDE II	**1.78***	**0.034***	**2.55***	**0.015***
rs3810 (G/**T**)*	STRRIDE I	**1.79***	**0.016***	1.79	0.687
	STRRIDE II	**2.02***	**0.012***	**3.49***	**0.005***
rs2427746 (A/**G**)*	STRRIDE I	0.95	0.838	0.95	0.455
	STRRIDE II	1.17	0.543	1.15	0.711
rs1049874 (T/**C**)*	STRRIDE I	1.11	0.635	1.11	0.92
	STRRIDE II	1.18	0.628	1.21	0.628

All models controlled for race, gender and intervention group. Significant effects are marked with asterisk (*) and indicated in bold.

Genetic Effects on mRNA Expression of ASAH1

We generated gene expression profiles using a subset of 60 individuals from both STRRIDE I and STRRIDE II. As shown in **Table 1**, demographic data for age, height, weight, and BMI were not different between the total cohort and the gene expression-profiled sub-cohort. Peak VO_2 at baseline was lower in the gene expression subgroup than the total population; however, it was not different between the subgroup and all subjects that completed the study. There are five probe sets for *ASAH1* on the U133 Plus 2.0 microarray, of which only two (213702_x_at and 210979_at) target the full length *ASAH1* transcript and are highly expressed in skeletal muscle. Expression of these two probe sets was significantly different between rs3810 genotypes, when tested with either an additive ($p = 0.006$ and $p = 0.007$ for probe sets 213702_x_at and 210979_at, respectively) or a dominant model ($p = 0.00007$ and $p = 0.0001$ for probe sets 213702_x_at and 210979_at, respectively). For rs3810, the TT group demonstrated between a 1.3 and 1.4 times reduction in *ASAH1* mRNA expression when compared with the GG group (**Figure 5**). Linkage disequilibrium between rs3810 and rs2898458 was nearly complete in the gene expression subgroup. Therefore, similar results were found for the relation between skeletal muscle *ASAH1* expression and rs2898458 genotype (data not shown). Linkage disequilibrium between rs3810 and rs7508 was somewhat lower at 78%, with only one GG subject (also homozygous for the minor allele for both other SNPs); however, the gene expression results for rs7508 were similar to those for rs3810 (not shown).

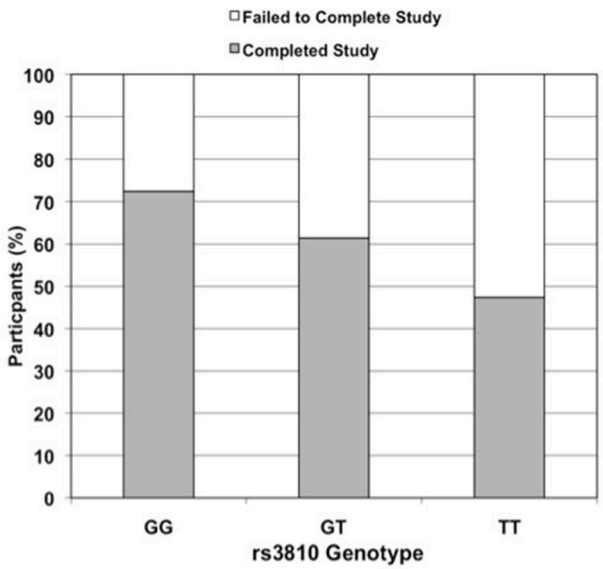

FIGURE 2 | Effect of rs3810 genotype on completion rate in STRRIDE II. Percent of subjects in STRRIDE II completing (grey filled bars) and failing to complete (open bars) the study intervention by rs3810 genotype (GG genotype $n = 87$, GT genotype $n = 114$, TT genotype $n = 38$; when controlled for race, gender, and exercise group effects, additive model OR = 2.0, $p = 0.01$ and dominant model OR = 3.5, $p = 0.005$). These findings were similar to those for rs2898458 and rs7508 (data not shown).

DISCUSSION

In the present study, we provide evidence suggesting that genetic variation within the acid ceramidase gene is associated with an individual's ability or willingness to persist in a newly prescribed exercise program. Three of six *ASAH1* SNPs (rs2898458, rs3810, and rs7508; all in LD) were associated with significantly increased risk of non-completion of an exercise intervention in two exercise training interventions — STRRIDE I and STRRIDE II. These SNPs were also associated with change in peak VO_2 among the completers; there also were differences in *ASAH1* gene expression such that genetic correspondence with gene expression was exactly consistent with less improvement in VO_2 with training and greater non-completion rates. Thus, these findings support

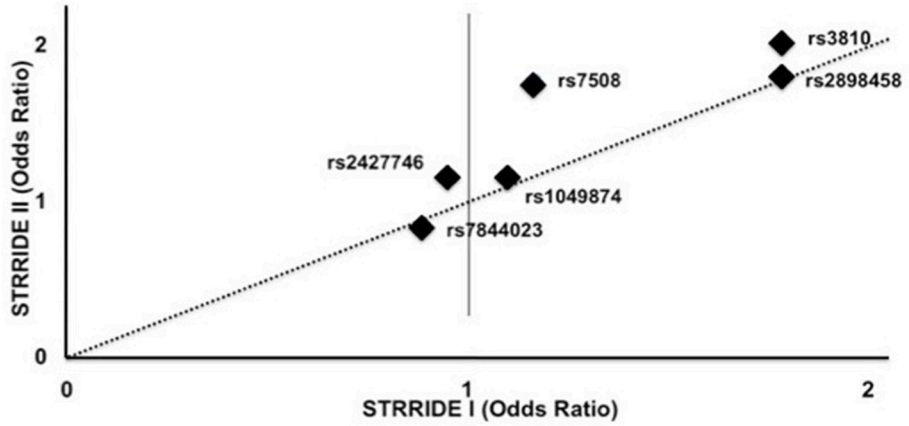

FIGURE 3 | Genotype effect on odds of failure to complete study by SNP in STRRIDE II vs. STRRIDE I. Additive model odds ratios for risk of study non-completion for each ASAH SNP for STRRIDE I (x axis) and STRRIDE II (y axis). Two SNPs significantly increased the odds of failure to complete both STRRIDE II and STRRIDE I: rs2898458 and rs3810. SNP rs7508 significantly increased the odds of study non-completion in STRRIDE II.

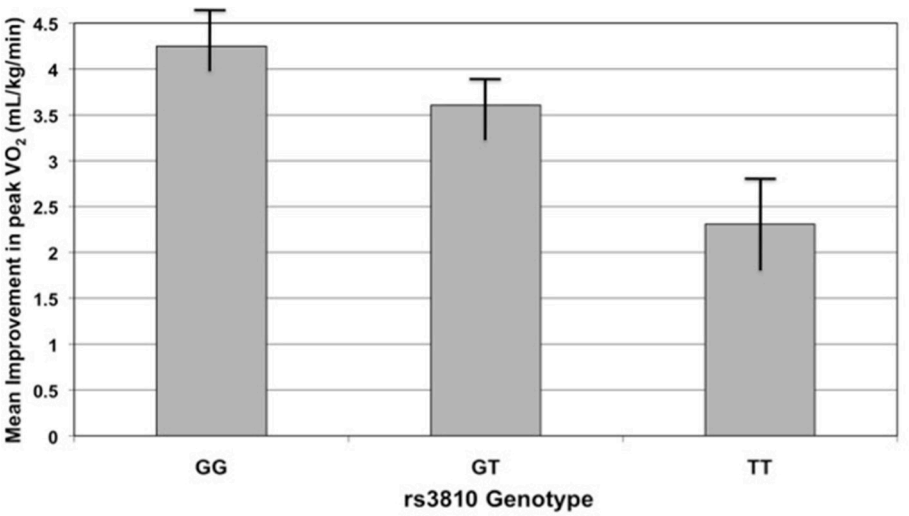

FIGURE 4 | Improvement in peak oxygen consumption (peak VO_2) with exercise training by rs3810 genotype. Improvement in peak VO_2 (mL/kg/min), measured as the difference in peak VO_2 before and after an exercise intervention, compared to rs3810 genotype. STRRIDE I and STRRIDE II datasets were combined for the analysis (GG genotype $n = 168$, GT genotype $n = 207$, TT genotype $n = 90$). The rs3810 genotype significantly predicted change in peak VO_2 ($p = 0.0185$), despite the fact that some groups differed in the intensity of the training stimulus.

plausible biological links between genetic variation in *ASAH1* and exercise behaviour.

Ceramide is produced either via hydrolysis of membrane sphingomyelin; or *de novo* from long- chain saturated fatty acids. In non-adipocytes, increased fatty acid deposition with subsequent conversion to ceramide has been proposed as a mechanism of lipotoxicity in type 2 diabetes, heart failure, and atherosclerosis (Zhou et al., 2000). Accumulation of ceramide initiates pro-apoptotic pathways converging on mitochondrial function—the major source of pro-apoptotic molecules (e.g., BCL1). (Birbes et al., 2002). Increased intracellular ceramide leads to the apoptosis of pancreatic beta cells and cardiac myocytes in obese rats leading to decreased function of both cell types (Unger et al., 1999; Zhou et al., 2000). Ceramide also directly inhibits both complex I and complex III of the mitochondrial respiratory chain in rat heart muscle *in vitro* (Gudz et al., 1997; Di Paola et al., 2000).

Acid ceramidase, ubiquitously expressed in somatic cells, plays a crucial role in the maintenance of cellular ceramide concentrations (Park and Schuchman, 2006). Acid ceramidase metabolizes ceramide into sphingosine and free fatty acids. Over-expression of the enzyme prevents the inhibitory effects of accumulated saturated fatty acids on insulin signalling(Chavez et al., 2005) while abnormally high expression of the enzyme has been reported in several human cancers (Park and Schuchman, 2006). Ceramide content is increased in skeletal muscle of

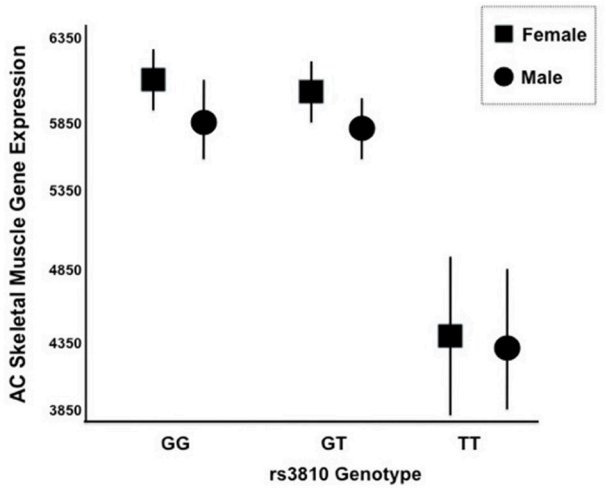

FIGURE 5 | mRNA Expression of ASAH1 Stratified by rs3810 Genotype. Two distinct Affymetrix probe sets indicated that ASAH1 mRNA expression is significantly different by rs3810 genotype using either full ($p = 0.006$–0.007) or dominant ($p = 0.01$–0.02) models, using sex and race as covariates. Females are represented by rectangles and males are represented by circles. TT individuals demonstrated 1.3–1.4 times lower skeletal muscle acid ceramidase expression than did GG individuals at baseline.

obese, insulin resistant humans (Adams et al., 2004) and endurance exercise reduces the content of ceramide in skeletal muscle in obese subjects with a concomitant improvement in insulin sensitivity (Bruce et al., 2006). Nonetheless, ceramide relationships to insulin sensitivity and changes with exercise are complex and incompletely understood: effects appear dependent on ceramide species, exercise duration and intensity, and underlying insulin sensitivity (Dobrzyn and Gorski, 2002a; Helge et al., 2004; Baranowski et al., 2008; Błachnio-Zabielska et al., 2008; Bergman et al., 2016). Further, in addition to acid ceramidase, ceramide regulation can occur via degradation by neutral and alkaline ceramidases or conversion to sphingomyelin.

It is important to recognize that we have not necessarily found functional acid ceramidase variants; rather, we only have identified and replicated associations implicating the acid ceramidase gene in the biological response. Nonetheless, in light of recognised acid ceramidase functions, it is intriguing that acid ceramidase polymorphisms were associated with reduced completion rates for and poorer peak oxygen consumption (peak VO_2) responses to exercise training. All three significant acid ceramidase SNPs were associated with differential improvements in peak VO_2 with exercise. While only one of the SNPs was significant in this association, one should note that post-intervention peak VO_2 measurements were available for exercise completers only. Were it feasible to include both exercise completers and non-completers in the post-intervention peak VO_2 analysis, the correlation between acid ceramidase minor allelic genotype and decreased improvement in oxygen consumption may have been stronger for all three genotypes.

There were significant differences in racial groups for non-completion rates, as well as large differences in allele frequencies for rs3810 and rs2898458; this raised the issue of the potential for allele frequency differences to confound the strong association observed for these variants. To evaluate the stability of the associations observed in the overall group, we performed race-stratified analyses for each study. In both whites and blacks, rs3810 demonstrated association with non-completion; the odds ratios for the same alleles in both races and both studies were very consistent. The non-significant *p*-values in the smaller black subgroup reflected differences in allele frequency and sample size. Although detailed evaluation of genetic variation across *ASAH1* will be required to identify functional SNPs, comparing the results for blacks and whites in light of the expected differences in linkage disequilibrium patterns provided specific support for rs3810 as the SNP of interest in *ASAH1*.

We routinely queried participants for their reasons when withdrawing from the study prior to completion. The reasons varied considerably: among others were time constraints; family issues; "a changed mind"; or unrelated medical problems. Clearly, many issues can affect compliance with behavioural interventions—including exercise—not explainable by genetics alone. However, the association between study non-completion and acid ceramidase genotypes remained significant despite the "noise" created by the subjects' social environment and personality traits; this implies that the biological relation might be even stronger than our findings indicate. Furthermore, the association of the phenotypic measure of peak VO_2 improvement with exercise compliance and acid ceramidase genotype points to a physiologic mechanism for exercise intolerance. Perhaps the genetically associated unresponsiveness to exercise training may consciously or subconsciously play into the willingness of individuals to continue to participate in an exercise program; this may be exacerbated by personality factors, life stress, or other environmental influences.

Personality characteristics may play into the interplay between genetics and exercise behaviour (Herring et al., 2014). Elements of the Big Five Personality Factors are associated with other lifestyle elements—dietary habits and smoking, among others—and also are associated with aerobic capacity (Terracciano et al., 2013) muscle strength (Tolea et al., 2012) and adherence to post-surgery rehabilitation (Hilliard et al., 2014). It would be important—and perhaps therapeutically useful—to know whether the half of individuals with the "drop-out genotype" that persisted with the intervention have a different personality profile than those that fail to complete the intervention. If genetic effects on exercise behaviour are mediated—at least in part—through personality factors; and if personality factors explain the variation in exercise adherence behaviour among those that are at genetic risk of poor adherence; then one might want to test whether personality factors can inform personalised strategies and messaging to increase adherence for those whose health would most benefit from increases in regular exercise.

To our knowledge this is the first report of the effects of acid ceramidase polymorphisms on exercise behaviour in overweight to mildly obese, insulin resistant subjects. Our findings should be validated in subsequent studies. Given resource constraints and sample availability, we were unable to quantify acid ceramidase enzyme activity, *per se*, within our subjects. We also did not measure whether acid ceramidase gene variants differentially affected skeletal muscle enzyme or ceramide content in these subjects—either prior to or in response to an exercise intervention. Future studies will be necessary to further elucidate the relation between common gene variants of acid ceramidase with skeletal muscle enzyme activity, ceramide content, and physical performance.

In conclusion, these data suggest that genetic variation within the acid ceramidase gene significantly affects exercise tolerance and completion of an exercise program. Acid ceramidase regulates ceramide content within skeletal and cardiac muscle; these effects are likely to be involved in muscle adaptations to exercise training. Due to the proven health benefits of regular exercise, characterization of individual exercise potential conferred by genetic variation prior to initiation of an intervention may be helpful in maximizing the adherence to exercise and therefore the health benefits accrued therefrom.

ETHICS STATEMENT

Informed consent was obtained under protocols approved by the Investigational Review Boards of Duke University and East Carolina University.

AUTHOR CONTRIBUTIONS

WK and MD conceived the science. WK and EPH financial support for the research. WK and LL wrote manuscript. WK, CS, JH, LL, ICS, and MH conducted experiment. LL, ERH, and MH performed analysis. WK, KH, and ICS edited manuscript.

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Acute Effects of High Intensity, Resistance, or Combined Protocol on the Increase of Level of Neurotrophic Factors in Physically Inactive Overweight Adults: The BrainFit Study

María A. Domínguez-Sanchéz[1], Rosa H. Bustos-Cruz[2], Gina P. Velasco-Orjuela[3], Andrea P. Quintero[3], Alejandra Tordecilla-Sanders[3], Jorge E. Correa-Bautista[3], Héctor R. Triana-Reina[4], Antonio García-Hermoso[5], Katherine González-Ruíz[6], Carlos A. Peña-Guzmán[7], Enrique Hernández[3], Jhonatan C. Peña-Ibagon[3], Luis A. Téllez-T[3], Mikel Izquierdo[8] and Robinson Ramírez-Vélez[3]*

[1] Grupo de Investigación Movimiento Corporal Humano, Facultad de Enfermería y Rehabilitación, Universidad de La Sabana, Chía, Colombia, [2] Evidence-Based Therapeutic Group, Clinical Pharmacology, Universidad de La Sabana, Bogotá, Colombia, [3] Centro de Estudios en Medición de la Actividad Física, Escuela de Medicina y Ciencias de la Salud, Universidad del Rosario, Bogotá, Colombia, [4] Grupo GICAEDS, Programa de Cultura Física, Deporte y Recreación, Universidad Santo Tomás, Bogotá, Colombia, [5] Laboratorio de Ciencias de la Actividad Física, el Deporte y la Salud, Universidad de Santiago de Chile, Santiago, Chile, [6] Grupo de Ejercicio Físico y Deportes, Facultad de Salud, Programa de Fisioterapia, Universidad Manuela Beltrán, Bogotá, Colombia, [7] Facultad de Ingeniería Ambiental, Grupo de Investigación INAM-USTA Universidad Santo Tomás, Bogotá, Colombia, [8] Department of Health Sciences, Public University of Navarra, Navarrabiomed, CIBER of Frailty and Healthy Aging (CIBERFES) Instituto de Salud Carlos III, Pamplona, Spain

The purpose of this study was to compare the neurotrophic factor response following one session of high-intensity exercise, resistance training or both in a cohort of physically inactive overweight adults aged 18–30 years old. A randomized, parallel-group clinical trial of 51 men (23.6 ± 3.5 years; 83.5 ± 7.8 kg; 28.0 ± 1.9 kg/m^2) who are physically inactive (i.e., <150 min of moderate-intensity exercise per week or IPAQ score of <600 MET min/week for >6 months) and are either abdominally obese (waist circumference ≥90 cm) or have a body mass index, BMI ≥25 and ≤30 kg/m^2 were randomized to the following four exercise protocols: high-intensity exercise (4 × 4 min intervals at 85–95% maximum heart rate [HRmax] interspersed with 4 min of recovery at 75–85% HRmax) (n = 14), resistance training (12–15 repetitions per set, at 50–70% of one repetition maximum with 60 s of recovery) (n = 12), combined high-intensity and resistance exercise (n = 13), or non-exercising control (n = 12). The plasma levels of neurotrophin-3 (NT-3), neurotrophin-4 (also known as neurotrophin 4/5; NT-4 or NT-4/5), and brain-derived neurotrophic factor (BDNF) were determined before (pre-exercise) and 1-min post-exercise for each protocol session. Resistance training induced significant increases in NT-3 (+39.6 ng/mL [95% CI, 2.5–76.6; p = 0.004], and NT-4/5 (+1.3 ng/mL [95% CI, 0.3–2.3; p = 0.014]), respectively. Additionally, combined training results in favorable effects on BDNF (+22.0, 95% CI, 2.6–41.5; p = 0.029) and NT-3 (+32.9 ng/mL [95% CI,

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Billy Sperlich, Universität Würzburg, Germany

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***Correspondence:**
María A. Domínguez-Sanchéz
mariads@unisabana.edu.co

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12.3–53.4; $p = 0.004$]), respectively. The regression analysis revealed a significant positive relationship between changes in BDNF levels and changes in NT-4/5 levels from baseline to immediate post-exercise in the combined training group ($R^2 = 0.345$, $p = 0.034$) but not the other intervention groups. The findings indicate that acute resistance training and combined exercise increase neurotrophic factors in physically inactive overweight adults. Further studies are required to determine the biological importance of changes in neurotrophic responses in overweight men and chronic effects of these exercise protocols.

Trial Registration: ClinicalTrials.gov, NCT02915913 (Date: September 22, 2016).

Keywords: neurotrophic factors, exercise, obesity, inactivity, plasticity

INTRODUCTION

The neurotrophin family of growth factors, comprised of nerve growth factors, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5), all of which were originally defined by their ability to support the survival, development, and function of neurons (Dechant and Neumann, 2003). Moreover, a metabotrophic role of nerve growth factor and BDNF has recently been implicated in the pathogenesis of metabolic related disorders, sensation, and energy homeostasis (Hristova, 2013). Likewise, the BDNF/tyrosine kinase receptor signaling pathway controls feeding and metabolism, and its dysfunction leads to severe obesity or subclinical inflammatory diseases such as metabolic syndrome, insulin resistance, and type 2 diabetes (Tonra et al., 1999; Nonomura et al., 2001; Cai et al., 2006; Rosenthal and Lin, 2014; Wei et al., 2017).

In contrast to metabolic related disorders-induced BDNF changes, Mercader et al. (2008) analyzed an NT-4/5 knockout mouse model and showed that this mice displays reduced food satiation and hyperphagic behavior resulting in an obese mouse when fed *ad libitum*. Furthermore, the infusion of NT-4/5 into the third ventricle of the brain reversed the obese phenotype, which suggests that NT-4/5 is involved in the activation of signaling cascades in the hypothalamic nuclei; these cascades are responsible for the control of food intake and energy expenditure.

Interestingly, physical exercise (particularly moderate to vigorous training) is an effective strategy to combat metabolic disorders due to its ability to influence body composition and some biomarkers, such as cholesterol and insulin resistance (Gibala and McGee, 2008; Buch et al., 2017). During high-intensity exercise (>80% of maximal oxygen intake), significant increases in neurotrophic factor have been observed (Marston et al., 2017). In this line, Saucedo-Marquez et al. (2015) showed that after a 20-min single session of either a continuous exercise protocol (at 70% of maximal work rate) or an high-intensity interval training (HIIT) protocol (at 90% of maximal work rate for periods of 1 min alternating with 1 min of rest), the BDNF levels were increased compared to the levels at rest. Tonoli et al. (2015) showed that a combined session of HIIT and continuous exercise increased serum BDNF and IGF-I levels in 10 participants with type 1 diabetes. Verbickas et al. (2017) showed that serum BDNF levels were decreased at 1–24 h after 200 drop jumps. Afzalpour et al. (2015) compared the effects of 6 weeks of HIIT and continuous training regimens on the levels of BDNF, glial cell line-derived neurotrophic factor (GDNF) and tumor necrosis factor alpha (TNF-α) in the rat brain. These authors showed that both HIIT and continuous training regimens significantly increased BDNF and GDNF concentrations with larger increases following HIIT than continuous training.

Other studies report increases in peripheral BDNF following acute resistance training (RT) (Yarrow et al., 2010; Marston et al., 2017), whereas others report no change (Correia et al., 2010; Goekint et al., 2010). These discrepancies can likely be explained by the wide range of characteristics of the participants (age, health status, diseases, etc.), study duration, intervention exercise program (i.e., jumping, treadmill, and/or elliptical, weight machines), and the extent of change in body composition across these studies. There are also reports of exercise-induced changes in NT-3 (Gómez-Pinilla et al., 2001; Johnson and Mitchell, 2003) and NT-4/5 (Chung et al., 2013) expression following focal cerebral ischemia or spinal cord injury in rats. However, there are no reports of exercise-induced changes in plasma NT-3 or NT-4/5 levels in humans. There is considerable information available concerning the endocrine response in lean individuals as a point of reference for future research using an inactive/overweight model.

Therefore, the aim of the present study was to compare the acute responses of the neurotrophic factors BDNF, NT-3, and NT-4/5 prior to and after one session of exercise comprising HIIT, RT, or both in a cohort of physically inactive overweight adults aged 18–30 years old. It was hypothesized that the combined training protocol would induce the highest metabolic perturbations and therefore the highest hormonal responses to a greater extent than RT group and HIIT alone.

Abbreviations: BDNF, brain-derived neurotrophic factor; neurotrophin-3, NT-3; NT-4/5, neurotrophin-4/5; HIIT, high-intensity interval training; GDNF, glial cell line-derived neurotrophic factor; TNF-α, tumor necrosis factor alpha; CEMA, in Spanish at the Centre of Studies in Physical Activity Measurements; RT, resistance training; WHO, world health organization; HR, heart rate; 1RM, one repetition maximum; BMI, body mass index; TEM, technical error of measurement; BIA, bioelectrical impedance analyser; VO2max, maximal oxygen intake.

METHODS

Study Design and Participants

The BrainFit Study is a single blind, randomized controlled 2 × 2 factorial trial (ClinicalTrials.gov ID: NCT02915913). The study was approved by the Medical Research Ethics Committee of The Universidad Nacional de Colombia (Code N° 018-223-16). Fifty-one men (aged 18–30 years), who were inactive (according to the International Physical Activity Questionnaire, IPAQ <150 min of moderate-intensity exercise per week or IPAQ score of <600 MET min/week for greater than 6 months), had abdominal obesity (defined as waist circumference [WC] ≥ 90 cm) or excess weight (defined as BMI ≥25 and ≤30 kg/m^2) were recruited from Bogota, Colombia. The latest International Diabetes Federation/National Heart, Lung, and Blood Institute/American Heart Association (IDF/NHLBI/AHA-2009) consensus stated that WC was measured according to Country/Specific values which, for Latin Americans, were set to be equal to South Asian parameters, specifically WC ≥90 cm for males (Alberti et al., 2009). Subjects were recruited between September 1, 2016, and June 30, 2017. Healthy and inactive men who were currently not taking drugs, tobacco, or any other medications were included in this study. A validated questionnaire, the "FANTASTIC" lifestyle (Ramírez-Vélez and Agredo, 2012), was used to collect comprehensive information about substance use via a personal interview with participants. At the beginning of every session, quality of sleep, smoking, alcohol, and caffeine consumption, before the experiment were documented. The final follow-up visit was in July 2017. Inclusion and exclusion criteria are provided in **Table 1**.

TABLE 1 | Inclusion/exclusion criteria.

Inclusion criteria	Exclusion criteria
a. Inactive: no participation in supervised exercise more than once a week for the previous 6 months, according to the IPAQ score of <600 MET min/week	a. Systemic infections
b. Central obesity: waist circumference ≥90 cm or excess weight: body mass index ≥25 and ≤30 kg/m^2	b. Weight loss or gain of >10% of body weight in the past 6 months for any reason
c. Interested in improving cardiovascular health and physical fitness	c. Currently taking medication that suppresses or stimulates appetite
d. Written informed consent	d. Uncontrolled hypertension: systolic blood pressure 160 mmHg or diastolic blood pressure 95 mmHg on treatment
e. Interested in improving cardiovascular health and physical fitness	e. Gastrointestinal disease, including self-reported chronic hepatitis or cirrhosis, any episode of alcoholic hepatitis or alcoholic pancreatitis within past year, inflammatory bowel disease requiring treatment in the past year, recent or abdominal surgery (e.g., gastrostomy)
	f. Asthma
	g. Diagnosed diabetes (type 1 or 2), fasting impaired glucose tolerance (blood glucose 118 mg/dL), or use of any anti-diabetic medications
	h. Currently taking antidepressant, steroid, or thyroid medication, unless dosage instable (no change for 6 months)
	i. Current exerciser (>30 min organized exercise per week)
	j. Indication of unsuitability of current health for exercise protocol (Physical Activity Readiness. Questionnaire, PARQ)
	k. Any other conditions which, in opinion of the investigators, would adversely affect the conduct of the trial

Recruitment

Consecutive males with either abdominal obesity or excess weight were recruited from different educational institutions (Universidad Nacional, CEMA-Universidad del Rosario, Universidad Santo Tomás, Universidad Manuela Beltrán, and Universidad de la Sabana) that receive referrals from both medical consultants and biomedical practitioners in the capital district of Bogotá and the Cundinamarca Department in the Andean region (Ramírez-Vélez et al., 2016). Subjects who are interested in participating were provided additional information and underwent the following procedures: (i) initially screened for pre-participation exercise using a cardiovascular and musculoskeletal checklist; (ii) baseline testing; (iii) a single isocaloric acute training protocol; and (iv) post-training testing. All participants were informed of the purpose and risk of the study before signing an informed consent form.

Blinding and Randomization Methods

Randomization into the four study arms was performed by CEMA at the University of Rosario, Bogotá, Colombia, using block randomization with a block size of four. After completing the baseline measurements, eligible participants were randomly assigned to either the control or one of the exercise training groups. Participants were randomly allocated using a computer-generated randomization code created before the data collection by an investigator not involved in the assessment or treatment of the participants. These procedures are also detailed in the study operations manual. Assessors were also blinded to each participant's treatment allocation.

Preliminary Analysis

Each of the volunteers participated in three randomized trials (HIIT, RT, HIIT+RT, and control [no exercise]), and the starting trial was randomized. At 48 h after the start of the training period, the 1RM was measured for six different exercises: bicep screw curl, triceps extension, dumbbell side lateral raise, military press, dumbbell squat, and dumbbell front lunge which was implemented based on similar procedures (Ramírez-Vélez et al., 2016). The 1RM was performed in six resistance exercises and

was conducted between 09:00 and 11:00 a.m.; the highest load of three attempts per exercise were reported. Total muscle strength was calculated as the sum of the six exercises. The 50–70% value of the 1RM was used to determine the work load during the single sessions for the experimental group.

Maximal oxygen intake (VO_2max) of inactive subjects was determined 24 h before acute intervention using a maximum treadmill exercise test (Precor TRM 885, Italy). Subjects completed an incremental maximal oxygen uptake test on a treadmill ergometer. A metabolic cart with an on-line gas collection system (COSMED K5 portable metabolic system, Rome, Italy) was used to measure VO_2max and carbon dioxide production data, and HR was continuously monitored with a HR monitor (A3, Polar Elector OY, Finland).

Exercise Training Protocol

The current exercise protocol was based on data derived from the Cardiometabolic HIIT-RT Study (ClinicalTrials.gov ID: NCT02715063). Details of methods had been previously published elsewhere (Ramírez-Vélez et al., 2016), however, the most relevant information is briefly described below.

1. *Control group*: Without exercise training.
2. *High-intensity interval training (HIIT) group:* All HIIT sessions were preceded with a 5-min warm-up and ended with a 4-min cooldown at a 65% heart rate maximal until the subject expended between 400 and 500 kcal. The HIIT protocol consisted of four bouts of 4-min intervals at 85–95% HR maximal interspersed with 4 min of active recovery at 75–85% HR maximal. Participants in the HIIT groups were instructed to reach their target HR for each interval within the first 2 min of the 4 min interval. We calculated the training energy expenditure with the consensus public health recommendations from the World Health Organization (WHO, 2004) and the US Department of Health and Human Services (Physical Activity Guidelines Advisory Committee, 2008). Heart-rate monitors (A3, Polar Elector OY, Finland) were used to adjust workload to achieve the target heart rate. In addition, a rating of perceived exertion was also measured during each exercise session (15–17 during high intensity and 11–13 during recovery), (**Figure 1A**).
3. *Resistance training (RT) group:* The RT session was initiated with ≈12–15 repetitions per set of six exercises that targeted all the major muscle groups at high intensity. A 60-s recovery was permitted as many times as needed according to subject's weight until the subject expended between 400 and 500 kcal at 50–70% one repetition maximum (1RM). The RT included both upper and lower body large-muscle exercises using weight machines (bicep screw curl, triceps extension, dumbbell side lateral raise, military press, dumbbell squat, and dumbbell front) (Ramírez-Vélez et al., 2016). Our exercise structure, is similar to utilized by Church et al. (2016) is considered moderate to vigorous intense and therefore it would be expected resulted in increased levels of peripheral BDNF, (**Figure 1B**).
4. *Combined training (HIIT+RT) group:* This group underwent both the HIIT and RT protocols, as described above, in that order. Therefore, the energy expenditure associated with the physical training prescribed for the vigorous intensity group was ≈400–500 kcal/session (Ramírez-Vélez et al., 2016).

Training Intensity and Energy Expenditure During the Exercise Session

In terms of exercise intensity, the actual intensity values were reported as the mean of HR measured in the HIIT and combined groups and as the average value of workload and repetitions determined in the acute session in RT group. The intensity of the HIIT or HIIT+RT group was based on the percentage of each individual's HRmax derived from a maximal treadmill test. The exercise training was 100% supervised. Research staff monitored and recorded compliance with target HR and energy expenditure during the sessions. Energy expenditure was estimated during exercise via indirect calorimetry using a COSMED K^5 portable metabolic system (Rome, Italy) assuming a non-protein respiratory exchange ratio (Graf et al., 2013). It was expected that the gradual increase in total energy expenditure would minimize fatigue, soreness, injuries, and attrition.

Blood Draws and Analysis

Participants arrived at the CEMA laboratory between 6:00 and 9:00 following a 10–12 h overnight fast. Participants were reminded to maintain standardized conditions (i.e., hydrated state and abstaining from caffeine and alcohol consumption for 36 h). Blood samples were drawn into a tube containing ∼1.8 mg EDTA-K^3 per mL blood for plasma measurements and a tube with polymer gel for serum measurements (Vacutainer, Becton Dickinson and Company 2017). Samples were handled according to Clinical Laboratory Improvement Amendments, which must be followed in order to achieve valid test results for accurate diagnoses (Centers for Medicare Medicaid Service, 2005). Finally, both samples were stored at −80°C for the analysis of plasma biomarkers of neuronal function using surface plasmon resonance (SPR) biosensors.

Surface Plasmon Resonance (SPR) Biosensors

SPR allows real-time monitoring of NT-3, NT-4/5, and BDNF (R&D Systems, Minnesota, USA) (Bustos et al., 2014). In a typical experiment, the signal reflection, measured at a fixed angular position, is evaluated as a function of time. The real-time strategy of quantification involved specific antibodies to the target proteins. All experiments were carried out at 25°C using a SPR Biacore 2000 automatic (Biacore, Uppsala, Sweden). HBS-EB buffer was used as running buffer at 5–60 μg/mL flow. Anti-neurotrophin-3, anti-neurotrophin-4 and anti-BDNF antibodies were immobilized onto the CM5 sensor surface at a concentration ranging from 10 to 50 μg/mL. Before immobilization of the antibodies, the sensor surface was activated via an amino-coupling chemistry kit K AN-50 Coupling Kit (GE Healthcare, Uppsala, Sweden). For the immobilization steps, the basic principles of assays using SPR were applied, such as preconcentration and binding assay with the analyte. Excess activated carboxyl groups were blocked with 1 M ethanolamine hydrochloride (pH 9.5). A reference or control

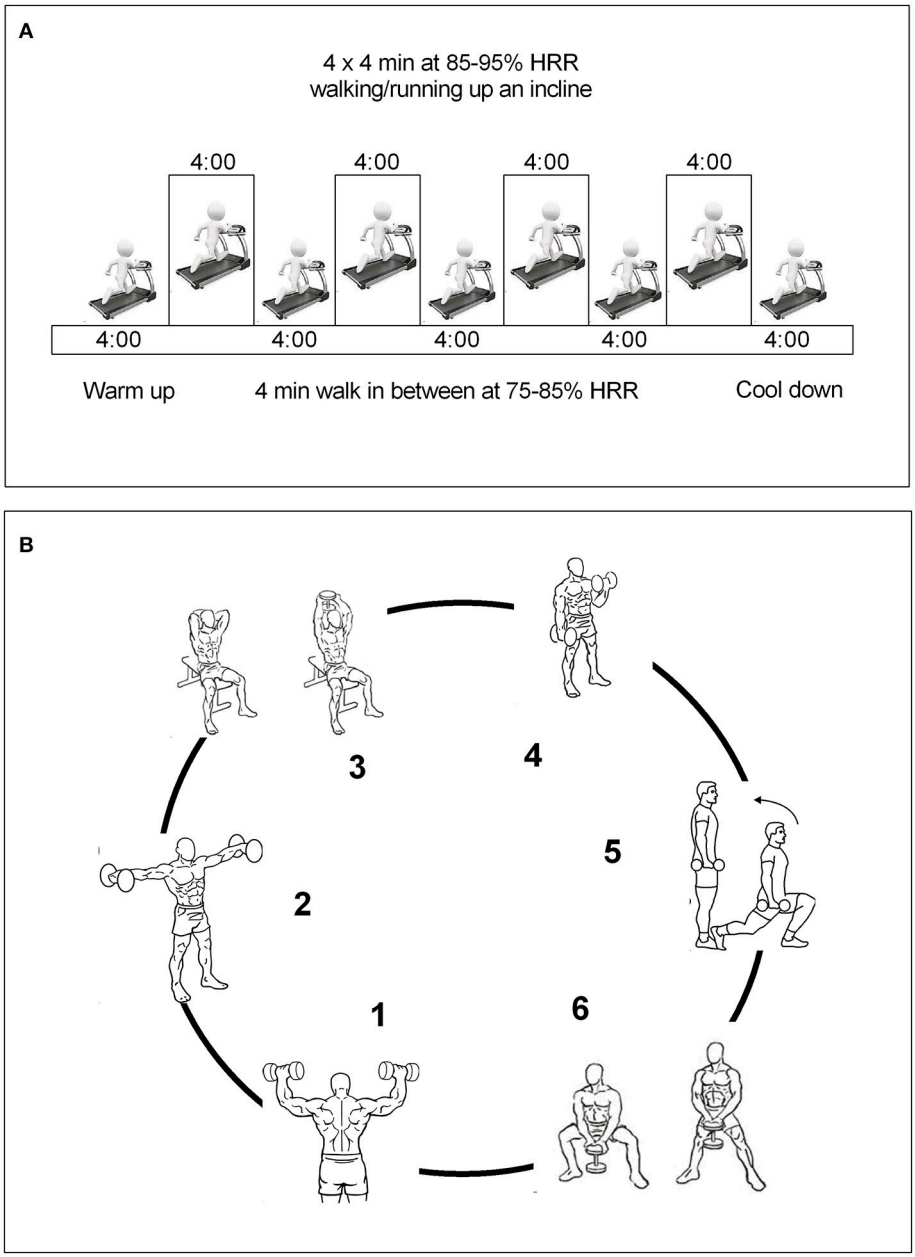

FIGURE 1 | Run-in training interventions. **(A)**, HIIT group; **(B)**, RT group. Combined training group were received both the HIIT and RT protocols as described above.

flow cell containing 50 μg/mL bovine serum albumin (Sigma-Aldrich, Saint Louis, MO) at pH 5.0 was used to subtract the instrument's systematic noise and drift (i.e., background response). After each injection of the analyte and standard samples to the binding assay, an optimal regeneration solution was injected as described in previous studies (Andersson et al., 1999; Kuroki and Maenaka, 2011). The percentage of surface regeneration was estimated using the following equation: [1 – (Rreg/Ro) × 100]. The complete regeneration was higher than 50%. Standard curves were constructed by diluting standards of recombinant NT-3, NT-4, and BDNF protein (Bustos et al., 2014). A range of 20–5,000 ng/ mL was used to obtain the curve (Bustos et al., 2014). The manufacturer declares intra- and inter-assay coefficients of variation of <10%.

Body Composition and Anthropometric Measures

Body fat mass was determined with a multifrequency bioelectrical impedance analyser (BIA) using tetrapolar whole body impedance (Model Seca® mBCA 514 Medical Body Composition Analyzer, Hamburg, Germany). A detailed description of the BIA technique can be found in a previous

study (Rodríguez-Rodríguez et al., 2016). The intra-observer technical error (% reliability) of the measurements was 95%.

The values of height and body mass were recorded in meters to the nearest 0.1 cm and 0.1 kg with an electronic height (Seca® 274, Hamburg, Germany) and weight scale (Model Seca® mBCA 514 Medical Body Composition Analyzer, Hamburg, Germany). BMI was calculated with the following formula: BMI = body weight (kg)/height squared (m^2) (WHO, 2000). Waist circumference (WC) was also measured to the nearest 1 mm with a flexible steel tape measure (Lufkin W606PM®, Parsippany, New Jersey, USA) placed midway between the lowest rib and the iliac crest while participants were in a standing position at the end of an exhalation in accordance with the International Society for the Advancement of Kinanthropometry guidelines (Marfell-Jones et al., 2012). The technical error of measurement values was < 2% for all anthropometric variables.

Statistical Analysis

To calculate the required sample size, we used the formula for the comparison of two means: $n = [A + B]^2 \times 2 \times SD^2/DIFF^2$, where n = the sample size required in each group, *SD* = standard deviation of the outcome variable and DIFF = size of the desired difference between groups. A and B depend on the desired significance level and desired power, respectively. Using estimates obtained from the literature (Saucedo-Marquez et al., 2015; Tonoli et al., 2015), a sample size of eight subjects in each group was needed to reach a power of 80% to detect a difference in means in the relative change of BDNF (10%Δ after 5 min of HIIT training, assuming an SD of 588 pg/mL using a two-sample *t*-test with a 0.05 two-sided significance level). Assuming a drop-out rate of 20%, the total minimal sample size has been increased to 12 subjects for each group. We believe this sample size is feasible and realistic based on other experience in experimental studies (Gómez-Pinilla et al., 2001; Johnson and Mitchell, 2003; Krabbe et al., 2007; Rasmussen et al., 2009; Seifert et al., 2010; Chung et al., 2013; Saucedo-Marquez et al., 2015).

Comparisons between differences of mean values of normally distributed variables between groups of exercise were tested using linear mixed-effects modeling for repeated measures over time using BDNF, NT-3, and NT-4 as the dependent variable and effects for time, group (HIT, RT, HIIT+RT, or control group), and time by group interaction including their baseline measurement and VO_2max the intervention time as co-variables, following the same procedures as in the intent-to-treat analyses. The significance level adopted to reject the null hypothesis was $p < 0.05$, and effect size for paired comparisons was reported as η^2 partial throughout; these values are interpreted as small (≥0.02), moderate (≥0.13), and large (≥0.26). Regression analyses were used to examine the relationship between changes in BDNF and other neurotrophic factors. Finally, goodness-of-fit tests were performed to determine whether the proportion of participants who showed higher final neurotrophic levels in each protocol was equal. Parametric datasets are summarized in text as the mean (*SD*), standard error of the mean (SEM) and 95% CI. All analyses were performed using the SPSS software package (Version 22, IBM, New York, USA).

RESULTS

Of the 70 participants who entered the run-in phase, 56 (80%) were randomized. Reasons for pre-randomization exclusion included excessive BMI, refusal to participate, or a medical condition (**Figure 2**). Five additional participants (2 from the control group, 2 from the RT group, and 1 from the combined group) were excluded from the blood sample analyses because their serum was technically inadequate.

Descriptive statistics for each sex group are shown in **Table 2**. No significant intergroup baseline differences were observed.

The concentration of BDNF, NT-3, and NT-4/5 at rest and immediately after the exercise, are presented in **Table 3**. The variables analyzed were similar at baseline. RT induced significant increases in NT-3 (+39.6 ng/mL [95% CI, 2.5–76.6; $p = 0.004$], and NT-4/5 (+1.3 ng/mL [95% CI, 0.3–2.3; $p = 0.014$]), respectively. Additionally, combined training results in favorable effects on BDNF (+22.0, 95% CI, 2.6–41.5; $p = 0.029$) and NT-3 (+32.9 ng/mL [95% CI, 12.3–53.4; $p = 0.004$]), respectively. In the per-protocol analyses, neither intervention significantly changed BDNF [$F_{(interaction)} = 1.412$; $\eta^2 = 0.083$], NT-3 [$F_{(interaction)} = 1.280$; $\eta^2 = 0.076$], and NT-4/5 [$F_{(interaction)} = 0.649$; $\eta^2 = 0.040$].

The regression analysis revealed a significant positive relationship between changes in BDNF levels and changes in NT-4/5 levels from baseline to immediate post-exercise in the combined training group ($R^2 = 0.345$, $p = 0.034$; **Figure 3C**) but not the other intervention groups. Additionally, no relationship was found between changes in BDNF levels and changes in NT-3 levels (**Figures 3A,B,D**).

DISCUSSION

The primary findings were that all three exercise protocols induced greater changes in neurotrophin levels compared to the levels in the baseline levels; however, the combined and RT protocols exhibited greater changes in BDNF, NT-3, and NT-4/5 than the HIIT group. Additionally, we observed a positive relationship between changes in the BDNF levels and changes in the NT-4/5 levels from baseline to immediately post-exercise in the combined training group. These data provide preliminary evidence regarding the role of acute exercise in increasing neurotrophic factors in humans (Krabbe et al., 2007; Rasmussen et al., 2009; Seifert et al., 2010; Saucedo-Marquez et al., 2015) and confirm the results observed in mice (Gómez-Pinilla et al., 2001; Johnson and Mitchell, 2003; Chung et al., 2013). In addition, it also suggests that an concurrent training protocol (high intensity intermittent exercise followed by strength training) may be more suitable for influencing neurotrophin levels than HIIT or RT alone.

Within the training protocols, we observed an 6.8% (Combined training), and 9.3% (RT) mean increase in plasma BDNF levels immediately post-exercise when compared with the baseline values. Studies that analyzed the acute effects of RT (Correia et al., 2010; Goekint et al., 2010; Yarrow et al., 2010; Church et al., 2016) or HIIT (Saucedo-Marquez et al., 2015; Tonoli et al., 2015) showed inconsistent results. Our RT protocol

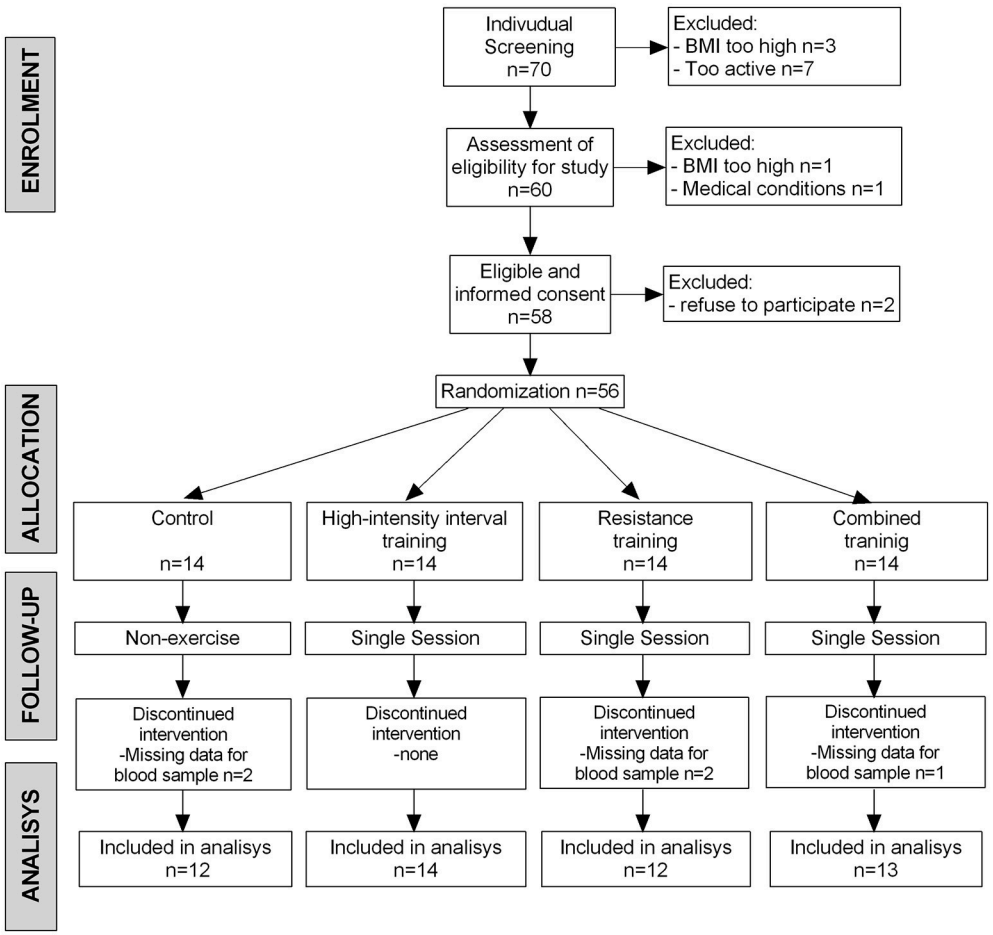

FIGURE 2 | The BrainFit Trial Flow Diagram. Those whose blood samples ($n = 5$) were technically inadequate and not analyzed. BMI, body mass index.

comprised exercises at an intensity between 50 and 70% of 1RM with 25–30 repetitions per exercise. Compared to the aerobic protocols, the RT session had relatively large resting periods in between the efforts. By contrast with our results, several studies demonstrated that a single RT session did not induce significant changes in blood BDNF levels in healthy inactive adults (Goekint et al., 2010; Yarrow et al., 2010). However, Church et al. (2016) and Marston et al. (2017) reported that the use of a to-fatigue hypertrophy-based RT protocol provides the necessary stimulus to increase peripheral serum BDNF levels.

The discrepancies observed between our results and these findings could be due to the population sampled since BDNF levels are negatively correlated to weight (Lommatzsch et al., 2005); therefore, the probability of increases could be greater in the overweight population included in our study. Regarding high-intensity exercise or combined exercise, a previous study in 10 inactive individuals with type 1 diabetes showed that acute HIIT protocols result in larger increases in serum BDNF concentrations than acute low- or moderate-intensity exercise protocols (Tonoli et al., 2015). Therefore, these studies have shown that various features of exercise stimuli (i.e., intensity, duration, and mode of activity) can affect BDNF levels. It seems that exercise and/or training temporarily elevate basal BDNF levels and possibly upregulate the cellular processing of this factor (Knaepen et al., 2010). In future studies, it could be interesting to investigate the effects of an intensive strength protocol on BDNF levels.

Of particular interest, we observed that changes in BDNF were significantly related to changes in NT-4/5 levels from baseline to immediately post-exercise in the combined training group. In literature, the %Δ [BDNF] concentrations after performing exercise vary from 11.7 to 41% (in both healthy and clinical populations) (Pedersen et al., 2009; Yarrow et al., 2010; Marston et al., 2017). These results are very much in line with results reported by Saucedo-Marquez et al. (2015), who showed that BDNF levels gradually increased over time, reaching maximal levels after 20 min of exercise and returned to baseline values after 10 min of recovery. However, this finding was also present in animal studies showing positive relationships between serum hippocampal NT-3 concentration and distance ran ($R^2 = 0.157$, $p < 0.001$) (Johnson and Mitchell, 2003). Similarly, Chung et al. (2013) suggested that NT-4/5 levels were altered in response to ischemic injury, and treadmill exercise plays a role in the changes of the levels of neurotrophins and their receptors.

TABLE 2 | Baseline participant characteristics by group training.

Characteristics	Group training			
	Control ($n = 12$)	HIIT ($n = 14$)	RT ($n = 12$)	HIIT+RT ($n = 13$)
Age, y	24.7 (3.4)	24.5 (3.7)	22.8 (3.1)	22.2 (3.4)
Weight, kg	88.6 (8.9)	81.7 (6.7)	83.9 (7.4)	80.6 (6.7)
Height, m	1.75 (0.05)	1.72 (0.05)	1.68 (0.18)	1.69 (0.05)
BMI, kg/m^2	28.7 (2.0)	27.4 (1.7)	27.8 (1.3)	28.1 (1.2)
Waist circumference, cm	97.9 (6.3)	95.3 (4.9)	94.1 (4.6)	96.9 (5.8)
Body fat percentage, %	28.7 (4.1)	26.2 (4.3)	27.0 (3.7)	28.1 (3.6)
VO_2max, ml·kg·min^{-1}	41.2 (17.3)	40.6 (16.7)	38.9 (10.5)	37.8 (13.6)
EE during exercise, Kcal	–	462.6 (74.9)	460.9 (86.7)	461.7 (59.1)
Bicep screw curl, 1RM	25.6 (11.6)	23.4 (7.9)	21.9 (10.3)	20.9 (6.9)
Triceps extension, 1RM	16.1 (5.7)	16.1 (5.3)	17.1 (4.9)	17.9 (4.0)
Dumbbell side lateral, 1RM	9.0 (1.9)	10.8 (3.6)	8.9 (1.9)	10.2 (3.3)
Military press, 1RM	25.8 (9.4)	23.0 (8.3)	22.8 (13.5)	19.0 (5.1)
Dumbbell squat, 1RM	47.8 (23.0)	55.0 (34.4)	53.3 (14.1)	52.8 (23.5)
Dumbbell front lunge, 1RM	28.4 (7. 7)	28.0 (16.2)	22.7 (6.6)	26.3 (10.7)
Total muscle strength, (kg; total of six exercises)	142.7 (43.7)	156.3 (53.4)	136.5 (34.6)	147.2 (38.5)
BDNF, ng/mL*	176.8 (37.6)	161.1 (24.7)	166.0 (30.5)	189.1 (27.0)
NT-3, ng/mL*	247.5 (20.5)	285.4 (41.5)	315.1 (28.6)	275.3 (38.3)
NT-4/5, ng/mL*	14.3 (1.7)	18.0 (1.7)	16.4 (1.8)	17.4 (1.7)

Data in mean (standard deviation) or (SEM). HIIT, high-intensity interval training; RT, resistant training; BMI, body mass index; EE, energy expenditure; VO_2max, cardiorespiratory fitness; 1RM, one repetition maximal; BDNF, brain-derived neurotrophic factor; NT-3, neurotrophin-3; NT-4/5, neurotrophin-4/5; (–), not applicable.*

However, treadmill exercise-mediated changes in the expression levels of NT-4/5 and tyrosine kinase B might participate in the recovery process in rats with brain damage. This supports the idea that increasing the levels of neurotrophic factors contributes to functional recovery (Keyvani et al., 2004).

The potential mechanism mediating the higher neurotrophin (i.e., BDNF) response to the different exercise protocols is currently unclear. However, BDNF is primarily produced in the brain, some of which crosses the blood-brain barrier and travels to the periphery where it can be measured in plasma and serum. Nevertheless, possible BDNF modulating factors such as lactate, cortisol, and intensity have been previously proposed. Wrann et al. (2013) proposed a novel biochemical pathway linking an exercise-induced secreted factor from skeletal muscle to BDNF gene expression in the brain, especially in the hippocampus. Interestingly, then RT or concurrent training protocol (HIIT followed by RT) would be the ideal protocol for elevating BDNF levels. On the basis of this model we speculate that skeletal muscle contractions during combined or RT protocol might be a possible trigger of this biochemical pathway to induce elevated BDNF levels in the brain. Another physiological mechanism is platelets, which have the ability to store BDNF and release it upon agonist stimulation depending on the specific need of BDNF in certain tissues (Fujimura et al., 2002). Although it remains unknown how exercise influences the platelets, one potential use of BDNF stored in platelets is thought to be in the repair of exercise-induced muscle damage (Saucedo-Marquez et al., 2015). However, further research is needed to confirm these mechanisms, especially in childhood obesity during and after weight-loss exercise programs.

On the other hand, several lines of evidence support a hypothesis whereby neurotrophins, especially BDNF (Dmitrzak-Weglarz et al., 2013), play an essential role in with the initiation and development of both atherosclerosis and metabolic syndrome (Chaldakov et al., 2003). In a previous study Krabbe et al. (2009) found that plasma levels of BDNF decreased in humans with type 2 diabetes, independently of obesity, suggesting that certain metabotrophic deficit due to hyponeurotrophinemia may operate in this metabopathy. Matthews et al. (2009) have shown that BDNF appears to be a major player not only in central metabolic pathways, but also as a regulator of metabolism in skeletal muscles. Studies of animal models and humans have shown that reduced expression of neurotrophins results in the glucose and lipid metabolism alterations (Tonra et al., 1999; Nonomura et al., 2001; Mercader et al., 2008; Dmitrzak-Weglarz et al., 2013). In general, studies have observed a correlation between low serum BDNF levels and high inflammatory protein levels, increased vascular dysfunction and more cardiovascular risk factors (Chaldakov et al., 2004; Dmitrzak-Weglarz et al., 2013). Current evidence indicates plasma BDNF is a proxy marker of BDNF production in the brain; however, BDNF can also be synthesized peripherally (Church et al., 2016). However, there are some gaps in the literature pertaining to obesity, exercise training, and subsequent neurotrophin responses (Pedersen et al., 2009).

There are several limitations and strengths that should be considered with respect to the design and outcomes of this study. Because this study was designed as a pilot study to inform the development of more elaborate trials, the sample size, and intervention duration of acute exercise are modest.

TABLE 3 | Intent-to-treat analysis of BDNF, NT-3, and NT-4/5 at baseline and changes after acute effect.

Characteristics	Mean (SEM		Δ change (*P* value)	From baseline to acute, Mean (95% CI)		$F_{interaction}$ (η^2 partial)
	Baseline	Acute		Within-group change	Between-group difference in change	
BDNF, ng/mL						
HIIT training, ($n = 14$)	161.0 (23.1)	172.1 (25)	+6.8 (0.134)	11.05 (3.8 to 26.0)	–	–
RT training, ($n = 12$)	166.0 (30.6)	181.5 (31.2)	+9.3 (0.066)	15.5 (−1.2 to 32.3)	–	–
Combined training, ($n = 13$)	189.1 (29.7)	211.2 (34)	+11.6 (0.029)	22.0 (2.6 to 41.5)	–	–
Control group, ($n = 12$)	176.7 (38.7)	177.9 (38.4)	+0.6 (0.804)	1.19 (−11.6 to 9.2)	–	–
Δ Combined vs. Δ control	–	–	–	–	20.8 (−0.1 to 41.8)	1.412 (0.083)
Δ HIIT vs. Δ control	–	–	–	–	9.8 (−10.7 to 30.4)	
Δ RT vs. Δ control	–	–	–	–	14.3 (−35.7 to 7.0)	
Δ Combined vs Δ HIIT	–	–	–	–	11.0 (−9.1 to 31.2)	
Δ RT vs. Δ HIIT	–	–	–	–	4.4 (−16.1 to 25.0)	
Δ Combined vs. Δ RT	–	–	–	–	6.5 (−14.4 to 27.5)	
NT-3, ng/mL						
HIIT training, ($n = 14$)	285.3 (46.3)	322.4 (41.5)	+12.5 (0.097)	37.4 (−7.7 to 81.7)	–	–
RT training, ($n = 12$)	315.1 (28.6)	354.6 (28.6)	+13.0 (0.038)	39.6 (2.5 to 76.6)	–	–
Combined training, ($n = 13$)	275.2 (33.7)	308.1 (38.9)	+11.9 (0.004)	32.9 (12.3 to 53.4)	–	–
Control group, ($n = 12$)	247.4 (26.4)	250.0 (25.3)	+1.0 (0.638)	2.5 (−8.9 to 14.0)	–	–
Δ Combined vs Δ control	–	–	–	–	30.4 (−12.4 to 73.2)	1.280 (0.076)
Δ HIIT vs Δ control	–	–	–	–	34.5 (−7.5 to 76.5)	
Δ RT vs Δ control	–	–	–	–	37.0 (−6.5 to 80.7)	
Δ Combined vs Δ HIIT	–	–	–	–	−4.1 (−45.3 to 37.0)	
Δ RT vs Δ HIIT	–	–	–	–	2.5 (−39.5 to 44.6)	
Δ Combined vs Δ RT	–	–	–	–	−6.6 (−49.5 to 36.1)	
NT−4/5, ng/mL						
HIIT training, ($n = 14$)	17.9 (2.56)	18 (2.32)	+0.5 (0.870)	0.1 (−1.5 to 1.8)	–	–
RT training, ($n = 12$)	16.4 (2.0)	17.5 (2.4)	+6.7 (0.014)	1.3 (0.3 to 2.3)	–	–
Combined training, ($n = 13$)	17.3 (1.9)	18.9 (2.5)	+9.2 (0.246)	1.5 (−1.2 to 4.3)	–	–
Control group, ($n = 12$)	14.2 (2.3)	14.8 (2.5)	+4.2 (0.175)	−0.6 (−0.3 to 1.5)	–	–
Δ Combined vs Δ control	–	–	–	–	0.9 (−1.4 to 3.3)	0.649 (0.040)
Δ HIIT vs Δ control	–	–	–	–	−0.4 (−2.8 to 1.9)	
Δ RT vs Δ control	–	–	–	–	0.7 (−1.7 to 3.2)	
Δ Combined vs Δ HIIT	–	–	–	–	1.4 (−0.8 to 3.7)	
Δ RT vs Δ HIIT	–	–	–	–	1.2 (−1.1 to 3.5)	
Δ Combined vs Δ RT	–	–	–	–	0.2 (−2.1 to 2.6)	

Data in standard error of the mean (SEM) or (95% CI). HIIT, high–intensity interval training; RT, resistant training; BDNF, brain–derived neurotrophic factor; NT-3, neurotrophin-3; NT-4/5, neurotrophin-4/5; (–), not applicable; Δ, within–group change. η^2 partial are interpreted as small (≥0.02), moderate (≥0.13), and large (≥0.26).

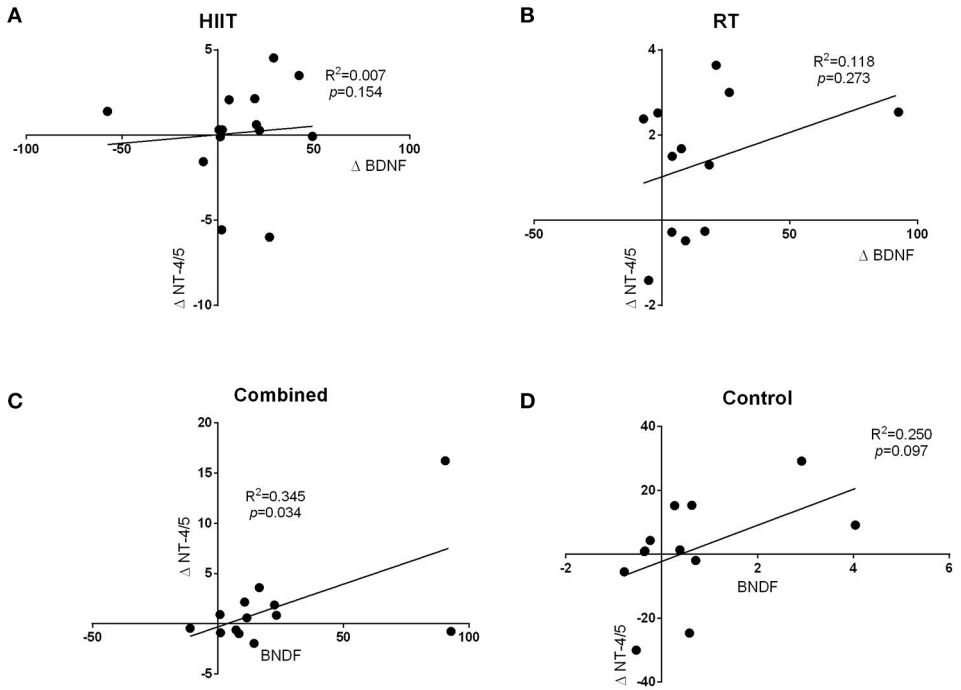

FIGURE 3 | The regression analysis revealed a significant positive relationship between changes in BDNF levels and changes in NT-4/5 levels from baseline to immediate post-exercise in the combined training group ($R^2 = 0.345$, $p = 0.034$; **C**). Additionally, no relationship was found between changes in BDNF levels and changes in NT-3 levels **(A,B,D)**.

This study was performed with physically inactive individuals, which could complicate the generalization of results to a more active overweight population; also, the study was performed with volunteers, which could lead to sampling bias. While our results argue that this level of physical activity is feasible for many, it remains to be seen whether the reported duration is the most efficacious.

To our knowledge, this is the first study to examine whether HIIT, RT, or HIIT+RT elicit different effects on plasma levels of NT-3, NT-4/5, and BDNF in inactive overweight adults. Interestingly, despite this evidence, there are few clinical trials that have directly evaluated the effects of sustained exercise regimens on the brain health of inactive adults (Saucedo-Marquez et al., 2015; Church et al., 2016; Barha et al., 2017; Dinoff et al., 2017). The strategy immobilized antibodies onto sensor surfaces for quantification of protein using biosensors, which is more accurate and fast compared to traditional assays (Heinrich et al., 2010; Wöllner et al., 2010; Hu et al., 2013; Tam et al., 2017). The determination of levels of neurotrophins is usually performed by ELISA immunoassays; however, the use of biosensors opens a new possibility in obtaining results in real time, label free with a high sensitivity and specificity.

CONCLUSION

Our findings indicate that the use of several exercise protocols (i.e., fatigue hypertrophy-based RT or combined training) provides the necessary stimuli to increase peripheral plasma neurotrophin levels among inactive overweight individuals. Based on our findings, however, the use of concurrent training may be considered in exercise programs to enhance the possibility of a potential metabotropic benefit for individuals due to increased expression of neurotrophic factors. Additional longitudinal studies should be performed to establish the effects of exercise and training on cognitive function in overweight adults over the long term.

NEW AND NOTEWORTHY

Identifying the training regimen that has the most beneficial effects on each parameter could potentially lead to enhanced precision in prescribing exercise training intensity to achieve optimal outcomes in this population.

The present study demonstrates that that acute resistance training and combined exercise increase neurotrophic factors in physically inactive overweight adults. Not all neurotrophic factors measured responded the same to this type of exercise, suggesting different regulatory mechanisms and time courses for induction.

AUTHOR CONTRIBUTIONS

MD-S, RB-C, and RR-V conceived and designed the project. EH, GV-O, MD-S, KG-R, and JC-B reviewed the literature studies

and conducted data extraction. MD-S and CP-G conducted data analyses. RR-V, HT-R, AG-H, and MI were responsible for data interpretation. RB-C, AQ, CP-G AT-S, MD-S, MI, and RR-V drafted the manuscript. JP-I, LT-T, and AT-S revised it critically for intellectual contributions. MD-S, RB-C, and RR-V coordinate the study development. All authors reviewed and edited the manuscript. All authors read and approved the final manuscript.

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