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

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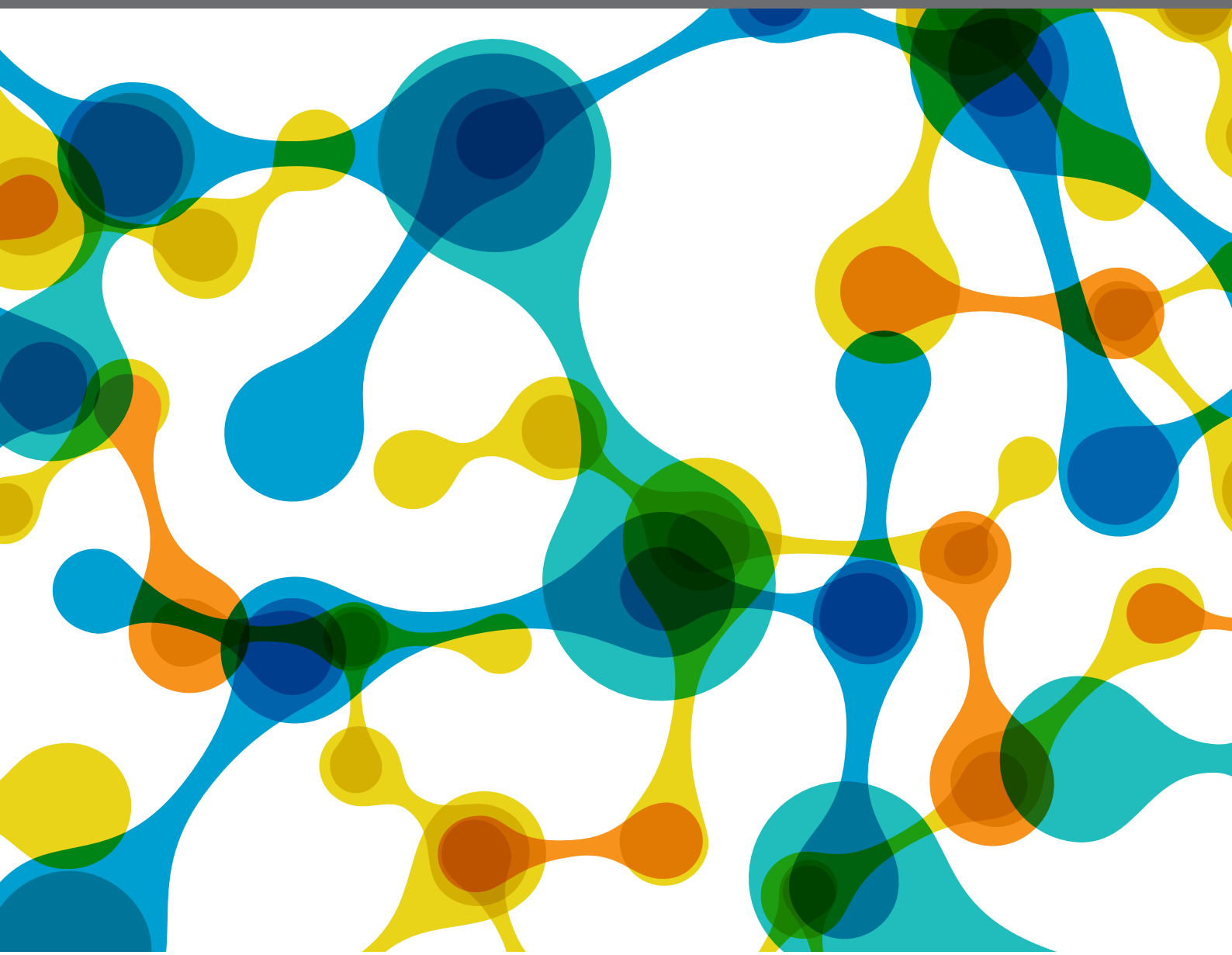

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PRECISION PHYSICAL ACTIVITY AND EXERCISE PRESCRIPTIONS FOR DISEASE PREVENTION: THE EFFECT OF INTERINDIVIDUAL VARIABILITY UNDER DIFFERENT TRAINING APPROACHES, VOLUME II

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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, Volume II

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Editorial on the Research Topic

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

The second volume of the Research Topic entitled "*Precision Physical Activity and Exercise Prescriptions for Disease Prevention: The Effect of Interindividual Variability Under Different Training Approaches*" has been successfully completed, as expected. As stated in the preface to the first volume, this Research Topic was initially intended to address a challenge in this field, but this topic is becoming, over time, an important cornerstone for scientists who are exploring the fascinating subject of "Precision Physical Activity and Exercise Prescriptions for Disease Prevention" (Ramírez-Vélez et al., 2017). This Research Topic consists of 10 articles, of which seven contain original data, one is a systematic review with meta-analysis and two are opinion/hypothesis articles.

We open the second volume of this with an interesting hypothesis and theory article described by Gentil et al., from the Federal University of Goiás, Brazil. The manuscript presents a useful overview of practical recommendations relevant to the use of resistance training for people who have been diagnosed with COVID-19 during different phases of disease, with a special focus on immune, respiratory, and cardiovascular systems.

Lazarus and Harridge's opinion article proposes a novel hypothesis called "The Interplay of Exercise and Physiological Heterogeneity as Drivers of Human Aging." Both authors discuss this hypothesis and related concepts in the context of the trajectory of healthy and non-healthy human aging. Here, the human aging process, interacting with lifestyle factors and heterogeneous physiologies, governs and modifies all processes in all humans all the time, yet its presence does not seem to merit a place in most physiology or medical texts.

de Santana et al., call for action of alternative assessments to understand the variability of the response and assist in planning new studies. The authors suggested that such a range could be

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based on the number of sets per muscle group, comprising at least 10 sets per week. Additionally, Omic sciences (e.g., transcriptomics, epigenomics, proteomics, and/or metabolomics) emerge as the next frontier to be explored in this field of knowledge that help to explain the complexity to phenomenon of exercise response heterogeneity.

Bonafiglia et al., systematic review lays the groundwork of the Research Topic, to determine the extent to which studies in the exercise training literature have adopted sound statistical approaches for examining individual responses to exercise training. Bonafiglia underlines the compelling need for prospective trials to identify studies that statistically estimated the presence of interindividual differences in trainability substantially in participant characteristics, training modes, and outcomes assessed. The authors addressed novel data better convince researchers to statistically estimate interindividual differences in trainability and consider error and an smallest worthwhile change or minimum clinically important difference in future clinical trials.

Castro et al., report that metabolic profile and pathways in blood serum and the skeletal muscle responses after 8-week of continuous endurance training (ET) or high-intensity interval training (HIIT), in a group of 70 men, young and sedentary. The main finding changed and impacted pathways by these metabolites were: arginine and proline metabolism, glycine, serine and threonine metabolism, and glyoxylate and dicarboxylate metabolism for both ET and HIIT programs; and additional alanine, aspartate and glutamate metabolism, arginine biosynthesis, glycolysis/gluconeogenesis, and pyruvate metabolism for ET. These results suggest that regulating the metabolism of amino acids and carbohydrates may be a potential mechanism for understanding the inter-individual variability of cardiorespiratory fitness in responses to ET and HIIT programs.

Gallegos-Carrillo et al., showed for the first time a pragmatic cluster randomized trial, in 4 Primary Health Care Units. Differences were observed in triglycerides, BMI, metabolic risk scores variables and depressive symptoms among exercise referral and brief physical activity counseling programs. In addition, differences in the brief physical activity group were observed according to level of adherence in blood pressure levels, central obesity and waist-to-hip ratio, depressive symptoms and the mental health component of health-related quality of life. These results reinforce the idea that usefulness of this physical activity programs in primary health care facilities.

Obesity is a major contributor to the development of type 2 diabetes (T2DM), with 80% of individuals being classified as obese. In this line, Andrade-Mayorga et al., illustrate the beauty and complexity the association of perilipin 1 (PLIN1; rs1052700 and rs2304795), lipoprotein lipase (rs283), and adrenoceptor beta 3 (rs4994) polymorphisms with high and low responders (LoRes) to fat mass reduction after 12-weeks of HIIT and dietary energy restriction in 30 adult women with overweight/obese. Their data suggest that rs1052700 (14995A>T) polymorphism of the PLIN1 gene is associated with a differential response to fat mass reduction after a 12-week HIIT intervention. In addition, women with the TT genotype of this genetic variant showed greater changes in fat mass than AA and AT genotypes. However, further studies are needed to confirm these findings. Subsequently, Andrade-Mayorga et al., illustrate the physiological effects and inter-individual variability on fat mass and other health-related and physical performance outcomes after 12 weeks of HIIT in overweight/obese adult women. This intervention caused an improvement in multiple health-related and physical performance outcomes, i.e., reductions in absolute fat mass, body fat percentage, total body mass, blood pressure, and increases in absolute/relative cardiorespiratory fitness. However, beyond the good average group responses found in the present study, a wide range of responses was appreciated in each study variable individually.

Magalhães et al., present the interindividual variability in fat mass loss in response to HIIT with resistance training, moderate continuous training with resistance training (MCT), and control group in adults with T2DM over a 1-year intervention. Their results suggest that the number of fat mass responders did not differ between the MCT or HIIT, compared to the control, following a 1-year exercise intervention in individuals with T2DM. However, low responders to fat mass may still derive reductions in arterial stiffness and structure.

Lastly, Delgado-Floody et al., reported improvements in obesity markers, metabolic risk factors, and endurance/muscle performance, and the interindividual variability after 20-weeks of two CT configurations (i.e., HIIT plus resistance training (RT), compared with RT plus HIIT) in 26 women with severe/morbid obesity. Considering the expensive and long treatments before bariatric surgery, the topic of interindividual variability to exercise training is of high interest and value.

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. It should be recognized that what is suitable for prevention may be entirely inadequate for treatment, as is also the case with pharmacological management of chronic diseases (Izquierdo et al., 2021). 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 (Ramírez-Vélez and Izquierdo, 2019). Concerns have been raised about the true magnitude of response variability as well as maximal trainability. Hypothesized reasons for non-response include insufficient training stimulus (i.e., intensity or specificity of intervention), sex-related differences in response to exercise, and baseline fitness levels. Additionally, the individual interaction of physiological, molecular (i.e., genetics, epigenetics, transcriptomics, and metabolic factors), and environmental factors are being investigated as potential mediators of the lack of a response to exercise in some participants (Izquierdo et al., 2021). Further investigation is warranted to evaluate whether response heterogeneity differs across population subtypes and with similar lifestyle modifications to move closer to a personalized lifestyle medicine that optimizes changes in clinical outcomes based on individual characteristics (Izquierdo et al., 2021). Given the importance of personalized exercise for a healthy aging for today's society, we consider that this

eBook will be of great scientific and social impact with the consequent transfer applications. We hope to continue the success of this Research Topic with a third edition in the near future.

AUTHOR CONTRIBUTIONS

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

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Practical Recommendations Relevant to the Use of Resistance Training for COVID-19 Survivors

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The novel coronavirus disease (COVID-19) has emerged at the end of 2019 and caused a global pandemic. The disease predominantly affects the respiratory system; however, there is evidence that it is a multisystem disease that also impacts the cardiovascular system. Although the long-term consequences of COVID-19 are not well-known, evidence from similar diseases alerts for the possibility of long-term impaired physical function and reduced quality of life, especially in those requiring critical care. Therefore, rehabilitation strategies are needed to improve outcomes in COVID-19 survivors. Among the possible strategies, resistance training (RT) might be particularly interesting, since it has been shown to increase functional capacity both in acute and chronic respiratory conditions and in cardiac patients. The present article aims to propose evidence-based and practical suggestions for RT prescription for people who have been diagnosed with COVID-19 with a special focus on immune, respiratory, and cardiovascular systems. Based on the current literature, we present RT as a possible safe and feasible activity that can be time-efficient and easy to be implemented in different settings.

Keywords: resistance exercise, rehabilitation, strength training, pulmonary rehabilitation, cardiac rehabilitation, coronavirus

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THE PROBLEM

The novel coronavirus disease (COVID-19) pandemic has posed a great threat to public health concern and safety (Wu et al., 2020; Zu et al., 2020). Caused by acute respiratory syndrome coronavirus 2 (or SARS-CoV-2), COVID-19 is characterized by respiratory distress and multisystem disease, which is frequently severe and might result in death (Kreutz et al., 2020). Many COVID-19 survivors who required critical care may develop psychological, physical, and cognitive impairments (Barker-Davies et al., 2020). There is evidence that coronaviruses may induce neurological impairments by invading the central nervous system and some patients may have symptoms like severe muscle pain (Li Y. C. et al., 2020). COVID results in relevant morbidity for 3–6 months (intermediate phase), and rehabilitation services and medical care might be needed for more than 12 months (chronic phase) (Barker-Davies et al., 2020).

Previous studies showed that survivors of acute respiratory diseases might have persistent functional disability and psychological symptoms for as much as 1 year after discharge

(Herridge et al., 2003; Tansey et al., 2007), with most of them showing extrapulmonary conditions, with muscle wasting and weakness being most frequent (Herridge et al., 2003). Moreover, many COVID-19 patients will need to be on intensive care units, which is associated with symptoms like dyspnea, anxiety, depression, impaired physical function, and poor quality of life for up to 12 months after discharge (Oeyen et al., 2010; Denehy and Elliott, 2012; Jackson et al., 2012). Among them, physical function is one of the factors least likely to recover to normal values as it is heavily affected by critical illness (Gerth et al., 2019). The cardinal manifestations include limb muscle weakness, muscle atrophy, and impairments in deep tendon reflexes (Li Z. et al., 2020). Neuromuscular weakness in the intensive care units can prolong the patient's mechanical ventilation time and hospitalization. Therefore, rehabilitation should commence in the critical care setting, since early exercise prevents neuromuscular complications and improves functional status in critical illness, being considered effective, safe, and feasible (Sosnowski et al., 2015; Barker-Davies et al., 2020). Moreover, rehabilitation programs starting within the post-acute phase (<30 days) seem to bring the most benefits (Barker-Davies et al., 2020).

Besides all the knowledge about intensive care management and recovery, there is a paucity of evidence-based recommendations regarding rehabilitation following COVID-19. Among the possible strategies for rehabilitating COVID-19 patients survivors, resistance training (RT) that conventionally consists of the voluntary muscle contractions against some kind of external resistance might be particularly interesting, since it has been shown to be a safe and feasible strategy to increase functional capacity in both acute and chronic respiratory conditions (Troosters et al., 2010; Liao et al., 2015; Li et al., 2019; Rice et al., 2020). Based on the current scientific evidence, RT can be safe, time-efficient, and easy to be implemented in almost anywhere and with minimal resources (Gentil et al., 2020b; Souza et al., 2020). Therefore, the present article aims to propose evidence-based and practical suggestions for the use of RT for people who have been diagnosed with COVID-19 during different phases of disease, with a special focus on immune, respiratory, and cardiovascular systems.

IMMUNE SYSTEM

The immune system works through the coordinated functions of many cells to protect the organism against opportunistic infections (Pedersen and Hoffman-Goetz, 2000). Therefore, preserving or improving its function is important for people who were affected by COVID-19. There are evidences of either immune surveillance or immunodepression in response to exercise (Pedersen et al., 1998; Peake et al., 2017; Nieman and Wentz, 2019); however, the specific effects of RT on immune function have not being extensively studied (Freidenreich and Volek, 2012). Interestingly, people involved in endurance training are more commonly affected by immunodepression and illness (Nieman, 2007) when compared to strength and power sports (Alonso et al., 2010, 2012; Horn et al., 2010; Timpka et al., 2017), which might be a favorable point to RT (Natale et al., 2003; Gentil et al., 2020b). In general, the association between exercise and body immune defenses follows a J-shaped curve (Pedersen et al., 1998; Peake et al., 2017; Nieman and Wentz, 2019), improving with moderate amounts of physical exercise and decreasing with excessive or low amounts of exercise (Pedersen et al., 1998; Peake et al., 2017; Nieman and Wentz, 2019). This complex relation is negatively influenced by many factors, such as higher energy expenditure (Spence et al., 2007; Rama et al., 2013), increased exercise volume (Peters and Bateman, 1983; Gleeson et al., 2013; Siedlik et al., 2016), and metabolic stress (Pedersen and Hoffman-Goetz, 2000). In this sense, an acute bout of exercise might induce a suppressive effect on lymphocyte proliferative responses, with long-duration (longer than 1 h) and high-intensity exercise exhibiting a moderate suppressive effect (Siedlik et al., 2016).

A study by Davis et al. (1997) analyzed the effects of physical exercise on susceptibility to respiratory infection by using a murine model. The exercise design was composed of three groups: no exercise, moderate short-term exercise (30 min), and prolonged exercise to voluntary fatigue (2.5–3.5 h). According to the results, exercising to fatigue resulted in greater mortality rate (41%) than either no exercise or short-term moderate exercise. Although mortality rate tended to be lower after short-term moderate exercise (9%) than no exercise (16%), there was no significant difference between conditions. The results also showed a decrease in antiviral resistance after strenuous exercise within the lungs, in conjunction with increased susceptibility to respiratory infection *in vivo*. Although there is paucity of data linking the transitory immune suppression after strenuous exercise with chronic immune system impairment and subsequently infection risk (Nieman and Wentz, 2019), it is reasonable to suggest that exercise-induced immune suppression may impair the clearance of pathogen in acute illness COVID-19 patients. Therefore, even after the acute phase of the disease, physical exercise should ensure the adequate restoration of immune defense.

For these reasons, it might be advisable to avoid strenuous activities and adopt a reduced total training RT volume/duration (<45 min) to preserve immune function and decrease the risk of complications, particularly when the immune response is still compromised (Gleeson et al., 2013; Peake et al., 2017). With that in mind, low-volume RT should be recommended. Here, it is important to note that training sessions lasting a few minutes have been suggested to promote muscle strength and size gains in different populations (Fisher J. et al., 2017; Souza et al., 2020). From a practical standpoint, previous studies showed that untrained young and older adults can obtain many health benefits (e.g., increased functionality and cardiovascular improvements) from minimal dose RT protocols involving two sets of three to four basic exercises with a training frequency of one or two sessions per week (Fisher et al., 2014; de Barbalho et al., 2017; Seguro et al., 2019; Souza et al., 2019; Dias et al., 2020).

It is important to consider that rises in epinephrine, cortisol, and sympathetic modulation seem to be related to immunosuppression induced by exercise (Pedersen and Hoffman-Goetz, 2000; Nieman and Wentz, 2019). In this

regard, previous studies have shown an association between elevated metabolic stress, cortisol levels, and immunosuppression in response to RT (Miles et al., 2003; Ramel et al., 2003; Krüger et al., 2011). Therefore, it might be interesting to avoid such responses in COVID-19 survivors under rehabilitation. According to previous studies, RT protocols with a few number of repetitions (≤6 repetitions) and long between-sets rest intervals (≥3 min) result in less pronounced increases in sympathetic activity, cortisol, and lactate levels (Kraemer et al., 1990; Smilios et al., 2003, 2007; Vale et al., 2018). Moreover, low-volume RT with few repetitions is less glycolytic (Knuiman et al., 2015). Therefore, it could prevent the concurrency for energy substrate and subsequent immunosuppression, since glucose is the main fuel of immune cells (Palmer et al., 2015).

Regarding time of the day, studies involving endurance activities showed that the acute increases in leukocytes were higher when exercise was performed during the night (6 PM) when compared to morning (9 AM), and it remained high for 1 h after exercise in a hot and humid weather (Boukelia et al., 2018). When comparing exercise during the morning and afternoon (9 AM vs. 4 PM) in a cold environment, Boukelia et al. (2017) found higher immune function and less pulmonary inflammation during afternoon exercise. We could not find specific studies with RT; however, it has been previously shown that plasma cortisol levels are increased during the morning (Hayes et al., 2010), which could suggest an impaired immune function. Therefore, the suggestion is to train in the afternoon or early night.

The basis of COVID-19 pathogenesis is associated with a delayed antiviral response followed by an immunological overreaction that results in an excessive proinflammatory state (Castelli et al., 2020). The levels of systemic inflammation might explain the severity of the disease, with the most affected patients presenting higher serum levels of proinflammatory cytokine, as well as reduced T lymphocytes count (Chen et al., 2020). Regulatory T lymphocyte (Treg) is also reduced in severely ill patients and seems to play an important role in COVID-19 pathogenesis, since it is associated with controlling autoimmune and proinflammatory response (Gladstone et al., 2020; Stephen-Victor et al., 2020). In this context, RT may contribute to control proinflammatory state (Chupel et al., 2017; Santiago et al., 2018; Lammers et al., 2020). Despite the fact that studies investigating the effect of RT on Treg cells are scarce (Dorneles et al., 2020), a previous study in murine model showed that RT can upregulate this immune marker (Souza et al., 2017). Moreover, regular practice of RT increases the levels of interleukin-10, an anti-inflammatory cytokine that is mainly produced by Treg cells (Chupel et al., 2017; Lammers et al., 2020).

RESPIRATORY SYSTEM

The high levels of proinflammation mediators and histopathological changes in the lungs in response to SARS-CoV-2 might induce apoptosis in pulmonary endothelial and epithelial cells, leading to impaired respiratory function such as acute respiratory distress (Castelli et al., 2020). Additionally, persistent proinflammatory state in severe COVID-19 patients is associated with fibroblast proliferation in the alveolar septum, resulting in pulmonary interstitial fibrosis (Zhang et al., 2020). Pulmonary diseases are commonly associated with loss of muscle mass and function (Steiner, 2007; Bone et al., 2017). The analysis of previous outbreaks of severe acute respiratory syndrome (SARS) revealed that 6–20% of the patients showed mild or moderate restrictive lung function consistent with muscle weakness 6–8 weeks after hospital discharge (Chan et al., 2003). This seems to persist for an even longer period as persistent pulmonary function impairment was present in 37% of the patients after recovery from SARS and their health status was also significantly worse compared with healthy subjects (Ong et al., 2005). Results from a cohort study showed significant impairment in lung capacity in 23.7% of SARS survivors 1 year after illness onset (Hui et al., 2005). Moreover, health status and exercise capacity were remarkably lower than those found in the normal population (Hui et al., 2005).

Previous studies showed that, in people with pulmonary diseases, low muscle strength is associated with physical inactivity (Osthoff et al., 2013) and is an independent predictor of morbidity and mortality independent of the degree of respiratory limitation (Swallow et al., 2007). Consequently, the key target in rehabilitation for pulmonary diseases should be improving locomotor muscle structure and function, as exercise results in reduced benefits on exertional ventilation, operating lung volumes, and respiratory muscle performance (Marillier et al., 2020). Moreover, the performance of physical exercise is advised as adjuvant non-pharmacological treatment during pulmonary fibrosis rehabilitation (Spruit et al., 2009).

RT has been suggested as an successful strategy for pulmonary rehabilitation, either performed alone or in conjunction with aerobic training, since it brings important increases in functional capacity (Liao et al., 2015; José and Dal Corso, 2016; Li et al., 2019). It is also important to highlight that exercise training during hospitalization due to acute respiratory conditions seems to bring important health and functional benefits, is well tolerated, and the adverse events are infrequent (Troosters et al., 2010; Rice et al., 2020). RT can be successfully performed as a stand-alone exercise strategy, without increasing adverse events in chronic obstructive pulmonary disease patients under pulmonary rehabilitation (Liao et al., 2015).

Considering that most people infected with SARS-CoV-2 could experience breathing difficulties, it is recommended to control the respiratory responses to exercise. One advantage of RT is that it might promote less cardiorespiratory stress (i.e., oxygen consumption and pulmonary ventilation) than aerobic exercise, even during maximal exercise testing (Houchen-Wolloff et al., 2014; Garnacho-Castaño et al., 2015; Albesa-Albiol et al., 2019). The manipulation of RT variables might further reduce the respiratory stress. Pulmonary ventilation and oxygen consumption increase with increased volume/duration (Haddock and Wilkin, 2006; Mookerjee et al., 2016; Garnacho-Castaño et al., 2018), lower rest intervals (Ratamess et al., 2007; Farinatti and Castinheiras Net, 2011), higher movement velocities (Mazzetti et al., 2011; Mukaimoto and Ohno, 2012; Buitrago et al., 2014), and higher number of repetitions (Scott et al., 2011; Ratamess et al., 2014). Therefore, training with

lower number of repetitions, higher interval between sets, and controlled movement velocity might be recommended (Buitrago et al., 2013).

CARDIOVASCULAR SYSTEM

Similar to other coronavirus infections, COVID-19 is associated with cardiac complications, especially arrhythmias, heart failure, and myocardial injury (Kochi et al., 2020; Madjid et al., 2020; Wang et al., 2020). Acute cardiac injury is higher in those with increased mortality, with severe disease, and requiring ventilatory support (Kochi et al., 2020; Madjid et al., 2020). Cardiac complications have been suggested to be multifactorial. It may be caused by hypoxia, viral myocardial injury, hypotension, ACE2-receptor downregulation, drug toxicity, or elevated systemic inflammation (Kochi et al., 2020). The proinflammatory mediators associated with COVID-19 can result in vascular inflammation, myocarditis, and arrhythmic complications (Kochi et al., 2020; Madjid et al., 2020). Another complication regarding cardiovascular system is the increased risk of thromboembolism as a consequence of coagulopathy and endothelial vascular dysfunction in critical illness COVID-19 patients (Goshua et al., 2020).

Patients diagnosed with COVID-19 should be fully assessed and, if necessary, additional investigations may include resting electrocardiogram (ECG), blood exams, 24 h ECG, cardiopulmonary, echocardiogram, cardiovascular magnetic resonance imaging, and exercise testing with the involvement of a cardiologist (Barker-Davies et al., 2020). In case of myocarditis, a period of 3–6 months of complete rest from strenuous exercise might be necessary, depending on the clinical severity illness duration (Pelliccia et al., 2019; Schellhorn et al., 2020). After returning, it is advisable to conduct periodic reassessment in the first 2 years due to an increased risk of silent clinical progression (Pelliccia et al., 2019).

RT has been shown to be safe and effective for several cardiac patients from different cardiac diseases and has been recommended as a core component of cardiac rehabilitation for many decades (McKelvie and McCartney, 1990; Verrill et al., 1992; Yamamoto et al., 2016). Some studies suggested that RT might be even safer than aerobic exercise, since it results in less myocardial stress and reduced hemodynamic responses in patients with heart diseases like controlled heart failure (Karlsdottir et al., 2002; Levinger et al., 2005), coronary arterial disease (Karlsdottir et al., 2002), and ischemic cardiomyopathy (McKelvie et al., 1995) and in patients in cardiac rehabilitation after myocardial infarction and percutaneous coronary intervention (Adams et al., 2010). Moreover, RT leads to improvements in cardiac autonomic control of diseased individuals (Bhati et al., 2019).

Cardiovascular stress might be more related to the duration of the exercise than with the load used, granting the use of higher loads and a lower number of repetitions. In this regard, Lamotte et al. (2005) reported higher levels of blood pressure and heart rate in response to RT using lower external loads and higher repetitions [four sets of 17 repetitions at 40% of the one-repetition maximum strength (1RM)] when compared with higher external loads and lower repetitions (four sets of 10 repetitions at 70% of 1RM) in 14 patients who participated in a rehabilitation program (e.g., bypass surgery, percutaneous coronary angioplasty, or valvular surgery). Similarly, Gjøvaag et al. (2016) reported higher levels of blood pressure and heart rate in patients with coronary arterial disease after performing 15RM with lower external loads than performing 4RM with higher external loads. Regarding autonomic modulation, Vale et al. (2018) showed that hypertensive women training with lower repetitions and higher external loads (6RM) showed less sympathetic activation and higher parasympathetic activation when compared to training with lower external loads and more repetitions (15RM). Therefore, in order to reduce cardiovascular stress during exercise, the recommended RT program should involve lower number of repetitions regardless of the load used.

One important feature in previous studies is that blood pressure and heart rate progressively increase over the sets, especially when the rest between sets is shorter (Gotshall et al., 1999; Lamotte et al., 2005; Gjøvaag et al., 2016). This suggests that one should consider performing a lower number of sets (one or two) and using higher rest between sets ($\geq$3 min). Other additional strategies to reduce cardiovascular stress is to give short pauses (i.e., 5 s) in the middle of the sets (da Silva et al., 2007; Rúa-Alonso et al., 2020), avoid performing repetitions until muscle failure (MacDougall et al., 1992), and exercise during the afternoon, since cardiac reactivity is lower (Jones et al., 2006; Boukelia et al., 2018) and there is a better blood pressure control (Jones et al., 2008) at this period of the day.

PRACTICAL RECOMMENDATIONS

RT might be performed in many settings, including acute hospitalization and rehabilitation scenarios. Previous studies have shown that RT performed during intensive care units might bring important benefits either alone (Morris et al., 2016; Barbalho et al., 2019; Veldema et al., 2019) or combined with other activities (Eggmann et al., 2018). Interestingly, the benefits of RT in intensive care unit patients have been reported even in the presence of mechanical ventilation (Eggmann et al., 2018).

Another important concern with COVID-19 is the neuropsychiatric sequalae. In addition to pandemic-associated psychological distress, the direct and indirect effects of the coronavirus on the human central nervous system might be related to neuropsychiatric disorders such mood changes, sleep disorders, depression, and anxiety (Khatoon et al., 2020; Steardo et al., 2020; Troyer et al., 2020). Studies investigating COVID-19 patients found a high level of post-traumatic stress and depressive symptoms in comparison with non-infected people (Vindegaard and Eriksen Benros, 2020). In this regard, there are consistent evidence that RT is associated with improvements in depression (Gordon et al., 2018), anxiety (Gordon et al., 2017), and sleep disorders (Kovacevic et al., 2018), including patients with chronic diseases (Ferreira et al., 2020) and during rehabilitation (McCartney, 1998; Vincent and Vincent, 2012; Chan and Cheema, 2016; Andrade et al., 2018;

Seguro et al., 2019). The potential benefits of RT for COVID-19 patients are illustrated in **Figure 1**.

RT programs commonly involve many exercises with the addition of isolated exercises for specific muscles, which might be too time-consuming. However, multi-joint exercises seem to be sufficient to improve muscle strength and hypertrophy in the muscles involved in the exercises (Gentil et al., 2015, 2017b; Paoli et al., 2017; Barbalho et al., 2020a,b) and there is no additional benefits in using single-joint exercises (Gentil et al., 2013; de França et al., 2015; Barbalho et al., 2020b). This allows the use of multi-joint exercises combined with low-volume programs, increasing feasibility and safety for most of the patients affected by COVID-19, hospitalized or not, including individuals with cardiometabolic diseases and frail elderly. Patients with COVID-19 that present severe body aches, sore throat, shortness of breath, chest pain, general fatigue, cough, or fever should avoid exercises between 2 and 3 weeks after the cessation of these symptoms. It is also recommended to avoid prolonged exhaustive or high-intensity exercise. These current restrictions to RT practice could be reviewed after cessation of the symptoms. COVID-19 patients that are asymptomatic should continue to exercise, as they would do normally. A pulmonary rehabilitation approach should be combined in the case on return from mild/moderate COVID-19 illness (Barker-Davies et al., 2020).

RT using non-traditional equipment such as elastic devices, which are low cost and portable, and can be performed in almost anywhere, might contribute to increase the possibilities for RT performance in many different settings, including intensive care units. Previous studies reported that RT using elastic bands or tubes resulted in similar muscle activation and mechanical stress (Aboodarda et al., 2011, 2016), strength gains (Martins et al., 2013), and improvements in functional capacity (Colado et al., 2010; Souza et al., 2019) when compared to traditional RT. Furthermore, RT might also be performed using body weight exercises as it promotes gains in muscle strength, hypertrophy, and body composition similar to traditional RT for many different populations, like middle-aged people with non-alcoholic fat liver disease (Takahashi et al., 2015, 2017), elderly people (Tsuzuku et al., 2017), and even young trained practitioners (Calatayud et al., 2015; Kikuchi and Nakazato, 2017).

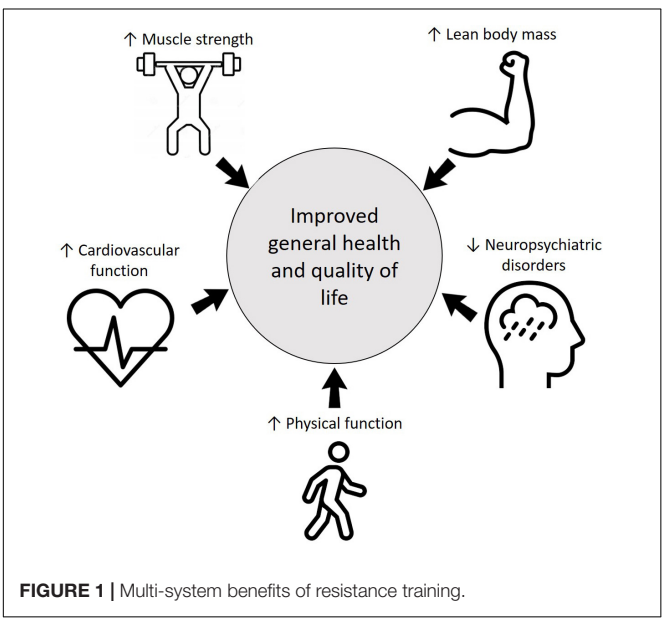

FIGURE 1 | Multi-system benefits of resistance training.

Another possible limitation in rehabilitation settings is the belief that RT has to be performed with moderate to high loads (ACSM, 2009; Kraemer et al., 2002), as it is commonly suggested that it would be necessary to use loads ≥60% of 1RM for optimal gains in strength and muscle mass (McDonagh and Davies, 1984; ACSM, 2009). However, previous studies have shown that low external load RT might bring increases in muscle fitness and hypertrophy that are similar to conventional approaches, when effort is high (Fisher J. P. et al., 2017; Steele et al., 2019). Previous studies in both trained (Morton et al., 2016) and untrained people (Mitchell et al., 2012; Assunção et al., 2016) reported that RT with low external load resulted in similar increase in muscle strength and hypertrophy when compared to high external load. This is particularly evident when the strength tests not similar to the situations trained (Fisher J. P. et al., 2017). The caveats for using low external load are that it would require a higher number of repetitions and longer exercise times, which can result in more negative impact on the immune system and a higher stress on respiratory and cardiovascular systems, as suggested above. Therefore, the cost–benefit of such adaptations might be analyzed individually.

Significant physiological stimulus can also be obtained with maximal or near-maximal voluntary muscle contractions performed without external load. In this regard, previous studies reported high levels of muscle activation when performing RT with the intention to maximally contract the muscles and no external load (Gentil et al., 2017a; Alves et al., 2020). A previous study reported equivalent gains in arm muscle hypertrophy after traditional and no external load RT in young men and women, using a contralateral training design (Counts et al., 2016). Positive outcomes in terms of hypertrophy and functionality have also been reported in intensive care units patients (Barbalho et al., 2019).

Particularly in aging people, the performance of high-velocity RT might be considered as an alternative strategy when the performance of high or low external load RT with high effort is not possible or recommended (Fragala et al., 2019). High-velocity RT may provide superior increases on functional capacity in comparison with conventional RT (Bottaro et al., 2007; Nogueira et al., 2009; Ramírez-Campillo et al., 2014). A previous study suggested that high-velocity RT might be a feasible and safe strategy to revert or prevent functional decline during acute hospitalization (Martínez-Velilla et al., 2019). Thus, the performance of few repetitions using high-velocity concentric muscle action combined with long rest intervals and/or intra-set short pauses could provide significant gains on functionality while preventing higher cardiovascular stress (Lamotte et al., 2010; Dias et al., 2020). Considering that the use of light to moderate loads (e.g., 30–60% of 1RM) are recommended to optimize muscle power (Fragala et al., 2019), this might be easily achieved with small implements such light dumbbells or

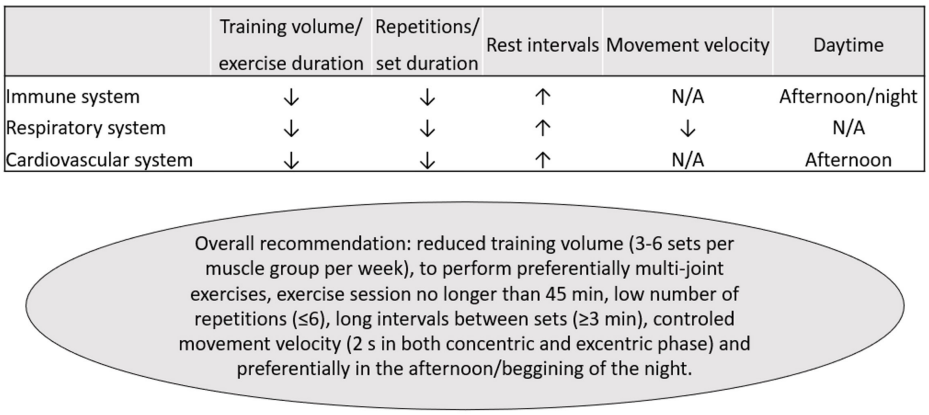

FIGURE 2 | Practical recommendations for resistance training in COVID-19 survivors. ↑, higher; ↓, lower. N/A, not available.

elastic devices. Therefore, equipment and implements should not be a barrier to implement RT programs during COVID-19 rehabilitation.

RT progression should be based on individual analysis, considering performance parameters and clinical symptoms. Initially, it is recommended that progression should be performed through increases in load, since higher number of sets and repetitions and lower rest intervals might impose unwanted risks. Therefore, the recommendation is to establish a repetition margin (i.e., 4–6RM) and increase load when the participant reaches the upper limit. When the patient reaches pre-COVID physical capacity, it would be interesting to re-examine for the possibility of restoring normal routine (Phelan et al., 2020).

FINAL CONSIDERATIONS

It is important to observe some general precautions for returning to exercise post-COVID-19, like monitoring temperature before training, starting with a muscle strengthening program prior to cardiovascular work, keeping social distancing, observing hygiene, adequate ventilation, and the use of masks when necessary (So et al., 2004; Gentil et al., 2020a). Another relevant point is the need to carefully evaluate clinical status and supervise patients that have been diagnosed with COVID-19, especially people with cardiac injuries (Barker-Davies et al., 2020), highlighting the need of a multidisciplinary approach. A subclinical myocardial injury may be present after clinical recovery from mild infections, even without cardiac symptoms or hospital admission. While the present article addresses RT for rehabilitation purposes, medical clearance is required. Therefore, a medical evaluation is recommended to exclude subclinical diseases before resuming high-intensity training or competition, eventually with exams such as transthoracic echocardiogram, maximal exercise testing, and 24 h Holter monitoring (Dores and Cardim, 2020; Wilson et al., 2020).

Considering the negligible chance of cardiac sequelae after asymptomatic infection or local symptoms of COVID-19, it is not necessary to perform pre-participation screening if a critical evaluation of signs and symptoms is negative and shows a complete recovery (Verwoert et al., 2020; Wilson et al., 2020). However, a pre-participation screening and cardiologist consultation may be considered for specific groups, including, but not limited to, people with pre-existent cardiovascular disease, elite athletes, and those with impaired recovery of exercise capacity.

For those with regional or symptoms not requiring hospitalization, it is strongly recommended to perform a pre-participation screening that includes physical examination, critical evaluation of symptoms, and a 12-lead ECG (Verwoert et al., 2020; Wilson et al., 2020). A cardiologist experienced in reading athletes' ECG should be consulted to differentiate between ECG changes due to exercise adaptation and ECG abnormalities suggestive of cardiac disease. This is necessary because 12-lead ECG is not the gold standard for the detection of myocarditis. It is also recommended to use cardiac biomarkers to detect myocarditis (Verwoert et al., 2020; Wilson et al., 2020). However, caution should be taken when using this strategy because most people do not have previously documented baseline measurements to compare with, and exercise might elevate the levels of these biomarkers, without clear-cut clinical implications (Verwoert et al., 2020). RT may be done after myocarditis if serum biomarkers of myocardial injury and left ventricular systolic function are normal and if 24 h ECG monitoring or exercise testing rules out relevant arrhythmias (Barker-Davies et al., 2020).

It is worthy to note that most of these screening recommendations refer to competitive athletes and high intense activities (Dores and Cardim, 2020; Verwoert et al., 2020; Wilson et al., 2020). Therefore, the specific limitations for performing RT should be individually analyzed and consider the specificities of each protocol. In this context, RT might be designed to be especially safe for people who have been diagnosed with COVID-19, in different stages of disease and recovery, by decreasing the risk of immunosuppression and reducing respiratory stress and cardiovascular risk. Interestingly, when combining the evidence in immune, pulmonary, and cardiovascular systems, the use of low volume/duration approaches and the manipulation of

training variables (moderate to high loads, short set duration, low number of sets, exercise choice, high rest intervals, and/or intra-set rest) might be particularly safe (**Figure 2**). RT might be also convenient as it can be performed with different implements (traditional machines, elastic devices, body weight exercises, or with no external load) and settings (in-hospital, exercise facilities, or home based), increasing its feasibility.

Finally, RT as an approach of the rehabilitation treatment should be individualized according to the patient's need, taking into consideration their comorbidities, symptoms of dyspnea, and psychological distress.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

PG and DS: conceptualization and writing the first draft. PG, CL, VC, WB, PV, RR-C, WM, and DS: writing, review, and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.637590/full#supplementary-material

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Physiological Effects and Inter-Individual Variability to 12 Weeks of High Intensity-Interval Training and Dietary Energy Restriction in Overweight/Obese Adult Women

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Background: Human adaptive response to exercise interventions is often described as group average and SD to represent the typical response for most individuals, but studies reporting individual responses to exercise show a wide range of responses.

Objective: To characterize the physiological effects and inter-individual variability on fat mass and other health-related and physical performance outcomes after 12 weeks of high-intensity interval training (HIIT) and dietary energy restriction in overweight/obese adult women.

Methods: Thirty untrained adult overweight and obese women (age = 27.4 ± 7.9 years; BMI = 29.9 ± 3.3 kg/m^2) successfully completed a 12-week supervised HIIT program and an individually prescribed home hypocaloric diet (75% of daily energy requirements) throughout the whole intervention. High and low responders to the intervention were those individuals who were able to lose ≥ 10 and < 10% of initial absolute fat mass (i.e., kilograms), respectively.

Results: The prevalence for high and low responders was 33% (n = 11) and 66% (n = 19), respectively. At the whole group level, the intervention was effective to reduce the absolute fat mass (30.9 ± 7.2 vs. 28.5 ± 7.2 kg; $p < 0.0001$), body fat percentage (39.8 ± 4.3 vs. 37.8 ± 4.9%; $p < 0.0001$), and total body mass (76.7 ± 10.1 vs. 74.4 ± 9.9 kg; $p < 0.0001$). In addition, there were improvements in systolic blood pressure (SBP; Δ% = −5.1%), diastolic blood pressure (DBP; Δ% = −6.4%), absolute VO_2peak (Δ% = +14.0%), relative VO_2peak (Δ% = +13.8%), peak power output (PPO; Δ% = +19.8%), anaerobic threshold (AT; Δ% = +16.7%), maximal ventilation (VE; Δ% = +14.1%), and peak oxygen pulse (O_2 pulse; Δ% = +10.4%). However, at the individual level, a wide range of effects were appreciated on all variables, and the magnitude of the fat mass changes did not correlate with baseline body mass or fat mass.

Conclusion: A 12-week supervised HIIT program added to a slight dietary energy restriction effectively improved fat mass, body mass, blood pressure, and cardiorespiratory fitness (CRF). However, a wide range of inter-individual variability was observed in the adaptative response to the intervention. Furthermore, subjects classified as low responders for fat mass reduction could be high responders (HiRes) in many other health-related and physical performance outcomes. Thus, the beneficial effects of exercise in obese and overweight women go further beyond the adaptive response to a single outcome variable such as fat mass or total body mass reduction.

Keywords: exercise, high-intensity interval training, overweight, interindividual variability, individual response, women

INTRODUCTION

Human adaptive response to exercise interventions is often described in general terms, assuming that the group average and SD are sufficient to represent the typical response for most individuals (Mann et al., 2014). However, studies reporting individual responses to exercise are usually heterogeneous, showing a wide range of responses to the interventions rather than a similar response (Bouchard and Rankinen, 2001; King et al., 2008; Scharhag-Rosenberger et al., 2012; Bonafiglia et al., 2016; Gurd et al., 2016; Parr et al., 2016; Álvarez et al., 2017a; de Lannoy et al., 2017; Sparks, 2017; Williamson et al., 2017; Chrzanowski-Smith et al., 2019; Ross et al., 2019). Thus, research attempting to quantify, predict, and explain inter-individual variability to exercise training has grown gradually (Chrzanowski-Smith et al., 2019). This heterogeneity has been linked to physiological, genetic, and epigenetic factors (Sparks, 2017; Hagstrom and Denham, 2018). Following this, recent literature has dichotomously classified individuals as either "responders/non-responders" or "high responders (HiRes)/low responders (LoRes)" using a pre-determined threshold. The most commonly used criteria are: clinical cut-off points (Mann et al., 2014; Parr et al., 2016; Álvarez et al., 2019), within-subjects coefficient of variation (CV) (Scharhag-Rosenberger et al., 2012; Astorino and Schubert, 2014), typical error of measurement (TE) (Ross et al., 2015; Montero and Lundby, 2017), or two times the typical error (2x TE) (Bouchard et al., 2012; Bonafiglia et al., 2016, 2018; Gurd et al., 2016; Raleigh et al., 2016; Álvarez et al., 2017a; de Lannoy et al., 2017; Astorino et al., 2018). Concerning exercise, most studies have focused on heterogeneity of the cardiorespiratory fitness (CRF) adaptations to training (Bouchard and Rankinen, 2001; Scharhag-Rosenberger et al., 2012; Ross et al., 2015, 2019; Williamson et al., 2017). Few studies explore the inter-individual variability in body composition and exercise-induced fat mass changes (King et al., 2008; Álvarez et al., 2017a; Andreato et al., 2019). This research topic is essential because overweight and obesity have emerged as the leading health concerns during the last decades (Heymsfield and Wadden, 2017), and it is expected that by 2025 global obesity prevalence will reach 18% in men and surpass 21% in women (NCD-RisC, 2016). The global combined prevalence of overweight and obesity [i.e., body mass index (BMI) $\geq$ 25 kg/m^2] is currently estimated to be over 38% (Ng et al., 2014). Furthermore, obesity is associated with expanding white adipose tissue (adipocyte hypertrophy) and visceral adiposity, producing metabolic dysregulation resulting from the associated pro-inflammatory and insulin-resistant phenotype (Vazquez-Carrera, 2016; Hepler and Gupta, 2017). Thus, adiposity is a strong predictor of morbidity and mortality (Verheggen et al., 2016). Obesity is usually treated through behavioral changes involving exercise and nutrition (Petridou et al., 2019). Exercise represents an effective strategy to reduce body mass and improve health, being the lack of exercise one of the main factors associated with obesity (Booth et al., 2012). Moreover, high-intensity interval training (HIIT) has proven to be superior to traditional exercise programs in reducing fat mass and improving CRF in adults with obesity (Turk et al., 2017). This finding is important because some results support the idea that women have particular metabolic characteristics such as greater reliance on fat oxidation than men during submaximal exercise. However, they also indicate that this greater fat oxidation is reached at higher exercise intensities in women than men (Cheneviere et al., 2011). Therefore, the present study aimed to characterize the physiological effects and inter-individual variability on fat mass and other health-related and physical performance outcomes after 12 weeks of HIIT in overweight/obese adult women.

MATERIALS AND METHODS

Study Design and Participants

A group of untrained adult overweight and obese women were studied, all referred by a physician to the supervised exercise program in our research center. Ethical approval for the study was provided by Scientific Ethics Committee at Universidad de La Frontera (Protocol N°112/16). The study was conducted according to the Helsinki Declaration. All volunteers received information about the protocol and provided written consent before the beginning of the study.

The inclusion criteria were: (a) women aged 18–45 years; (b) diagnosed with overweight or type 1 or 2 obesity (BMI between 25 and 39.9 kg/m^2); (c) untrained (not involved in regular physical activity or exercise program during the previous 6 months); (d) pre-menopausal women; and (e) previously screened by a physician professional. The exclusion criteria

were: (a) previously diagnosed diseases, such as diabetes mellitus, hypertension, myocardial infarction, and class III obesity; (b) receiving pharmacologic corticoids, metformin, or other drugs that may affect metabolism; (c) smoking habit; (d) history of bariatric surgery; (e) untreated hypothyroidism; and (f) skeletal muscle disabilities or a specific indication to avoid exercise by medical reasons. Attendance to a minimum of 70% (26/36 sessions) of the exercise program duration was fixed as the cut-off point for individual compliance for subjects included in the final statistical analyses. A structured medical history record and physical examination were performed on 105 adult women for enrolment purposes. Forty-three subjects met all the inclusion criteria, and 30 of them were finally studied. This study used a non-probabilistic sample aiming to separate high and low responders to a 12-week exercise program. In this study, HiRes to exercise intervention were those individuals who were able to lose ≥ 10% of initial absolute fat mass (i.e., kilograms), and LoRes were individuals who lost < 10%, similar to previous studies that have also used clinical cut-off points for this classification (Milagro et al., 2013; Parr et al., 2016). The study design is shown in **Figure 1**. Anthropometry/body composition, endurance performance, resting blood pressure, fasting glucose and insulin, and lipids levels, were assessed before and after the 12-week follow-up. Participants performed a supervised progressive exercise program three times a week on non-consecutive days, and it consisted of high-intensity cycling intervals interspersed with recovery inactive periods. Dietary intake was considered a control variable. To this purpose, all subjects were assessed and had nutrition counseling by the study nutritionist and instructed to follow an individually prescribed home hypocaloric diet (75% of daily energy requirements) throughout the whole intervention.

Anthropometric/Body Composition and Cardiovascular Measurements

The initial assessment was carried out to record socio-demographic, physical, and physiological characteristics. Body mass, fat mass, body fat percentage, and fat-free mass (FFM) were assessed with the subject barefoot, wearing underclothes, and no metal objects using a Tanita™ foot-foot bioelectrical impedance analyzer (BIA; Tanita Corporation, model BC-541, Japan). Its prediction formula has been previously validated, showing high reproducibility (Jebb et al., 2000). Height was

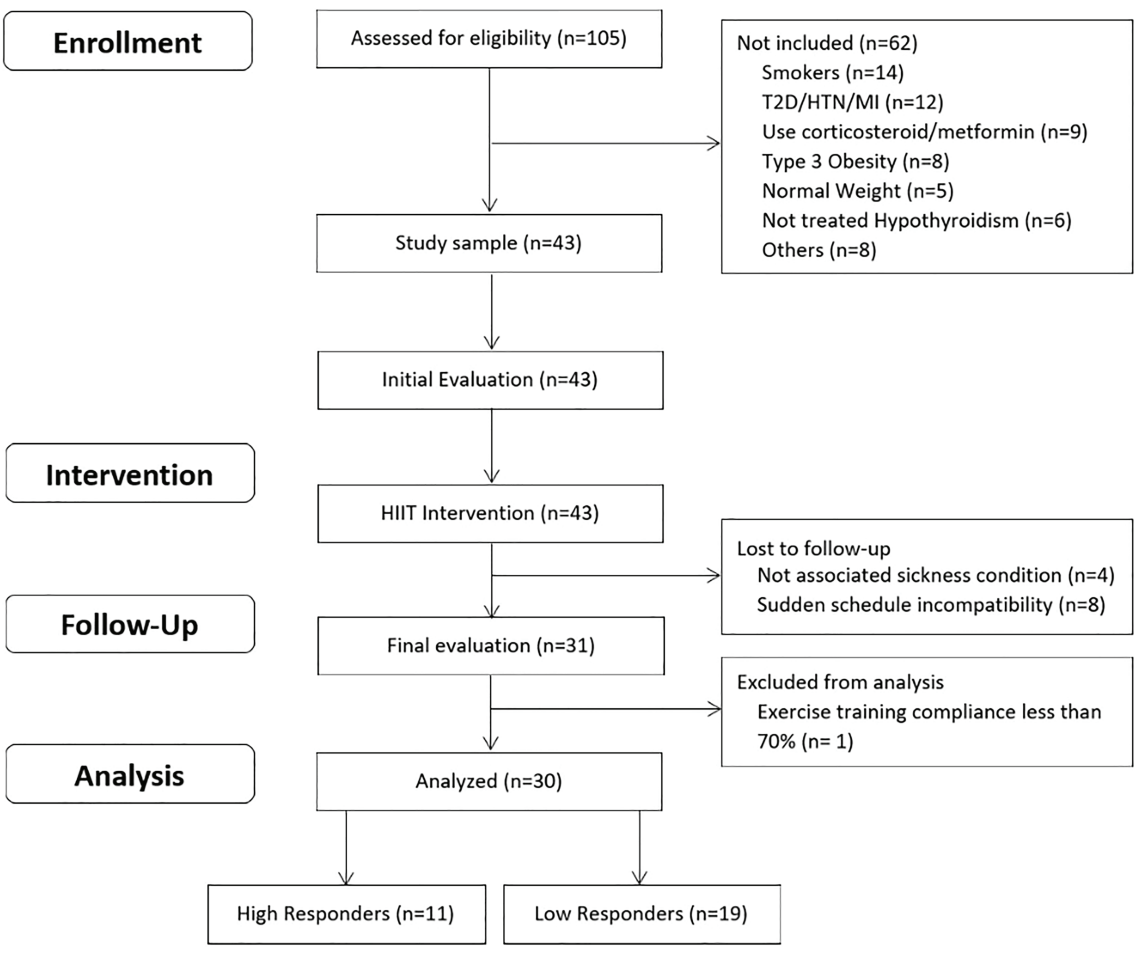

FIGURE 1 | Study design.

measured without shoes to the nearest millimeter with a stadiometer (Seca model 213, Hamburg, Germany). BMI was calculated using the formula weight divided by height squared (kilograms per square meter). The systolic and diastolic blood pressures (DBPs) were determined using an automatic monitor (Omron HEM-7114; Omron Healthcare) in duplicate and after 15 min of complete rest with the subjects in a supine position. The heart rate (HR) was measured at rest in similar conditions using a telemetric heart rate sensor (Polar model V800, Finland).

Endurance Performance Assessment/ Incremental Exercise Test

Endurance performance was assessed 1 week before and after the 12-week intervention during an incremental exercise test designed to obtain peak oxygen consumption (VO_2peak). In brief, the VO_2peak test consisted of free-wheel pedaling for 2 min at 70–80 RPM on a cycle ergometer (Lode Corival, Groningen, The Netherlands), followed by an initial 50 W load for 2 min and 25 W increments every 2 min until the participant reached volitional fatigue. Gas exchange was collected throughout the test using an indirect calorimeter/ ergospirometer system (Ultima CPX™ metabolic system, Medgraphics, Minnesota, United States), which was calibrated before the exercise test. Additionally, it was measured the peak power output (PPO), anaerobic threshold (AT), respiratory exchange ratio (RER), peak oxygen pulse (O_2 pulse; VO_2peak/HRmax during the exercise test), ventilation (VE), and respiratory rate (RR). HR was monitored with a continuous telemetric HR sensor (Polar model V800, Finland) throughout the whole test.

Exercise Training Intervention

Participants performed a 12-week supervised HIIT program, with training sessions three times a week on non-consecutive days. The exercise session consisted of 1-min cycling at a high intensity (workload during each interval was set to achieve muscle failure at the end of 1-min exercise period and reaching ~85–100% maximal heart rate obtained during the incremental exercise test), followed by a 2-min inactive resting period (sitting on the cycle ergometer), and repeated 10 times (*1x2x10* protocol; 1:2:10 to work: rest: repetitions, respectively). In summary, the total duration of one session of the *1x2x10* protocol was 30 min, with 10 min of effective exercise training, and without a warm-up or cool-down period. All exercise sessions were individually supervised to achieve muscle fatigue at each exercise interval as the primary indicator of intensity together with a continuous heart rate monitoring (Polar V800, Polar™, Finland) in order to supervise that the chronotropic response was expected according to previously found with the same HIIT exercise protocol (Andrade-Mayorga et al., 2020). Load progression was defined as the gradual increase in workload developed when the subject failed to reach muscle failure at the end of the 60-s exercise interval, monitored individually on a series-by-series and session-by-session basis. Thus, the load progression increased in parallel to the increment in the work capacity of each individual.

Dietary Assessment and Hypocaloric Diet

Participants were instructed to follow an individually designed hypocaloric diet [75% of estimated energy requirements (EERs)], equivalent to 1,354 ± 114.5 kcal/day, throughout the 12-week study period to control the dietary intake. A 24-h diet recall and a modified food choice questionnaire (7 days) were applied at baseline (Salvador Castell et al., 2015). Total energy expenditure (TEE) was estimated using the factorial method based on their reported daily physical activity (FAO/WHO/UNU, 2004; Levine, 2005). In brief, basal metabolic rate (BMR) was estimated using the Mifflin–St. Jeor equation (Cancello et al., 2018), additional caloric requirements were determined based on each subject's physical activity level (PAL), which were used to calculate the PAL index, TEE, and the 75% EER. Subjects had individual monthly meetings with the nutritionist during the 3-month intervention to encourage compliance. Dietary compliance was assessed using an instrument adapted from the Perceived Self-Regulatory Success in Dieting Scale (Meule et al., 2012), where each subject's perceived adherence was quantified using a five-point Likert scale. The nutritional intervention excluded the use of nutritional supplements.

Statistical Analysis

GraphPad Prisma statistical software 7.0 (San Diego, CA, United States) was used. The normal distribution of all the variables was tested using the D'Agostino-Pearson test. All the continuous variables were expressed as mean ± SD. The differences between quantitative variables were analyzed by paired *t*-test (intra-group differences pre- and post-intervention) or unpaired *t*-test for independent groups (between-groups difference within time points). Gardner-Altman estimation plots, which show individual values, means, and effect size with a 95% CI, were developed using Estimation Statistics for Data Visualization (Ho et al., 2019). The correlation between the magnitude of the fat mass responses and baseline anthropometric measures was calculated using Pearson correlation coefficient with 95% CIs. The level of significance used in all comparisons was $p < 0.05$.

RESULTS

From the initial cohort, 30 adult women (age = 27.4 ± 7.9 years; BMI = 29.9 ± 3.3 kg/m^2) successfully completed the 12-week intervention. All participants tolerated the exercise program well, and there were no injuries during the intervention. The baseline demographic, physical, and physiological characteristics of the participants are presented in **Table 1**.

At the group level, the intervention induced a reduction of absolute fat mass (i.e., kilograms) by 7.8% (30.9 ± 7.2 vs. 28.5 ± 7.2 kg; $p < 0.0001$; **Figure 2A**). The effect size of this group reduction in absolute fat mass was a mean difference of −2.4 kg (95%CI −3.23 to −1.76; **Figure 2B**). In addition, this group reduction in fat mass was also evidenced as a decrease in body fat percentage (fat mass divided by total body mass, multiplied by 100; 39.8 ± 4.3 vs. 37.8 ± 4.9%; $p < 0.0001$). On an individual level, wide

TABLE 1 | Baseline demographic, physical, and physiological characteristics of the participants.

Variable	All (*N* = 30)	Low responders (*N* = 19)	High responders (*N* = 11)	*p* value (LoRes vs. HiRes)
Demography				
Age (years)	27.4 ± 7.9	27.6 ± 8.6	27.2 ± 7.2	0.898
Physical Activity				
Physical activity level index	1.39 ± 0.08	1.38 ± 0.08	1.41 ± 0.09	0.437
Metabolic and Nutritional				
BMR (kcal/day)	1,295 ± 54.3	1,283 ± 55.7	1,314 ± 48.1	0.143
TEE (kcal/day)	1805 ± 152.7	1,778 ± 159.9	1852 ± 133.4	0.140
75% EER (kcal/day)	1,354 ± 114.5	1,333 ± 119.9	1,389 ± 100.1	0.140
Anthropometry/Body composition				
Body mass (kg)	76.7 ± 10.1	77.2 ± 9.8	76.1 ± 11.1	0.788
BMI (kg/m^2)	29.9 ± 3.3	30.6 ± 3.3	28.8 ± 3.0	0.159
Fat mass (kg)	30.9 ± 7.2	31.6 ± 6.9	29.8 ± 8.0	0.533
Body fat percent (%)	39.8 ± 4.3	40.5 ± 4.0	38.6 ± 4.9	0.246
FFM (kg)	45.9 ± 3.5	45.6 ± 3.5	46.3 ± 3.5	0.610
Cardiorespiratory fitness				
VO_2peak (ml·min^{-1})	1,800± 249	1,750 ± 230	1,888 ± 266	0.146
VO_2peak/FFM (ml·kg^{-1}·min^{-1})	39.2 ± 3.9	38.3 ± 3.6	40.7 ± 3.9	0.106
Cardiovascular				
O_2 pulse (ml/beat)	10.3 ± 1.5	10.2 ± 1.6	10.4 ± 1.2	0.683
Systolic BP (mmHg)	110.2 ± 8.7	110.9 ± 8.4	108.9 ± 9.4	0.575
Diastolic BP (mmHg)	73.6 ± 7.5	73.2 ± 8.7	74.2 ± 5.2	0.739
Mean BP (mmHg)	85.8 ± 7.2	85.7 ± 7.9	85.8 ± 6.2	0.996

Data are shown as mean and ± SD. LoRes, low responders; HiRes, high responders; BMR, basal metabolic rate; TEE, total energy expenditure; EER, estimated energy requirement; BMI, body mass index; FFM, fat-free mass; VO_2peak, peak oxygen consumption; and BP, blood pressure.

inter-individual variability was found for absolute fat mass reduction (**Figure 2C**), allowing to separate individuals who lost ≥ 10% of initial absolute fat mass (i.e., kilograms) and individuals who lost < 10% of initial absolute fat mass, and classify them as HiRes or LoRes, respectively. The prevalence for HiRes was 33% (n = 11; **Figure 2C**, gray bars) and LoRes was 66% (n = 19; **Figure 2C**, black bars). The reduction in absolute fat mass in HiRes was 18.3% (Δ:−4.7 kg; 29.8 ± 8 vs. 25.2 ± 6.9 kg; $p < 0.0001$) and in LoRes was 3.6% (Δ:−1.1 kg; 31.6 ± 6.9 vs. 30.5 ± 6.7 kg; $p < 0.0001$), and the between-groups comparisons in the post-intervention values were statistically significant ($p < 0.05$). It is noteworthy that HiRes and LoRes groups were statistically similar in the demographic, physical, and physiological variables assessed at baseline (**Table 1**). Moreover, there was no difference in hypocaloric diet compliance between HiRes and LoRes (3.6 ± 0.5 vs. 3.3 ± 3.5 points; $p = 0.3211$). Numbers on top of each column in **Figure 2C** represent the identification number of each subject according to their order of magnitude in the individual response to reduce fat mass after the intervention. These numbers and the classification as HiRes or LoRes to reduce fat mass were maintained in the subsequent figures to identify the individual response of these same subjects in the other study variables. Furthermore, it is important to mention that the magnitude of the fat mass changes did not correlate with baseline body mass ($r = -0.221$, $p = 0.242$) or fat mass ($r = -0.069$, $p = 0.716$).

At the group level, there was a reduction in total body mass (76.7 ± 10.1 vs. 74.4 ± 9.9 kg; $p < 0.0001$; **Figure 3A**). The effect size of this group reduction in body mass was a mean difference of −2.36 kg (95%CI −3.43 to −1.61). Individual changes in body mass from highest to lowest response are presented in **Figure 3B**, with HiRes represented with gray bars and LoRes with black bars (the subject number is the same as **Figure 2C**). The reduction in body mass in HiRes was 6.3% (76.1 ± 11.1 vs. 71.3 ± 9.9 kg; $p < 0.0001$) and in LoRes was 1.3% (77.2 ± 9.8 vs. 76.2 ± 9.7 kg; $p = 0.0004$) but without statistically significant difference between groups observed in the post-intervention values. Moreover, there were no significant pre-post changes in FFM (45.9 ± 3.5 vs. 45.9 ± 3.5 kg; $p = 0.8813$).

Related to CRF, for the whole group, there was an increased absolute VO_2peak by 14.0%. Paired t-tests revealed differences between baseline and after intervention measures for absolute VO_2peak (1,800 ± 249 vs. 2,094 ± 328 ml/min; $p < 0.0001$; **Figure 4A**) and for VO_2peak relative to FFM (VO_2peak/FFM; 39.2 ± 3.9 vs. 45.5 ± 5.6 ml/kg/min; $p < 0.0001$; **Figure 4C**). The effect size of this group increment in absolute VO_2peak was a mean difference of +294 ml/min (95%CI 213–374), and for VO_2peak/FFM was +6.35 ml/kg/min (95%CI 4.57–8.14). Individual changes in absolute and relative VO_2peak are presented in **Figures 4B,D**, respectively. VO_2peak relative to total body mass was not used considering pre-post differences in total body mass. The increment in absolute VO_2peak in HiRes was 14.9%

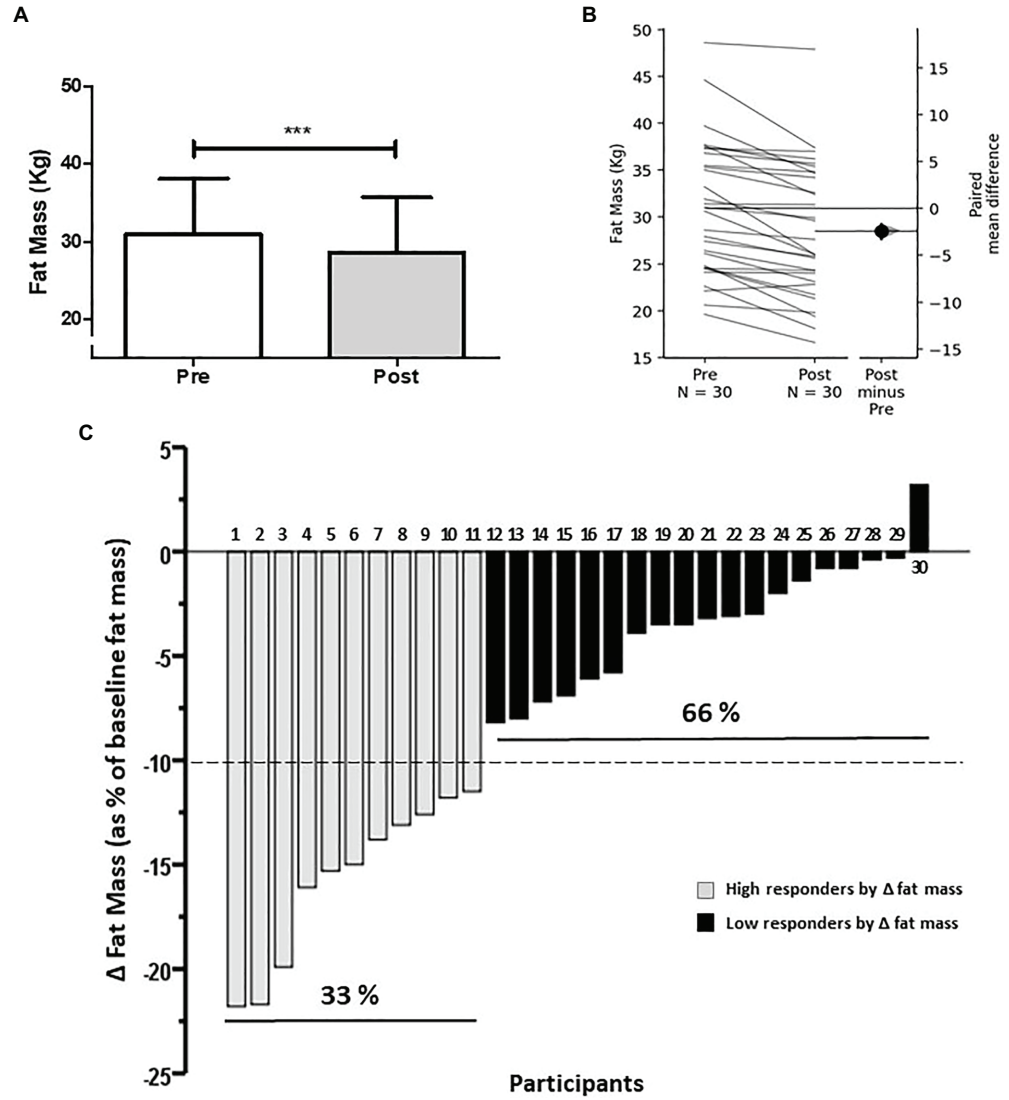

FIGURE 2 | Average changes **(A)** and individual response **(B,C)** to decrease absolute fat mass after 12-week intervention (**A**: absolute fat mass; **B**: Gardner Altman Plot with individual responses on the left axes as a line graph and the mean difference between groups on floating axes on the right; and **C**: individual changes in delta fat mass as % of baseline absolute fat mass). Numbers on the bars in panel **(C)** represent the order of magnitude in the individual response to reduce fat mass. High and low responders are identified in relation to the ability to reduce fat mass. ***$p < 0.0001$.

(1,888 ± 266 vs. 2,169 ± 322 ml/min; $p = 0.0004$) and in LoRes was 17.2% (1,750 ± 230 vs. 2,051 ± 332 ml/min; $p < 0.0001$) but without statistically significant difference between groups observed in the post-intervention values. Furthermore, there were significant improvement in exercise performance, measured as increases in PPO by 19.8% (128.3 ± 17.0 vs. 160.0 ± 24.2 W; $p < 0.0001$), AT by 16.7% (58.0 ± 11.9 vs. 69.6 ± 14.2 W; $p = 0.0025$), and maximal ventilation (VEmax) by 14.1% (66.3 ± 15.2 vs. 77.2 ± 13.7 L/min; $p = 0.0021$). There was no pre-post change in maximal respiratory rate (RRmax; 36.9 ± 8.2 vs. 39.2 ± 5.5 breath/min; $p = 0.05$).

Within the cardiovascular adaptations to 12-week exercise intervention, a 10.4% increase was observed in the group average for O_2 pulse (10.3 ± 1.5 vs. 11.5 ± 1.6 ml/beat; $p < 0.0001$; **Figure 5A**), which is a measure of maximum oxygen consumed per heartbeat. The effect size of this group increment in peak O_2 pulse was a mean difference of +1.2 ml/beat (95%CI 0.91–1.65]. Individual changes in peak O_2 pulse are presented in **Figure 5B**. In addition, decreases of 5.1 and 6.4% were observed for systolic blood pressure (SBP; 110.2 ± 8.7 vs. 104.9 ± 8.4 mmHg; $p = 0.02$; **Figure 5C**), and DBP (73.6 ± 7.5 vs. 69.2 ± 6.8 mmHg; $p = 0.0003$; **Figure 5E**), respectively. The effect size of this group reduction in SBP was a mean difference of −5.3 mmHg (95%CI −3.83 to −6.87), and for DBP was −4.4 mmHg (95%CI −2.57 to −6.63). Individual changes in SBP and DBP are presented in **Figures 5D,F**, respectively. The reduction in SBP in HiRes was 2.7% (108.9 ± 9.4 vs. 106.0 ± 9.8 mmHg;

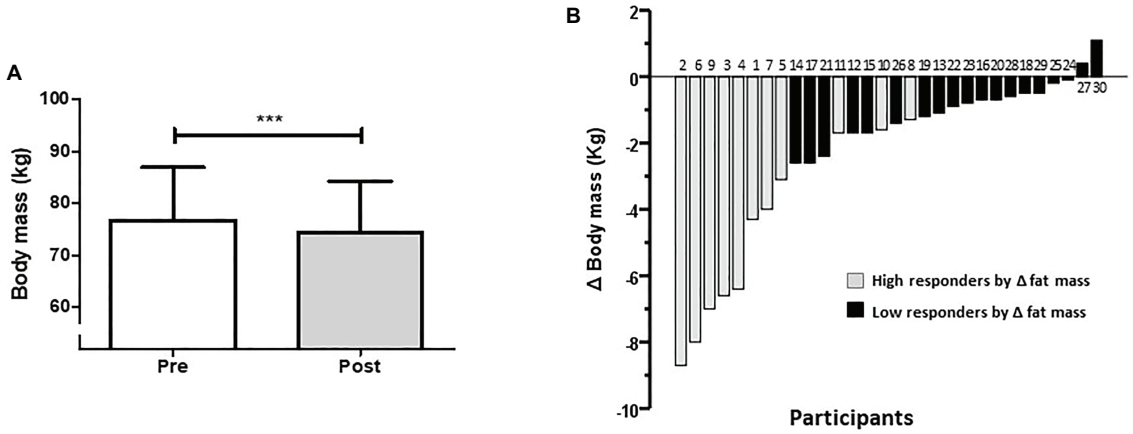

FIGURE 3 | Average changes **(A)** and individual response **(B)** to decrease body mass after the 12-week intervention. Subjects with a high or low response to reduce their fat mass are represented with gray and black bars, respectively. Numbers on the bars **(B)** represent the order of magnitude in the individual response to reduce fat mass. High and low responders are identified in relation to the ability to reduce fat mass. ***$p < 0.0001$.

p = 0.357) and in LoRes was 5.6% (110.9 ± 8.4 vs. 104.7 ± 9.1 mmHg; p = 0.03) with a significant difference only in the LoRes group. When comparing reductions in DBP levels in the HiRes and LoRes groups, no differences were found.

DISCUSSION

We characterized the physiological effects and inter-individual variability on fat mass and other health-related and physical performance outcomes after 12 weeks of HIIT in overweight/obese adult women. The uniqueness of the present study was that it shows the inter-individual variability to HIIT in overweight/obese young adult women, classified as LoRes or HiRes for a particular variable, i.e., absolute fat mass reduction, in response to a specific supervised 12-week exercise program added to a slight dietary energy restriction. This intervention caused an improvement in multiple health-related and physical performance outcomes, i.e., reductions in absolute fat mass (Δ% = −7.8%, equivalent to −2.4 kg), body fat percentage (Δ% = −5.0%), total body mass (Δ% = −3.1%), SBP (Δ% = −5.1%), DBP (Δ% = −6.4%), and increases in absolute VO_2peak (Δ% = +14.0%), relative VO_2peak (Δ% = +13.8%), PPO (Δ% = +19.8%), anaerobic threshold (Δ% = +16.7%), maximal ventilation (Δ% = +14.1%), and O_2pulse (Δ% = +10.4%). These results agree with several reviews and meta-analyses demonstrating that HIIT interventions allow multiple beneficial effects for health in a time-efficient manner, both in adults with obesity (Turk et al., 2017; Rugbeer et al., 2021) and lifestyle-induced cardiometabolic diseases (Gibala et al., 2012; Weston et al., 2014; Batacan et al., 2017; Maillard et al., 2018; Viana et al., 2019). Moreover, the meta-analysis by Dupuit et al. (2020) shows that cycling HIIT programs over 8 weeks are most effective in reducing fat and body mass in women before menopause (Dupuit et al., 2020). Correspondingly, the systematic review and meta-analysis by Johns et al. (2014) reported that programs combining exercise and diet are more effective in the short and long term for behavioral weight management (Johns et al., 2014). Also, the systematic review and meta-analysis by Clark (2015) reported that the combination of exercise and diet is more effective in producing changes in body composition, recommending interventions that generate large metabolic stress (induced by high levels of endurance exercise or resistance training) and not focused on energy imbalance in overweight adults (Clark, 2015). The reduction of fat mass after behavioral interventions is essential, as shown in the recent systematic review by Abe et al. (2021), where changes in total body fat have been associated with changes in visceral and subcutaneous adipose tissue following caloric restriction, caloric restriction plus exercise, and exercise alone interventions (Abe et al., 2021). However, beyond the good average group responses found in the present study, a wide range of responses was appreciated in each study variable individually.

Regarding the cut-off point used to classify subjects with a high or low response to the reduction of their absolute fat mass (kg), mention that there are different methods to make this differentiation (clinical cut-off points, within-subjects coefficient of variation, typical error of measurement, or two times the typical error) but without a consensus on which is the most appropriate method to differentiate these groups. The criterion used in this study was similar to previous studies, given an expected biological expression (Milagro et al., 2013; Parr et al., 2016). Parr et al. (2016) used similar cut-off points to evaluate circulating microRNA in the bloodstream (c-miR) before and after a 16-week intervention with exercise and diet in obese/overweight subjects. They observed that HiRes subjects (>10% reduction) have lower c-miR-935 expression pre- and post-intervention, knowing that c-miR-935 could be involved in modulating the expression of different metabolism-related genes (Parr et al., 2016). Similarly, Milagro et al. (2013) used a 5% cut-off point to assess differential expression of different miRNA in blood cells between HiRes and LoRes groups, but in a short-term (8 weeks) diet intervention without

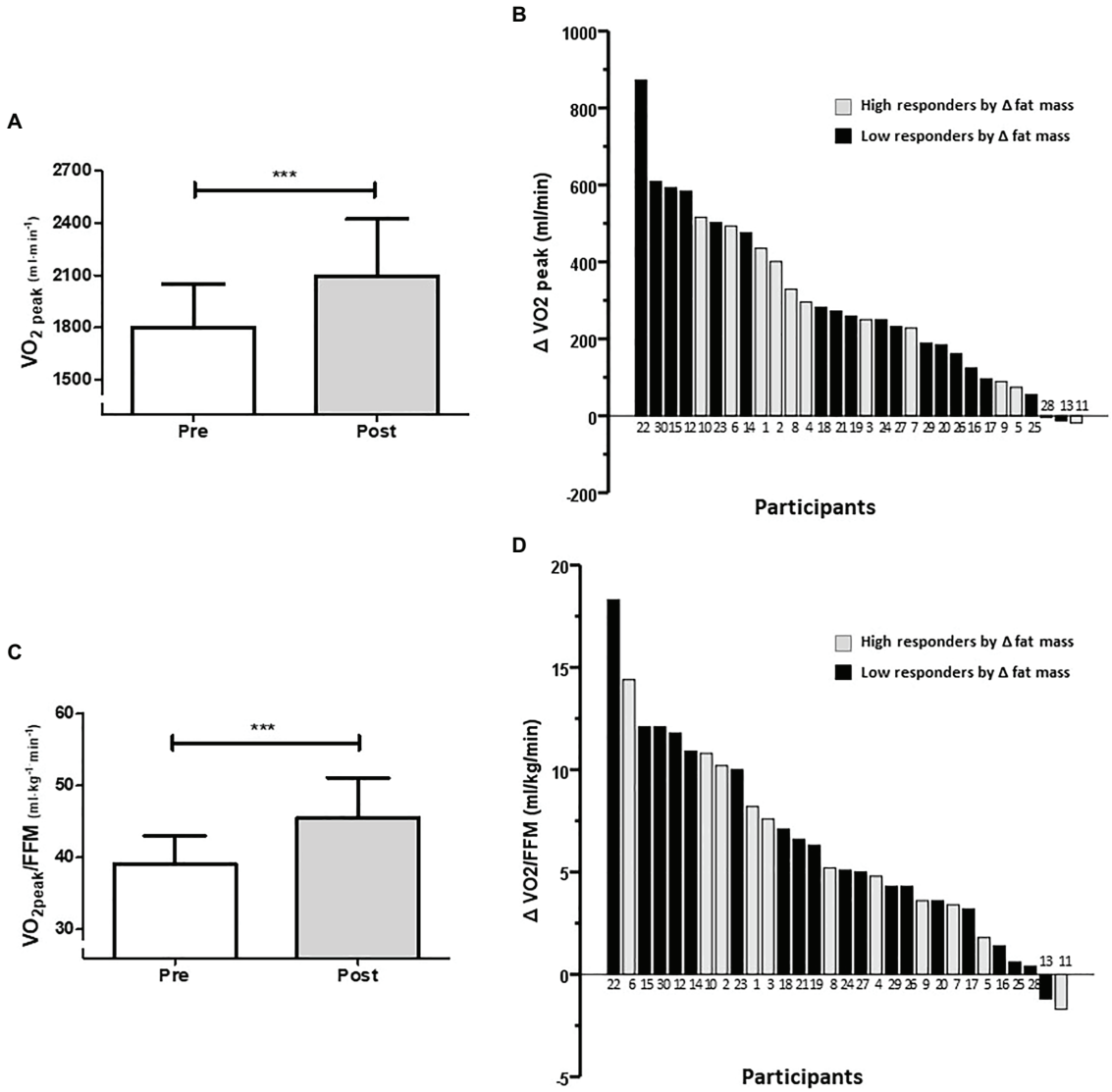

FIGURE 4 | Average changes **(A,C)** and individual response **(B,D)** to increase cardiorespiratory fitness (CRF) after 12-week intervention (**A,B**: absolute VO_2peak; **C,D**: VO_2peak relative to fat-free mass). Numbers below the bars **(B,D)** represent the order of magnitude in the individual response to reduce fat mass. High and low responders are identified in relation to the ability to reduce fat mass. ***$p < 0.0001$.

exercise training in excess body weight women (Milagro et al., 2013). Furthermore, the relevance of the cut-off point used in this study is supported by a systematic review comparing the effects of hypocaloric diet versus exercise (Verheggen et al., 2016), where the authors demonstrated that in the absence of weight loss, body fat reduction was much higher with exercise (6.1%) than diet (1.1%). Therefore, a 10% absolute fat mass reduction is a reasonably strict cut-off point to judge the failure/success of a 12-week intervention with exercise and diet.

Most of the previous studies reporting inter-individual variability have shown the prevalence of non-responders for a single outcome variable but without identifying the individual response of each subject for the primary variable and comparing it with the other study variables. For this study, the modification of fat mass was considered the primary outcome variable since it is a strong predictor of morbidity and mortality (Verheggen et al., 2016). Therefore, it was interesting for us to observe the individual response of each subject on other physical performance and health-related study variables, maintaining their classification as high-responder or low-responder for fat mass reduction and their corresponding identification number in the other study variables. Thus, subjects with more remarkable changes in fat mass (HiRes; **Figure 2C**, gray bars) were also those who had a more considerable reduction in total body mass (**Figure 3B**, gray bars); however, this was not the case for the rest of variables studied. Similar inter-individual variability to reduce fat mass has been observed in previous studies reporting the prevalence of non-responders after a HIIT intervention in subjects with cardiometabolic disorders (Álvarez et al., 2017b) and after moderate-intensity continuous training in overweight and obese subjects (Álvarez et al., 2012).

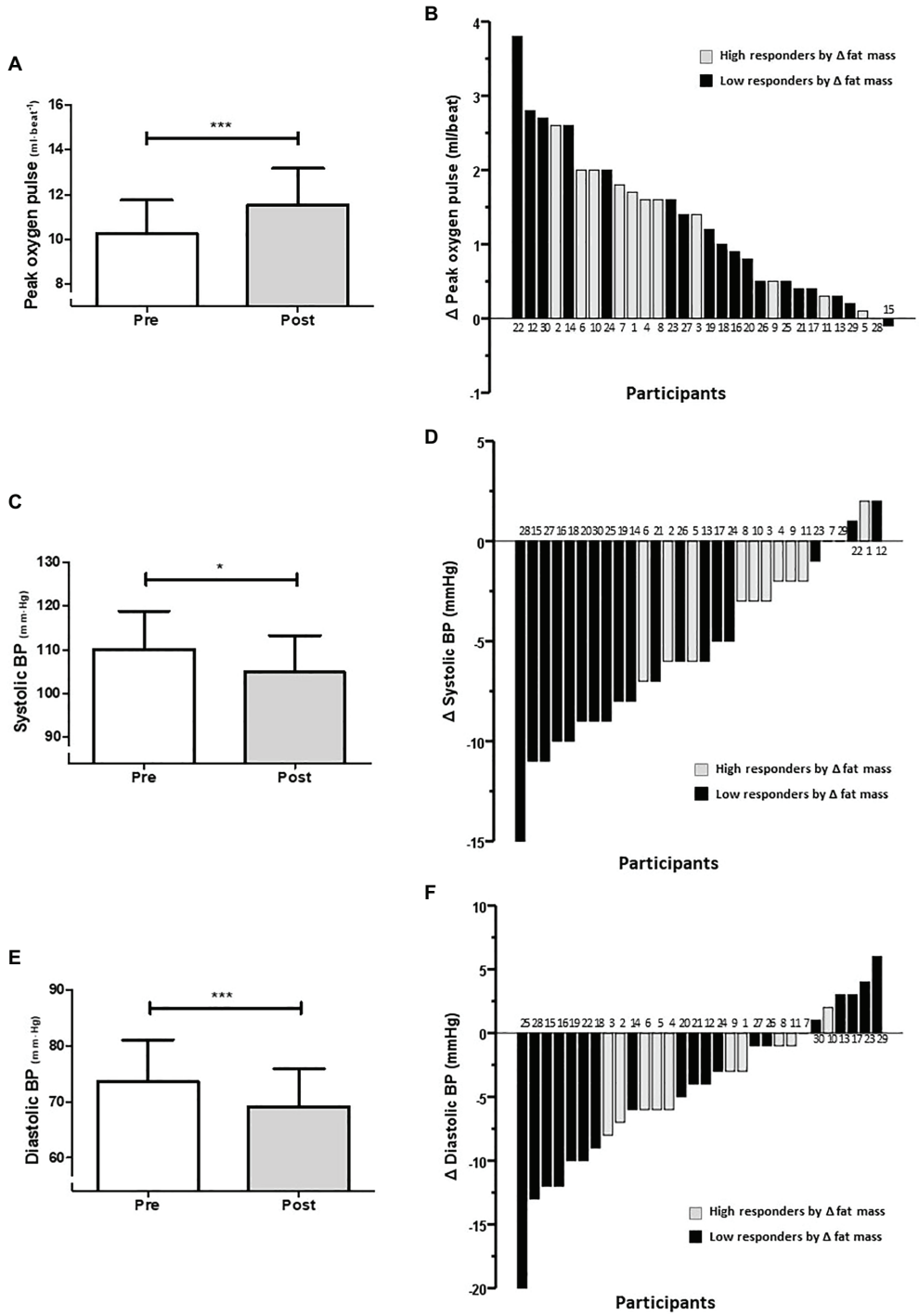

FIGURE 5 | Average changes **(A,C,E)** and individual response **(B,D,F)** in cardiovascular outcomes after 12-week intervention [**A,B**: peak oxygen pulse; **C,D**: systolic blood pressure (SBP); and **E,F**: diastolic blood pressure (DBP)]. Numbers above and below the bars **(B,D,F)** represent the order of magnitude in the individual response to reduce fat mass. High and low responders are identified in relation to the ability to reduce fat mass. ***$p < 0.0001$; *$p < 0.05$.

On the other hand, HIIT produces significant increases in CRF (Weston et al., 2014), which is highly relevant since improving VO_2peak reduces the risk of all-cause mortality and cardiovascular events (Kodama et al., 2009). Nevertheless, we observe that improvement in CRF (i.e., VO_2peak) after our 12-week intervention occurs with wide inter-individual variability, and this seems to occur independently of the high or low fat-mass response (**Figure 4B**, black bars). This pattern did not change when the VO_2peak was normalized by FFM (**Figure 4D**, black bars). Since Bouchard et al. (1999) published their article, inter-individual

variability of VO_2peak in response to different exercise interventions has been one of the most widely reported topics (Bouchard et al., 1999). However, according to Ross et al. (2015), the frequency of non-responders decreases until it disappears; with increasing intensity and duration of a 24-week exercise program (Ross et al., 2015). Correspondingly, Montero and Lundby (2017) reported that CRF non-responders prevalence is abolished by increasing the dose of exercise in a 6-week endurance training program (Montero and Lundby, 2017).

Similarly, the individual responses for cardiovascular outcomes after the 12-week intervention showed a wide variability to increase the peak O_2 pulse (**Figure 5B**). This improvement can be interpreted as a reflection of a greater stroke volume during maximum exercise (Mezzani, 2017), suggesting cardiac adaptations. Wide individual variability in reducing systolic and DBP (**Figures 5D,F**) were appreciated. It is worth mention that regardless that all participants in the present study were normotensive, they still showed improvements in their blood pressure levels, in agreement with a meta-analysis by Cornelissen and Smart (2013). They found that different exercise modalities effectively reduce blood pressure in normotensive and hypertensive subjects (Cornelissen and Smart, 2013). Thus, we could speculate that improvements were produced on endothelial function after the 12-week HIIT and diet intervention.

Among the numerous factors explaining the wide interindividual variability on exercise-induced fat and body mass loss, it can be cited: sex, ethnicity, muscle fiber type, body fat depot, diet, physical activity, sedentary behaviors, stress, sleep deprivation, adenovirus-36, and others (Boutcher and Dunn, 2009). Additionally, genetic and epigenetic factors could be playing a vital role in this exercise response heterogeneity (Sparks, 2017; Williamson et al., 2017; Hagstrom and Denham, 2018).

Our results corroborate the understanding that although there are high and low responders to exercise interventions, this classification is appropriate only for a single parameter and a specific exercise protocol. In this regard, Barbalho et al. (2017) study demonstrated that all participants in a 12-week resistance training showed improvements in at least one study outcome; therefore, there were no non-responders (Barbalho et al., 2017). This topic is genuinely relevant because it can motivate overweight and obese people to exercise, particularly when a time-efficient exercise strategy is used (Lidegaard et al., 2016). Thus, exercise modalities such as HIIT cause greater enjoyment than traditional programs of moderate-intensity continuous training (Thum et al., 2017) and in a time-efficient manner (Gillen and Gibala, 2014). Consequently, participants in these programs must be informed about the improvements in the different physical and health-related outcome variables during and after the interventions beyond reducing fat mass or body mass. This critical information will increase their adherence and motivation to behavioral changes to improve their health status.

The strengths of our study include the rigorous design of the exercise protocol monitored individually on a series-by-series and session-by-session basis during the 12-week program. Another strength was the individually prescribed home hypocaloric diet, as most similar studies acknowledge a lack of dietary control and only report that subjects were encouraged to maintain their usual diet during the exercise intervention period. On the other hand, one of its limitations was the lack of a no-intervention control group. Another limitation was using a BIA to measure body composition variables, as this is not considered the "gold standard" method.

In conclusion, a 12-week supervised HIIT program added to a slight dietary energy restriction effectively improved fat mass, body mass, blood pressure, and CRF. However, a wide range of inter-individual variability was observed in the adaptative response to the intervention. Furthermore, subjects classified as LoRes for fat mass reduction could be HiRes in many other health-related and physical performance outcomes. Thus, the beneficial effects of exercise in obese and overweight women go beyond the adaptive response to a single outcome variable such as fat mass or total body mass reduction.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Scientific Ethics Committee at Universidad de La Frontera, Temuco, Chile. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors have read the manuscript and agreed with the content. OA-M, ED, and LS conceived and designed the study. OA-M and NM-M performed the experiments. OA-M and ED analyzed the data. ED and LS contributed reagents, materials, and analysis tools and reviewed and edited the manuscript. OA-M wrote the paper. All authors contributed to the article and approved the submitted version.

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Effects of Four Lipid Metabolism-Related Polymorphisms on Body Composition Improvements After 12 Weeks of High-Intensity Interval Training and Dietary Energy Restriction in Overweight/Obese Adult Women: A Pilot Study

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Background: Polymorphisms in lipid metabolism-related genes have been associated with obesity and body composition, but these have been scarcely described concerning the magnitude of the response to exercise interventions in the overweight/obese population.

Objective: To evaluate the association of perilipin 1 (*PLIN1*; rs1052700 and rs2304795), lipoprotein lipase (rs283), and adrenoceptor beta 3 (rs4994) polymorphisms with high and low responders (LoRes) to fat mass reduction after 12 weeks of high-intensity interval training (HIIT) and dietary energy restriction in overweight/obese adult women. In addition, we examined the effect of these genetic variants on body composition changes.

Methods: Forty-three unrelated overweight/obese adult women were incorporated and genotyped, of which 30 women (age = 27.4 ± 7.9 years; BMI = 29.9 ± 3.3 kg/m^2) successfully completed the 12-week supervised HIIT program plus an individually prescribed home hypocaloric diet.

Results: An association was observed between the *PLIN1* rs1052700 polymorphism with high and LoRes (χ^2 = 8.138; 2 *df*; p = 0.01). Moreover, after the intervention, the carriers of TT genotype of *PLIN1* rs1052700 as compared to AA and AT showed a greater reduction in absolute fat mass (Δ: –5.1 ± 1.8 vs. – 1.8 ± 1.4 vs. – 2.1 ± 2.3 kg; p = 0.04). The effect size of this fat mass reduction between TT and AT genotypes was a mean difference of –3.01 kg [95%IC – 4.88– – 1.1], and between TT and AA genotypes was –3.29 kg [95%IC – 4.86– – 1.65]. No differences were observed for other polymorphisms investigated.

Conclusion: These results suggest that the rs1052700 (14995A>T) polymorphism of the *PLIN1* gene is associated with a differential response to fat mass reduction after a

12-week intervention in overweight/obese adult women. In addition, women with the TT genotype of this genetic variant showed greater changes in fat mass than AA and AT genotypes. However, further studies are needed to confirm these findings.

Keywords: genetic polymorphisms, exercise, high-intensity interval training, obesity, inter-individual variability, women

INTRODUCTION

Obesity and overweight have become major global health problems over the past few decades (Heymsfield and Wadden, 2017) because the expansion of white adipose tissue (adipocyte hypertrophy) and visceral adiposity leads to metabolic dysregulation as a result of their associated pro-inflammatory phenotype (Vazquez-Carrera, 2016; Hepler and Gupta, 2017). Importantly, there is a global gender disparity in obesity, with a higher prevalence in women than men in all world regions (Ahmad et al., 2016). Specifically, obese women have a higher risk of developing type 2 diabetes (T2D), and these women have a 44% greater risk of cardiovascular disease than men (Hu, 2003; Peters et al., 2014), as well as a higher risk of morbidity and mortality from T2D, cardiovascular diseases, cancer, and other obesity-related conditions (Ahmad et al., 2016). Behavioral interventions, such as exercise and nutrition, have been essential in managing obesity and overweight because of their contribution to reducing fat and body mass (Petridou et al., 2019). However, studies reporting individual responses to exercise have shown a wide range of responses to the interventions rather than a similar response (Bouchard and Rankinen, 2001; King et al., 2008; Scharhag-Rosenberger et al., 2012; Bonafiglia et al., 2016; Gurd et al., 2016; Parr et al., 2016; Alvarez et al., 2017; de Lannoy et al., 2017; Sparks, 2017; Williamson et al., 2017; Chrzanowski-Smith et al., 2019; Ross et al., 2019). Recent evidence shows that genetic and epigenetic factors could contribute significantly to the inter-individual variability in response to exercise interventions (Sparks, 2017; Williamson et al., 2017; Hagstrom and Denham, 2018). Regarding genetic factors, although some single-nucleotide polymorphisms (SNPs) in lipid metabolism-related genes have previously been associated with obesity and body composition, they have been scarcely described concerning the magnitude of the response to exercise interventions in the overweight/obese population. In the present study, four candidate gene polymorphisms related to lipid metabolism and fat mass response after exercise training were studied as: rs2304795 and rs1052700 variants of the perilipin 1 (*PLIN1*) gene, rs283 variant of the lipoprotein lipase (*LPL*) gene, and rs4994 variant of the Adrenoceptor beta 3 (*ADRB3*) gene. In this sense, *PLIN1* gene has recently emerged as a candidate gene to explain part of the inter-individual differences in cardiovascular and metabolic risk factors (Jenkins et al., 2010). The different isoforms of the perilipin protein encoded by the *PLIN* gene are proteins that coat lipid droplets and regulate intracellular lipolysis (Bickel et al., 2009). The *LPL* gene encodes the *LPL* enzyme, which plays a fundamental role in lipid metabolism since it is the rate-limiting enzyme for the hydrolysis of triglycerides (TG). For this reason, it has been related to plasma lipid levels and the development of obesity (Xie et al., 2010; Gao et al., 2015). In addition, an association has been found between the rs283 polymorphism of the *LPL* gene and blood lipids levels, with higher levels of high-density lipoprotein-cholesterol in the carriers of GG genotype (Sarzynski et al., 2011). This same GG genotype was more sensitive to reducing fat mass, insulin resistance, and plasma triglyceride level induced by 4 weeks of supervised physical exercise in Asian adolescents with obesity (Gao et al., 2015). *ADRB3* gene encoded the human β3-adrenergic receptor (β3AR), expressed mainly in adipose tissue, plays a role in determining the basal metabolic rate (BMR) through its ability to stimulate lipolysis and thermogenesis (Kahara et al., 2002). Therefore, a dysfunction in the β3AR may increase the risk of developing obesity and insulin resistance (Zhan and Ho, 2005). The rs4994 (Trp64Arg) polymorphism of the *ADRB3* gene has shown a tendency to have a low resting metabolic rate, abdominal obesity, insulin resistance, and the development of T2D. Therefore, it has been widely studied as a potential genetic factor associated with the development of obesity but with inconclusive results (Nakashima et al., 2013). A study in healthy young Japanese men found that subjects carrying the Arg64 allele of the *ADRB3* gene have a reduction in fatty acid oxidation at rest and during acute physical exercise (Morita et al., 2009). Thus, the present study aimed to evaluate the association of lipid metabolism-related polymorphisms (*LPL* rs283, *PLIN1* rs2304795, *PLIN1* rs1052700, and *ADRB3* rs4994) with a high or low response to fat mass reduction after 12 weeks of high-intensity interval training (HIIT) in overweight/obese adult women. Additionally, we examine whether body composition changes are influenced by these polymorphisms.

MATERIALS AND METHODS

Study Design and Participants

A group of unrelated adult overweight and obese women was studied. Participants were recruited from the community and referred by a physician to the supervised exercise program in our research center. The study was conducted following the Declaration of Helsinki Ethical Principles for Medical Research involving human subjects. Ethical approval for the study was provided by the Scientific Ethics Committee based at Universidad de La Frontera (Study Protocol N°112/16). All volunteers received information about the protocol and provided written consent before the beginning of the study.

The inclusion criteria were as: a) unrelated women aged 18–45 years; b) diagnosed with overweight or type 1 or 2 obesity [Body mass index (BMI) between 25 and 39.9 kg/m^2];

c) untrained (not involved in regular physical activity or exercise program during the previous 6 months); d) pre-menopausal women; and e) previously screened by a physician. The exclusion criteria were as: a) previously diagnosed diseases, such as diabetes mellitus, hypertension, myocardial infarction, and class III obesity; b) receiving pharmacologic corticoids, metformin, or other drugs that may affect metabolism; c) smoking habit; d) history of bariatric surgery; e) untreated hypothyroidism; and f) skeletal muscle disabilities or a specific indication to avoid exercise by medical reasons. A minimum of 70% (26/36 sessions) attendance at the exercise program was required for study participants to be included in the final statistical analyses. A structured medical history record and physical examination were performed on 105 adult women for enrolment purposes. Forty-three subjects met all the inclusion criteria and were finally studied. This study used a non-probabilistic sample aiming to separate high and low responders (LoRes) to a 12-week exercise program. Subjects who successfully completed the 12 weeks of intervention (N = 30) were classified as high or LoRes according to their greater or lesser reduction in fat mass, similar to previous studies that have also used clinical cutoff points for this classification (Milagro et al., 2013; Parr et al., 2016). Thus, high responders (HiRes) to fat mass reduction after the 12-week intervention were those individuals who were able to lose ≥10% of initial absolute fat mass (i.e., kilograms), and LoRes were individuals who lost <10%. Body composition, endurance performance, resting blood pressure, fasting glucose, and insulin were assessed before and after the 12-week follow-up. All the molecular analyses were performed after the 12-week intervention. The study design is shown in **Figure 1**, and the study protocol is shown in **Figure 2**.

Body Composition Assessment and Cardiovascular Measurements

The initial assessment was carried out to record socio-demographic, physical, and physiological characteristics. Body mass, absolute fat mass, and fat-free mass (FFM) were assessed with the subject barefoot, wearing underclothes, and no metal objects, using a Tanita™ foot-foot bioelectrical impedance analyzer (BIA) (Tanita Corporation, model BC-541, Japan). Its prediction formula has been previously validated against a four-compartment model, showing high reproducibility and a residual standard deviation of 3.3% for body fat in women (Jebb et al., 2000). Stature was measured without shoes to the nearest mm with a stadiometer (Seca model 213, Hamburg, Germany). BMI was calculated using the formula body mass divided by stature squared (kilograms per square meter). The systolic and diastolic blood pressures were determined using an automatic monitor (Omron HEM-7114; Omron Healthcare) in duplicate and after 15 min of complete rest with the subjects in a supine position.

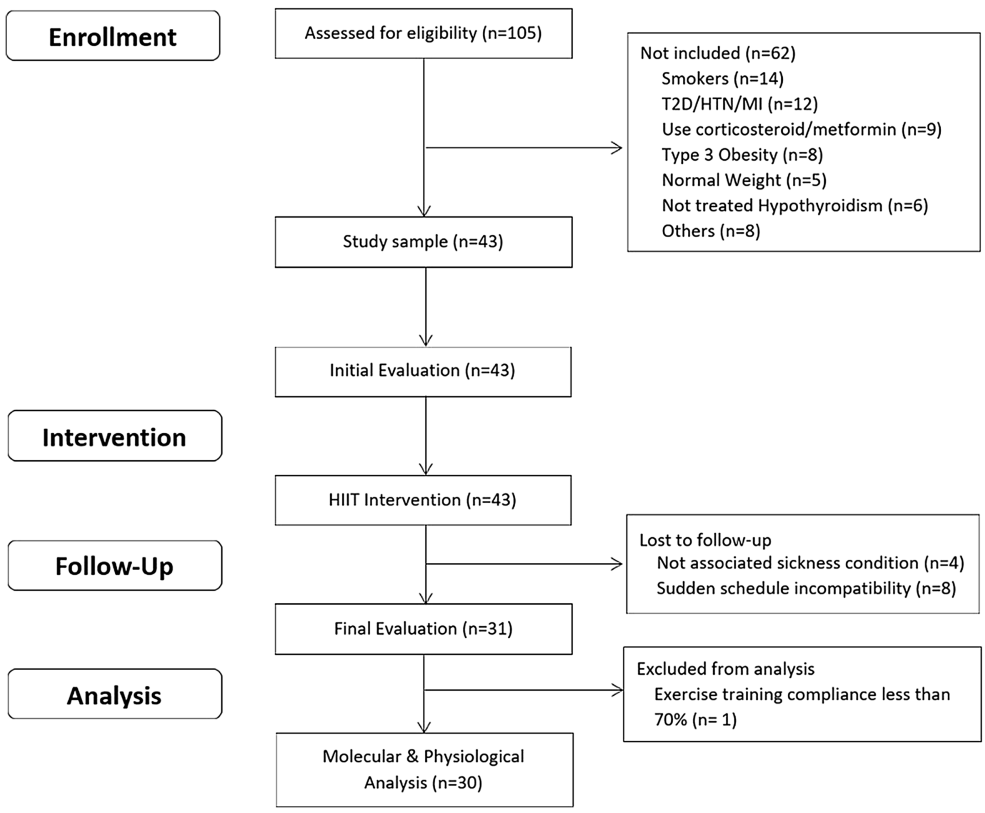

FIGURE 1 | Study design.

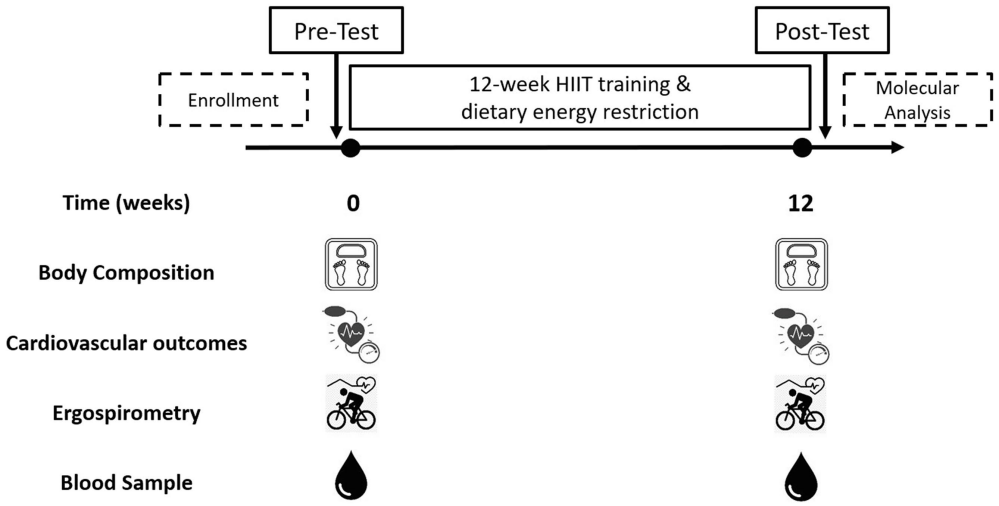

FIGURE 2 | Study protocol. Pre-test: initial assessments; Post-test: final assessments.

Endurance Performance Assessment

Endurance performance was assessed 1 week before and after the 12-week intervention during an incremental exercise test designed to obtain peak oxygen consumption (VO_2peak). In brief, the VO_2peak test consisted of free-wheel pedaling for 2 min at 70–80 RPM on a cycle ergometer (Lode Corival, Groningen, The Netherlands), followed by an initial 50 watts load for 2 min and 25 watts increments every 2 min until the participant reached volitional fatigue. Gas exchange was collected throughout the test using an indirect calorimeter/ergospirometer system (Ultima CPX™ metabolic system, Medgraphics, Minnesota, United States), calibrated (gas and volume) before the exercise test. Measurements performed were the peak power output (PPO), anaerobic threshold (AT), respiratory exchange ratio (RER), peak oxygen pulse (O_2 pulse; VO_2peak/HRmax during the exercise test), ventilation (VE), and respiratory rate (RR). Heart rate (HR) was monitored with a continuous telemetric HR sensor (Polar model V800, Finland) throughout the whole test.

Exercise Training Intervention

Participants performed a 12-week supervised HIIT program, with training sessions three times a week on non-consecutive days. The exercise session consisted of 1-min cycling at a high intensity (workload during each interval was set to achieve muscle failure at the end of 1-min exercise period and reaching ~85–100% maximal heart rate obtained during the incremental exercise test), followed by a 2-min inactive resting period (sitting on the cycle ergometer), and repeated 10 times (1x2x10 protocol; 1:2:10 to work: rest: repetitions, respectively). In summary, the total duration of one session of the 1x2x10 protocol was 30 min, with 10 min of effective exercise training, and without a warm-up or cool-down period. All exercise sessions were individually supervised to achieve muscle fatigue at each exercise interval as the primary indicator of intensity together with a continuous heart rate monitoring (Polar V800, PolarTM, Finland) in order to supervise that the chronotropic response was the expected according to previously found with the same HIIT exercise protocol (Andrade-Mayorga et al., 2020). Load progression was defined as the gradual increase in workload developed when the subject failed to reach muscle failure at the end of the 60-s exercise interval, monitored individually on a series-by-series and session-by-session basis. Thus, the load progression increased in parallel to the increment in the work capacity of each individual.

Dietary Assessment and Hypocaloric Diet

Participants were instructed to follow an individually designed hypocaloric diet [75% of estimated energy requirements (EERs)], equivalent to 1354 ± 114.5 kcal/day, throughout the 12-week study period to control the dietary intake. A 24-h diet recall and a modified food choice questionnaire (7 days) were applied at baseline (Salvador Castell et al., 2015). Total energy expenditure (TEE) was estimated using the factorial method based on their reported daily physical activity (FAO/WHO/UNU, 2004; Levine, 2005). In brief, BMR was estimated using the Mifflin-St. Jeor equation (Cancello et al., 2018), additional caloric requirements were determined based on each subject's physical activity level (PAL), which were used to calculate the PAL index, TEE, and the 75% EER. Subjects had individual monthly meetings with the nutritionist during the 3-month intervention to encourage compliance. Dietary compliance was assessed using an instrument adapted from the Perceived Self-Regulatory Success in Dieting Scale (Meule et al., 2012), where each subject's perceived adherence was quantified using a five-point Likert scale. The nutritional intervention excluded the use of nutritional supplements.

Blood Analyses

Blood samples (5 ml) were collected to analyze plasma glucose and insulin in the early morning after 12 h overnight fasting, and they were immediately placed on ice and centrifuged at

3000 rpm for 15 min at −4°C. Plasma samples were directly transferred to pre-chilled microtubes and stored at −20°C for later analysis. Fasting plasma glucose was analyzed by enzymatic colorimetric methods using an auto-analyzer (Wiener Metrolab 2300, Wiener Lab, Argentina). The fasting insulin was analyzed by ELISA using the Human Insulin ELISA Kit (Catalog # KAQ1251, Invitrogen, Thermo Fisher Scientific Inc., Waltham, MA, United States).

DNA Genotyping

Genomic DNA was extracted from blood leukocytes by optimized salting out procedure (Salazar et al., 1998). Genotyping of *LPL* rs283, *PLIN1* rs2304795, *PLIN1* rs1052700 (14995A>T), and *ADRB3* rs4994 polymorphisms were performed by real-time polymerase chain reaction (qPCR), using TaqMan® SNP Genotyping Assays (Life Technologies, CA, United States). PCR assays contained 12.5 µl of TaqMan® Genotyping Master Mix (2X; Life Technologies CA, United States), 1.25 µl of TaqMan® SNP Genotyping Assay (20X; catalog numbers: 4351379, 4351379, 4351379, and 4351379), and 2 µl of DNA (25 ng) diluted in nuclease-free water. The thermal cycling protocol was initiated with a cycle for 10 min at 95°C and followed by 40 cycles at 95°C for 15 s and 60°C for 1 min using standard conditions for a real-time system (Life Technologies). Genotyping was performed using the allelic discrimination plot issued after PCR amplification in the StepOne software v. 2.2 (Life Technologies). No template controls were included per triplicate in each genotyping experiment plate. Genotyping was randomly repeated on 20% of the samples for quality control purposes without finding differences.

Statistical Analysis

GraphPad Prism statistical software 7.0 (San Diego, CA, United States) was used. Chi-square test (χ^2) was used to analyze differences in genotype distribution and allelic frequencies and verify Hardy–Weinberg equilibrium. The normal distribution of all the variables was tested using the D'Agostino-Pearson test. All the continuous variables were expressed as mean ± standard deviation (SD). The differences between quantitative variables were analyzed by paired *t*-test for paired data (intra-group differences before and after intervention) or unpaired *t*-test for independent groups (between-groups difference within time points). Cumming estimation plots, which show individual values, means, and effect size with a 95% CI, were developed using Estimation Statistics for Data Visualization (Ho et al., 2019). Kruskal-Wallis test was used to compare the body composition changes among the different genotypes. Differences between two specific genotypes were evaluated with the Mann-Whitney *U* test. The level of significance used in all the comparisons was $p < 0.05$.

RESULTS

Forty-three unrelated overweight/obese adult women volunteered for the study, of which 30 women (age = 27.4 ± 7.9 yrs; BMI = 29.9 ± 3.3 kg/m^2) successfully completed the 12-week intervention and were finally analyzed. All participants well tolerated the exercise program, and there were no injuries during the intervention. Demographic, physical, and physiological variables measured before and after the intervention of the HiRes and LoRes groups are presented in **Table 1**. At baseline, the LoRes and HiRes groups are similar in age (27.6 ± 8.6 vs. 27.2 ± 7.2 years; p = 0.897), PAL index (1.38 ± 0.08 vs. 1.41 ± 0.09; p = 0.143), BMR (1,283 ± 55.7 vs. 1,314 ± 48.1 kcal/day; p = 0.437), TEE (1778 ± 159.9 vs. 1852 ± 133.4 kcal/day; p = 0.140), and 75% EER (1,333 ± 119.9 vs. 1,389 ± 100.1 kcal/day; p = 0.140; **Table 1**). Diet compliance measured after the intervention was similar between groups (3.3 ± 0.5 vs. 3.6 ± 0.5 points; p = 0.3211; **Table 1**). The prevalence for high (HiRes) and low (LoRes) responders to absolute fat mass reduction was 33% (n = 11) and 66% (n = 19), respectively. The intervention applied in this study effectively improved several variables, showing significant changes in both groups (LoRes and HiRes) in reducing body mass, absolute fat mass (kg), % body fat, and BMI. In addition, the 12-week intervention was effective in increasing VO_2peak and VO_2peak relative to lean mass (VO_2peak/FFM) similarly in both groups (LoRes and HiRes; **Table 1**). FFM did not change post-intervention in either group (**Table 1**).

However, it is noteworthy that the reduction in absolute fat mass (kg) in HiRes was 18.3% (29.8 ± 8 vs. 25.2 ± 6.9 kg; p<0.0001) and LoRes was 3.6% (31.6 ± 6.9 vs. 30.5 ± 6.7 kg; p<0.0001; **Table 1**). The effect size of this reduction in absolute fat mass was −4.65 kg [95.0%CI − 5.66– − 3.88] for the HiRes group and was −1.09 kg [95.0%CI − 1.49– − 0.705] for the LoRes group (**Figure 3**). When comparing the magnitude of change (Δ) in absolute fat mass between the LoRes and HiRes groups, this difference was statistically significant (−1.1 ± 0.9 vs. −4.7 ± 1.6 kg; $p < 0.0001$; **Table 1**). Similarly, differences were found between LoRes and HiRes in the magnitude of changes (Δ) in body mass (−1.0 ± 0.9 vs. −4.8 ± 2.7 kg; $p < 0.0001$), % body fat (−0.9 ± 0.9 vs. −3.8 ± 1.1%; $p < 0.0001$), and BMI (−0.4 ± 0.4 vs. − 1.8 ± 1.0 kg/m^2; p = 0.01) following the 12-week intervention (**Table 1**).

Physical performance, physiological, and cardiovascular variables measured pre- and post-12-week intervention of the LoRes and HiRes are listed in **Table 2**. Concerning performance indicator variables during the incremental exercise test, there were improvements in most variables measured post-intervention, but no differences between LoRes and HiRes groups. There were increases in both groups in peak oxygen pulse (Peak O_2pulse; LoRes: 10.2 ± 1.6 vs. 11.4 ± 1.6 ml/min; p = 0.0002/ HiRes: 10.4 ± 1.2 vs. 11.8 ± 1. 7 ml/min; p = 0.0002), (PPO; LoRes: 126.3 ± 17.6 vs. 156.6 ± 24.8 W; p = 0.0001/HiRes: 131.8 ± 16.2 vs. 165.9 ± 23. 1 W; p = 0.0002), and anaerobic threshold (AT; LoRes: 57.4 ± 11.7 vs. 69.1 ± 16.6 W; p = 0.0068/ HiRes: 59.1 ± 12.6 vs. 70.5 ± 10.1 W; p = 0.03). In addition, in the LoRes group, maximum ventilation (VEmax; 63.5 ± 9.7 vs. 77.4 ± 14.1 ml/min; p = 0.001) and maximum respiratory rate (RRmax; 36.7 ± 6.9 vs. 40.0 ± 5.4 cycles/min; p = 0.01) increased (**Table 2**). In relation

TABLE 1 | Demographic, physical, and physiologic variables measured before and after intervention by low (LoRes) and high (HiRes) responders to fat mass reduction after the 12-week intervention.

	Low Responders (n = 19)			High Responders (n = 11)			Intra-group p-value (pre vs. post)*		Between groups p-value (within same time point)#		P-value Δ¥
	Pre	Post	Δ	Pre	Post	Δ	LoRes	HiRes	Pre	Post	
Age (years)	27.6 ± 8.6	–	–	27.2 ± 7.2	–	–	–	–	NS	–	–
PAL index	1.38 ± 0.08	–	–	1.41 ± 0.09	–	–	–	–	NS	–	–
BMR (kcal/day)	1,283 ± 55.7	–	–	1,314 ± 48.1	–	–	–	–	NS	–	–
TEE (kcal/day)	1778 ± 159.9	–	–	1852 ± 133.4	–	–	–	–	NS	–	–
75% EER (kcal/day)	1,333 ± 119.9	–	–	1,389 ± 100.1	–	–	–	–	NS	–	–
Diet compliance (pts)	–	3.3 ± 0.5	–	–	3.6 ± 0.5	–	–	–	–	NS	–
Body weight (kg)	77.2 ± 9.8	76.2 ± 9.7*	−1.0 ± 0.9	76.1 ± 11.1	71.3 ± 9.9*	−4.8 ± 2.7¥	**0.0004**	**0.0001**	NS	NS	**<0.0001**
Fat mass (kg)	31.6 ± 6.9	30.5 ± 6.7*	−1.1 ± 0.9	29.8 ± 8	25.2 ± 6.9*,#	−4.7 ± 1.6¥	**<0.0001**	**<0.0001**	NS	**0.04**	**<0.0001**
% Body fat (%)	40.5 ± 4	39.6 ± 3.9*	−0.9 ± 0.9	38.6 ± 4.9	34.7 ± 5.1*,#	−3.8 ± 1.1¥	**0.0003**	**<0.0001**	NS	**0.0068**	**<0.0001**
BMI (kg/m^2)	30.6 ± 3.3	30.2 ± 3.3*	−0.4 ± 0.4	28.8 ± 3	27 ± 2.8*,#	−1.8 ± 1.0¥	**0.0003**	**0.0001**	NS	**0.01**	**<0.0001**
FFM (kg)	45.6 ± 3.5	45.8 ± 3.5	0.1 ± 0.7	46.3 ± 3.5	46.2 ± 3.7	−0.1 ± 1.8	NS	NS	NS	NS	NS
VO2peak (ml/min)	1750 ± 230	2051 ± 332*	301.2 ± 240.2	1888 ± 266	2,169 ± 322*	281.1 ± 177.3	**<0.0001**	**0.0004**	NS	NS	NS
VO2peak/FFM (ml kg^{-1} min^{-1})	38.3 ± 3.6	44.7 ± 5.6*	6.4 ± 5.0	40.7 ± 3.9	46.9 ± 5.4	6.2 ± 4.6	**<0.0001**	**0.001**	NS	NS	NS

*intra-group difference pre- vs. post-intervention.

#between-groups difference within same time points.

¥difference between average delta values.

Data are presented as mean ± SD. LoRes, low responders; HiRes, high responders; PAL, physical activity level; BMR, basal metabolic rate; TEE, total energy expenditure; EER: estimated energy requirement; BMI, body mass index; FFM, fat-free mass; and VO_2peak, maximal oxygen consumption. Bold values denote significant differences.

FIGURE 3 | Cumming plot with individual responses to decrease absolute fat mass after the 12-week intervention by low (left) and high (right) responders. The raw data (kg) are plotted on the upper axes; each paired set of observations is connected by a line. On the lower axes, each paired mean difference is plotted as a bootstrap sampling distribution. Mean differences are depicted as dots; 95% confidence intervals are indicated by the ends of the vertical error bars. LoRes-1, initial assessment of low responders; LoRes-2, final assessment of low responders; HiRes-1, initial assessment of high responders; and HiRes-2, final assessment of high responders.

to cardiovascular variables at rest, there was a decrease of diastolic blood pressure (LoRes: 73.2 ± 8.7 vs. 74.2 ± 5.2 mmHg; p = 0.001/HiRes: 74.2 ± 5.2 vs. 70.6 ± 5.8 mmHg; p = 0. 005) and mean blood pressure (LoRes: 85.7 ± 7.9 vs. 80.7 ± 7.3 mmHg; p = 0.001/HiRes: 85.8 ± 6.2 vs. 82.4 ± 6.6 mmHg; p = 0.002) in LoRes and HiRes groups, and a reduction of systolic blood pressure only in the LoRes group (110.9 ± 8.4 vs. 104.7 ± 9.1 mmHg; p = 0.03; **Table 2**). Importantly, all these changes occurred, although none of the study participants had arterial hypertension. About metabolic parameters, no differences were found in fasting glycemia, but there was an inter-group difference in blood fasting insulin after the intervention, where the LoRes group showed higher values than the HiRes group (11.9 ± 2.3 vs. 9.4 ± 2.2.2 μU/dl; p = 0.04; **Table 2**).

Genotypes distribution and relative frequency of alleles for *PLIN1* rs1052700, *PLIN1* rs2304795, *LPL* rs283, and *ADRB3* rs4994 gene polymorphisms are shown in **Table 3**. All polymorphisms evaluated were in Hardy-Weinberg equilibrium (**Table 3**).

TABLE 2 | Physical performance, cardiovascular, and metabolic variables measured before and after intervention by low (LoRes) and high (HiRes) responders to fat mass reduction after the 12-week intervention.

	Low Responders (n = 19)			High Responders (n = 11)			Intra-groups p-value (pre vs. post)		Between-groups P-value (within time point)		P-value Δ[¥]
	Pre	Post	Δ	Pre	Post	Δ	LoRes	HiRes	Pre	Post	
Peak O2 Pulse (ml/min)	10.2 ± 1.6	11.4 ± 1.6[*]	1.2 ± 1.1	10.4 ± 1.2	11.8 ± 1.7[*]	1.4 ± 0.8	**0.0002**	**0.0002**	NS	NS	NS
PPO (W)	126.3 ± 17.6	156.6 ± 24.8[*]	30.3 ± 13.4	131.8 ± 16.2	165.9 ± 23.1[*]	34.1 ± 20.2	**0.0001**	**0.0002**	NS	NS	NS
AT (W)	57.4 ± 11.7	69.1 ± 16.6[*]	10.5 ± 15.2	59.1 ± 12.6	70.5 ± 10.1[*]	11.4 ± 13.1	**0.0068**	**0.03**	NS	NS	NS
VEmax (ml/min)	63.5 ± 9.7	77.4 ± 14.1[*]	13.9 ± 13.0	71.3 ± 21.3	76.9 ± 13.5	5.6 ± 16.6	**0.001**	NS	NS	NS	NS
RRmax (breath/min)	36.7 ± 6.9	40.0 ± 5.4[*]	3.3 ± 5.6	37.4 ± 10.6	37.8 ± 5.6	0.5 ± 6.6	**0.01**	NS	NS	NS	NS
RERmax	1.27 ± 0.1	1.28 ± 0.1	0.01 ± 0.15	1.28 ± 0.1	1.27 ± 0.1	0.02 ± 0.17	NS	NS	NS	NS	NS
Systolic BP (mmHg)	110.9 ± 8.4	104.7 ± 9.1[*]	−6.7 ± 4.5	108.9 ± 9.4	106 ± 9.8	−3.4 ± 2.1[*]	**0.03**	NS	NS	NS	**0.03**
Diastolic BP (mmHg)	73.2 ± 8.7	68.7 ± 7.6[*]	−5.1 ± 6.8	74.2 ± 5.2	70.6 ± 5.8[*]	−4.1 ± 3.7	**0.001**	**0.005**	NS	NS	NS
Mean BP (mmHg)	85.7 ± 7.9	80.7 ± 7.3[*]	−5.6 ± 5.4	85.8 ± 6.2	82.4 ± 6.6[*]	−3.9 ± 2.8	**0.001**	**0.002**	NS	NS	NS
Pulse pressure (mmHg)	37.7 ± 7.2	36.1 ± 7.8	−1.6 ± 6.1	34.7 ± 6.4	35.4 ± 7	0.7 ± 3.2	NS	NS	NS	NS	NS
Fasting glucose (mg/dl)	91.2 ± 10.1	89.4 ± 9.3	−1.8 ± 7.7	89.5 ± 7.2	89.1 ± 7.5	−0.4 ± 6.7	NS	NS	NS	NS	NS
Fasting insulin (μU/dl)	11.9 ± 2.3	11.9 ± 1.6	−5.0 ± 6.8	10.7 ± 2.6	9.4 ± 2.3[*]	−6.4 ± 6.2	NS	NS	NS	**0.04**	NS

[*]*intra-group difference pre- vs. post-intervention.*
[¶]*between-groups difference within same time point.*
[¥]*difference between average delta values.*
Data are presented as mean ± SD. O_2 pulse, oxygen pulse; PPO, peak power output; AT, anaerobic threshold; VEmax, peak ventilation; RRmax, peak respiratory rate; RERmax, peak respiratory exchange ratio; and BP, blood pressure.
Bold values denote significant differences.

An association between the distribution of *PLIN1* rs1052700 genotypes polymorphism with LoRes and HiRes groups was found (χ^2 = 8.138; 2 *df*; p = 0.01; **Table 4**). The OR was 2.8 [95%CI 0.93–8.45] for being LoRes among A allele carriers. However, when performing an analysis with a genetic dominance model, comparing AA+AT vs. TT genotypes, we found significant differences between groups evaluated with a Fisher's exact test (p = 0.012) and an RR of 1.571 [95%CI 1.57–1.63], which could be interpreted as an increased relative risk of subjects carrying AA or AT genotypes to be classified as "low responders" for their post-intervention fat mass reduction. On the other hand, no associations in the distribution of genotypes or allele frequency for *PLIN1* rs2304795, *LPL* rs283, or *ADRB3* rs4994 polymorphisms were found (**Table 4**).

Following the 12-week intervention, carriers of the TT genotype of the rs1052700 variant of the *PLIN1* gene, compared to AA and AT genotypes, showed a greater reduction in absolute fat mass (Δ: − 5.1 ± 1.8 vs. − 1.8 ± 1.4 vs. − 2.1 ± 2.3 kg; p = 0.04; **Table 5**). The effect size of this fat mass reduction between TT vs. AT genotypes was a mean difference of −3.01 kg [95%IC − 4.88– − 1.1], and when comparing the TT vs. AA genotypes, the effect size was −3.29 kg [95%IC − 4.86– − 1.65]. No differences were found in modifications of body mass, BMI, FFM, maximal oxygen consumption (VO_2peak), or VO_2peak relative to FFM (VO_2peak/FFM) when comparing the different genotypes of the rs1052700 variant of the *PLIN1* gene (**Table 5**). Finally, no differences were found in the modifications of the variables studied after the intervention when comparing the genotypes of the *PLIN1* rs2304795, *LPL* rs283, and *ADRB3* rs4994 polymorphisms.

DISCUSSION

We evaluated the association of *PLIN1* (rs1052700 and rs2304795), *LPL* (rs283), and *ADRB3* (rs4994) polymorphisms with high and LoRes to fat mass reduction after 12 weeks of HIIT and dietary energy restriction in overweight/obese adult women. Additionally, we examined whether body composition changes are influenced by these polymorphisms.

In our study, the LoRes and HiRes groups had multiple improvements in body composition and cardiorespiratory fitness after 12 weeks of intervention, where both groups decreased their fat mass, % body fat, body mass, and BMI (**Table 1**). In addition, they increased their absolute VO_2peak and VO_2peak relative to lean mass (**Table 1**). About changes in body composition, the reductions in absolute fat mass, body mass, % body fat, and BMI, were of greater magnitude in the HiRes group (**Table 1**), which is consistent with the research design where the primary criterion for classification of low and HiRes was the reduction in absolute fat mass (i.e., kilograms) less than or greater than 10%, respectively. Thus, our results showed a differential absolute fat mass modification, reducing 18.3 and 3.6% for HiRes and LoRes, respectively. All these improvements are consistent with that reported in the meta-analysis by Turk et al. (2017), showing multiple benefits of HIIT compared to traditional exercise on health-related physiological parameters

TABLE 3 | Genotype distribution and relative allelic frequencies for studied lipid metabolism-related polymorphisms in all subjects (*N* = 43).

Gene/Polymorphism	Genotypes			Alleles		H-W
PLIN1/rs1052700	AA: 47% (20)	AT: 42% (18)	TT: 12% (5)	A: 0.674 (58)	T: 0.326 (28)	χ^2 = 0.094, 1 *df*, *p* = 0.759
PLIN1/rs2304795	AA: 44% (19)	AT: 44% (19)	TT: 12% (5)	A: 0.663 (57)	T: 0.337 (29)	χ^2 = 0.005, 1 *df*, *p* = 0.939
LPL/rs283	CC: 63% (27)	CT: 37% (16)	TT: 0% (0)	C: 0.814 (70)	T: 0.186 (16)	χ^2 = 2.247, 1 *df*, *p* = 0.134
ADRB3/rs4994	AA: 72% (31)	AG: 21% (9)	GG: 7% (3)	A: 0.826 (71)	G: 0.174 (15)	χ^2 = 3.210 1 *df*, *p* = 0.073

H-W, Hardy–Weinberg equilibrium. Number in parenthesis indicates number of individuals from the total sample studied (N = 43).

TABLE 4 | Genotype distribution and relative allelic frequencies for studied lipid metabolism-related polymorphisms by low and HiRes to fat mass reduction after the 12-week intervention (*N* = 30).

Gene/Polymorphism	Genotype and Allele	Low Responders (*N* = 19)	High Responders (*N* = 11)	*P*-value
PLIN1/rs1052700 *	A/A A/T T/T	47% (9) 53% (10) 0% (0)	36% (4) 28% (3) 36% (4)	χ^2 = 8.138; 2 *df*; **p = 0.01***
	A T	0.737 (28) 0.263 (10)	0.500 (11) 0.500 (11)	χ^2 = 3.436; 1 *df*; *p* = 0.063
PLIN1/rs2304795	A/A A/G G/G	42% (8) 37% (7) 21% (4)	36% (4) 64% (7) 0% (0)	χ^2 = 3.445; 2 *df*; *p* = 0.179
	A G	0.605 (23) 0.395 (15)	0.682 (15) 0.318 (7)	χ^2 = 0.352; 1 *df*; *p* = 0.553
LPL/rs283	C/C C/T T/T	63% (12) 37% (7) 0% (0)	73% (8) 27% (3) 0% (0)	χ^2 = 0.287; 1 *df*; *p* = 0.592
	C T	0.816 (31) 0.184 (7)	0.864 (19) 0.136 (3)	χ^2 = 0.229; 1 *df*; *p* = 0.632
ADRB3/rs4994	A/A A/G G/G	79% (15) 16% (3) 5% (1)	82% (9) 18% (2) 0% (0)	χ^2 = 0.61; 2 *df*; *p* = 0.737
	A G	0.868 (33) 0.132 (5)	0.909 (20) 0.091 (2)	χ^2 = 0.224; 1 *df*; *p* = 0.636

**genotype distribution differences. PLIN1, perilipin 1 protein coding gene; ADRB3, adrenoceptor beta 3 protein coding gene; LPL, lipoprotein lipase protein coding gene; and df, degree of freedom. Number in parenthesis indicates number of individuals from by low- and high-responders' groups. Bold values denote significant differences.*

TABLE 5 | Body composition and cardiorespiratory fitness deltas after 12-week exercise intervention according to genotypes for rs1052700 PLIN1 gene polymorphism.

	Genotype AA (*N* = 13)	Genotype AT (*N* = 13)	Genotype TT (*N* = 4)	*P*-value
Δ Body mass (kg)	−1.9 ± 2.1	−1.9 ± 2.4	−5.4 ± 3.1*,#	0.07
Δ Fat mass (kg)	−1.8 ± 1.4	−2.1 ± 2.3	−5.1 ± 1.8*,#	**0.04**
Δ Body fat (%)	−1.6 ± 1.4	−1.7 ± 1.7	−4.1 ± 1.6*,#	0.05
Δ BMI (kg/m^2)	−0.8 ± 0.7	−0.7 ± 0.9	−2.1 ± 1.2*,#	0.06
Δ FFM (kg)	−0.1 ± 1.3	+0.2 ± 1.1	−0.3 ± 1.7	0.76
Δ VO2peak (ml/min)	231.3 ± 197.5	335.9 ± 240	360.3 ± 187.2	0.39
Δ VO2peak/FFM (ml/kg/min)	5.3 ± 4.8	6.9 ± 5.1	8.2 ± 3.2	0.46

**Difference between TT and AA genotypes evaluated with Mann-Whitney U test.*
#Difference between TT and AT genotypes evaluated with Mann-Whitney U test.
Data are presented as mean ± SD. Δ, magnitude of change after the intervention; BMI, body mass index; and FFM, fat-free mass. Bold values denote significant differences evaluated with the Kruskal-Wallis test.

in adults with obesity (Turk et al., 2017), and with the systematic review and meta-analysis by Viana et al. (2019) that showed a 28.5% greater reduction in absolute fat mass (kg) with HIIT interventions compared to MICT (Viana et al., 2019). In addition, recent systematic reviews and meta-analyses have shown more significant benefits in reducing fat mass and controlling body mass with interventions that combine exercise and nutrition (Johns et al., 2014; Clark, 2015), which is consistent with our results.

Regarding the improvements in VO_2max and exercise performance variables during the incremental exercise test in both LoRes and HiRes groups (**Table 2**), this is consistent with previously published studies, where endurance performance has improved in women after a similar 16-week HIIT program (Alvarez et al., 2018). There is strong evidence demonstrating increases in VO_2max following HIIT interventions (Gibala et al., 2012; Milanović et al., 2015), which is corroborated by the findings in our cohort of obese women. Clark et al. (2019) reported increases in VO_2max following a 6-week HIIT intervention in obese women but showed no changes in peak O_2 pulse, PPO, AT, RER, or VE (Clark et al., 2019). However, it should be considered that being a short duration program may not be sufficient to achieve these exercise-associated adaptations.

About cardiovascular changes, the reductions in blood pressure in both groups (**Table 2**), independent of being non-hypertensive subjects, are consistent with what has been previously reported in older women following 10-week resistance training (Nascimento et al., 2018) but inconsistent with other studies showing changes only in the group of hypertensive subjects following similar HIIT interventions (Olea et al., 2017; Álvarez et al., 2018). Therefore, we could hypothesize that this decrease in blood pressure values in normotensive women occurs due to the reduction of their fat mass, as there are studies that show the association between higher adiposity and increased blood pressure levels in different populations (Malden et al., 2019).

The genotypes distribution and relative frequency of alleles found for the *PLIN1* rs1052700, *PLIN1* rs2304795, *LPL* rs283, and *ADRB3* rs4994 polymorphisms (**Table 3**), to the best of our knowledge, are the first reports in the Chilean population. It is noteworthy that an association was found in the distribution of genotypes of the rs1052700 polymorphism of the *PLIN1* gene with the LoRes and HiRes groups, wherein the LoRes group there was no presence of the TT genotype (**Table 4**).

Previously, the *PLIN1* rs1052700 polymorphism has been associated with body fat and waist circumference in Caucasian women but not men (Qi et al., 2004). Additionally, this same polymorphism was associated with T2D but not obesity in the Iranian population (Saravani et al., 2017) and increased risk of diabetes in Chinese adults with elevated waist circumference (Yu et al., 2013).

Among the most remarkable findings of the present study, we have the greater reduction in absolute fat mass shown by the carriers of the TT genotype of the rs1052700 variant of the *PLIN1* gene compared to the AA and AT genotypes (**Table 5**). These results are concordant with that found by Aller et al. (2017), where they reported an association between the TT genotype of the *PLIN1* rs1052700 polymorphism and the reduction of body mass ≥5% following 3 months of a multidisciplinary intervention with dietary advice, psychological counseling, and increased physical activity in a Caucasian obese population (Aller et al., 2017). In addition, a haplotype of two SNPs of the *PLIN1* gene, 13041A>G (rs2304795) and 14995A>T (rs1052700), has previously been associated with obesity risk (Qi et al., 2004) and with response to a 6-month endurance exercise intervention (Jenkins et al., 2010). The *PLIN1* 13041A/14995A haplotype which is present in ~ 50–55% of the white Caucasian population and has been associated with a better cardiorespiratory phenotype, body composition, and metabolism before and after an aerobic exercise intervention in Caucasian subjects compared to non-AA haplotype subjects (Jenkins et al., 2010). The Jenkins et al. (2010) study shows that a 6-month endurance exercise intervention improved body composition in AA and non-AA haplotype groups, but the elevated fat mass was maintained in non-AA carriers after training (Jenkins et al., 2010). Conversely, in our study, the highest magnitude of response to reduce absolute fat mass had by subjects of the TT genotype. It was also observed that, although no significant differences, there is a tendency to find greater reductions in body mass, % body fat, and BMI in the TT genotype (**Table 5**), which should be explored in larger sample sizes.

Finally, we know that the different isoforms of the perilipin are proteins that coat lipid droplets and regulate intracellular lipolysis (Bickel et al., 2009). Their phosphorylation state regulates access to triglycerides (TG) in the lipid droplet core by lipases, such as hormone-sensitive lipase and adipose triglyceride lipase, where TG hydrolysis increases with perilipin phosphorylation (Brasaemle et al., 2000). Therefore, we could hypothesize that this higher response of subjects with TT genotype could be due to a differential effect of this genotype on the phosphorylation/dephosphorylation state of perilipin. To confirm this hypothesis, future mechanistic studies that take into account these genotypes are required.

The strengths of our study include the rigorous design of the exercise protocol monitored individually on a series-by-series and session-by-session basis during the 12-week program and the individually prescribed home hypocaloric diet. Another strength was using real-time quantitative PCR for SNP genotyping. On the other hand, one of its main limitations was the small sample size, which prevents us from presenting more solid conclusions. However, this did not restrict the identification of differential response to fat mass reduction after the 12-week intervention according to *PLIN1* rs1052700 genotypes. Therefore, our preliminary data should be interpreted in light of the limited cohort evaluated. Other limitations were the lack of a non-intervention control group and the use of a BIA to measure body composition variables, as this is not considered the "gold standard" method. However, BIA has a demonstrated reliability which is crucial for the pre-post comparisons.

In conclusion, these results suggest that the rs1052700 (14995A>T) polymorphism of *PLIN1* is associated with a differential response to fat mass reduction after a 12-week intervention in overweight/obese adult women. In addition, women with the TT genotype of this genetic variant showed greater changes in fat mass than AA and AT genotypes. However, further studies are needed to confirm these findings.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Scientific Ethics Committee, Universidad de La Frontera, Temuco, Chile. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors have read the manuscript and agreed with the content. OA-M, ED, and LS conceived and designed the study. OA-M performed the intervention and experiments and wrote the paper. OA-M and ED analyzed the data. ED and LS contributed the reagents/materials and analysis tools and reviewed and edited the manuscript.

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HYPOTHESIS AND THEORY
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A Hypothesis: The Interplay of Exercise and Physiological Heterogeneity as Drivers of Human Ageing

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As the inherent ageing process affects every facet of biology, physiology could be considered as the study of the healthy human ageing process. Where biological health is affected by lifestyle, the continual and continuing interaction of this process with physical activity and other lifestyle choices determine whether the ageing trajectory is toward health or disease. The presentation of both these states is further modified in individuals by the interaction of inherent physiological heterogeneity and the heterogeneity associated with responses and adaptions to exercise. The range of heterogeneity in healthy physiology is circumscribed by the necessity to conform to that of the human species. Our hypothesis is that, when sufficient exercise is present, these multiple interactions appear to produce an ageing profile that, while functional ability is in decline, remains synchronous, coherent, and integrated throughout most of life. In the absence of sufficient physical activity, physiology over time is gradually deteriorating toward the production of a lifestyle disease. Here, the ageing process, interacting with individual physiological heterogeneity, probably determines the age of presentation of a disease as well as the order of presentation of subsequent diseases. In this article, we discuss this hypothesis and related concepts in the context of the trajectory of healthy and non-healthy human ageing.

Keywords: ageing, exercise, health, physiology, lifestyle

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INTRODUCTION

A common belief has been that "to grow old is to be sick" (Rowe and Kahn, 1999), but for a large segment of the older population, when the link between age and health decline is evaluated, chronological age is not a relevant marker for understanding, measuring, or experiencing healthy ageing (Yang and Lee, 2010; Lowsky et al., 2014).

The WHO defines healthy ageing as "the process of developing and maintaining the functional ability that enables wellbeing in older age" (WHO, 2015). While there are multiple definitions of healthy ageing, for the purposes of this article, we define it as being free of the diseases that are largely due to lifestyle influences. These lifestyle factors include physical inactivity/lack of exercise/sedentary behavior, poor nutrition, smoking and alcohol consumption, and which can result in cardiovascular disease, type II diabetes, and some cancers. However, healthy ageing can also be influenced by multiple factors including gender, race, income, educational level, and socioeconomic environment (Braveman, et al., 2011). In the arena circumscribed

by diseases affiliated to lifestyle, this definition of healthy ageing encompasses the parameters as defined by the WHO definition of health (WHO, 2015). Healthy ageing is thus par excellence, reflected in the maintenance of whole-body integrated function (Lazarus and Harridge, 2017) despite an overall decline in physical capability and intrinsic capacity. In addition, while acknowledging that differences between definitions for "physical activity"/"inactivity"/"sedentary behavior" and "exercise" exist, for the purposes of this article, we have simply defined those that expend sufficient energy to be healthy as exercisers and those that do not as non-exercisers.

Many authors have drawn attention to the influence of lifestyle, particularly sufficient physical activity or exercise on the diseases that occur as people age (Fiaterone et al., 1990; Pedersen and Saltin, 2006, 2015; Booth et al., 2012; Lazarus and Harridge, 2017). The integrative mechanisms of healthy ageing will not be best understood by using a patho-physiological approach that is systems based. This approach is slanted toward understanding and treating disease processes. Because exercise affects most physiological functions and processes (Morris and Heady, 1953; Nixon et al., 1976; Wilson and Tanaka, 2000; Heinonen et al., 2014; Sjøgaard et al., 2016; Duggal et al., 2018; Lazarus et al., 2018; Mailing et al., 2019; Joanisse et al., 2020; Kern et al., 2020), it is unlikely for healthy ageing to occur against the backdrop of an absence of physical activity. The effects of exercise on these processes are not monolithic (Sparks, 2017). Recently, four categories of regulation encompassing physiology, age and exercise have been proposed (Lazarus and Harridge, 2018; Lazarus et al., 2019). Category A consists of those processes that decreases by age alone, category B are those processes that are affected by age but can be modified by exercise, category C has processes that are unaffected by ageing but they too can be modified by exercise, and category D reside processes that are independent of both age and exercise. As people age the interactions of categories A, B, C, and D will be in constant flux (Lazarus et al., 2018). Thus, flow diagrams that depict any age-related changes in signaling or metabolic interactions are reflective of the state of physiology at a given age/moment in time.

The removal of exercise is an intervention that will impact negatively on all systems. As two categories of regulation (B and C), will be negatively affected directly by the absence of exercise, the overarching affect is that interactions between all four categories will be different between exercisers and non-exercisers. The same ageing process now interacting with a less optimal physiology produces the lifestyle diseases, whose incidence increases with age. These diseases can thus probably best be categorized as exercise deficiency diseases (Booth et al., 2012; Lazarus et al., 2018). While the development of disease may take time to manifest, it is possible to detect the markers of incipient disease relatively early in the sedentary.[1] Non-exercisers may not be ill, but they are not healthy. It has been suggested that physical inactivity is the biggest health problem of the 21st century (Blair, 2009).

[1]www.nhlbi.nih.gov

Heterogeneity is a ubiquitous characteristic of the presentation of both pathological and healthy human physiology (Chareonthaitawee et al., 2001; Aird, 2011; Lynch and Watt, 2018). Indeed, in health most systems will operate within reasonably well-defined constraints and have normative values (e.g., blood glucose, insulin, markers of liver function, etc.). While physiological functional and integrative indices can very markedly between individuals even when they show the similar physiognomy (e.g., in indices such as VO_{2max} and muscle function). The heterogeneity of phenotype is partly genetic but more generally reflects the underlying differences in the response and adaptation of physiological systems to external factors and in particular exercise (Walter et al., 2012; Lazarus and Harridge, 2018; Said et al., 2019). In non-exercisers, a lack of exercise imposes a different yet related heterogeneity; in that, all the same processes are now operating in an exercise deficient environment (Sparks, 2017). Although there is a very large literature of the role of heterogeneity in human disease and particularly in the generation of cancers (Klocke and Wittenberg, 1969; McClellan and King, 2010; Sominsky et al., 2020), there is sparse literature on the specific impact of heterogeneity on the presentation of human ageing. In a physiological spectrum ranging from healthy ageing, through sedentariness with its associated non-optimal physiology and continued into disease, indices may mirror the effects of this spectrum. For example, the range of VO_{2max} values produced by varying (e.g., through type, intensity, and duration) the exercise stimulus and the correlation of these values with individual health and disease markers is well documented (Blair et al., 1989; American College of Sports Medicine, 2014; Gries et al., 2018). The ageing process interacting with lifestyle factors and physiological heterogeneity, continuously and continually moderates and influences all physiological processes and systems. It is therefore legitimate to regard physiology as the science concerned with investigating the inherent ageing process. These points form the basis of an overarching hypothesis, which can be summarized as being that: when sufficient exercise is present, the multiple interactions of categories A, B, C, and D produce an ageing profile that remains essentially synchronous, coherent, and integrated throughout most of life. But, in the absence of sufficient exercise, physiology over time will deteriorate toward the production of a lifestyle disease. Thus, the ageing process interacts with the physiological heterogeneity present in categories A, B, C, and D, which will determine the age of presentation of a disease as well as the order of presentation of subsequent diseases. Below, we take three different groups of older individuals who differ in their exercise levels to expand on these points.

HYPOTHESIS: INTEGRATING THE INTERPLAY OF AGEING, EXERCISE, AND HETEROGENEITY IN MASTER ATHLETES

Master athletes represent a group of people who exercise regularly and intensely in later life (Ransdell et al., 2009; Rittweger et al., 2009) and at a point well over that required

for healthy ageing (above their individual Set Point, Lazarus and Harridge, 2017). When performance times of running, cycling (Baker and Tang, 2010), and swimming (Donato et al., 2003) are followed over five decades, a clear pattern emerges. This pattern (essentially a curve with acceleration in the rate of decline during the eighth decade) is independent of sporting discipline and phenotype and applies to both men and women. The curves of decreasing performance time with age show no evidence of disruption by disease and were sufficiently similar for Baker and Tang (2010) to derive an equation that had a good fit for master running performance times across the complete spectrum of official distances available in flat races. The self-selection criteria for each discipline are rigorous. Athletes must have the body phenotype that is best suited to a given discipline are undertaking similar types of exercise training and at maximum training loads (commensurate with their age) as they seek to maximize their performance. In addition, their nutritional intake must be able to sustain their requirements both for energy expenditure and for the maintenance of healthy physiological function across all organs. While they also most probably have the necessary social and economic systems in place to allow them to keep them mentally focused on their tasks. When all these criteria are fulfilled, it has been hypothesized that these decreasing performance times are driven by the inherent human ageing process (Rittweger et al., 2009; Lazarus and Harridge, 2017). From the above, four important conclusions emerge. Firstly, these criteria remove the heterogeneity of decreases in performance times that might have been expected in a cross-sectional study (Donato et al., 2003). Secondly, the diversity of discipline and distance make no difference to the projection of the curves. Thirdly, the curve is independent of body shape and gender. Fourthly, this suggests that the human ageing process is probably the same in all humans (Lazarus and Harridge, 2017). This hypothesis is re-enforced by the fact that most people arrive at the same end point, i.e., death at around the end of the ninth decade (Office for National Statistics, UK, 2017).

If the ageing process is generating the shape of the performance curve, then any person who decides to participate in a discipline, in which training loads are at maximal for their physiological make up, should generate the same drop off in their performance curve if placed in competition (Lazarus and Harridge, 2018). Granted their times would be poor because they could be unsuited to the discipline chosen, but it is the ageing process that is driving the profile of the curves and not physical prowess.

It is important to realize that similar declining performance time curves need not necessarily be the resultant of the homogenization of the physiology of those taking part as many factors determine performance. It is the combination of these constituents, albeit at differing magnitudes, that determine final competitive outcomes. However, the ranges of magnitudes are confined within limits that are species dependent. Thus, the strict self-selection criteria result in a cohort of competitors who are not homogeneous physiologically, but who have a range of heterogeneities that are circumscribed to give similar performance times when subjected to exercise at maximum intensity.

HYPOTHESIS: INTEGRATING THE INTERPLAY OF AGEING, EXERCISE, AND HETEROGENEITY IN HABITUAL EXERCISERS

Having considered competitive athletes, the next step is to consider individuals who are very physically active and could be termed as "dedicated exercisers" (Bhella et al., 2014), but who are not competitive athletes. We recently undertook a well-controlled cross-sectional study on older cyclists (Pollock et al., 2015). Cyclists from 55 to 79 years were studied. In this study, volume of exercise, but not intensity, was measured. However, it was highly likely they were exercising at an exercise load that was sufficient, as indicated by their measured VO_{2max} values (American College of Sports Medicine, 2014), to offset the diseases associated with inactivity and above their set point for healthy ageing, but likely below that of a competitive athlete (Lazarus and Harridge, 2017). Under these conditions, an analysis of VO_{2max} data has shown that people of the same age could have markedly different VO_{2max} levels (Lazarus and Harridge, 2010; Pollock et al., 2015). The different VO_{2max} levels can be indicative of different exercise loads (Wilson and Tanaka, 2000), but other factors including genetics and specificity of training, are also at work causing differences between the same aged individuals and final VO_{2max} values (Magel et al., 1975; Bouchard et al., 1986; Noakes, 2003; Lundby et al., 2016). Thus, even at the same exercise training loads, heterogeneity in values can be demonstrated. Because of all these factors, it is not surprising that it was also shown that exercising people of different ages can exhibit the same VO_{2max} values (Pollock et al., 2015). These are important findings because they emphasize that health, as defined by being free of diseases due to lifestyle, can be maintained by a range of exercise loads that start at individual thresholds sufficient to counter sedentary diseases, their set points (Lazarus and Harridge, 2017) continue all the way up to maximum exercise loads. The physiological indices of all cyclists were exhibiting the sum of the spectrum of values produced by the dose dependent effect of exercise (Wilson and Tanaka, 2000; Bhella et al., 2014). All cross-sectional studies on relevant physiological indices will display this dose effect of exercise, and this will contribute to any inherent heterogeneity between individuals. Longitudinal studies on any individual, who is exercising above their set point, will over the period of study, show the heterogeneity of functions even if the absolute exercise load changes relative to age (Lambert et al., 2002). Thus, even when training at the same exercise loads relative to age, the ageing process will ensure that values of indices in the individual will fall.

Viewed from the perspectives stated above, the relation between biological age and chronological age carries a different connotation. A 70-year-old exercising at his or her set point (Lazarus and Harridge, 2017) is likely to be healthy, as defined by being free of the diseases of lifestyle and likely have a VO_{2max} value that will confirm that prediction (American College of Sports Medicine, 2014). The chronological age and

biological ages of the 70-year-old are exactly where they should be for health. Now take another 70-year-old exercising at higher intensity. All other factors being equal, VO_{2max} will be higher. This 70-year-old is not more healthy or ageing more optimally but has a training-related enhancement of his/her cardio-respiratory fitness. In terms of biological health both individuals are matched because the dose threshold of exercise, necessary to ward off the diseases of inactivity, has been passed and the ageing trajectories (all other things being similar) no different.

A further point to consider on the subject of heterogeneity is that of differences in the tissue response to exercise within any given individual. While some organs will benefit directly from exercise (e.g., increased use of contracting muscle) others, such as the brain, also benefit, probably from the consequent increases in blood flow. One of the growing areas of interest is how exercise is beneficial to cognitive function during ageing (Northey et al., 2018). However, the rates at which adaptations to exercise occur or the rate at which different organs "age" under optimal circumstances remains to be determined.

HYPOTHESIS: INTEGRATING THE INTERPLAY OF AGEING AND HETEROGENEITY IN NON-EXERCISERS

As discussed, there are two regulatory mechanisms that are directly affected by exercise (Lazarus and Harridge, 2017; Lazarus et al., 2018). These are, firstly, category B, in which processes are age-affected but in which exercise can ameliorate the trajectory of change. Secondly, category C in which those mechanisms not affected by age but can be positively modified by exercise would also be adversely affected.

In non-exercisers interactions of categories A, B, C, and D will now be radically different from exercisers. For example, VO_{2max} levels will be lower across all ages when compared to exercising counterparts (Gries et al., 2018). If the development of disease is caused by falling outside normative threshold values, then the initial threshold to be broken will depend on the physiological make-up of the individual. Because of individual heterogeneity, it is not possible to predict which of the lifestyle-associated diseases will be the first to present. In addition, the presentation of a particular disease will not strictly follow ageing. It is not the ageing process that is the prime etiological factor underlying disease presentation, but the interaction of a universal ageing process on an exercise deficient physiology (Fried et al., 2001; Chiu and Wray, 2010; Lazarus et al., 2018).

HYPOTHESIS: THE INTERACTION OF REGULATORY CATEGORIES A, B, C, AND D WITH EXERCISE, AGE, AND INTRINSIC HETEROGENEITY

Healthy ageing is the product of the interaction of many factors. Firstly, there are the interactions of categories A, B, C, and D. These in turn can be modified. Categories A and B are age dependent and will therefore change over time. However, because category B is positively influenced by exercise the relationship between A and B will undergo further change. Category C that is independent of age is also modified by exercise. Category D is independent of age and exercise. However, overlying all these different properties of the categories is inherent human physiological heterogeneity. We have depicted this schematically in **Figure 1**. Thus, the interaction of age, exercise, and human heterogeneity will ensure that the relationships between processes residing in any of the four categories will be undergoing continuous change both in them and in relation to their interactions with other categories. All these processes are operating on a global scale and affect all physiological systems. To seek a single index, which will define the mechanism of these effects across the whole of physiology, borders on the futile. There is no magic index, as has been postulated for some diseases (Jackson and Schoenwaelder, 2003; Benfeito et al., 2013; Venkata and Ram, 2019), that will suffice as the definitive marker of healthy ageing.

HEALTHY AND NON-HEALTHY PHENOTYPES

The removal of exercise will affect two of the four regulatory categories. The outcomes of the regulatory interaction will begin to diverge between exercisers and non-exercisers from the time that exercise is removed. In exercisers the outcome, over time, is a physiology that is coherent and integrated while shrinking under the influence of age. In non-exercisers the outcome is eventually disease (**Figure 1**). Physiological regulation is now geared to preserving function in the face of a disease process. The regulatory mechanisms underpinning the two phenotypes will be following different trajectories. The physiology of non-exercisers should not be used as exemplars of health. All physiological parameters measured in this group of people will probably represent either incipient ill health at the best or disease at the worst. By the same token, the addition of exercise to a previously inactive scenario will have positive effects on the two categories and thus the regulation and interaction of all four. The known improvements in physiological and clinical indices as a result of exercise training interventions are well-known. However, what remains to be determined is at what age category A indices have diminished such that the interventions have come too late to be able to put an individual on a track that would match their optimal ageing trajectory.

SUMMARY

This hypothesis article has attempted to provide a logical foundation for unraveling the multifaceted and evolving conundrum that is the human ageing process. We have

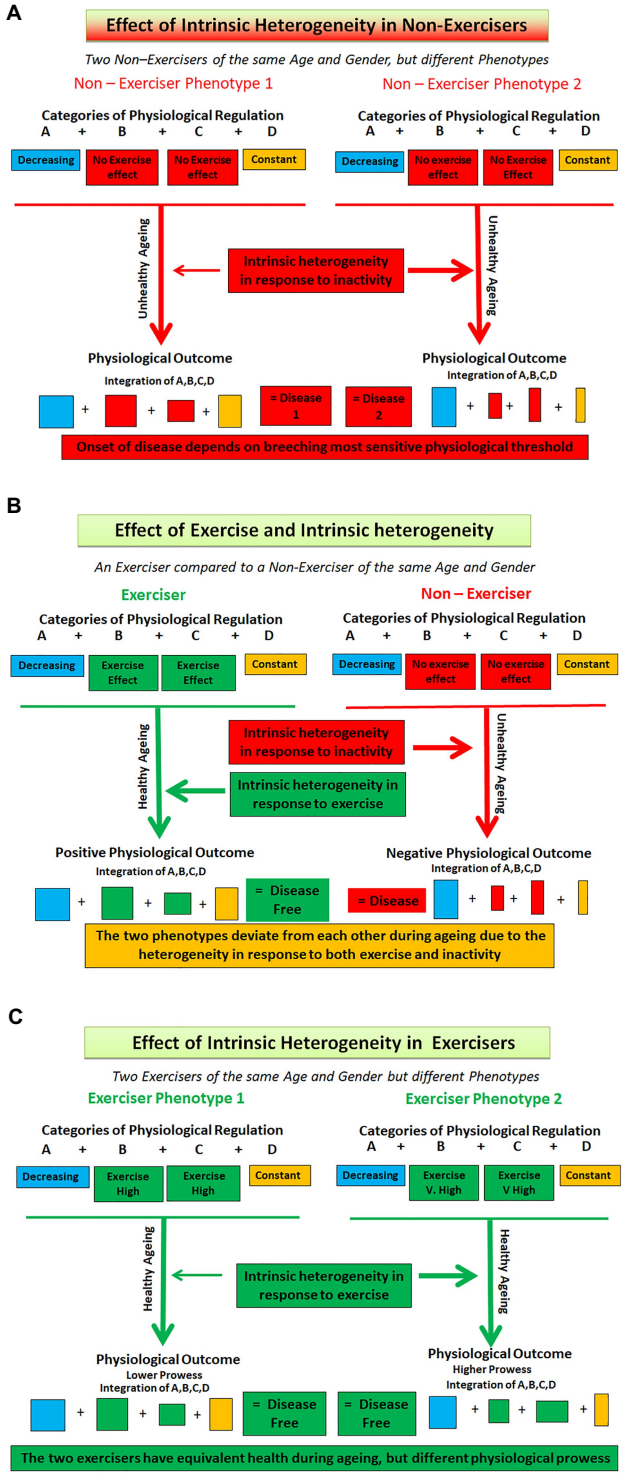

FIGURE 1 | Depicting interaction of exercise and heterogeneity in exercisers and non – exercisers of the same age and gender. **(A)** Removal of exercise. Here a disease model is shown. Both individuals are non-exercisers, but different phenotypes are produced because of the effects of intrinsic heterogeneity in all categories and because Categories B and C are negatively affected by a lack of exercise. **(B)** Comparing the presence of exercise (Left hand side) with no exercise. Here the differences between the two individuals

(Continued)

FIGURE 1 | are not only due to exercise (Categories B and C) but also because of the intrinsic heterogeneity in the interactions of all four categories. **(C)** Here two exercisers undertaking the same levels of exercise are shown. Different phenotypes are produced because of the differing effects of exercise on categories B and C and because of the intrinsic heterogeneity and subsequent differences in the interactions of all four categories of regulation. The coloured boxes represent the four categories of regulation (A, B, C, and D), with the size of each box representing the heterogeneity within each category.

proposed that the decline in physiological function is determined by four interacting categories of regulation which overtime manifests itself as a continually changing physiological landscape. These categories have the role of exercise and an acknowledgement of the heterogeneity as core components. Healthy ageing, defined here as being free from diseases of lifestyle, only occurs when exercising at set point and above. The investigation of master athletes who are at the maximum end of that exercising spectrum will give no greater insight into the mechanisms underlying the healthy human ageing process than investigating those exercising at set point. It is not possible to be healthier than healthy.

There is no evidence that an increased proficiency of exercise discipline alters the health trajectory of any individual. However, master athletes will give insight into the effects of age on performance and by default on integrative physiological function. The removal of exercise causes a disintegration of ageing physiology such that disease is an almost inevitable outcome. In all cross-sectional studies only a snapshot of the effect of age, exercise, and heterogeneity on physiological indices and systems is obtained. Over time, exercise and heterogeneity interact, causing constant flux in complex biochemical, molecular, and physiological processes. A healthy, exercising 70-year-old not only differs from a healthy exercising 50-year-old in chronological age but also in having a physiology that has had to adapt to ageing and lifestyle interactions, so that integrity, co-ordination, and synchronization are maintained by a shrinking physiology. How this integrity is maintained awaits discovery. The human ageing process, interacting with lifestyle factors and heterogeneous physiologies, governs and modifies all processes in all humans all the time, yet its presence does not seem to merit a place in most physiology or medical texts.

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

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Interindividual Variability in Fat Mass Response to a 1-Year Randomized Controlled Trial With Different Exercise Intensities in Type 2 Diabetes: Implications on Glycemic Control and Vascular Function

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Purpose: Little is known about the interindividual variability in fat mass (FM) loss in response to high-intensity interval training (HIIT) and moderate continuous training (MCT) in individuals with type 2 diabetes mellitus (T2DM). Moreover, the impact on health-related outcomes in those who fail to reduce FM is still unclear. The aims of this investigation were (1) to assess if the individuals with T2DM who FM differed across MCT, HIIT, and control groups over a 1-year intervention and (2) to assess the changes on glycemic control and vascular function in the exercising patients who failed to lose FM.

Methods: Adults with T2DM were randomized into a 1-year intervention involving a control group ($n = 22$), MCT with resistance training (RT; $n = 21$), and HIIT with RT ($n = 19$). FM was assessed using dual-energy X-ray absorptiometry and a change in total body FM above the typical error was used to categorize FM responders. Glycemic control and vascular stiffness and structure were assessed. A chi-square test and generalized estimating equations were used to model the outcomes.

Results: Both MCT ($n = 10$) and HIIT ($n = 10$) had a similar proportion of individuals who were categorized as high responders for FM, with the percent change in FM on average $-5.0 \pm 9.6\%$ for the MCT and $-6.0 \pm 12.1\%$ for the HIIT, which differed from the control group ($0.2 \pm 7.6\%$) after a 1-year intervention ($p < 0.05$). A time-by-group interaction for carotid artery intima-media thickness (cIMT) (p for interaction = 0.042) and lower-limb pulse wave velocity (LL PWV; p for interaction = 0.010) between those categorized as low FM responders and the control group. However, an interaction was observed between the high responders for FM loss and controls for both brachial and carotid hemodynamic indices, as well as in cIMT, carotid distensibility coefficient, carotid beta index, and LL

PWV (p for interactions <0.05). No interactions were found for glycaemic indices (p for interaction >0.05).

Conclusion: Our results suggest that the number of FM responders did not differ between the MCT or HIIT, compared to the control, following a 1-year exercise intervention in individuals with T2DM. However, low responders to FM may still derive reductions in arterial stiffness and structure.

Clinical Trial Registration: Comparing Moderate and High-intensity Interval Training Protocols on Biomarkers in Type 2 Diabetes Patients (D2FIT study) – number: NCT03144505 (https://clinicaltrials.gov/ct2/show/NCT03144505).

Keywords: arterial stiffness, Carotid artery intima-media thickness, exercise intervention, peak wave velocity, high-intensity interval training, moderate continuous training

INTRODUCTION

Obesity is a major contributor to the development of type 2 diabetes (T2DM), with 80% of individuals being classified as obese (Goedecke and Micklesfield, 2014). Several investigations have shown that obesity is associated with insulin resistance and the development of cardiovascular disease in individuals with T2DM (Dube et al., 2011), whereas weight loss, particularly induced by a reduction in fat mass (FM), is a paramount strategy for optimizing glycemic control (Lean et al., 2018) and reducing manifestations of cardiovascular pathology, such as arterial stiffness and structure (Cardoso and Salles, 2016).

Exercise has been shown to be an effective strategy for decreasing body fat although the type, frequency, duration, and intensity most effective for reducing adiposity remain debated in individuals with T2DM (Dube et al., 2011; De Nardi et al., 2018). In short to medium-term interventions, high-intensity interval training (HIIT) has been proposed as a time efficient training method that may induce greater reductions in FM when compared to moderate-intensity continuous training (MCT) in individuals with T2DM (Liu et al., 2019). However, all of these exercised-based interventions rely on group mean effects for FM loss, which provides no information about the interindividual variability of FM changes in response to HIIT and MCT in individuals with T2DM (Chrzanowski-Smith et al., 2020). A previous investigation comparing continuous aerobic training at different intensities during a 24-week intervention period showed that in obese adults, there was a higher number of individuals in the higher intensity exercise group achieving a clinically important reduction in visceral adipose tissue (<0.28 kg), when compared to those in the moderate-intensity group (Brennan et al., 2020a). Whether HIIT affects the proportion of individuals with T2DM who are likely to achieve a clinically meaningful FM reduction following a long-term intervention is unknown.

Regardless of the alteration in the exercise characteristics, there still remains a portion of individuals who do not achieve clinical meaningful FM loss (Stephens and Sparks, 2015). Nevertheless, irrespective of reductions in FM, heterogeneity in the effects of exercise on cardiometabolic outcomes exist, such that improvements in glycemic control and vascular function have been found independent of FM (Tanaka et al., 2000; Gaesser et al., 2011; Hawkins et al., 2014). Although FM plays a major role in the pathophysiology of T2DM, exercise can work through other pathways to induce beneficial changes in two of the most impacted systems of this disease, being glycemic control and vascular function. In fact, we recently have shown that regardless of the cardiorespiratory fitness (CRF) response to 1-year of exercise, favorable changes in vascular structure and function were found (Hetherington-Rauth et al., 2020a). On this matter, no longitudinal randomized controlled trial (RCT) with different exercise intensities using an ecological approach has yet analyzed the glycemic and vascular benefits in patients who do not achieve meaningful fat loss.

Therefore, the aims of this investigation were 2-fold: (1) to compare the response to FM loss following 1 year of MCT or HIIT in individuals with T2DM and (2) to assess whether individuals who failed to attain exercise-derived clinically meaningful reductions in FM still improved their cardiovascular risk profile by improving glycemic control and reducing local and regional manifestations of cardiovascular pathology, such as arterial stiffness and structure.

MATERIALS AND METHODS

Subjects

The current investigation assessed individuals with T2DM who took part in a 1-year exercise RCT (D2FIT study) with three distinct arms: a non-exercise control group, a MCT with resistance training (RT) group, and a HIIT with RT group (ClinicalTrials.gov registration no. NCT03144505). The randomization proceeded with a 1:1:1 allocation ratio between the three intervention groups by a researcher external to the D2FIT study and blinded

Abbreviations: Body mass index, BMI; Brachial systolic blood pressure, SBP; Cardiorespiratory fitness, CRF; Carotid mean arterial pressure, cmap; Carotid systolic blood pressure, csbp; Diastolic blood pressure blood pressure, DBP; Dual-energy X-ray absorptiometry, DXA; Fat mass, FM; Glycated hemoglobin, hba1c; Heart rate reserve, HRR; High-intensity interval training, HIIT; Homeostasis model assessment, HOMA; Individual response standard deviation, SD_{IR}; Intima-media thickness, IMT; Mean arterial pressure, MAP; Moderate-intensity continuous training, MCT; Physical activity, PA; Pulse wave velocity, PWV.

to the enrolment process, using computer-generated list of random numbers. The study design and methodology of D2FIT study have been previously published (Magalhaes et al., 2019a).

The primary outcome of D2FIT study concerned changes in glycated hemoglobin (HbA1c), which was assessed at baseline and at the end of the intervention period (i.e., 1 year). Participants were recruited in Lisbon, Portugal between February 2014 and July 2016. Eligible criteria included as: adults previously diagnosed with T2DM (American Diabetes, A, 2020); aged 30–75 years; no major micro-and macrovascular complication from T2DM; body mass index <48 kg/m^2; and no physical limitation preventing individuals from exercising. Power and sample size calculations (G-Power, Version 3.1.3, Düsseldorf, Germany) were based on a predicted HbA1c change of 0.66 units with an SD of 1.2 units, $\alpha = 0.05$, 1-$\beta = 0.80$, and an expected dropout rate of 10% (Boule et al., 2001). A total of 80 individuals were selected and randomized, however, for the current analysis only participants who completed the 1-year investigation ($n = 62$) were considered.

Written informed consent was obtained from all participants prior to screening. The D2FIT study protocol was reviewed and approved by the Ethics Committee of the Portuguese Diabetes Association (approval number: 07/17/2013).

Exercise Intervention

Exercise prescription and session time were standardized based on physical activity (PA) guidelines (U.S. Department of Health and Human Services, 2008) to achieve a weekly target of 41.84 kJ/kg (10 kcal/kg) for both the MCT and HIIT group.

Throughout the intervention, individuals from both groups received a structured periodization of the exercise program with an individualized and supervised intensity of training based upon heart rate reserve (HRR). A full detailed description of the periodization protocol can be found elsewhere (Magalhaes et al., 2019a).

Participants in the HIIT with RT ($n = 19$) and MCT with RT groups ($n = 21$) exercised 3 days per week. The MCT group performed continuous cycling on a cycle ergometer (Monark Ergometric 828e, Vansbro, Sweden) at 40–60% of HRR throughout the intervention. The HIIT group performed 1 min of cycling at 90% of HRR, followed by a 1 min rest period at 40–60% of HRR (1:1 exercise:rest ratio). Following the aerobic training component, participants from both groups underwent a specific RT including one set of 10–12 repetitions of upper- and lower-limb exercises. The intensity of all trainings was monitored using a heart rate monitor (Polar T-31, Bethpage, NY, United States) worn on the participant's chest.

The control group had an initial orientation session with standard counseling regarding general PA guidelines, with an additional session every month where thematic sessions were held in order to discuss topics, such as nutrition or PA as a retention strategy.

Anthropometry and Body Composition

Participants weight and height were measured according to standardized procedures (Lohman et al., 1988).

Total FM was estimated using dual-energy X-ray absorptiometry (DXA; Hologic Explorer-W, Waltham, United States). Following the protocol for DXA described by the manufacturer, a laboratory technician positioned the participants, performed the scans, and executed the analyses according to the operator's manual. The %CV in our laboratory is 1.7 for FM and 0.8 for lean mass (Santos et al., 2013).

Hemodynamic Indices

Brachial systolic blood pressure and diastolic blood pressure (bDBP) were measured using an automated oscillometric cuff (HEM-907-E; Omron, Tokyo, Japan) following the participant lying 15 min in the supine position. Carotid systolic blood pressure (cSBP) and carotid diastolic blood pressure were measured using ultrasound scanner equipped with a linear 13 MHz probe (MyLab One, Esaote, Italy). The mean arterial pressure (MAP) was calculated using the formula: MAP = DBP + [1/3(SBP − DBP)].

Local Carotid Artery Intima-Media Thickness

Carotid artery intima-media thickness (cIMT) was measured on the far wall of the right carotid artery using an ultrasound scanner equipped with a linear 13 MHz probe (MyLab One, Esaote, Italy; Hoeks et al., 1997). Distension curves were acquired within a segment of the carotid artery ~1 cm before the flow divider.

Carotid Arterial Stiffness Indices

After 15 min in a supine position, an ultrasound scanner equipped with a linear 13 MHz probe (MyLab One) was placed ~1 cm before the carotid artery bifurcation on the right side of the body and used to calculate pulse wave velocity (PWV; m/s), carotid distensibility coefficient (DC; 1/Kpa), and stiffness index β. A detailed description can be found elsewhere (Hetherington-Rauth et al., 2020b).

Regional PWV

The distance between the carotid and femoral and radial and distal posterior tibial arteries were measured using applanation tonometry and values were directly inserted into the Complior Analyse software (ALAM Medical, Paris, France). PWV values obtained from measurements of the carotid to femoral artery, carotid to radial artery, and carotid to distal posterior tibial artery were taken as indices of aortic and peripheral arterial stiffness for upper (UL) and lower limbs (LL), respectively. A detailed description can be found elsewhere (Hetherington-Rauth et al., 2020b).

Laboratory Measurements

Fasting blood samples were collected for the assessment of glucose, insulin, and HbA1c before a mixed meal tolerance test and 30 and 120 min after beginning of meal consumption (two bottles of boost complete nutritional drink) for glucose and insulin. Samples were drawn into chilled, heparinized tubes and centrifuged rapidly to avoid glycolysis. Plasma glucose was measured by photometry (auto analyzer Olympus AU640, Beckman Coulter). Plasma insulin was analyzed using electrochemiluminescence immunoassays (Liaison, Diasorin).

HbA1c was analyzed by immunoassay (auto analyzer Hb9210 Premier A. Menarini Diagnostics). The homeostasis model assessment (HOMA) variables were estimated using the HOMA2 calculator.[1]

Physical Activity

Both the control and the exercise groups wore an accelerometer every 3 months to access their physical activity and sedentary behavior (ActiGraph, GT3X+ model; Fort Walton Beach, FL, United States). Participants were asked to wear the accelerometer on the right hip, close to the iliac crest, for 7 consecutive days. The device activation, download, and processing were performed using the software, Actilife (v.6.9.1; ActiGraph). Data were recorded using the raw mode with a 100 Hz frequency and posteriorly downloaded into 15 s epochs. Troiano et al. cut-points and validation criteria were used for data analysis (Troiano et al., 2008).

Identifying Individual Exercise Fat Mass Responders

Currently, there are no accepted guidelines for the percent of FM loss considered to be clinically meaningful (Brennan et al., 2019). Therefore, we considered someone who had a FM loss greater than the typical error (TE) as clinically meaningful. The TE was calculated from the SD of the differences in FM over 1 year in the control group ($TE = SD_{diff}/\sqrt{2}$), as described by Hopkins (2000) and used by others (Walsh et al., 2020; Brennan et al., 2020a). The TE represents the technical error of measurement as well as the within-subject variability caused by changes in behavioral/environmental factors across an intervention (Bonafiglia et al., 2018). The TE for FM in our study was 1.73 kg (i.e., ~−6% FM from baseline). Hence, any individual with a FM loss >1.73 kg was considered to be a high responder and individuals with FM ≤1.73 kg were considered low responders.

Statistical Analysis

Descriptive statistics, including measures of central tendency (mean) and variability (SD) for normally distributed variables and median (interquartile range) for skewed variables, were used to describe baseline descriptive characteristics. Depending on normality and variable type, a one-way ANOVA with Bonferroni adjustment for multiple comparisons, Kruskal–Wallis test, χ^2 test, or Fisher's exact test were used to compare baseline measures between groups.

Differences in the proportion of individuals in the control, MCT, and HIIT groups who reduced FM after 3, 6, and 12 months of intervention were assessed using a chi-square test.

Generalized estimating equations were used to assess outcomes indicative of glycaemic control and vascular structure and function, while adjusting for sex, baseline moderate-to-vigorous PA (MVPA), number of training sessions completed, and percent changes in MAP (only in models assessing differences in arterial stiffness and structure indices). A linear distribution with an identity link function for the response was assumed and an autoregressive model with a robust estimator was used for the working correlation matrix and covariance matrix, respectively. Finally, the maximum likelihood estimate was set to the data to calculate the parameter estimation and the lowest value for the goodness of fit was used for comparisons between models.

A Bonferroni post-hoc test was used to estimate the between- and within-group effects. A linear distribution for the response was assumed and an autoregressive correlation matrix was set to the data.

A value of p of <0.05 was considered statistically significant. Data analyses were performed using IBM SPSS Statistics version 22.0 (SPSS, Chicago, IL, United States) and STATA version 13.1 (StataCorp, College Station, TX, United States).

RESULTS

Table 1 describes baseline values between high responders ($n = 20$; ΔFM >TE), low responders ($n = 20$; ΔFM ≤TE), and the control group ($n = 22$). Out of the 62 participants (45% female), 53% were categorized as obese, and 31% were overweight. At baseline, we found no differences ($p \geq 0.05$) between the low and high responders, except for MVPA, in which high responders had higher values when compared to the controls ($p < 0.05$). On the other hand, and considering the Canadian physical activity guidelines (Ross et al., 2020), all groups spent a considerable amount of time in sedentary pursuits as shown by accelerometer data and time spent watching TV.

At the end of the 1-year intervention, 20 individuals in the exercise groups (10 from MCT and 10 from HIIT) decreased their FM above the TE threshold, compared with only two individuals in the control group ($p > 0.05$; **Table 2**).

Figure 1 shows the individual changes in FM loss for each participant in the control (**Figure 1A**), MCT (**Figure 1B**), and HIIT groups (**Figure 1C**) after 1-year relative to the TE cutoffs.

Table 3 depicts the within- and between-group changes in glycemic control, hemodynamic indices, and indices of vascular stiffness and structure in control and FM responder groups. The high FM responders had favorable changes in vascular structure and stiffness indices as indicated by the time-by-group interaction observed between the cIMT (p for interaction <0.001), carotid DC (p for interaction = 0.016), beta stiffness index (p for interaction = 0.035), carotid PWV (p for interaction = 0.038), and LL PWV (p for interaction = 0.021) with the control group. Favorable changes were also observed on hemodynamic indices between the high FM responder group and controls (p for interactions <0.05), except for cSBP and carotid mean arterial pressure (cMAP; p for interactions >0.05). Moreover, for the hemodynamic indices, similar interactions were observed between the high FM responder and low FM responder groups (p for interactions <0.05). Although no interactions in hemodynamic indices were observed between the low FM responders and the controls ($p > 0.05$), there still was a time-by-group interaction between the low FM responders and the controls in vascular structure (cIMT: p for interaction = 0.042) and vascular stiffness (LL PWV: p for

[1]https://www.dtu.ox.ac.uk/homacalculator/

TABLE 1 | Baseline descriptive characteristics of control group and exercise groups who either reduced or did not reduce their total body FM.

	Control (*n* = 22)	Low responders (*n* = 20)	High responders (*n* = 20)	Value of *p*
	Mean (SD)	Mean (SD)	Mean (SD)	
Age (years)	60.8 ± 7.5	57.5 ± 8.9	59.6 ± 7.0	0.39
Gender (F:M)	11:11	8:12	9:11	0.81
Weight (kg)	84.6 ± 15.4	82.1 ± 18.1	80.5 ± 11.3	0.67
Height (cm)	164.5 ± 9.5	165.0 ± 8.8	163.9 ± 8.1	0.93
Body mass index (kg/m^2)	31.7 ± 4.7	30.5 ± 6.0	30.6 ± 5.2	0.73
Time from DM dx	5.9 ± 5.4	7.8 ± 4.9	7.5 ± 5.2	0.44
Hypertensive medication (%)	54.5	45.0	50.0	0.82
Oral hypoglycemic medication	95.5	95.0	90.0	0.83
% Trainings completed (%)	NA	74.2 ± 20.7	77.5 ± 22.4	0.63
MCT (n): HIIT (n)	NA	11:9	10:10	0.75
Baseline MVPA (min/d)[a]	22.1 ± 16.0	31.0 ± 18.6	46.9 ± 30.8*	0.003
Sedentary time (min/d)[a]	570.1 ± 147.4	603.5 ± 67.2	570.3 ± 86.6	0.527
TV viewing time (min/d)	199.3 ± 141.2	195.3 ± 108.7	186.8 ± 143.1	0.952
HbAIC (%)[a]	6.9 ± 1.1	7.3 ± 1.4	6.9 ± 1.1	0.54
HbAIC (mmol/L)[a]	51.7 ± 11.8	55.9 ± 15.7	52.1 ± 12.3	0.54
Fasting glucose (mg/dL)[a]	8.0 ± 1.8	9.8 ± 3.7	8.5 ± 3.6	0.18
VO_{2peak} (ml/min/kg)	25.2 ± 5.6	25.5 ± 5.1	25.7 ± 6.1	0.95
bMAP (mmHg)	91.6 ± 9.0	96.2 ± 13.0	99.3 ± 10.5	0.08
Total body FM (kg)	29.9 ± 6.8	28.1 ± 9.4	28.2 ± 8.5	0.73
Total body % FM (%)	35.5 ± 6.3	33.9 ± 6.7	34.7 ± 7.7	0.73
Total body lean soft tissue (kg)	52.3 ± 11.4	51.6 ± 11.0	49.9 ± 7.4	0.73
bSBP (mmHg)	127.4 ± 17.3	133.3 ± 18.5	136.1 ± 16.7	0.28
bDBP (mmHg)	73.8 ± 6.7	78.0 ± 11.7	81.0 ± 9.3	0.06

DM, diabetes mellitus; bSBP, brachial systolic blood pressure; bDBP, brachial diastolic blood pressure; FM, fat mass; HbAIC, glycated hemoglobin; HIIT, high-intensity interval training; MAP, mean arterial pressure; MCT, moderate continuous training.
[a]*Median (interquartile range).*
**significantly different from control, p<0.05.*

TABLE 2 | Proportion of individuals in experimental groups who reduced body fat mass at different time points.

	Control	MCT	HIIT	Value of *p*
High responders, n (%)				
3 months	3 (14.3)	8 (38.0)	5 (26.3)	0.22
6 months	4 (19.1)	8 (38.1)	6 (35.3)	0.41
12 months	2 (9.1)	10 (47.6)	10 (52.6)	0.004

MCT, moderate continuous training; HIIT, high-intensity interval training; FM, fat mass; and TE, technical error. Values presented as absolute and percentage.

interaction = 0.010). No interactions were observed between the high and low FM responders and the controls for glycemic outcomes (p for interactions >0.05).

DISCUSSION

To the best of our knowledge, this is the first investigation to address the response rate to changes in FM following a long-term intervention with both MCT and HIIT in individuals with T2DM. Following a 1 year of exercise, we found that the proportion of individuals who attained meaningful changes in FM (high responders) differed between the exercise and the control groups, but no differences were found between the MCT and HIIT. Moreover, those considered low responders still had favorable changes on vascular structural and stiffness indices, such as cIMT and LL PWV. Despite the benefits observed in low responders, individuals with higher FM losses had superior benefits, not only on cIMT and LL PWV, but also on other stiffness indices and hemodynamic outcomes, which may have favorable implications on the progression of diabetes related macrovascular complications.

With a similar approach, in an obese population, Brennan et al. (2020a) aimed to determine the effect of different exercise intensities on the proportion of individuals who had meaningful reductions in total and abdominal adipose tissue (i.e., responders) following 24 weeks of intervention. Their results suggested that increasing exercise amount and/or intensity may increase the proportion of individuals who achieve clinically meaningful visceral adipose tissue reductions. In our study, we observed a difference in the proportion of high responders in both the MCT and the HIIT group when compared to the control; however, no differences were found between exercise intensities. The lack of differences between our exercise intensities (i.e., MCT *vs.* HIIT), as opposed to those observed in Brennan et al. (2020a), may be due to the longer length of our intervention period (1 year *vs.* 24 weeks) and the intensity of our exercise protocol (HIIT at 90% HRR *vs.* continuous vigorous exercise at >75% VO_{2max}), both of which may have led to higher experienced physiological and psychological fatigue by the HIIT group toward the end of our intervention, impairing their overall 1-year exercise outcomes compared to MCT. In fact, the population differences between studies (T2DM *vs.* obese adults) may also explain why HIIT did not have a higher proportion of responders compared to the MCT, given that individuals with T2DM are known to have a reduced peak workload capacity, peak oxygen assumption, oxygen pulse, and ventilatory efficiency, thus potentially inhibiting the additional effects of a more demanding exercise protocol (e.g., HIIT; Nesti et al., 2020).

When assessing the number of high responders in each group over the length of the intervention, we did not find differences in either of the exercise groups and the controls at the 3- and 6-month mark. This is likely due to the fact that, for ethical reasons, our control group had monthly sessions, where topics of nutrition and PA were discussed, hence increasing their odds of being categorized as high responders. Nevertheless, at the 12-month mark, there were differences between the proportion

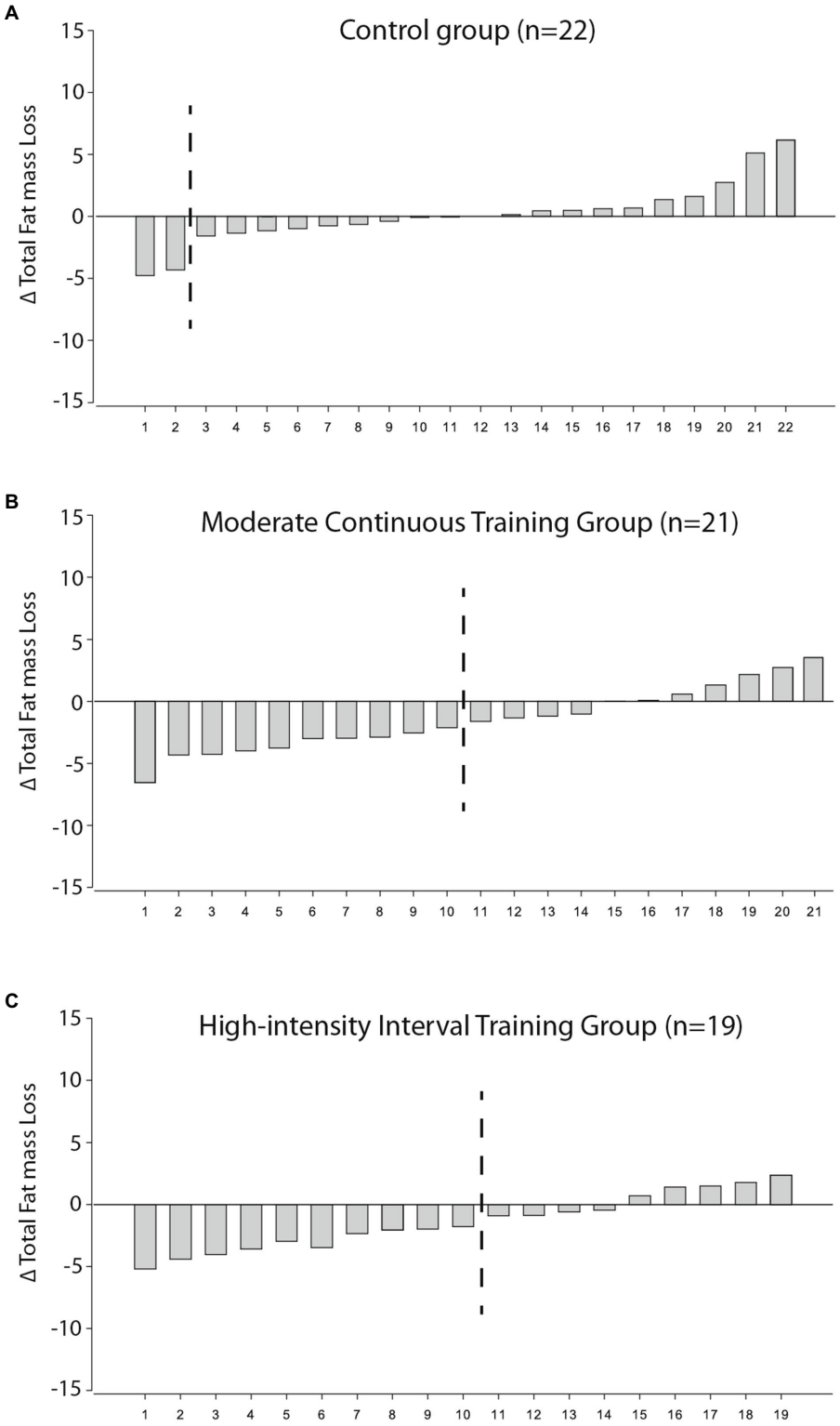

FIGURE 1 | Individual response changes for total FM loss in the control **(A)**, MCT **(B)**, and HIIT group **(C)** according to 90% CI SWC cutoffs. Those on the left of the dashed black line are the participants who were considered high responders according to ΔFM loss>TE. TE, typical error.

TABLE 3 | Within- and between-group changes in vascular health of control and low and high FM responders' groups.

Outcome	Control ($n = 22$)		Low FM Responders ($n = 20$)		High FM Responders ($n = 20$)		Low FM Responders * Control	High FM Responders * Control	High FM Responders * Low FM Responders
	Baseline	12 months	Baseline	12 months	Baseline	12 months	β (95% CI)	β (95% CI)	β (95% CI)
Glycemic indices									
Fasting glucose (mmol/mol)	7.3 ± 1.8	7.8 ± 1.8	9.8 ± 3.7	9.9 ± 4.6	8.5 ± 3.6	8.2 ± 3.6	0.03 (−0.08, 0.13)	−0.01 (−0.11, 0.10)	0.03 (−0.09, 0.15)
IAUC Glucose	7.3 ± 3.7	7.7 ± 3.6	7.9 ± 3.8	9.0 ± 3.9	7.6 ± 4.2	7.8 ± 3.7	0.05 (−0.13, 0.24)	−0.01 (−0.18, 0.15)	0.07 (−0.13, 0.27)
IAUC Insulin	751.9 ± 546.5	703.6 ± 647.7	493.6 ± 301.9	575.1 ± 493.0	443.1 ± 388.3	502.5 ± 502.8	15.91 (−4.78, 36.59)	17.83 (−3.65, 39.30)	−1.20 (−17.55,13.71)
HOMA2-IR	2.4 ± 1.6	2.4 ± 1.7	2.2 ± 1.4	2.1 ± 1.8	1.5 ± 0.6	1.5 ± 0.7	−0.001 (−0.07, 0.07)	−0.01 (−0.06, 0.07)	−0.03 (−0.05, 0.05)
HgAIC (mmol/mol)	51.7 ± 11.8	54.7 ± 11.2	55.9 ± 15.7	58.9 ± 16.5	52.1 ± 12.3	52.9 ± 14.8	0.13 (−0.36, 0.63)	−0.07 (−0.56, 0.45)	0.19 (−0.22, 0.60)
Hemodynamic índices									
bSBP (mmHg)	127.4 ± 17.3	131.5 ± 19.2	133.3 ± 18.5	138.3 ± 19.6	136.1 ± 16.7	129.6 ± 15.6	0.10 (−0.62, 0.83)	−0.90 (−1.60, −0.20)*	1.01 (0.34, 1.67)*
bDBP (mmHg)	73.8 ± 6.7	74.0 ± 11.0	78.0 ± 11.7	78.4 ± 7.1	81.0 ± 9.3	75.6 ± 8.2†	0.05 (−0.41, 0.51)	−0.48 (−0.90, −0.07)*	0.53 (0.07, 0.99)*
bMAP (mmHg)	91.5 ± 9.0	93.0 ± 11.8	96.2 ± 13.0	98.1 ± 9.6	99.3 ± 10.5	93.5 ± 9.6	0.06 (−0.45, 0.57)	−0.63 (−1.09, −0.17)*	0.69 (0.20, 1.19)*
cSBP (mmHg)	121.9 ± 25.3	121.2 ± 19.3	121.0 ± 20.0	127.6 ± 21.1	122.7 ± 14.0	117.6 ± 15.2	0.61 (−0.44, 1.66)	−0.40 (−1.33, 0.52)	1.01 (0.31, 1.71)*
cDBP (mmHg)	73.8 ± 6.7	74.0 ± 11.0	78.0 ± 11.7	78.4 ± 7.1	81.0 ± 9.3	75.6 ± 8.2	0.05 (−0.41, 0.51)	−0.48 (−0.90, −0.07)*	0.54 (0.07, 0.99)*
cMAP (mmHg)	89.8 ± 11.8	89.8 ± 12.2	92.3 ± 13.4	98.4 ± 10.4	94.9 ± 9.8	89.6 ± 9.5	0.23 (−0.36, 0.82)	−0.47 (−0.96, 0.02)	0.70 (0.20, 1.20)*
Vascular stiffness and structure indices									
Carotid IMT (mm)	714.9 ± 130.7	751.2 ± 119.4*	740.8 ± 203.9	723.3 ± 156.7	704.2 ± 139.7	673.9 ± 116.6*	−4.34 (−8.53, −0.15)*	−5.40 (−8.27, −2.53)*	1.01 (−3.35, 5.47)
Carotid PWV (m/s)	7.5 ± 1.3	8.1 ± 1.9†	7.6 ± 1.5	7.9 ± 1.7	7.7 ± 1.3	7.6 ± 1.5	−0.03 (−0.07, 0.02)	−0.05 (−0.11, −0.01)*	003 (−0.02, 0.07)
Carotid DC (1/Kpa)	1.8 ± 0.6e^{-2}	1.5 ± 0.6e-2*†	0.02 ± 0.8e^{-2}	0.02 ± 0.8e^{-2}	0.02 ± 0.7e^{-2}	0.02 ± 0.8e^{-2}	1.80e^{-4} (−4.91e1^{-5}, 4.09e^{-5})	3.47e^{-4} (6.48e^{-5}, 6.28e^{-4})*	−1.67e^{-5} (−4.45e^{-5}, 1.11e^{-5})
Carotid β	11.9 ± 4.5	14.2 ± 6.8†	11.9 ± 4.9	13.5 ± 3.4†	12.2 ± 4.2	12.1 ± 4.2	−0.06 (−0.23, 0.10)	−0.19 (−0.34, −0.04)*	0.13 (−0.04, 0.29)
Aortic PWV (m/s)	13.1 ± 4.8	14.0 ± 4.3	13.0 ± 3.0	13.5 ± 3.4	13.3 ± 4.2	114.5 ± 4.5	−0.03 (−0.17, 0.11)	0.02 (−0.11, 0.15)	−0.05 (−0.17, 0.07)
UL PWV (m/s)	9.2 ± 1.9	9.3 ± 1.5	9.5 ± 1.6	9.0 ± 1.7	10.0 ± 2.2	9.2 ± 2.4	−0.05 (−0.16, 0.06)	−0.08 (−0.20, 0.04)	0.03 (−0.09, 0.15)
LL PWV (m/s)	9.0 ± 1.8	10.3 ± 1.7†	9.9 ± 2.1	9.6 ± 2.0	10.1 ± 2.4	9.7 ± 2.6	−0.13 (−0.24, −0.03)*	−0.15 (−0.27, −0.02)*	−0.01 (−0.09, 0.12)
Total body FM (kg)	29.9 ± 6.8	30.1 ± 7.6	28.1 ± 9.4	28.6 ± 9.5	28.2 ± 8.5	24.8 ± 8.4†	−0.04 (−0.06, 0.14)	−0.26 (−0.36, −0.16)*	0.30 (0.22, 0.38)*

*β=unstandardized β adjusted for sex, total number of trainings completed, and baseline MVPA and age; unstandardized β for carotid structure and stiffness and regional arterial stiffness indices additionally adjusted for percent change in MAP IAUC, incremental area under the curve; FM, fat mass; CRF, cardiorespiratory fitness; bSBP, brachial systolic blood pressure; cSBP, carotid systolic blood pressure; bDBP, brachial diastolic blood pressure; cDBP, carotid diastolic blood pressure; MAP, mean arterial pressure; IMT, intima-media thickness; PWV, pulse wave velocity; DC, distensibility coefficient; UL, upper limb; and LL, lower limb.*Between-group changes significant at $p < 0.05$; † Within-group changes significant at $p < 0.05$.*

of high responders in both the MCT and HIIT group compared to control, which is partly in line with our previously published main outcomes, where a time-by-group interaction for total FM loss was observed at the 1-year mark in the MCT group, but not for the HIIT group (Magalhaes et al., 2019a). These results put in perspective the importance of looking not only at the mean effects of an exercise intervention, but also to the added value of exploring the individual responses to exercise.

We have previously reported the main results of the D2FIT study, where glycemic and vascular health outcomes varied depending on the exercise intensity (Magalhaes et al., 2019a,b). As part of our secondary aim, we further explored whether individuals classified as low responders for FM loss could still benefit from the exercise intervention. We observed no time-by-group interaction for all of the glycemic control outcomes when comparing the high and low responders against the control group. Conversely, we found that being categorized as a low responder did not preclude individuals from having improvements in vascular and stiffness indices (i.e., cIMT and LL PWV) when compared to the controls. Despite benefits in vascular function being independent of FM loss (Tanaka et al., 2000; Hawkins et al., 2014), those classified as high responders had superior benefits in vascular health, as observed by the time-by-group interaction on cIMT, carotid DC, beta stiffness index, carotid PWV, and LL PWV, in addition to improvements in blood pressure parameters when compared to controls. Indeed, a positive relationship between obesity and blood pressure and risk for hypertension has been thoroughly documented (Hubert et al., 1983; Fogari et al., 2010). In our investigation, only the high responders had a time effect on their DBP, with a reduction of ~6 mmHg, which is noteworthy given the estimated 15–27% reduced incidence of CVD with a decrease of 3.0 mmHg in DBP (Appel et al., 1997). Plausible mechanisms, such as improvements in the sympathetic nervous system and the renin–angiotensin–aldosterone system, may explain the favorable changes in hemodynamic outcomes observed in FM high responders (Kurukulasuriya et al., 2011; DeMarco et al., 2014).

Although improvements in glycemic control with exercise are a main driving factor for the favorable changes in structural and functional vascular indices (MacDonald et al., 2020), neither the low responders or high responders improved their glycemic control when compared to the control group. Thus, other mechanisms may be responsible for the actual improvements observed in vascular parameters in both groups. Indeed, the action of exercise itself can improve vascular function through increasing cardiac output, hence affecting systemic blood flow and impacting endothelial shear stress, which increases the forces exerted on the arterial wall that lead to the production of nitric oxide (Green et al., 2017).

Lastly, similar to the results of this study, we previously observed a similar pattern when considering CRF response to this same exercise intervention, where a low responder to CRF was not precluded from improvements in vascular health (Hetherington-Rauth et al., 2020a). Given that both CRF and FM are two of the most used clinical measurements indicative of the successfulness of an exercise intervention, our current results extend those previously published on CRF by showing that vascular benefits can still be obtainable with exercise in individuals with T2DM who lack reductions in total FM. Nevertheless, individuals classified as high responders for FM loss seem to have superior vascular health benefits, compared to low responders, which was not observed when classifying responders based on CRF.

In an era of personalized lifestyle-based medicine and with an increase in the number of interventions focused on the interindividual variability of several cardiometabolic risk factors in response to exercise (Alvarez et al., 2017; Solomon, 2018; Brennan et al., 2019; Ross et al., 2019; Hetherington-Rauth et al., 2020a; Brennan et al., 2020b), there is a clear need to have a control group to differentiate the inevitable within-subject random variability due to biological error and the technical error of measurement from the variability resulting from the exercise intervention. A major strength of the D2FIT study was its long-term RCT design in individuals with T2DM, which follows the recent consensus statement recommendations, highlighting the importance of using a control group (Ross et al., 2019; Padilla et al., 2021).

The present investigation is not without limitations. The baseline differences observed for the time spent in MVPA may have contributed to the variability observed between the responders and non-responders; however, the results remained unchanged after adjusting for baseline MVPA levels. Due to the nature of this secondary analysis, the originally isoenergetic MCT and HIIT groups were rearranged based on their response rate for FM loss, which could have led to an un-matched exercise volume between the low and high responders. Despite these changes, the proportion of participants from the MCT and HIIT groups, as well as the percent trainings completed, was similar between the low and high responders.

There is still a potential risk to misclassify responders using the TE, which could be reduced by incorporating the 90% confidence intervals on top of the TE, as suggested by Bonafiglia et al. (2018). Nevertheless, for our investigation, this approach would be too conservative, given our high TE derived from the different FM losses observed in the control group, which were likely due to the length and the design of our study (for ethical reasons and participant retention, the control group had monthly sessions on how to improve several aspects of diabetes care, including exercise and diet). However, even when using the TE approach, we still observed variability in the responses to exercise training for FM loss at the 1-year mark with an average percent FM loss of ~13% in the high FM responder group. In fact, this is considerably large considering that in general a 5% loss of body weight is considered to be clinically meaningful for the improvement of cardiometabolic risk factors (Jensen et al., 2014).

In conclusion, a 1-year exercise intervention of either a MCT or HIIT protocol combined with RT had a superior proportion of T2DM individuals who were classified as high responders when compared to the control group. Moreover, individuals who did not improve their body FM following the 1-year intervention still had beneficial adaptations on

vascular structure and stiffness indices. Still, high responders to FM loss had additional improvements in vascular health and blood pressure. Practitioners should not overlook the other benefits on vascular health that can arise from exercise in those who are classified as low responders to FM loss. Nevertheless, FM loss is still an important outcome of exercise interventions to further reduce the progression of CVD in individuals with T2DM.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because data sharing was not included in the Ethics Committee proposal. Requests to access the datasets should be directed to lbsardinha@fmh.ulisboa.pt.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Portuguese Diabetes Association (approval number: 07/17/2013). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LS and JM contributed to the conception and design of the study. JM, PJ, IC, GR, DH-N, and XM were responsible for data collection and acquisition. MH-R and EC were responsible for data analysis and interpretation. JM and MH-R drafted the manuscript. AS, EC, LS, JM, PJ, IC, DH-N, GR, and XM contributed to reviewing and editing the manuscript. All authors contributed to the article and approved the submitted version.

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The Effects of Two Different Concurrent Training Configurations on Markers of Metabolic Syndrome and Fitness in Women With Severe/Morbid Obesity: A Randomised Controlled Trial

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Concurrent training (CT), characterised by combining both aerobic and resistance training modalities within the same session, is recognised to improve metabolic syndrome (MetS) markers, but little is known about the effects of different configurations (i.e., order) of these exercise modalities on MetS markers and the interindividual responses. The purpose of the present study was to describe the effects, and the interindividual variability, of 20 weeks of two CT configurations (i.e., high intensity interval training (HIIT) plus resistance training (RT), compared with RT plus HIIT) in women with severe/morbid obesity. Overall, 26 women with severe/morbid obesity were assigned either to HIIT + RT [$n = 14$, mean and 95%CI, 45.79 (40.74; 50.83) or RT + HIIT ($n = 12$), 33.6 (25.30; 41.79) years]. MetS-related outcomes were waist circumference (WC, cm), systolic (SBP, mmHg) and diastolic (DBP, mmHg) blood pressure, high-density lipoprotein cholesterol (HDL-c), triglycerides (Tg), and fasting plasma glucose (FPG). Secondary outcomes were other anthropometrics, body composition, lipids, muscle strength, and the six-minute walk test (6Mwt). There were significant differences in the prevalence of nonresponders (NRs) only for WC comparing HIIT + RT 2 (18.1%) vs. RT + HIIT group 5 (50.0%), $p < 0.0001$, but not for SBP 4 (27.2%) vs. 4 (40.0%), DBP 8 (72.7%) vs. 7 (70.0%), FPG 8 (72.7%) vs. 9 (90.0%), HDL-c 7 (63.6%) vs. 8 (80.0%), and Tg 7 (63.6%) vs. 8 (80.0%), all $p > 0.05$. Additionally, the RT + HIIT group showed significant reductions in WC (Δ –3.84 cm, $p = 0.015$), SBP (Δ –8.46 mmHg, $p = 0.040$), whereas the HIIT+RT group elicited significant reductions only in SBP (Δ –8.43 mmHg, $p = 0.022$). The HIIT + RT promoted a lower prevalence of NRs than the RT + HIIT configuration on WC, and overall, there were slightly more beneficial training-induced effects on markers of MetS in the RT + HIIT group compared to the HIIT + RT group.

Keywords: concurrent training, morbid obesity, exercise training, metabolic syndrome, exercise order, interindividual variability

INTRODUCTION

Morbid obesity, defined as a body mass index (BMI) of ≥40 kg/m^2 (class III obesity), is a chronic disease with life-threatening cardiometabolic consequences such as elevated blood pressure [systolic (SBP) or diastolic BP (DBP)], fasting plasma glucose (FPG), triglycerides (Tg), and low high-density lipoprotein cholesterol (HDL-c), all summarised as metabolic syndrome (MetS; Baffi et al., 2016), substantially increasing the rates of total mortality, with most of the excess deaths due to heart disease, cancer, diabetes, and important life expectancy reductions compared with normal weight peers (Kitahara et al., 2014). Moreover, morbid obesity has been associated with impairments of cardiorespiratory fitness and muscle strength, limiting the capacity to perform activities of daily living (Pazzianotto-Forti et al., 2020). Additionally, this fact increases the economic costs associated with healthcare in this population (Espallardo et al., 2017).

Due to the multi-factorial aetiology of morbid obesity, such as the genetic load (e.g., the *FTO* gen), and other environmental factors, including mainly lifestyle determinants such as physical activity, exercise training participation, and diet as the main modulators, the application of lifestyle strategies have been proposed prior to alternative surgical intervention in these populations (Bächler et al., 2013; Espallardo et al., 2017). In this sense, exercise training such as the resistance training (RT), defined as any exercise that causes voluntary skeletal muscle contraction by using external weights including dumbbells and metal bars (Schoenfeld et al., 2017), is a known non-pharmacotherapy strategy for improving muscle strength and functional capacity in obese patients undergoing bariatric surgery (Huck, 2015). Similarly, high intensity interval training (HIIT), defined as several and brief bouts of high-intensity effort usually by cycling/running, interspersed by recovery periods (Gibala et al., 2012; Delgado-Floody et al., 2020), has produced strong evidence for the improvement of cardiometabolic risk factors for type 2 diabetes mellitus, arterial hypertension, central arterial stiffness, vascular function, and cardiorespiratory fitness (Ramírez-Vélez et al., 2019). Thus, HIIT might have protective effects against the development of cardiometabolic diseases including populations with poor glucose control and high blood pressure in comparison to moderate-intensity continuous training (MICT; Campbell et al., 2019). Thus, following RT or HIIT alone, unique physiological adaptations have been described for improving, for example, muscle strength, the endurance performance by walking test, as well as beneficial metabolic improvements at MetS markers including fasting glucose reductions, increases at HDL-c, and decreases in Tg in populations with higher adiposity (de Matos et al., 2018).

Thus, in individuals with morbid obesity for example, exercise training has proven to be effective for inducing clinically significant weight loss (5–10%; Gerber et al., 2015), and for the reduction of cardiovascular risk (Delgado-Floody et al., 2019), in accordance with the standard recommendations for these cohorts prior to bariatric surgery. Additionally, there are also other benefits such as the increase of skeletal muscle mass, the reduction of body fat, and better glucose control by the lowering of FPG, and lipids regulation (i.e., increases of HDL-c, and decreases of Tg; Han and Lean, 2016). Briefly, 12 weeks of concurrent training (CT), defined as a combination of both MICT/plus RT, decreased body weight (by ~7.3 kg), blood pressure, and FPG in this cohort (Marc-Hernández et al., 2019). In addition, part of our preliminary findings have shown that 20 weeks of RT decreases MetS risk factors in morbidly obese patients, showing a low inter-individual variability in those patients with greater adiposity, revealing that with more adiposity alteration, the benefits of RT are also more visible (Delgado-Floody et al., 2019). However, some inconsistences, which are directly related with the 'order' (i.e., starting the CT session with MICT followed by RT, or vice versa) of the CT session, have been described after CT. For example, some literature reports that by starting the CT exercise session by RT, participants can get more benefits and improve their physical fitness markers (i.e., increases of upper body strength and neuromuscular markers; Murlasits et al., 2018). However, in contrast, other studies have reported that starting CT with MICT/or HIIT does not alter physiological adaptations in similar outcomes (Wilhelm et al., 2014). Other evidence shows that starting CT with RT exercises first clearly promotes greater lower-body strength gains and neuromuscular economy (Cadore et al., 2013). By contrast, other reports claim no more benefits by starting with MICT/HIIT or RT to physical fitness markers in populations of athletes (Eddens et al., 2018), and additionally, no other benefits for decreasing body fat using one or another exercise modality when starting the CT session (Cadore et al., 2012). However, little is known about the interindividual variability to exercise training (IVET) in relation to different orders of sessions of CT in morbidly obese populations, and in health-related outcomes, such as MetS markers. Briefly, IVET means that some subjects achieve benefits after training, and are termed responders (Rs), while others exhibit a worsened or unchanged response, that are commonly known as nonresponders (NRs; Bouchard et al., 2012). With regard to the causes of IVET, genetic (Stephens et al., 2015) and environmental factors, including the mode of exercise training (Alvarez et al., 2017), have been described. In addition, considering the health-related benefits of CT including MICT/or HIIT plus RT in terms of physical fitness, and the metabolic markers in different populations, as well as taking into account the previous discrepancies of the order session in relevant literature, it is necessary to

Abbreviations: CT, Concurrent training; MetS, Metabolic syndrome; HIIT, High-intensity interval training; RT, Resistance training; WC, Waist circumference; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; HDL-c, High-density lipoproteins; Tc, Total cholesterol; LDL-c, Low-density lipoproteins; Tg, Triglycerides; FPG, Fasting plasma glucose; 6Mwt, Six minutes walking test; IVET, Interindividual variability to exercise training; TE, Technical error of measurement; Rs, Responders; NRs, Nonresponders; MICT, Moderate-intensity continuous training; BMI, Body mass index; CRF, Cardiorespiratory fitness; HGS, Handgrip strength; SMM, Skeletal muscle mass; LM, Lean mass.

investigate the exercise modalities interaction as a precision medicine for improving MetS markers. The purpose of the present study was to describe the effects, and the interindividual variability of 20 weeks CT in different orders by HIIT plus RT compared with another group doing RT plus HIIT in women with severe/morbid obesity at risk of MetS.

MATERIALS AND METHODS

Study Design and Participants

This study is a parallel-group randomised controlled trial in which 34 women with morbid obesity were randomly allocated to one of the two similar CT exercise programmes. The exercises were then applied in different session orders by the two groups (HIIT+RT, $n=17$), and resistance training plus high-intensity interval training (RT + HIIT, $n=17$). The sample size was calculated by G*Power software, and by using the observed delta changes in FPG after previous CT exercise interventions of −4.0 mg/dl, and a standard deviation of 1.0 mg/dl (Álvarez et al., 2019). Thus, a sample with a minimum of four cases per group (minimum sample of $n=8$), gave us an alpha error of $\alpha=0.05$, and a $\beta=0.80$. All participants were informed of the pre–post procedures and of the possible risks/benefits potentially involved with participation in the study, after which they signed an informed consent. The study follows the CONSORT guidelines for randomised trials, was developed in accordance with the Declaration of Helsinki (2013), and has been approved by the Ethical Committee of the Universidad de La Frontera, Temuco, Chile (DI18-0043 Project).

Eligibility criteria were as follows; (i) being a candidate for bariatric surgery (ii) aged between 18 and 60 years (iii) being medically authorised, and (iii) with a BMI ≥40 kg/m^2 or ≥35 kg/m^2 with additional comorbidities (i.e., diabetes, hypertension, insulin resistance) controlled by pharmacotherapy according to the Chilean requirements for morbidly obese patients in order to be a candidate for bariatric surgery (Carrasco et al., 2005). Exclusion criteria were; (i) having physical limitations preventing the performance of exercise (e.g., restricting injuries of the musculoskeletal system) (ii) having exercise-related dyspnoea or respiratory alterations (iii) having chronic heart disease with any worsening in the last month, and (iv) adhering to less than 80% of the total interventions (these results were excluded from the statistical analyses). After the enrolment stage, 43 ($n=43$) participants were assessed for eligibility and nine ($n=9$) were not included according to the inclusion criteria. After the lost followed up participants ($n=8$) for data analysis, 26 ($n=26$) participants were part of the final sample size as follows; HIIT+RT group [$n=14$, mean and (95%CI); 45.79 (40.74; 50.83) or to RT + HIIT group ($n=12$), 33.6 (25.30; 41.79) years old]. Clinical trial number registration is NCT04932642. The study design is shown in (**Supplementary Material 1**).

Interindividual Variability With Regard to Concurrent Exercise Training

Following previous criteria applied in exercise training interventions (Bouchard et al., 2012), the IVET was categorised as responders (Rs), and nonresponders (NRs), using the typical error measurement (TE). Thus, we used previous TE × 2 calculated for WC (0.50 cm × 2), SBP (4.01 mmHg × 2), DBP (2.49 mmHg × 2), HDL-c (2.5 mg/dl × 2), Tg (12.3 mg/dl × 2), using the known equation: $TE = D_{diff} / \sqrt{2}$, where SD_{diff} is the variance (standard deviation) of the difference scores observed between the two repetitions of each test. The NRs for all the MetS outcomes were defined as those individuals who failed to demonstrate a decrease or increase (in favour of beneficial changes) that was greater than twice the TE away from zero.

Metabolic Syndrome Markers

The MetS markers were screened using standard criteria (Alberti et al., 2009). All participants were instructed to arrive at the laboratory with an overnight fasting of 8–10 h, being measured between 08:00 and 9:00 in the morning. These conditions were taken at baseline (pre-test), and at post intervention (post-test). Blood samples were taken with an extraction of ~5 ml, in order to determine the MetS outcomes; FPG, HDL-c, and triglycerides (Tg), as well as the additional markers, total cholesterol (Tc), and low-density lipoprotein cholesterol (LDL-c). Briefly, the samples were placed on ice and centrifuged at 2000 × g for 5 min at 4°C. Plasma samples were immediately transferred to pre-chilled microtubes and stored at −20°C for the following analysis. The measurement of plasma glucose, total cholesterol, and triglycerides were analyzed by enzymatically standard kits (Wiener Lab, Inc., Rosario, Argentina) using automatic equipment (Metrolab2300 Plus™, Metrolab Biomed, Inc., Buenos Aires, Argentina). The HDL-c was analyzed by enzymatic methods after phosphotungstate precipitation (Demacker et al., 1997), and LDL-c was estimated by the Friedewald formula (Friedewald et al., 1972).

The SBP and DBP measurements were carried out according to the standard criteria (Mancia et al., 2014). Blood pressure was measured in the sitting position after 5 min of rest. Two recordings were made, and the mean of the measurements was used for statistical analysis with an OMRON™ digital electronic BP monitor (model HEM 7114, Chicago, IL, United States). Previously to this measurement, we asked the participants to not smoke, or drink meals at least 30 min prior to measurement. Waist circumference (WC) was assessed with a tape measure calibrated in centimetres (Adult SECA™, United States) at the upper hipbone and the top of the right iliac crest, with a non-elastic measuring tape in a horizontal plane around the abdomen at the level of the iliac crest. The tape was snug, but did not compress the skin and was parallel to the floor. The measurement was made at the end of a normal expiration (National Institutes of Health et al., 2000).

Body Composition and Anthropometrics Parameters

The body composition and anthropometrics parameters were measured after fasting (>8 h). Body mass (kg), and body fat (% and kg), skeletal muscle mass (kg), and lean mass (kg) were measured using a digital bio-impedance scale (TANITA™, model 331, Tokyo, Japan) and height (m) was measured using a SECA™ stadiometer (model 214, Hamburg, Germany), with subjects in light clothing and without shoes. BMI was calculated

as the body mass divided by the square of the height (kg/m^2). The BMI was determined to estimate the degree of obesity (kg/m^2) using the standard criteria for obesity and severe/morbid obesity classification (Sturm, 2007).

Six-Minutes Walking Test

The day after the metabolic measurements, the physical condition of the participants in both groups was measured by endurance and muscle strength testing. First, a six-minute walking test (6Mwt) was used to estimate CRF. The test was performed in a closed space on a flat surface (30 m long), with two reflective cones placed at the ends to indicate the distance. During the test, participants were assisted with instructions from an exercise physiologist (de Souza et al., 2009).

Handgrip Strength

Handgrip strength (HGS) was assessed using a digital dynamometer (Baseline™ Hydraulic Hand Dynamometers, United States), which has been used in previous studies (Norman et al., 2011). Two attempts were made, measuring each hand, and the best result from each was selected. As previously, the mean value obtained was taken as the total score (Norman et al., 2011). Using this data we calculated other outcomes such as the HGS and its variation ratio by body mass [HGS/BM], skeletal muscle [HGS/SMM], and lean mass [HGS/LM].

Concurrent Training Intervention

The CT programme had two sections of HIIT and RT, which were applied in different orders to the two experimental groups; HIIT+RT, and RT + HIIT. Before the starting of each exercise-group, both HIIT+RT, and RT + HIIT participated in four familiarization sessions that included (i) knowledge of all measurements, exercise-machine, and instructions during the exercise program (ii) exercising in cycling, weights, and metal bars (iii) applying a few of the exercises of HIIT in 2–3 intervals, and RT in 2–3 sets of exercises, in order to know the configuration of each exercise, and (iv) applying 50–70% of their CT program, independent of their group. Following this, first, in the HIIT+RT group, the HIIT section consisted of 60 s of maximum intensity exercise using a magnetic resistance static bicycle (Oxford™ Fitness, model BE-2701, Chile), followed by 60–120 s of passive recovery with the bicycle totally off. This was repeated four to seven times according to the weekly schedule (Delgado-Floody et al., 2019). The intensity of the exercise was measured on the Borg scale of 1–10 of perceived exertion and the participants worked at a level of between 6 and 9 points.

Second, in the RT section, three out of four RT exercises were included (according to the planning week), targeting the following different muscle groups: (1) forearm (2) knee flexors and extensors (3) trunk (4) chest (5) shoulder elevators (6) horizontal shoulder flexors (7) extensors, and finally (8) plantar flexors. These exercises were performed in three sets of as many repetitions (continuous concentric/eccentric voluntary contraction) as possible in 60 s, followed by 60–120 s of passive recovery, as previously reported (Álvarez et al., 2019). To estimate the intensity of work in the different RT exercise, the maximum dynamic muscular strength (1RM) was estimated indirectly through the Brzycki formula (Brzycki, 1993), with fewer than 12 maximum repetitions. The RT + HIIT group performed the same training programme as the HIIT+RT group (described above) but the order of the HIIT and RT exercises were reversed (i.e., first RT and then HIIT). This was called the RT + HIIT group. The exercise programme compounds of the CT regime applied can be found in **Table 1**.

Statistical Analyses

Data are presented as the mean and (95%CI) in tables, as mean with (±) standard error in **Figures 1–3**, and as individual delta in **Figure 4** for identification of Rs and NRs. Normality and homoscedasticity assumptions for all data were analyzed using the Shapiro–Wilk and Levene's test, respectively. In the HIIT+RT group, the Tg outcome, as well as in the RT + HIIT group, LDL-c, and lean mass outcomes were analyzed by the *Wilcoxon* non-parametric test. For training-induced changes, the student's *t*-test was used to identify differences at baseline, while a repeated measure two-way ANOVA was applied to assess the occurrence of an actual training effect; namely, $p < 0.05$ for the interaction (time × group) on the main MetS (WC, SBP, DBP, FPG, HDL, and Tg, as well as to the secondary outcomes). A Sidack's *post hoc* test was used for multiple comparisons. Additionally, the Eta partial squared for interaction (Time × Group) was assessed by η^2 obtained from the ANCOVA with small ($\eta^2 = 0.01$), medium ($\eta^2 = 0.06$), and large ($\eta^2 = 0.14$) effects defined according to Lakens (2013). The prevalence of NRs was described using the comparisons by percentage between both experimental groups using a Chi square test χ^2. All statistical analyses were performed with SPSS statistical software version 23.0 (SPSS™ Inc., Chicago, IL). The alpha level was set at $p < 0.05$ for statistical significance.

RESULTS

Baseline Characteristics

There were significant differences between the groups ($p = 0.004$) in terms of age with 45.79 (95% CI; 40.74 to 50.83) in the HIIT+RT vs. 33.55 (95% CI; 25.30 to 41.79) in the RT + HIIT group. There were no other differences at baseline (**Table 2**).

Training-Induced Changes on Mets Markers and Secondary Outcomes

With regard to anthropometric and body composition and considering the absolutes values, in the RT + HIIT group there were significant changes between pre- and post-training measurements in outcomes relating to BMI: 42.72 (39.77; 45.63) vs. 41.87 (38.73; 45.01) kg/m^2, $p = 0.050$, WC: 118.67 (110.43; 126.91) vs. 114.83 (106.31; 123.35) cm, $p = 0.015$, of skeletal muscle mass 52.21 (49.06; 55.37) vs. 51.00 (48.53; 53.48) kg, $p = 0.015$, and the lean mass of this group: 54.99 (51.66; 58.31) vs. 53.78 (51.18; 56.38) kg, $p = 0.022$ (**Table 2**). There were no other changes of the RT + HIIT or HIIT+RT groups (**Table 2**).

Comparing the HIIT+RT and RT + HIIT groups with regard to delta (Δ) changes from pre to post test, there were no

TABLE 1 | Characteristics of the high-intensity interval training, and of the resistance training scheme, both compounds of a concurrent training programme of 20 weeks, applied to two different groups; CT applied in order HIIT+RT, and CT applied in order RT+HIIT.

Concurrent training (*)											
Weeks	HIIT					RT					
	E N°	Intensity (Borg subjective perception)	Time (min)	Sets	Rest (min)	E N°	Intensity (%1RM)	Reps	Time (min)	Sets	Rest (min)	Duration (min)
1–2	3	6–7	1	4	2.0	3	40	20–25	1	3	2.0	45
3–4	3	6–7	1	4	2.0	3	40–50	25–30	1	3	2.0	45
5–6	4	6–7	1	5	1.5	4	45–50	30	1	3	1.5	45
7–8	4	7–8	1	5	1.5	4	45–50	30–35	1	3	1.5	45
9–10	4	7–8	1	5	1.5	4	50–55	25–30	1	3	1.5	45
11–12	4	7–8	1	6	1.5	4	50–55	25–30	1	3	1.5	45
13–14	4	7–8	1	6	1.0	4	50–55	30	1	3	1.0	45
15–16	4	8	1	6	1.0	4	50–55	30–35	1	3	1.0	45
17–18	4	8	1	7	1.0	4	55	30–35	1	3	1.0	45
19–20	4	8–9	1	7	1.0	4	55–60	30–35	1	3	1.0	45

(*) The present HIIT and RT dosage, components of the CT scheme, are valid to both experimental HIIT+RT, and RT+HIIT groups. (HIIT) High-intensity interval training. (RT) Resistance training. (E N°) number of intervals (HIIT groups) or number of total exercises (RT group).

TABLE 2 | Training-induced changes on anthropometric and body composition in morbid obesity patients after 16 weeks of concurrent training applied in two different concurrent training session orders; HIIT + RT, or CT applied as RT+HIIT.

Outcomes	Time	Groups		Baseline †p value
		HIIT + RT	RT+HIIT	
(*n* =)				
Age (y)		45.79 (40.74; 50.83)	33.55 (25.30; 41.79)	***p* = 0.004**
Height (m)		1.55 (1.51; 1.59)	1.59 (1.55; 1.62)	*p* = 0.188
Anthropometric/Body composition outcomes				
Body mass (kg)	Pre	99.30 (87.45; 111.15)	108.12 (99.84; 116.40)	*p* = 0.109
	Post	98.09 (86.24; 109.93)	105.64 (97.05; 114.22)	
	p =	*p* = 0.160	***p* = 0.015**	
Body mass index (kg/m^2)	Pre	40.95 (37.00; 44.89)	42.72 (39.77; 45.63)	*p* = 0.217
	Post	40.23 (36.49; 44.01)	41.87 (38.73; 45.01)	
	p =	*p* = 0.065	***p* = 0.050**	
Waist circumference (cm)	Pre	114.22 (106.24; 122.18)	118.67 (110.43; 126.91)	*p* = 0.246
	Post	112.27 (104.31; 120.23)	114.83 (106.31; 123.35)	
	p =	*p* = 0.147	***p* = 0.015**	
Body fat (%)	Pre	46.95 (43.72; 50.19)	48.95 (47.27; 50.63)	*p* = 0.161
	Post	46.65 (43.90; 49.40)	49.05 (47.00; 51.10)	
	p =	*p* = 0.413	*p* = 0.807	
Body fat (kg)	Pre	47.59 (39.17; 56.01)	52.85 (47.49; 58.21)	*p* = 0.143
	Post	46.55 (38.58; 54.51)	52.21 (46.04; 58.39)	
	p =	*p* = 0.118	*p* = 0.388	
Skeletal muscle mass (kg)	Pre	49.08 (45.54; 52.62)	52.21 (49.06; 55.37)	*p* = 0.093
	Post	49.91 (45.02; 52.79)	51.00 (48.53; 53.48)	
	p =	*p* = 0.677	***p* = 0.015**	
Lean mass (kg)	Pre	51.70 (47.98; 55.42)	54.99 (51.66; 58.31)	*p* = 0.094
	Post	51.51 (47.42; 55.60)	53.78 (51.18; 56.38)	
	p =	*p* = 0.622	*p* = 0.081¥	

Data are shown as mean and (95%CI). Groups are described as; (HIIT+RT) order session of high-intensity interval training plus resistance training, (RT+HIIT) order session of resistance training plus high-intensity interval training. (†) Between-group baseline comparisons were measured by a General Lineal Model, using a Univariant test. Within-group comparisons by pre-post time were analyzed by Repeated Measures using 2-way ANOVA (Group × Time). (¥) Analyzed by non-parametric Wilcoxon test. Bold values denote significant differences at p ≤ 0.05. All analyses were adjusted by age, height, and BMI.

significant differences between groups at ΔBM kg, ΔBF%, ΔWC, ΔBF kg, ΔLM, and ΔSMM (**Figures 1A–F**).

With regard to cardiovascular and metabolic outcomes, and considering the absolutes values, there were significant changes in the HIIT+RT group from pre- to post-training measurements

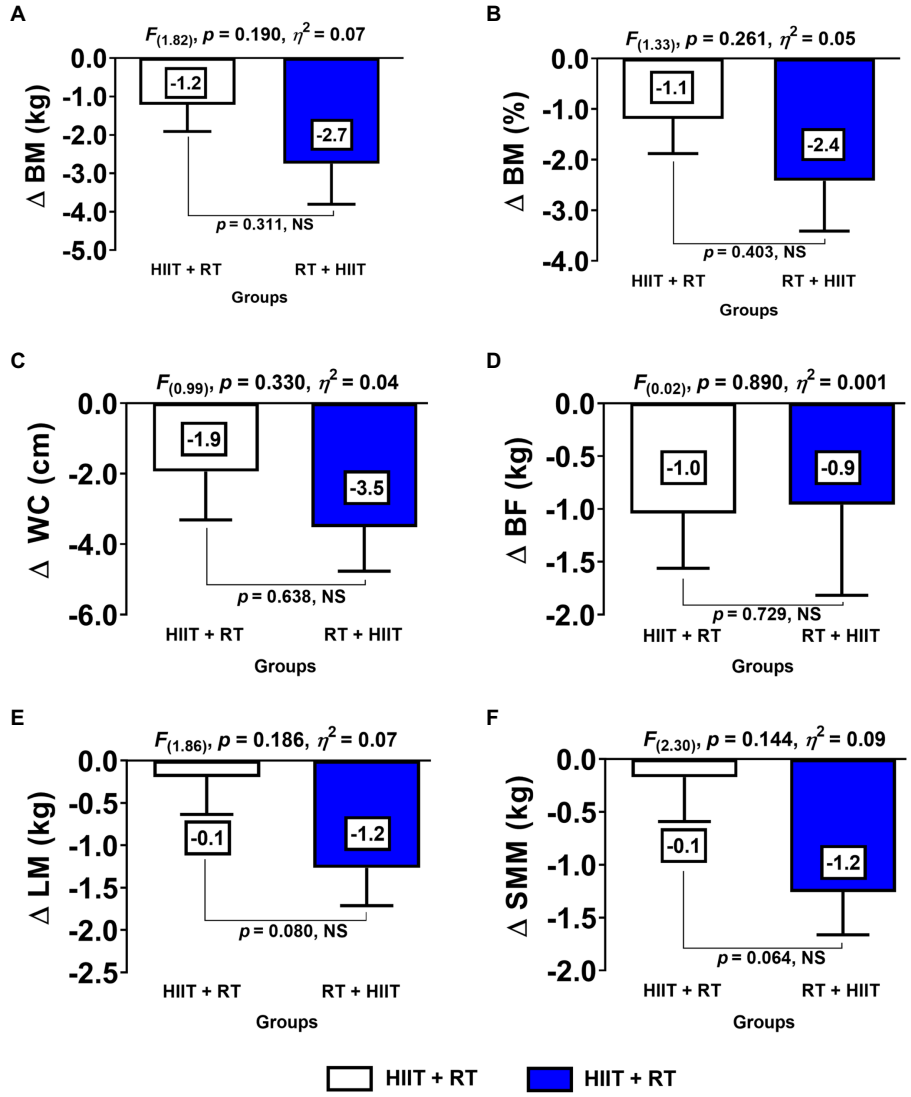

FIGURE 1 | Changes in body composition and anthropometric parameters for each experimental exercise group. (HIIT+RT) High-intensity interval training plus resistance training order group. (RT + HIIT) Resistance training plus high-intensity interval training order group. (BM) body mass (BF) body fat (SMM) skeletal muscle mass (LM) lean mass (WC) waist circumference. (Δ) denotes delta changes from pre–post intervention. (NS) denotes no significant differences.

in SBP: 138.57 (128.50; 148.64) vs. 130.14 (123.20; 137.08) mmHg, $p = 0.022$, to pre and post-test, respectively, (**Table 3**). No other pre- or post-training changes were observed in other outcomes in this group at these parameters. Similarly, for the RT + HIIT group, there were significant training-induced changes of SBP from 136.36 (125.74; 146.98) vs. 129.90 (122.85; 132.96) mmHg, $p = 0.040$, and of total cholesterol from 177.09 (161.31; 192.87) vs. 162.09 (148.27; 175.90) mg/dl, $p = 0.017$ (**Table 3**). No other pre- or post-training changes were observed in other outcomes in this group at these parameters.

Comparing the HIIT+RT and RT + HIIT group with regard to delta (Δ) changes from pre- to post-test, there were no significant differences between groups at ΔSBP, ΔDBP, ΔTc, ΔLDL-c, ΔHDL-c, ΔTg, and ΔFPG (**Figure 2**).

Training-Induced Changes on Fitness

With regard to physical fitness outcomes, and considering absolute values, there were significant changes in the HIIT+RT group from pre- to post-training measurements in the 6Mwt from pre 499.28 (426.58; 571.98) vs. 637.14 (573.87; 700.40) m, $p < 0.0001$ (**Table 4**). Similarly, in the RT + HIIT group, there were significant changes in this outcome from pre 533.63 (491.33; 575.94) vs. 597.27 (505.49; 689.04) m, $p = 0.048$ (**Table 4**). No other significant pre- or post-training changes were detected in both groups at these parameters.

Comparing the HIIT+RT and RT + HIIT group in terms of delta (Δ) changes from pre- to post-test, there were no significant differences between groups in ΔHGS, ΔHGS/BM ratio, ΔHGS/SMM ratio, ΔHGS/LM ratio, ΔHDL-c, and Δ6Mwt (**Figure 3**).

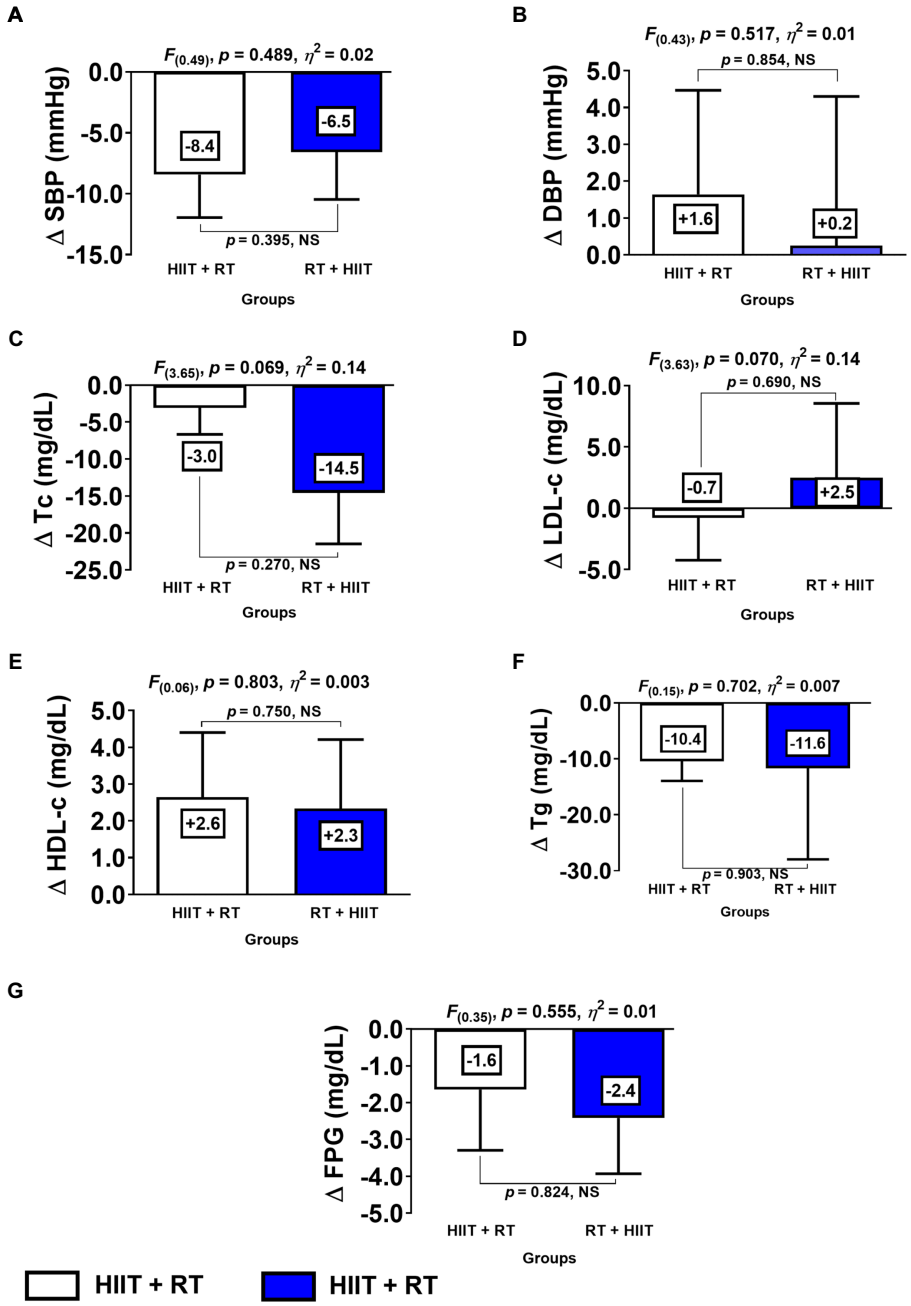

FIGURE 2 | Changes of metabolic syndrome makers for each experimental exercise-group. (HIIT+RT) High-intensity interval training plus resistance training order group. (RT + HIIT) Resistance training plus high-intensity interval training order group. (SBP) systolic blood pressure (DBP) diastolic blood pressure (FPG) fasting plasma glucose (Tc) total cholesterol (LDL-c) low-density lipids (HDL-c) high-density lipids, and (Tg) triglycerides. (Δ) denotes delta changes from pre to post intervention. (NS) denotes no significant differences.

Interindividual Variability on Mets Markers After HIIT+RT or RT + HIIT Session Orders of CT

There was a different prevalence of NRs in improving (i.e., decreasing) WC comparing HIIT+RT 2 (18.1%) vs. RT + HIIT group 5 (50.0%), $p < 0.0001$ (**Figure 4A**). The two groups can be compared as follows: HIIT+RT vs. RT + HIIT group SBP 4 (27.2%) vs. 4 (40.0%; **Figure 4B**), DBP 8 (72.7%) vs. 7 (70.0%; **Figure 4C**), FPG 8 (72.7%) vs. 9 (90.0%; **Figure 4D**), HDL-c 7 (63.6%) vs. 8 (80.0%; **Figure 4E**), and Tg 7 (63.6%) vs. 8 (80.0%; **Figure 4F**), there were no significant differences in these outcomes.

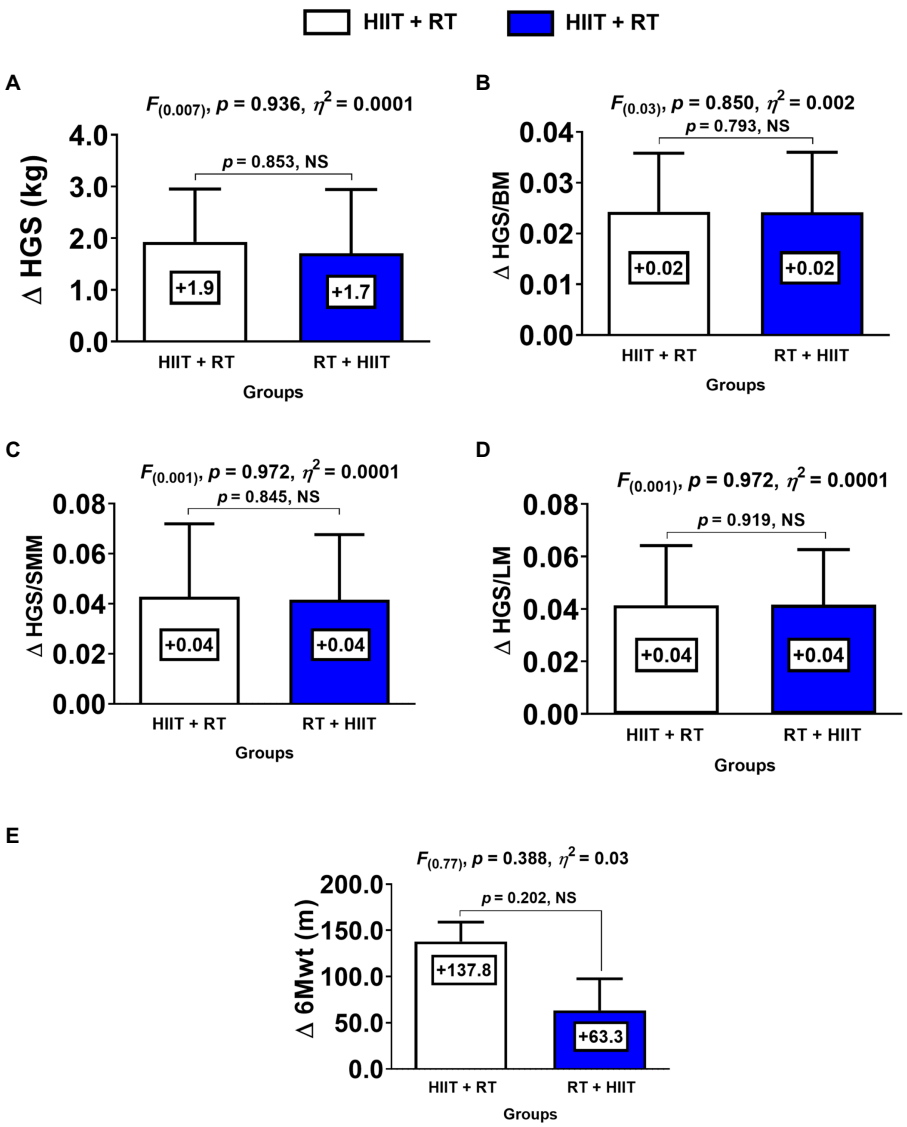

FIGURE 3 | Changes in physical fitness for each group. (HIIT+RT) High-intensity interval training plus resistance training order group. (RT + HIIT) Resistance training plus high-intensity interval training order group. (HGS) handgrip strength (BM) body mass (SMM) skeletal muscle mass (LM) lean mass (6Mwt) six-minute walking test. (Δ) denotes delta changes from pre to post intervention. (NS) denotes no significant differences.

DISCUSSION

The purpose of the present study was to describe the effects and the interindividual variability of 20 weeks of HIIT+RT compared with another group doing RT + HIIT in a sample of women with severe/morbid obesity who were at risk of MetS. The main results of this study were that: (i) considering the MetS outcomes, the WC adiposity marker had significantly less prevalence of NRs in the HIIT+RT compared to the RT + HIIT order session after 20 weeks of CT (**Figure 4A**) (ii) when both order session groups demonstrated significant improvements in MetS markers, such as at SBP (**Figure 2A**), or in secondary outcomes such as 6Mwt, for example (**Figure 4E**), there were no differences by group. Additionally, considering the overall training-induced changes for both HIIT+RT and RT + HIIT groups, a slight advantage in the RT + HIIT group was observed favouring more beneficial effects than the HIIT+RT group (**Tables 2** and **3**).

Although there are only a minor number of studies reporting the Rs and NRs phenomenon, there is little knowledge about this topic exploring exercise methodologies such as CT variations in clinical populations like the morbidly obese. Along these lines, and from our previous experience with obesity patients, for example, after an RT programme (20 weeks, eight exercises), morbid obesity patients showed an NR prevalence of 42.8% compared with obesity patients (85.4%). Interestingly, however,

TABLE 3 | Training-induced changes on cardiovascular and plasma markers in morbid obesity patients after 20 weeks of concurrent training applied in two different order session; CT as HIIT + RT, or CT applied as RT + HIIT.

Outcomes	Time	Groups		Baseline †p value
		HIIT + RT	RT + HIIT	
Cardiovascular/metabolic outcomes				
Systolic blood pressure (mmHg)	Pre	138.57 (128.50; 148.64)	136.36 (125.74; 146.98)	$p = 0.741$
	Post	130.14 (123.20; 137.08)	127.90 (122.85; 132.96)	
	p =	$\mathbf{p = 0.022}$	$\mathbf{p = 0.040}$	
Diastolic blood pressure (mmHg)	Pre	83.57 (77.02; 90.11)	91.54 (83.40; 99.68)	$p = 0.085$
	Post	85.21 (80.74; 89.68)	90.27 (85.01; 95.52)	
	p =	$p = 0.614$	$p = 0.728$	
Fasting plasma glucose | (mg/dl)	Pre	99.71 (86.51; 112.91)	97.45 (86.62; 106.88)	$p = 0.961$
	Post	98.07 (87.73; 108.40)	95.18 (88.00; 102.35)	
	p =	$p = 0.307$	$p = 0.213$	
Total cholesterol (mg/dl)	Pre	181.35 (163.65; 199.06)	177.09 (161.31; 192.87)	$p = 0.817$
	Post	178.28 (157.47; 199.10)	162.09 (148.27; 175.90)	
	p =	$p = 0.558$	$\mathbf{p = 0.017}$	
Low-density lipoprotein cholesterol (mg/dl)	Pre	124.78 (111.02; 138.54)	101.18 (78.09; 124.26)	$p = 0.068$
	Post	124.00 (110.93; 137.06)	104.09 (90.35; 117.82)	
	p =	$p = 0.868$	$p = 0.509^{¥}$	
High-density lipoprotein cholesterol (mg/dl)	Pre	54.71 (47.89; 61.52)	49.18 (43.44; 54.92)	$p = 0.151$
	Post	57.35 (50.84; 63.86)	51.45 (45.78; 57.12)	
	p =	$p = 0.153$	$p = 0.271$	
Triglycerides (mg/dl)	Pre	117.42 (72.85; 162.00)	123.81 (94.74; 152.88)	$p = 0.749$
	Post	107.00 (65.57; 148.42)	124.18 (88.14; 160.21)	
	p =	$\mathbf{p = 0.004^{¥}}$	$p = 0.966$	

Data are shown as mean and (95%CI). Groups are described as; (HIIT + RT) order session of high-intensity interval training plus resistance training, (RT + HIIT) order session of resistance training plus high-intensity interval training. (†) Between-group baseline comparisons were measured by General Lineal Model, using a Univariant test. Within-group comparisons by pre-post time were analyzed by Repeated Measures using 2-way ANOVA (Time × Group). (¥) Analyzed by non-parametric Wilcoxon test. Bold values denote significant differences at $p \leq 0.05$. All analyses were adjusted by age, height, and BMI.

TABLE 4 | Training-induced changes on strength and endurance performance in morbid obesity patients after 16 weeks of concurrent training applied in two different concurrent training session modalities; CT as HIIT + RT, or CT applied as RT + HIIT.

Outcomes	Time	Groups		Baseline †p value
		HIIT + RT	RT + HIIT	
Strength/endurance performance				
HGS (kg)	Pre	26.28 (22.51; 30.05)	29.72 (23.61; 35.83)	$p = 0.263$
	Post	28.21 (23.60; 32.81)	31.18 (25.87; 36.48)	
	p =	$p = 0.090$	$p = 0.249$	
Ratio HGS/ skeletal muscle mass	Pre	0.53 (0.47; 0.59)	0.57 (0.44; 0.71)	$p = 0.560$
	Post	0.57 (0.49; 0.65)	0.61 (0.49; 0.74)	
	p =	$p = 0.076$	$p = 0.295$	
Ratio HGS/ body mass	Pre	0.26 (0.23; 0.29)	0.28 (0.20; 0.35)	$p = 0.792$
	Post	0.29 (0.22; 0.36)	0.30 (0.23; 0.37)	
	p =	$\mathbf{p = 0.045}$	$p = 0.106$	
Ratio HGS/ lean mass	Pre	0.50 (0.44; 0.56)	0.54 (0.42; 0.67)	$p = 0.558$
	Post	0.54 (0.47; 0.62)	0.58 (0.46; 0.70)	
	p =	$p = 0.075$	$p = 0.131$ |	
6 min walking test (m)	Pre	499.28 (426.58; 571.98)	533.63 (491.33; 575.94)	$p = 0.463$
	Post	637.14 (573.87; 700.40)	597.27 (505.49; 689.04)	
	p =	$\mathbf{p < 0.0001}$	$\mathbf{p = 0.048}$	

Data are shown as mean and (95%CI). Groups are described as: (HIIT + RT) order session of high-intensity interval training plus resistance training, (RT + HIIT) order session of resistance training plus high-intensity interval training. (HGS) Handgrip muscle strength. (†) Between-group baseline comparisons were measured by a General Lineal Model, using a Univariant test. Within-group comparisons by pre-post time were analyzed by Repeated Measures using 2-way ANOVA (Group × Time). Bold values denote significant differences at $p \leq 0.05$. All analyses were adjusted by age, height, and BMI.

morbid obesity patients showed higher benefits of decreasing WC Δ −10.1 than their obese peers Δ −2 to −6 cm (Delgado-Floody et al., 2019). In this sense, the evidence shows the apparent role of the previously reported 'health status' factor in the prevalence of NRs, where populations of women with more disease clearly experience more benefits after exercise than peers with a minor degree of disease (Alvarez et al., 2017). However, one of the most intriguing results revealed in the present study regarding the minor prevalence of NRs for decreasing WC in the HIIT+RT 2 (18.1) vs. RT + HIIT 5 (50.0%; **Figure 4A**) is shown by contrast with the minor degree of reduction of WC in HIIT+RT Δ −1.2 vs. RT + HIIT Δ −2.7 cm (**Figure 1C**). Although this difference is more than approximately twofold of WC reduction and was non-significant between groups, we could presume that independent of showing a higher prevalence of NRs for decreasing WC in the CT order session of RT + HIIT rather than the HIIT+RT group (**Figure 4A**), the training-induced changes are independent of the NRs phenomenon.

Additionally, a study conducted in overweight and obese subjects showed that 6 weeks of HIIT (20-min protocol, consisting of 4 min of cycling at 15% of maximum anaerobic

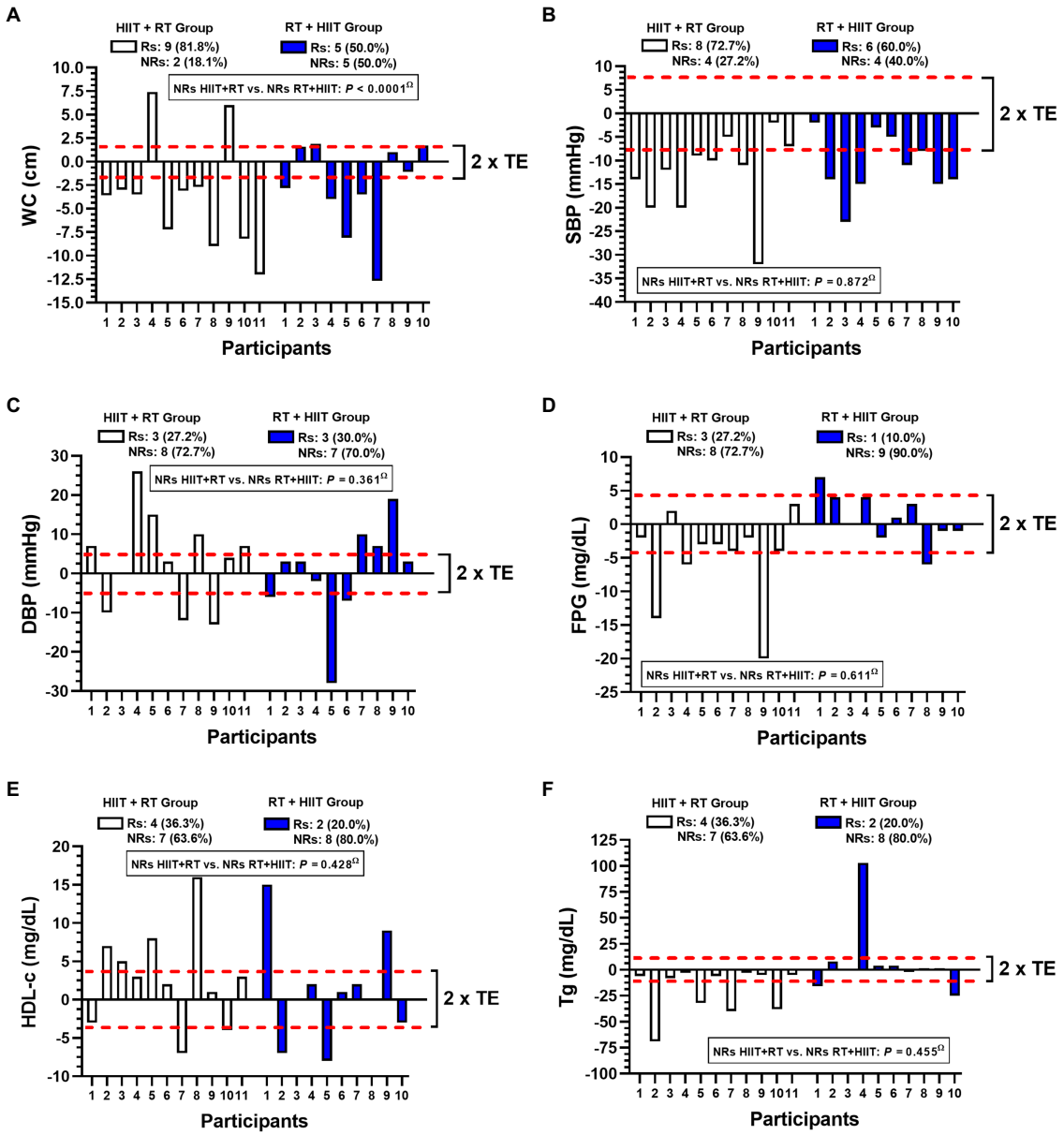

FIGURE 4 | Inter-individual variability for a different concurrent training order session in morbid obesity patients in MetS outcomes. Abbreviations: WC; waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL-c high-density lipids; and Tg, triglycerides. (Rs) denotes 'responders'. (NRs) denotes non-responders to improve MetS outcomes considering modifications more than 2 technical errors. Bold values denote significant differences among frequencies of NRs between HIIT+RT vs. RT + HIIT group.

power [Max-AP] followed by 30 s at 85% of Max-AP) decreased BF% Δ −0.88%, and BMI Δ −0.26 kg/m^2 (Fisher et al., 2015). Another study by Alvarez et al. (2018) reported that 16 weeks of HIIT (7–10 × 1 min exercise with 2 min of rest) reduced body mass Δ −3.3 kg, BMI Δ −1.4% kg/m^2, and BF% Δ −5.8% in a prehypertensive and overweight/obese cohort of women.

Additionally, a 12-week RT programme (three times weekly, 60 min/session, 17 strength exercises at 60–70% 1RM intensity) reported a significant reduction in both SBP Δ −12.3 mmHg, and DBP Δ −11.2 mmHg in obese men (Klimcakova et al., 2006). Although the authors have not reported the IVET as Rs and NRs, these blood pressure benefits are in accordance with the previous findings of our research team of Δ −10.4 mmHg at SBP, and Δ −7.0 mmHg at DBP, in morbidly obese patients after RT, where interestingly, these blood pressure benefits occurred independently of a weight loss (Delgado-Floody et al., 2019). Along similar lines, part of the exercise capacity for improving vasculature was the subject of a recent study conducted in young obese women which compared a HIIT group (4 × 4 min

at 85–95% of HR_{max}, interspersed with 3-min periods of active recovery at 65–75% of HR_{max}) with an MICT group (41 min at 65–75% of HR_{max}). This showed that both exercise protocols significantly reduced carotid–femoral pulse wave velocity by $\Delta -0.37$ and $\Delta -0.35$, respectively. Furthermore, significant reductions in brachial SBP $\Delta -6.3$, and central SBP $\Delta -6.6$ mmHg were observed after HIIT (de Oliveira et al., 2020). Thus, the authors concluded that HIIT and MICT reduced arterial stiffness in obese young women, therefore showing salutary benefits as an antihypertensive nondrug therapy. Another study which investigated the effects of 6 weeks of HIIT (10 × 1 min intervals at 90–100% peak workload) or MICT (30 min at 65–75% peak HR) on blood pressure and aortic stiffness in males who were overweight/obese, reported that HIIT was effective for reducing BP Δ ~3–5 mmHg, also there were MICT-induced moderate reductions in diastolic blood pressure (peripheral; Δ −3.4 mmHg and central; Δ −3 mmHg; Clark et al., 2020). From our previous experience, a 16 week-HIIT programme (7–10 × 1 min exercise with 2 min of rest) reduced SBP Δ −8.0 mmHg, and DBP Δ −5.8 mmHg in sedentary and overweight/obese women (Alvarez et al., 2018). Thus, the mechanisms by which exercise training decreases blood pressure have been explained in part by a reduction of arterial stiffness, an improvement of endothelial mediated vasodilation, a reduction in vascular peripheral resistance, an increase of the novel peptide apelin by the nitric oxide plasma levels, and a decrease in sympathetic nervous activity (Alvarez et al., 2018) to name a few. Likewise, WC, SBP, and DBP are important parts of the MetS in which both HIIT and RT have been shown to benefit populations with higher adiposity.

In other secondary outcomes, only the RT + HIIT group reported changes in Tc (**Table 3**). In this sense, a 16 week HIIT programme (7–10 × 1 min exercise with 2 min of rest) reduced Tc Δ −8 mg/dl, LDL-c Δ −2.6 mg/dl, Tg Δ −13.9 mg/dl, and increased HDL-c Δ +5 mg/dl in overweight/obese women (Alvarez et al., 2018). Following our experiences, similarly 20 weeks of RT (three sessions/week, 4–8 exercise) using free weights reduced Tc Δ −7.5 mg/dl in adults with obesity or morbid obesity (Delgado-Floody et al., 2019). Thus, at this level of adiposity, our present findings are in accordance with previous benefits of HIIT or RT in decreasing WC (a marker of visceral subcutaneous fat), as well as other fat sources such as the lipoproteins Tc, LDL-c, and Tg.

Both groups (HIIT+RT and RT+ HIIT) showed significant changes of the CRF marker 6Mwt, without a difference between the groups (**Figure 2E**). In this sense, for example, 34 sessions of HIIT (3–7 repetitions of 3 min bouts of high-intensity walking [100% of $V\,O_{2peak}$], interspersed by 1.5 min walking at low intensity) improved the $V\,O_{2peak}$ Δ+16% in obese subjects (Vaccari et al., 2020). Interestingly, RT has also been shown to improve CRF in some clinical populations. Following this, a study comparing 8 weeks of RT (the programme started with two sets of 18–20 maximal repetitions and progressed to three sets of 10–14 maximal repetitions with a rest of 1–2 min between sets) and CT (30 min of MICT 40–70 HR reserve and 30 min of RT per session) showed that the CRF was increased in RT Δ +1.5 ml/kg/min and CT Δ +7.7 ml/kg/min in obese adults (Schroeder et al., 2019). Considering that in the present study both HIIT+RT and RT + HIIT order session groups increased their performance in the 6Mt by Δ +137.8 and Δ +63.3 m, respectively, (**Figure 3E**), together with the overall training-induced changes of RT at level of both MetS and secondary outcomes, we can presume that in a CT intervention for clinical populations, the RT scheme can play a major role in the health-related benefits of morbidly obese patients.

A limitation of this study was that the foods habits (diet) of the participants during the intervention were not measured. By contrast, a strength of this study was that we included not only MetS markers but also different outcomes such as anthropometric, body composition, strength, and CRF fitness markers, as well as highlighting the severely/morbidly obese clinical cohort with whom we developed our research project. Considering the expensive and long treatments before bariatric surgery, the topic of IVET is of high interest and value.

In conclusion, the HIIT+RT intervention promotes a smaller prevalence of NRs than the RT + HIIT order session of CT. Overall there is a slight advantage in the RT + HIIT group in favour of more beneficial training-induced effects compared to the HIIT+RT group.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Committee of the Universidad de La Frontera, Temuco, Chile (ACTA N°071_18). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PD-F and CA: conceptualization, formal analysis, and writing -review and editing. PD-F and CA: methodology. MR-P: software. CA: validation. CA and PD-F: formal analysis. PD-F, CA, and AS-M: investigation. PD-F, CA, AS-M, CM-S, CV, and FC-N: data curation. AS-M, MR-P, CV, and DJ-M: writing -original draft preparation. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.694798/full#supplementary-material

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OPINION
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Strength Training Volume to Increase Muscle Mass Responsiveness in Older Individuals: Weekly Sets Based Approach

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INTRODUCTION

Currently, strength training (ST) is widely recommended to promote healthy aging. This reflects efforts made over the past three decades through which the role of ST as a sarcopenia countermeasure, preventing physical disability and other poor outcomes, has become evident. However, in recent years, considerable attention has been directed to the phenomenon of ST response heterogeneity. Different adaptive patterns among individuals submitted to the same intervention have increasingly led scholars to conventionally label non-responders or low responders those who do not respond appropriately, either for lack of meaningful improvements or even for worsening parameters. In the latter case, such individuals are specifically classified generally as adverse responders since they present responses in opposite direction to a threshold theoretically or empirically determined (Bouchard et al., 2012; Hecksteden et al., 2018). Although there is still no consensus on the definition of non-responders or low responders to ST, showing that their study is in its infancy, an interesting review by Atkinson et al. (2019) suggests that the categorization of a given response should be rationalized by the researcher after analyzing which threshold is clinically or practically relevant, changing the notion that minimal detectable change is imperative to determine who respond or not to an intervention.

In this scenario, the phenomenon of non- or low-responsiveness indirectly favored the development of a more restrictive and less important perspective regarding the role of ST. However, reaffirming the ST relevance in aging, Churchward-Venne et al. (2015) have claimed that there are no older non-responders. In that study, it has been shown that all older adults submitted to ST improve at least one analyzed parameter (e.g., functional capacity and muscle strength), defended its widespread application. As suggested by Pickering and Kiely (2019), there are likely no individuals totally unresponsive to training. Nevertheless, the reason why some individuals show less expressive results than others in apparently homogeneous samples remains unclear, especially concerning muscle hypertrophy.

It can be noticed in the study conduct by Churchward-Venne et al. (2015) that more than 35 and 30% of the older individuals had a maximum lean mass increase of 0.5 kg after 12 and 24 weeks of ST, respectively. In addition, about 20% had a decrease in lean mass, regardless of protocol duration. Despite the inherent physiological changes of aging, Ahtiainen et al. (2016) demonstrate that the variability of muscle mass response to ST is not affected by age. Although such changes may indeed lead older individuals to a decreased skeletal muscle tissue sensitivity to anabolic stimuli (Yang et al., 2012), it prohibits putting on account of aging the occurrence of inexpressive morphological adaptations in response

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to training exclusively. Regarding this muscle mass response heterogeneity, while some scholars (Atkinson et al., 2019; Dankel and Loenneke, 2020) recommend cautiousness when claiming its existence, emphasizing that individual differences need to be attested in studies that consider the random error, verified from a matched control group, many others widely recognize it (Hubal et al., 2005; Davidsen et al., 2011; Sparks, 2017; Stec et al., 2017; Camera, 2018; Räntilä et al., 2021). Although more studies need to be conducted to demonstrate the true variability of the response, the body of evidence indicates that such heterogeneity should not be ignored. In this context, it is inevitable to admit that more efforts should be made to clarify the reasons behind the low-responsiveness of some individuals, which, in turn, might assist in planning more effective ST programs.

VOLUME: A KEY POINT?

The proper design of an ST program involves the management of several variables. Among all, most of them are closely related to intensity and volume. By intensity, it is recognized as the load lifted in a given movement, calculated basically by the percentage of the maximum load lifted only once [e.g., percentage of one-repetition maximum (1RM)]. The training volume, in turn, is traditionally determined by multiplying the number of sets, repetitions, and load. It is noted that the amount of load lifted directly influences the number of repetitions performed (Spiering et al., 2008). Because of the close relationship between repetitions and load, the number of sets performed plays an independent role during the ST progression management and training volume measurement.

Currently, some scholars have considered volume the most effective ST variable to promote morphological changes (Figueiredo et al., 2018). Mattocks et al. (2017) demonstrated that subjects submitted to very high-intensity strength training, simulating 1RM test sessions, achieved the same strength gains as those who trained at a higher volume. However, only the group that trained with higher volume presented muscle hypertrophy. From a molecular point of view, higher exercise volume positively affects myofibrillar protein synthesis and anabolic signaling and is critical for the degree of p70S6k and S6 phosphorylation following a resistance exercise bout (Burd et al., 2010a,b; Terzis et al., 2010). Additionally, it appears that the type of contraction is not more important than volume to cause molecular changes after an exercise session (Garma et al., 2007). A meta-analysis on the influence of ST on the lean mass of the older individuals found that more significant results were particularly related to protocols where more total sets were performed during the training session (Peterson et al., 2011).

From this evidence, it would be reasonable to expect greater effectiveness of experimental protocols on morphological as higher training volume is applied. Several studies comparing the performance of a single set with multiple sets have demonstrated the superiority of performing multiple sets in both young and older individuals (Kramer, 1997; Radaelli et al., 2014a,b). However, the meta-analysis conducted by Krieger (2010) minimizes the relevance of the volume. In this review, the authors concluded that multiple sets are, in fact, better than a single set but performing four to six sets is not superior to performing two to three sets. While providing valuable information, this body of evidence partially explains why increasing the training volume for more robust physiological responses is not a unanimous strategy (Souza et al., 2018). By the way, it seems that there is a subtle scientific orientation in establishing a minimum dose of exercise by which older people can benefit from ST to the detriment of the search for an optimal dose (Fisher et al., 2017). This is understandable because less time spent on training sessions may increase adherence to the intervention.

These conflicting perspectives raise some unavoidable and related issues. First, does an inadequate training volume explain the unresponsiveness of some older individuals? Consequently, is it possible to establish an optimal volume to maximize hypertrophy in this population? To the best of our knowledge, only one study attempted to answer directly whether training volume is important for the variability of response to ST in older people, despite the large body of studies on this topic (Nunes et al., 2021b). Nunes et al. (2021b) verified that the non-responsiveness condition was not changed after the training volume increase. However, one should be cautious to make conclusions based on this evidence, since it is an analysis of retrospective data in which randomization was not specifically performed to provide a comparative analysis of training volume. On the other hand, Scarpelli et al. (2020) showed that a suboptimal training volume could hamper muscle hypertrophy responses in trained young individuals. This issue is yet to be impulsed by the interesting protocol designed by Montero and Lundby (2017), in which it was observed that all individuals classified as non-responders after 6 weeks of aerobic training responded positively after the additional 2 weeks with higher training volume. Although the intervention of this study has been aerobic training, one can speculate that non-responders to ST also may achieve better adaptations naturally by increasing the training volume.

WEEKLY SETS PER MUSCLE GROUP

Despite current recommendations on ST for older people (Fragala et al., 2019), which suggests prescribing two to three sets per exercise based on the previously discussed evidence, the optimal training volume to increase muscle mass responsiveness in older individuals is still a scientific challenge. It is proposed here that the findings observed in the literature may be interpreted based, specifically, on the number of weekly sets per muscle group to allow a more comprehensive analysis on this issue, since from this perspective the number of exercise and weekly frequency are already taken into account, avoiding misleading conclusions. It is worth mentioning that these factors usually differ among studies comparing single and multiple sets, which may partially be responsible for obtaining different results. Thus, this approach would contribute to a greater connection among the findings, uniformity in experimental design conception, and practicality in the training

prescription, emphasizing that few studies analyzed volume from this perspective.

In this regard, an interesting meta-analysis elaborated by Schoenfeld et al. (2017) provides one of the main clues on this matter. They investigated the dose–response relationship between weekly ST volume and muscle mass gains analyzing studies that compared protocols with low and high training volumes. It was noted that ST tends to promote better results with higher doses, further concluding that 10 weekly sets per muscle group or more may be necessary to maximize muscle hypertrophy. Even though this review was not limited to the older individuals, this is consistent with the review proposed by Peterson et al. (2011), although they did not specifically address the number of weekly sets per muscle group. It can be inferred that one of the main strengths of the analysis by Schoenfeld et al. (2017) was to indicate that there may be a training volume threshold to be reached. Importantly, this finding demands a more critical appraisal of current literature since some clinical trials claiming to apply a high training volume may be less than the supposedly required.

While a reduced number of weekly sets may be insufficient to promote more significant hypertrophy, excessive sets may produce prolonged muscle damage and consequently, decrease adaptive response in older people chronically (Hamada et al., 2005; Ferri et al., 2006; Fell and Williams, 2008; Sorensen et al., 2018). Yet, the dose–response of volume on muscle damage in older individuals has to be determined. In this regard, it was observed that 15 weekly sets per muscle group causes more ultrastructure muscle damage in older than young women after a 9-week intervention (Roth et al., 2000) but not in older men when compared to young (Roth et al., 1999). Although one may argue that the high intensity proposed in these studies may have influenced the results, it has been shown that high and low-intensity training with equal volume produces the same level of muscle damage in young people (Paschalis et al., 2005). It is reasonable to suppose that some older people, especially women, do not respond adequately to training due to overtraining, which in turn makes the previous results (Krieger, 2010) understandable since increasing training volume (2 to 3 vs. 4 to 6 sets a week) does not necessarily improve response. Therefore, it appears that there is an upper threshold for ST volume in older individuals still have to be confirmed. This observation may be particularly relevant in designing training programs for frail individuals and others with chronic diseases since higher training doses could be more harmful.

In this scenario, training weekly frequency should not be neglected. Dankel et al. (2017) have hypothesized that higher training frequencies may induce a higher hypertrophic response by promoting optimal successive increase in protein synthesis rate. Based on the evidence that there is a plateau in protein synthesis response to increased training volume (Kumar et al., 2012; Martín-Hernández et al., 2013), there would be no advantage in performing a high training volume in a single session. However, it is not possible to make inferences in this regard from the current evidence. A meta-analysis conducted by Schoenfeld et al. (2019) concluded that the ST frequency does not meaningfully impact muscle hypertrophy when the volume is equated. Concerning older individuals, recently Pina et al. (2019) found no difference in lean mass response between individuals who trained two or three times a week after a 12-week sets-equated ST. On the other hand, Zaroni et al. (2019) found that high-frequency training (five vs. one time per week) may confer a superior hypertrophic response in young individuals. Regarding the comparison of protocols, Nunes et al. (2021a) argued that weekly set-equated do not necessarily mean volume-equated when there are marked differences in the weekly frequency ($\geq$3) since a reduction in the number of repetitions performed in groups with low frequency is observed due to fatigue experienced during the sessions. Nevertheless, some studies that compared protocols with markedly different frequencies with weekly sets-equated did not find differences in the increase in muscle hypertrophy (Gomes et al., 2019; Saric et al., 2019).

We recognize that further research needs to be carried with the older population in this sense, as previously suggested (Dankel et al., 2017). Meanwhile, the number of weekly sets needed to enhance muscle hypertrophy in low-responsive older individuals could be achieved by following the ST current position, which recommends a frequency of two to three times per week, comprising one to two exercise for each muscle group per session (Fragala et al., 2019), depending, however, on sets number per exercise, in which it must be individually adjusted during the exercise program.

WHAT IS NEXT?

To provide more specific recommendations regarding the proper range of weekly sets per muscle group to increase responsiveness to ST in older people, it is first necessary that the scientific community does not ignore the heterogeneity of the exercise response, reflected, often, by neglecting individual responses in clinical trials. This simple but significant perspective may contribute greatly to the characterization of the non-responsiveness phenomenon and its prevalence, especially with respect to muscle mass. In particular, there should be an effort to define the minimal clinically important difference regarding muscle hypertrophy in older individuals to support responsiveness categorization, since such difference hardly coincides with the minimum detectable change (Atkinson et al., 2019). Thus, clinical trials assessing relevant clinical outcomes together with hypertrophy response at different times of intervention are required. Second, as a few reviews addressing the dose–response effects of training volume in the older individuals were inconclusive (Steib et al., 2010; Nicola and Catherine, 2011; Silva et al., 2014; Borde et al., 2015) or did not assess morphological adaptations (Rhea et al., 2003), systematic reviews and meta-analysis on the dose–response relationship between weekly sets per muscle group, rather than exercise general sets or weekly frequency, and morphological adaptations in this population are encouraged. It is worth mentioning, none of the reviews cited above considered or measured individual responses. Third, dose–response should be investigated through within-subject experimental protocols and long-term interventions. On the latter, if, on the one hand,

the duration of the intervention has been considered one of the most relevant ST variables for elderly individuals (Nicola and Catherine, 2011; Radaelli et al., 2014b), on the other hand, prolonged interventions could minimize the influence of the training volume on the hypertrophic response (Da Silva et al., 2018; Teodoro et al., 2019). In future investigations, it is imperative to separate the true interindividual response variance from other sources of variance, since the individual observed changes are the sum of the change caused by the training program, plus the change that would have occurred in the absence of intervention (in the control group), plus measurement error and day-to-day biological variability (Ross et al., 2019). We also emphasize controlling confounders such as concerning the dietary pattern including energy balance and protein intake, given their potential role on muscle mass maintenance (Houston et al., 2008; Hanach et al., 2019; Landi et al., 2019). In addition, possible differences between lower and upper-body muscle group's responses to training should be considered, as there is evidence that high-volume training seems to be more efficient for lower-body muscles (Radaelli et al., 2014b). Lastly, alternative assessments are necessary to understand the variability of the response and assist in planning new studies. In this sense, omic sciences (e.g., genomics, proteomics, and metabolomics) emerge as the next frontier to be explored in this field of knowledge (Tanaka et al., 2016; Picca et al., 2019). From them, diverse dimensions of the organism (e.g., metabolic and genetic) are analyzed simultaneously and, when associated with training responses, can create network maps that help to explain the complexity of morphological adaptations to ST.

CONSIDERATIONS

Strength training is an important strategy against poor outcomes during aging. In order to enable that older individuals plenty achieve positive adaptations, training variables need to be carefully manipulated. In this context, although it is recognized that the training volume is fundamental for muscle hypertrophy, there is no consensus on the magnitude of its influence. Consequently, the contribution level of the volume training for increasing responsiveness to ST in older individuals is lacking. It is noticeable that establishing such a volume is a challenge to be addressed. While future studies are expected to clarify this issue, it can be hypothesized, based on current evidence, that there should be a more specific range of training volume that older people can respond more adequately. We suggested that such a range could be based on the number of sets per muscle group, comprising at least 10 sets per week. Evidently, this does not mean that older individuals do not respond to other training volumes (e.g., <10), but only that some non-responders or low-responders may have better morphological adaptations in a specific range. In practical terms, after an initial adaptation period, the training volume can be allocated in this range and adjusted according to individual responses to achieve better results. To reach this number of sets, one must carefully select exercises for each muscle group and weekly frequency. In addition, reporting ST volume based on weekly sets per group muscle in future studies may facilitate literature analysis as it already includes the aforementioned variables.

AUTHOR CONTRIBUTIONS

DS contributed to the conception of the study and wrote the first draft of the manuscript. AC and CC revised the original manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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Association Between Changes in Serum and Skeletal Muscle Metabolomics Profile With Maximum Power Output Gains in Response to Different Aerobic Training Programs: The Times Study

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Purpose: High heterogeneity of the response of cardiorespiratory fitness (CRF) to standardized exercise doses has been reported in different training programs, but the associated mechanisms are not widely known. This study investigated whether changes in the metabolic profile and pathways in blood serum and the skeletal muscle are associated with the inter-individual variability of CRF responses to 8-wk of continuous endurance training (ET) or high-intensity interval training (HIIT).

Methods: Eighty men, young and sedentary, were randomized into three groups, of which 70 completed 8 wk of intervention (> 90% of sessions): ET, HIIT, or control. Blood and vastus lateralis muscle tissue samples, as well as the measurement of CRF [maximal power output (MPO)] were obtained before and after the intervention. Blood serum and skeletal muscle samples were analyzed by 600 MHz ^{1}H-NMR spectroscopy (metabolomics). Associations between the pretraining to post-training changes in the metabolic profile and MPO gains were explored via three analytical approaches: (1) correlation between pretraining to post-training changes in metabolites' concentration levels and MPO gains; (2) significant differences between low and high MPO responders; and (3) metabolite contribution to significantly altered pathways related to MPO gains. After, metabolites within these three levels of evidence were analyzed by multiple stepwise linear regression. The significance level was set at 1%.

Results: The metabolomics profile panel yielded 43 serum and 70 muscle metabolites. From the metabolites within the three levels of evidence (15 serum and 4 muscle metabolites for ET; 5 serum and 1 muscle metabolites for HIIT), the variance in MPO gains was explained: 77.4% by the intervention effects, 6.9, 2.3, 3.2, and 2.2% by changes in skeletal muscle pyruvate and valine, serum glutamine and creatine phosphate, respectively, in ET; and 80.9% by the intervention effects; 7.2, 2.2, and 1.2% by changes in skeletal muscle glycolate, serum creatine and creatine phosphate, respectively, in

HIIT. The most changed and impacted pathways by these metabolites were: arginine and proline metabolism, glycine, serine and threonine metabolism, and glyoxylate and dicarboxylate metabolism for both ET and HIIT programs; and additional alanine, aspartate and glutamate metabolism, arginine biosynthesis, glycolysis/gluconeogenesis, and pyruvate metabolism for ET.

Conclusion: These results suggest that regulating the metabolism of amino acids and carbohydrates may be a potential mechanism for understanding the inter-individual variability of CRF in responses to ET and HIIT programs.

Keywords: trainability, cardiorespiratory fitness, metabolomics, metabolites, responsiveness

INTRODUCTION

Physical inactivity and low levels of cardiorespiratory fitness (CRF) are currently considered a threat to public health. As a consequence, international agencies recommend that adults accumulate $\geq$150 min·wk^{-1} of moderate-intensity cardiorespiratory exercise training, $\geq$75 min·wk^{-1} of vigorous-intensity cardiorespiratory exercise training, or a combination of moderate- and vigorous-intensity exercise to achieve a total energy expenditure of $\geq$500–1000 MET·wk^{-1}, in an attempt to minimize the occurrence of non-communicable diseases (Garber et al., 2011; Riebe et al., 2018). However, several previous investigations demonstrate a wide variability of individual responses to standardized doses of exercise. There is a substantial number of individuals who do not show clinically important increases in CRF after completing traditional continuous endurance training (ET) or alternative high-intensity interval training (HIIT) programs conducted under current recommendations for physical activity practices (Bouchard and Rankinen, 2001; Ross et al., 2015a; Williams et al., 2019, 2021; Bonafiglia et al., 2021).

In the past decade, with the technological advance of the comprehensive methods that make up the omic sciences (Tanaka et al., 2016; Kelly et al., 2020) new opportunities to investigate the integrative mechanisms of the variability of individual CRF responses to exercise have emerged (Sanford et al., 2020). So far, there are few genomic (Bouchard et al., 2011; Sarzynski et al., 2017; Williams et al., 2021), proteomic (Robbins et al., 2021), transcriptomic (Timmons et al., 2010; Keller et al., 2011; Dias et al., 2015) and metabolomic (Lewis et al., 2010; Huffman et al., 2014; Castro A., et al., 2019) studies involving the use of omic methods to study the variability of responses to exercise.

Particularly, metabolomics is a powerful metabolic phenotyping technology that allows to identify and quantify metabolites that reflect the biochemical activity underlying different physiological conditions and complex phenotypes (Rinschen et al., 2019; Wishart, 2019a), such as CRF (Castro A., et al., 2019; Castro et al., 2021). In this sense, nuclear magnetic resonance (NMR) spectroscopy is known for its reproducibility, nondestructive nature and minimal sample preparation (Wishart, 2019b), being one of the most widely employed metabolomics platforms for detecting and quantifying metabolites and their metabolic pathways related to exercise, physical activity, and health (Duft et al., 2017b; Kelly et al., 2020). Based on a metabolomic approach, Huffman et al. identified, in different training programs, that the improvement in CRF is associated with changes in the concentrations of acetyl-heavy chains and intermediates of the citric acid cycle in the skeletal muscle, accompanied by changes in the expression of genes related to the pathways of production of these metabolites (Huffman et al., 2014). More recently, Castro et al. showed a panel of baseline serum and skeletal muscle metabolites associated with inter-individual response variability of CRF (TIMES Study), suggesting involvement of amino acid metabolism and translation processes in ET and HIIT programs, and carbohydrate metabolism in ET program (Castro A., et al., 2019).

Although the study of the variability of responses to training has received some attention in the last 4 decades (Bouchard et al., 1980, 2011; Lortie et al., 1984; Bouchard and Rankinen, 2001; Kohrt et al., 2004; Ross et al., 2015a, 2019; Castro A., et al., 2019; Williams et al., 2019; Bonafiglia et al., 2021; Meyler et al., 2021), as we know, the metabolic determinants of inter-individual variability of CRF in response to different aerobic training programs, especially under an integrated view of the adaptations in blood serum and skeletal muscle tissue, remain largely unknown. Understanding through which metabolic pathways individuals improve CRF in different aerobic training programs may be useful to guide new studies on the mechanisms related to the variability of CRF responses and pave the way for novel CRF-enhancing strategies in exercise training routine. Therefore, the aim of this study was to investigate whether changes in the metabolic profile and metabolic pathways of blood serum and skeletal muscle tissue are associated with the inter-individual variability of CRF responses to ET and HIIT programs.

METHODS

The sample, study design, and exercise training protocol of the TraInability and MEtabolomicS study (TIMES study) have been described in details previously (Castro A., et al., 2019).

Participants

A total of eighty healthy and sedentary young Caucasian men, which seventy were defined as completers [age: 23.7 $\pm$ 3.0 yr; height: 1.74 $\pm$ 0.06 m; body mass: 75.2 $\pm$ 8.8; body fat: 20.0 $\pm$

7.4%; body mass index (BMI): 24.8 ± 2.5 kg·m^{-2}], from the TIMES study, were used for analysis (Castro A., et al., 2019). Briefly, participants were sedentary and did not engage in regular exercise defined as 30 min.wk^{-1} at an energy expenditure of 6 METS or more in the previous 4 months (Riebe et al., 2018; Castro A., et al., 2019; Castro et al., 2021). All participants were free from diabetes (fasting glucose > 7.0 mmol·L−1), hypertension (blood pressure > 140/90 mmHg), dyslipidemia (based on medication use), severe obesity (defined as body mass index > 33 kg·m^{-2}), smoking, metabolic disorders, heart diseases, musculoskeletal problems interfering with exercise or significant chronic respiratory conditions (Castro A., et al., 2019).

Written informed consent was obtained from each participant. The study was approved by the University's Research Ethics Committee (Number: 2.717.688; CAAE: 52997216.8.0000.5404) and included in the Brazilian Clinical Trials Registry (ensaiosclinicos.gov.br; RBR-3rh38g).

Study Design

Prior to the intervention, blood and vastus lateralis muscle tissue samples were obtained. These evaluations were preceded by 12 h of fasting following a standardized meal. After 72 h, the body composition was evaluated (full body plethysmography), followed by cardiorespiratory assessment and retest 48 h later (Skinner et al., 1999). Seventy-two h after the last pre-training evaluation, 80 participants were randomized into three groups, with a 3:3:1 allocation ratio, and of the total sample, 70 completed the eight wk of intervention (exercise adherence > 90%): ET (n = 30), HIIT (n = 30) and Control (CO, n = 10). This unequal randomization strategy was used to ensure adequate sample size for correlation analysis and subsequent analysis of high and low responders in the intervention groups. After the 4th wk of intervention, cardiorespiratory assessment was performed to adjust the training's intensity. In the end, after 48 h of the last training session, the assessments referred to in the pre-training moment were repeated (Castro A., et al., 2019).

Standardization of Meals Prior to Data Collection

The night before the blood collections and muscle biopsies (12 h before), the participants consumed a standardized and balanced meal (60% carbohydrate, 25% lipid and 15% protein), with an energy value corresponding to 30% of the total individual energy expenditure estimated in order to avoid effects of dietary variations on the metabolic profile of the blood and muscle tissue samples (Peake et al., 2014; Shrestha et al., 2015; Castro et al., 2020).

Blood and Muscle Tissue Samples

The venous blood and muscle tissue samples were collected between 7 am and 10 am. After the blood samples' collection, they were kept at rest in a serological tube for 30 min, and then centrifuged at 5000 rpm for 10 min. Afterwards, the serum aliquots were obtained and stored in a freezer at −80°C. Following the blood collections, tissue biopsies of the dominant lower limb's vastus lateralis muscle were performed according to the procedure described above (Shanely et al., 2014). Prior to the tissue's extraction, the area was shaved and cleaned with an antiseptic. A small area over the selected region was anesthetized with 2% xylocaine, injected subcutaneously. After anesthesia, a small incision (∼5 mm) was made up to the muscle fascia using a surgical scalpel. The biopsy needle was then inserted into the muscle (∼3 cm) to obtain the sample. After the tissues' removal, the incision was closed and covered with bandages. After extraction, all samples were cleaned (free of blood and excess connective tissue), aliquoted, immediately frozen in liquid nitrogen, and stored at −80°C for further analysis.

Body Composition Assessment

The participants were instructed to drink only water and not to consume food or exercise 2 h prior to the assessment. For plethysmography measurements, the participants were asked to wear only trunks and a shower cap, without shoes and metallic accessories. Body mass and height were measured using a digital scale and stadiometer (BOD POD, Cosmed, Chicago, USA), respectively. Body density was then assessed using a full-body plethysmograph calibrated according to the manufacturer's recommendations (BOD POD®; Body Composition System; Life Measurement Instruments; Concord, CA) (McCrory et al., 1995). In all evaluations, the ambient temperature and the humidity conditions were maintained between 20–22°C and ∼60%, respectively, without significant variations in atmospheric pressure. Based on these data, body density was converted to fat percentage using the Siri equation (Siri, 1993).

Cardiorespiratory Assessment

Cardiorespiratory assessment was performed during an incremental test until exhaustion using a cycle ergometer with electromagnetic braking (Corival 400, Quinton® Instrument Co., Groningen, Holland). Heart rate (HR) was continuously monitored by a cardio-frequency monitor (S810, Polar, Keple, Finland). The subjective perception of effort was recorded at the final 15 s, using the 6–20 Borg scale (Borg and Linderho, 1967). Before and after each test, the cycle ergometer was calibrated according to the manufacturer's recommendations. After 5 min of rest on the ergometer cycle, the incremental test started, with a 3-min warmup at 50 W, followed by 25 W.min^{-1} increments (Buchfuhrer et al., 1983). The pedaling cadence was maintained between 70−80 rpm. The test was interrupted when the participant was unable to continue and/or did not maintain a minimum cadence of 70 rpm despite verbal encouragement (Thompson et al., 2013).

The resting HR values were estimated from the average of the values recorded during the 5-min rest (Swain and Leutholtz, 1997). The maximum HR (HR_{MAX}) was defined as the mean value in the test's final 10 s. The reserve HR (HRR) was estimated by subtracting the values at rest from the respective maximum values achieved in the incremental test (Swain and Leutholtz, 1997; Lounana et al., 2007). MPO was estimated as W+[25·(t/60)], where W is the last load reached and t is the number of seconds in the test's final load (Kuipers et al., 1985). The highest MPO value recorded between tests was considered for the analyses and defined as the measure of CRF (Castro A., et al., 2019; Castro et al., 2021). The within-test coefficient of

variations (CV) and intraclass correlation coefficient (ICC) were 2.8% and 0.98, respectively.

Additionally, an incremental validation step was conducted based on the HR_{MAX} achieved in relation to the HR_{MAX} expected, considering all the tests performed by the participants. As previously reported, the intra-participant standard deviation for HR_{MAX} derived from repeated measures is expected to be around 4 bpm (Skinner et al., 1999). Thus, the cutoff value corresponding to twice the intra-participant standard deviation, or 8 bpm, was used as a validation criterion. In the case of the pre-training tests, the highest MPO was retained as reference. In the post-training moment, for the tests to be validated and the participant considered for further analysis, the HR_{MAX} achieved could not have been 8 bpm higher than the HR_{MAX} obtained in the pre-training test. When this criterion was not met, the participant was excluded from the study (Castro A., et al., 2019).

Training Protocol

Throughout the training program, the exercise intensity was individualized and customized for each participant based upon HRR. Both ET and HIIT programs were designed to obtain the same exercise volume in total and by session. A complete and detailed description of training volume balancing between groups can be found elsewhere (Castro A., et al., 2019).

The training program was carried out on an ergometer cycle (U1x, Matrix, Brazil), with 40 min per session, for eight wk, divided into Stage 1 (first four wk) and Stage 2 (last four wk). At the end of Stage 1, a cardiorespiratory assessment was performed to adjust the training intensity to be prescribed in Stage 2. For ET, the participants exercised for 40 min at 70% of HRR, three times a wk, in Stage 1; and for 40 min at 75% of HRR, four times a wk, in Stage 2. For HIIT, the participants exercised for 40 min, with 5 min at 50% of HRR, followed by five 4-min intervals at 90% of HRR (effort phase) interspersed with 3-min intervals at 50% of HRR (recovery phase), three times a week, in Stage 1; and 5 min at 60% of HRR, followed by five 4-min intervals at 90% of HRR interspersed with 3-min intervals at 60% of HRR, four times a week, in Stage 2. For the control, the participants were instructed not to perform physical exercises for eight wk. After four wk, the control participants were contacted again to remind them about the importance of remaining sedentary and to schedule the tests for the end of the eight-wk period.

All training sessions were supervised by an experienced professional to ensure that the target HR and pedaling cadence (70–80 rpm) were maintained. All training sessions were carried out in a reserved environment, with temperature controlled between 21–23°C.

Preparation of Blood Samples for Metabolomics

Prior to the analysis of the blood samples, 3 kDa filters (Amicon Ultra) were washed with 500 μl of Milli-Q H_2O, followed by centrifugation at 14,000 rpm and 4°C for 10 min. After the fifth wash, spin was performed (inversion of the filter and rotation at 8,000 rpm for 5 s) to eliminate any remnants of Milli-Q H_2O. Subsequently, 500 μl of blood serum were added to the filter and centrifuged at 14,000 rpm and 4°C for 45 min. After this period, the filtered serum (250 μl) were diluted in an deuterium oxide solution (290 μl D_2O, 99.9 %; Cambridge Isotope Laboratories Inc., Massachusetts, USA) containing a phosphate buffer (60 μl, Monobasic Sodium Phosphate, NaH_2PO_4 – H_2O-137.99 g/mol; Dibasic Sodium Phosphate, Na_2HPO_3 – 141.96 g/mol; 0.1 M, pH 7.4), 0.5 TMSP-d4 (3-(trimethylsilyl)-2,2′,3,3′-tetradeuteropropionic acid from Sigma-Aldrich), and added to a 5 mm NMR tube (Wilmad Standard Series 5 mm, Sigma-Aldrich®) for immediate acquisition of the spectra on the spectrometer (Duft et al., 2017a; Castro A., et al., 2019).

Preparation of Muscle Tissue Samples for Metabolomics

The muscle tissue samples were processed following the Le Belle protocol (Belle et al., 2002) adapted by Castro et al. (Castro A., et al., 2019). Firstly, the samples (∼40 mg) were weighed and added to a cold methanol/chloroform solution (2:1 v/v, total of 2.5 ml), after which they were homogenized on ice (3×30-s repetitions, interspersed with 10-s pauses) and sonicated for 3 min, with 10-s pauses between minutes. Subsequently, a cold chloroform/Milli-Q water solution (1:1 v/v, total of 2.5 ml) was added to the samples, which were then briefly stirred (to form an emulsion) and centrifuged at 4°C for 30 min (2000 *g*). The top phase of the mixture (methanol, water and polar metabolites) was collected and completely dried in a vacuum concentrator (miVac Duo Concentrator, Genevac, UK). The remaining solid phase was rehydrated in 0.6 ml deuterium oxide containing phosphate buffer (0.1 M, 7.4 pH) and 0.5 mM of TMSP-d4. Finally, the samples were added to a 5 mm NMR tube (Wilmad Standard Series 5 mm, Sigma-Aldrich®) for immediate acquisition of the spectra on the spectrometer.

Acquisition of Spectra and Quantification of Metabolites

To obtain and quantify the metabolites via metabolomics, the spectra were acquired from the serum and skeletal muscle tissue samples at the National Biosciences Laboratory (LNbio – http://lnbio.cnpem.br/) using the VnmrJ software (Varian NMR Systems) and an Inova Agilent NMR spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA), operating at a resonance frequency of ^1H 600 MHz and a constant temperature of 298 K (25°C). A total of 256 free induction decays (FID) with 32-k data points over a spectral width of 8,000 Hz were used, with an acquisition time of 4 s and 1.5-s intervals between scans (relaxation delay). The spectral phase and baseline corrections, as well as the identification and quantification of the metabolites present in the samples, were performed using the Suite 7.6 Chenomx NMR software (Chenomx Inc., Edmonton, AB, Canada), with TMSP-d4 (concentration known) as a reference for quantifying the concentrations of other metabolites. All NMR spectra were processed with 0.5 Hz line broadening (lb) to smooth out the noise in the spectral signals. To inhibit any bias, the samples were randomly profiled by a blinded evaluator. Metabolites (methanol and ethanol) involved in the reagents used in the samples' collection and preparation were not considered for analysis. In addition, in relation to serum

metabolites included in the study (Castro A., et al., 2019), the median within-test CV and ICC were 8.5% (range: 3–23%) and 0.98 (range: 0.79–1.00), respectively.

Statistical Analysis

For all variables, the data distributions were checked for major deviation from normality. Logarithmic transformations (log_2) were used to improve normality of distributions when appropriate (skewness values > 3.0). All transformed data were presented in their original scale for easier interpretation.

Pearson's correlation test was used to analyze the association of the changes (Δ, post-training values - pretraining values) in the metabolites' concentration levels and participant's physical characteristics with MPO gains. Afterwards, the ET and HIIT groups were fragmented, separately, into new subgroups, based on the first tercile (low responders, LRE) and third tercile (high responders, HRE) of the distribution of MPO gains in response to training (Castro A., et al., 2019). Comparisons at the pretraining and of the pre- to post-training changes between the LRE and HRE groups were performed using Student's *t*-test for independent samples. These analyses were performed using the PASW statistics software version 18.0 (SPSS, Chicago, IL), and the significance level adopted was 1% for these hypothesis-generating analyses, assuming that a Bonferroni correction would be too conservative, leading to a high rate of false-negative results. In addition, this approach was supplemented by estimating the effect size and 95% confidence interval for each comparison between LRE and HRE. Thus, when the confidence intervals did not cross zero, the differences were considered significant (Nakagawa and Cuthill, 2007). This approach helped to minimize the occurrence of type II error in the study.

For the identification of metabolic pathways altered by training associated with MPO gains, based on all correlational analyses performed, the metabolites that showed a nominal correlation of $r \geq |0.2|$ were retained for pathway over-representation and pathway topology analyses (Castro A., et al., 2019; Castro et al., 2021). Pathway analyses were based on the "Homo sapiens" library using Hypergeometric Test for Over Representation Analysis and Relative-Betweenness Centrality for Test Pathway Topology Analysis, as previously described (Xia and Wishart, 2010). The significance level was adjusted considering a false discovery rate of 0.1 (Benjamini and Ochberg, 1995) for the purpose of corrections due to multiple tests (van den Oord and Sullivan, 2003).

Finally, to determine the main altered metabolites associated with the MPO gains, only those supported by three levels of evidence were selected: (1) correlation with MPO gains ($r \geq |0.2|$); (2) significant difference between HRE and LRE; and (3) contributing to significant metabolic pathways associated with MPO gains. Afterwards, multiple linear regression models with stepwise selection were used to determine the variance in MPO gains explained by the changes observed in each serum and/or skeletal muscle metabolites retained by the three levels of evidence and to point out the key metabolites. To validate the models, the assumption of multicollinearity of measures between the independent variables was assessed by the variance

TABLE 1 | Pearson's correlation coefficients (r) for the association between MPO gains and the pretraining to post–training changes in serum metabolites' concentration levels metabolic levels in the TIMES study.

Serum metabolites[#]	ET ($n = 30$)	HIIT ($n = 29$)
	Δ MPO	**Δ MPO**
Amino acids		
Alanine	−0.04	−0.06
Asparagine	**−0.39***	−0.10
Glutamine	**−0.51****	**−0.26**
Glycine	**−0.41***	−0.14
Histidine	**−0.33**	0.02
Isoleucine	−0.16	**−0.31**
Lysine	−0.01	0.13
Methionine	**−0.48****	0.16
Phenylalanine	**−0.20**	**0.23**
Proline	−0.03	−0.19
Threonine	**−0.41***	−0.04
Tyrosine	−0.05	0.11
Valine	**−0.23**	**−0.31**
Carboxylic Acids		
Betaine	**−0.35**	**−0.20**
Creatinine	**−0.32**	0.05
Guanidinoacetate	**−0.34**	**−0.45***
N,N–Dimethylglycine	−0.18	−0.03
Ornithine	**−0.50****	**−0.22**
Succinate	**−0.39***	**−0.31**
Creatine	−0.09	**−0.54****
Creatine phosphate	**−0.48****	**−0.48***
Formate	0.11	**0.29**
Fatty acids		
2–hydroxy–isocaproate	−0.18	**−0.24**
2–hydroxy–isovalerate	−0.05	−0.14
Methylsuccinate	−0.08	−0.07
O–Acetylcarnitine	**−0.64****	**−0.20**
Hydroxy Acids		
3–hydroxybutyrate	**−0.41***	**−0.31**
Lactate	0.04	0.05
Glycolate	**−0.32**	−0.02
Imidazopyrimidines		
Hypoxanthine	−0.16	−0.05
Xanthine	**−0.41***	**−0.24**
Organic Carbonic Acids		
N–methylhydantoin	−0.07	-0.17^{LT}
Urea	−0.08	**−0.23**
Organic Oxygen Compounds		
Glycerol	**−0.31**	−0.15
Carnitine	**−0.42***	**−0.29**
Choline	**−0.30**	−0.01
Citrate	**−0.29**	0.03
Dimethyl sulfone	−0.05	−0.02
Trimethylamine	**−0.25**	**0.24**
Propylene glycol	**−0.45***	**−0.21**

(Continued)

TABLE 1 | Continued

Serum metabolites[#]	ET ($n = 30$)	HIIT ($n = 29$)
	Δ MPO	Δ MPO
Unclustered		
Dimethylamine	−0.12	0.05
Inosine	−0.13	−0.14
Pyruvate	−0.12	−0.15

*ET, Endurance training; HIIT, High–intensity interval training; MPO, Maximal power output; Δ, post–training values – pretraining values. *P < 0.05. **P < 0.01. [LT]Data log transformed before analysis. Values in bold are correlation coefficients (r) ≥ |0.2|. [#]The metabolites' chemical taxonomy was based on the classes and subclasses of the Human Metabolome Database.*

inflation factor (VIF 1); the normality of residue distribution was determined by inspecting the frequency histograms; and the global influence of each case in the model was analyzed by inspecting the standardized residues and Cook's distance (Field, 2009).

RESULTS

Participants

The information regarding the correlations between the pretraining participant's characteristics and MPO gains were described in a previous publication (Castro A., et al., 2019). Briefly, there were no significant correlations between pretraining age, body mass, body fat percentage, BMI, and MPO with MPO gains for ET or HIIT programs (P > 0.01 for all).

Association Between Changes in Metabolites Concentration Levels and MPO Gains

There were no significant correlations between MPO gains and pretraining to post-training changes in: body mass (ET: $r = 0.26$, $P = 0.165$, $n = 30$; HIIT: $r = 0.22$, $P = 0.258$, $n = 30$); body fat (ET: $r = -0.17$, $P = 0.380$, $n = 30$; HIIT: $r = -0.04$, $P = 0.849$, $n = 29$); and fat-free mass (ET: $r = 0.29$, $P = 0.119$, $n = 30$; HIIT: $r = 0.36$, $P = 0.054$, $n = 29$).

Of the 43 metabolites quantified in blood serum, 24 and 19 showed correlation coefficients (r) $\geq$ |0.2| for the association between MPO gains and the pretraining to post-training changes in metabolites' concentration levels of ET and HIIT programs, respectively (**Table 1**). For ET, the most correlated metabolites were glutamine, methionine, ornithine, creatine phosphate, and o-acetylcarnitine ($P < 0.01$ for all), asparagine, glycine, threonine, succinate, 3-hydroxybutyrate, xanthine, carnitine, and propylene glycol ($P < 0.05$ for all), while for HIIT, the most correlated were creatine ($P < 0.01$), guanidinoacetate and creatine phosphate ($P < 0.05$ for both). These correlations were moderate (ET: $0.39 \leq r \leq 0.64$; HIIT: $0.45 \leq r \leq 0.54$) and negative for all serum metabolites.

Of the 70 metabolites quantified in the skeletal muscle, 42 and 18 showed correlation coefficients (r) $\geq$ |0.2| for the association between MPO gains and the pretraining to post-training changes

TABLE 2 | Pearson's correlation coefficients (r) for the association between MPO gains and the pretraining to post–training changes in skeletal muscle metabolites' concentration levels in the TIMES study.

Skeletal muscle metabolites[#]	ET ($n = 29$)	HIIT ($n = 28$)
	Δ MPO	Δ MPO
Alcohols and Polyols		
Ethylene glycol	−0.01	0.09
Myo–inositol	−0.15	−0.06
Amino acids		
Alanine	**−0.41***	0.03
Anserine	**−0.43***	0.09
Beta–Alanine	**−0.24**[LT]	0.17
Glutamate	**−0.31**[LT]	0.06
Glutamine	−0.19[LT]	0.09
Glycine	**−0.42*** [LT]	0.05
Histidine	**−0.50**** [LT]	0.16
Isoleucine	**−0.29**	−0.07
Leucine	**−0.21**	−0.17
Phenylalanine	**−0.35**	**0.21**
Proline	**−0.32**[LT]	−0.16
Threonine	**−0.45*** [LT]	0.11
Tyrosine	**−0.47*** [LT]	−0.12
Valine	**0.34**	0.19
Carboxylic Acids		
Acetate	**−0.34**	−0.18
Betaine	0.05	0.19
Citrate	0.09	**0.36**
Creatine	**−0.26**[LT]	−0.18
Creatine phosphate	0.08[LT]	**0.22**
Creatinine	**−0.28**	−0.11
Formate	−0.18 [LT]	−0.09
Fumarate	**−0.33**	−0.14
Glutathione	−0.17	**0.25**
Isobutyrate	**−0.61**** [LT]	**−0.36**
Isocitrate	**−0.21**	**0.23**
Maleate	0.08	0.11
Malonate	**−0.26**[LT]	0.03
N,N–Dimethylglycine	−0.18	0.12
N–Acetylaspartate	−0.10	**0.21**
N–Acetylglutamine	−0.04	0.08
Nicotinate	**0.32**	**0.53****
Ornithine	**−0.48****	−0.16
Succinate	−0.17 [LT]	0.09
π-Methyl–histidine	−0.13	0.02
τ-Methyl–histidine	0.06	**−0.35**[LT]
Fatty acids		
2–Hydroxy–isocaproate	**−0.35**[LT]	0.12
3–Hydroxy–isovalerate	−0.12[LT]	**0.36**
O–Acetylcarnitine	**−0.22**[LT]	**−0.26**
Hydroxy Acids		
Glycolate	**0.42***	**0.68****
Lactate	**−0.39***	−0.08
Imidazopyrimidines		

(Continued)

TABLE 2 | Continued

Skeletal muscle metabolites[#]	ET ($n = 29$)	HIIT ($n = 28$)
	Δ MPO	Δ MPO
3–Methylxanthine	**−0.48****	0.11
Oxipurinol	−0.08[LT]	0.11
Theophylline	**−0.33**	0.03
Nucleosides and Nucleotides		
ADP	−0.05	**0.22**
AMP	**−0.52****	−0.19
ATP	**−0.27**[LT]	0.05
NAD+	−0.04	**0.27**
NADP+	**−0.22**[LT]	0.11
Organic Oxygen Compounds		
2–Phosphoglycerate	**−0.44***	**−0.42***
Glucose	**−0.50****	−0.14
Glycerol	−0.01	−0.11
Organic Nitrogen Compounds		
Carnitine	**−0.37***	−0.06
Choline	0.19	0.16
Dimethylamine	**−0.32**[LT]	0.13
Histamine	**−0.56**** [LT]	**−0.43***
Methylamine	**−0.34**[LT]	**−0.34**
N–Nitrosodimethylamine	**−0.42***	−0.15
Trimethylamine	**−0.31**[LT]	0.07
Trimethylamine N–oxide	**−0.40*** [LT]	0.18
Tartrate	0.19	0.12
Unclustered		
2–Hydroxyphenylacetate	**−0.31**	−0.10
Acetamide	−0.07	**0.38**
Carnosine	−0.14	−0.01
Dimethyl sulfone	−0.02	0.05
Niacinamide	**−0.47****	−0.16
Pyrimidine	**−0.20**	−0.04
Pyruvate	**−0.55****	0.00
Taurine	−0.03[LT]	0.02

*ET, Endurance training; HIIT, High–intensity interval training; MPO, Maximal power output; Δ, post–training values – pretraining values. *P < 0.05. **P < 0.01. [LT]Data log transformed before analysis. Values in bold are correlation coefficients (r) ≥|0.2|. [#]The metabolites' chemical taxonomy was based on the classes and subclasses of the Human Metabolome Database.*

in metabolites' concentration levels of ET and HIIT programs, respectively (**Table 2**). For ET, the most correlated metabolites were isobutyrate, histamine, pyruvate, AMP, glucose, histidine, 3-methylxanthine, ornithine, niacinamide ($P < 0.01$ for all), tyrosine, threonine, 2-phosphoglycerate, anserine, glycolate, glycine, N-nitrosodimethylamine, alanine, trimethylamine N-oxide, lactate and carnitine ($P < 0.05$ for all), while for HIIT, the most correlated were glycolate, nicotinate ($P < 0.01$ for both), histamine and 2-phosphoglycerate ($P < 0.05$ for both). These correlations were moderate (ET: $0.37 \leq |r| \leq 0.61$; HIIT: $0.42 \leq |r| \leq 0.68$) and negative for all these skeletal muscle metabolites, except for glycolate in both training programs and nicotinate in HIIT, which were positive.

Differences Between Low and High Responders (LRE and HRE)

As demonstrated in previous study (Castro A., et al., 2019), there were no baseline differences between LRE and HRE for age, height, body mass, body fat, BMI, HR_{MAX} and MPO in the ET and HIIT programs ($P > 0.01$).

After ET, the pretraining to post-training changes for HRE showed a reduction in the serum levels of 3-hydroxybutyrate, asparagine, betaine, carnitine, citrate, creatine phosphate, creatinine, glutamine, glycine, glycolate, guanidinoacetate, histidine, methionine, O-acetylcarnitine, ornithine, propylene glycol, succinate, threonine, trimethylamine and xanthine, as well as lower increase in choline and glycerol levels compared to pretraining to post-training changes of LRE. The effect size (Cohen's *d*) for these comparisons was classified as wide, ranging from 0.99 to 1.98 (**Table 3**). In the skeletal muscle, the pretraining to post-training changes for HRE showed a higher reduction in the levels of histamine and isobutyrate, increase of glycolate and valine, and a reduction of 3-methylxanthine and pyruvate (effect size: 1.14 to 1.29) compared to LRE (**Table 4**).

After HIIT, the pretraining to post-training changes for HRE showed a reduction in the serum levels of carnitine, creatine, creatine phosphate, guanidinoacetate, propylene glycol and succinate, and a lower increase in valine levels compared to LRE. The effect size for these comparisons was classified as wide, ranging from 1.09 to 1.63 (**Table 3**). In the skeletal muscle, HRE showed an increase in the levels of acetamide, glycolate and nicotinate (effect size: −1.17 to −2.27) and a reduction of isobutyrate levels (effect size: 1.37) compared to LRE (**Table 4**).

Metabolic Pathways

For pathway analysis, pretraining to post-training changed metabolites that were correlated at $r \geq |0.2|$with MPO gains were used, separately for serum (ET: 24 metabolites; HIIT: 19 metabolites) and skeletal muscle (ET: 42 metabolites; HIIT: 18 metabolites) in each exercise program. A total of 18 distinct and significantly changed pathways were identified and related to MPO gains, at a false discovery rate of 0.1. From these pathways, only 6 in serum (aminoacyl-tRNA biosynthesis, arginine and proline metabolism, arginine biosynthesis, butanoate metabolism, glycine serine and threonine metabolism, and valine leucine and isoleucine biosynthesis) and 4 in skeletal muscle (alanine aspartate and glutamate metabolism, citrate cycle, glyoxylate and dicarboxylate metabolism, histidine metabolism) were similar between ET and HIIT programs. The complete list of significant pathways and their related metabolites for each training program are summarized in detail in **Supplementary Table 1** and **Figure 1**.

Summary of Key Altered Metabolites Associated With MPO Gains

The altered metabolites associated with gains in MPO were identified based on three levels of evidence, previously described: (1) correlation with MPO gains ($r \geq |0.2|$); (2) significant

TABLE 3 | Comparison of the pretraining to post–training changes in serum metabolites' concentration levels between low responders (LRE) and high responders (HRE) to the ET and HIIT programs in the TIMES study.

Metabolites (mM)#	ET									Metabolites (mM)#	HIIT								
	LRE (n = 10)			HRE (n = 10)			ES	95%	CI		LRE (n = 9)			HRE (n = 10)			ES	95%	CI
3-Hydroxybutyrate	0.1124	±	0.1501	−0.0393	±	0.0889*	**1.23**	**0.22**	**2.24**	2-Hydroxy-isocaproate	0.0210	±	0.0341	−0.0002	±	0.0171	0.80	−0.19	1.79
Asparagine	0.0212	±	0.0210	−0.0030	±	0.0163**	**1.29**	**0.27**	**2.31**	3-Hydroxybutyrate	0.1011	±	0.2216	−0.0228	±	0.1942	0.60	−0.38	1.57
Betaine	0.0239	±	0.0293	−0.0076	±	0.0117**	**1.41**	**0.37**	**2.45**	Betaine	0.0197	±	0.0414	0.0039	±	0.0133	0.53	−0.44	1.50
Carnitine	0.0256	±	0.0280	−0.0051	±	0.0127**	**1.41**	**0.37**	**2.45**	Carnitine	0.0139	±	0.0211	−0.0037	±	0.0092*	**1.10**	**0.08**	**2.13**
Choline	0.0045	±	0.0027	0.0004	±	0.0033**	**1.35**	**0.33**	**2.38**	Creatine	0.0057	±	0.0102	−0.0118	±	0.0112**	**1.63**	**0.53**	**2.73**
Citrate	0.0504	±	0.0741	−0.0127	±	0.0256*	**1.14**	**0.14**	**2.14**	Creatine phosphate	0.0041	±	0.0059	−0.0022	±	0.0032**	**1.34**	**0.29**	**2.40**
Creatine phosphate	0.0023	±	0.0058	−0.0020	±	0.0020*	**1.00**	**0.01**	**1.98**	Formate	−0.0117	±	0.0112	0.0018	±	0.0155*	−0.98	−1.99	0.03
Creatinine	0.0563	±	0.0682	−0.0080	±	0.0262	**1.24**	**0.23**	**2.26**	Glutamine	0.1092	±	0.1504	0.0020	±	0.0804	0.90	−0.10	1.91
Glutamine	0.1128	±	0.1875	−0.1211	±	0.1757**	**1.29**	**0.27**	**2.31**	Guanidinoacetate	0.0138	±	0.0295	−0.0189	±	0.0209*	**1.29**	**0.24**	**2.34**
Glycerol	0.1507	±	0.1081	0.0012	±	0.0484**	**1.78**	**0.69**	**2.88**	Isoleucine	0.0242	±	0.0212	0.0047	±	0.0235	0.87	−0.13	1.87
Glycine	0.1584	±	0.1875	−0.0356	±	0.0988**	**1.29**	**0.28**	**2.31**	O–Acetylcarnitine	0.0043	±	0.0065	0.0011	±	0.0059	0.53	−0.44	1.50
Glycolate	0.0145	±	0.0175	−0.0021	±	0.0062*	**1.26**	**0.25**	**2.28**	Ornithine	0.0140	±	0.0241	0.0000	±	0.0198	0.64	−0.34	1.62
Guanidinoacetate	0.0304	±	0.0376	−0.0078	±	0.0205*	**1.26**	**0.25**	**2.28**	Phenylalanine	−0.0002	±	0.0143	0.0068	±	0.0146	−0.48	−1.45	0.48
Histidine	0.0344	±	0.0382	−0.0074	±	0.0268*	**1.27**	**0.25**	**2.28**	Propylene glycol	0.0041	±	0.0053	−0.0012	±	0.0043*	**1.09**	**0.07**	**2.12**
Methionine	0.0091	±	0.0099	−0.0039	±	0.0087**	**1.40**	**0.36**	**2.43**	Succinate	0.0057	±	0.0081	−0.0010	±	0.0027*	**1.13**	**0.11**	**2.16**
O–Acetylcarnitine	0.0029	±	0.0022	−0.0013	±	0.0020**	**1.98**	**0.85**	**3.11**	Trimethylamine	0.0001	±	0.0017	0.0006	±	0.0007	−0.45	−1.42	0.51
Ornithine	0.0267	±	0.0199	−0.0073	±	0.0213**	**1.65**	**0.58**	**2.73**	Urea	0.1478	±	0.2013	−0.0014	±	0.3778	0.49	−0.48	1.45
Phenylalanine	0.0136	±	0.0157	−0.0025	±	0.0179*	0.96	−0.02	1.93	Valine	0.1111	±	0.1066	0.0148	±	0.0557*	**1.15**	**0.12**	**2.18**
Propylene glycol	0.0051	±	0.0071	−0.0015	±	0.0042*	**1.14**	**0.14**	**2.14**	Xanthine	0.0030	±	0.0105	−0.0034	±	0.0097	0.63	−0.34	1.61
Succinate	0.0045	±	0.0077	−0.0037	±	0.0053*	**1.23**	**0.22**	**2.24**										
Threonine	0.0566	±	0.0664	−0.0214	±	0.0519**	**1.31**	**0.29**	**2.33**										
Trimethylamine	0.0015	±	0.0024	−0.0003	±	0.0011*	**0.99**	**0.01**	**1.98**										
Valine	0.1089	±	0.1515	0.0039	±	0.1110	0.79	−0.17	1.75										
Xanthine	0.0171	±	0.0128	−0.0033	±	0.0110**	**1.71**	**0.63**	**2.79**										

The data are presented as mean ± standard deviation.

ET, Endurance training; HIIT, High–intensity interval training; LRE and HRE were stratified from the 1st and 3rd terciles, respectively, of the gains in maximal power output (MPO) in response to the ET and HIIT programs. ES, effect size (Cohen's d); #Metabolites with correlation coefficient (r) ≥ |0.2|for the association between MPO gains and pretraining to post–training changes in serum metabolites' concentration levels.

***P < **0.01** for independent t–test. *P < **0.05** for independent t–test. Values in bold are ES and 95% CI that did not cross zero.*

TABLE 4 | Comparison of the pretraining to post–training changes in skeletal muscle metabolites' concentration levels between low responders (LRE) and high responders (HRE) to the ET and HIIT programs in the TIMES study.

Metabolites ($mM.g^{-1}$)	ET		ES			Metabolites ($mM.g^{-1}$)	HIIT		ES		
	LRE ($n = 10$)	HRE ($n = 10$)		95%	CI		LRE ($n = 9$)	HRE ($n = 8$)		95%	CI
2–Hydroxy–isocaproate[LT]	−0.9767 ±0.0490	−1.1171±0.2971	0.66	−0.29	1.61	2–Phosphoglycerate	−0.1949 ± 1.3958	−1.4458 ± 1.6225	0.83	−0.23	1.89
2–Hydroxyphenylacetate	−0.0044±0.0217	−0.0291±0.0634	0.52	−0.42	1.46	3–Hydroxy–isovalerate	0.0018 ± 0.0641	0.0530 ± 0.1377	−0.49	−1.52	0.54
2–Phosphoglycerate	0.2978±1.4179	−2.1872±3.4615	0.94	−0.04	1.92	ADP	−0.0067 ± 0.0154	0.0003 ± 0.0100	−0.53	−1.57	0.50
3–Methylxanthine	0.0045±0.0525	−0.0701±0.0756*	**1.15**	**0.15**	**2.15**	Acetamide	−0.0257 ± 0.0277	0.0049 ± 0.0240*	**−1.17**	**−2.27**	**−0.07**
Acetate	−0.0936±0.1599	−0.3411±0.4866	0.68	−0.27	1.64	Citrate	−0.0579 ± 0.1036	0.0472 ± 0.2108	−0.65	−1.69	0.40
Alanine	−0.1011±0.6762	−1.3267±2.3226	0.72	−0.24	1.67	Creatine phosphate	−4.9562 ± 4.9418	0.3420 ± 7.8008	−0.82	−1.88	0.24
AMP	0.0167±0.0914	−0.0665±0.0875	0.93	−0.05	1.91	Glutathione	−0.0468 ± 0.0691	0.0246 ± 0.1175	−0.75	−1.81	0.30
Anserine	0.0266±0.0771	−0.0894±0.2401	0.65	−0.30	1.60	Glycolate	−0.3983 ± 0.5146	0.5652 ± 0.7211**	**−1.56**	**−2.71**	**−0.40**
ATP[LT]	−1.0135±0.0398	−1.0593±0.1739	0.36	−0.57	1.30	Histamine	0.0045 ± 0.1524	−0.0952 ± 0.1977	0.57	−0.47	1.61
Beta–Alanine[LT]	−1.0054±0.1144	−1.1512±0.5371	0.38	−0.56	1.31	Isobutyrate	0.0591 ± 0.0677	−0.0264 ± 0.0557*	**1.37**	**0.24**	**2.50**
Carnitine	−0.3709±0.9981	−1.2760±1.4129	0.74	−0.22	1.70	Isocitrate	−0.0574 ± 0.2519	0.0590 ± 0.1322	−0.57	−1.60	0.47
Creatine[LT]	−0.0487±3.0419	−1.4351±4.0936	0.38	−0.55	1.32	Methylamine	0.0248 ± 0.0724	−0.0192 ± 0.0636	0.64	−0.40	1.69
Creatinine	−0.0081±0.0789	−0.1002±0.1285	0.86	−0.10	1.83	N–Acetylaspartate	−0.0224 ± 0.0273	−0.0070 ± 0.0236	−0.60	−1.64	0.44
Dimethylamine[LT]	−0.9836±0.0227	−1.0350±0.1187	0.60	−0.35	1.55	NAD+	0.0019 ± 0.0596	0.0666 ± 0.1037	−0.78	−1.83	0.28
Fumarate	0.0157±0.0310	−0.0046±0.0503	0.49	−0.45	1.43	Nicotinate	−0.0251 ± 0.0156	0.0081 ± 0.0157*	**−2.13**	**−3.40**	**−0.86**
Glucose	0.0424±0.3745	−0.4060±0.7525	0.75	−0.20	1.71	O–Acetylcarnitine	0.1582 ± 0.4713	−0.0505 ± 0.4058	0.47	−0.56	1.50
Glutamate[LT]	−0.4999±0.7289	−1.0001±1.3018	0.47	−0.47	1.41	Phenylalanine	−0.0310 ± 0.0225	−0.0151 ± 0.0561	−0.38	−1.41	0.64
Glycine[LT]	−1.1648±0.4299	−1.7293±0.9048	0.80	−0.17	1.76	τ–Methylhistidine[LT]	−1.1472 ± 0.6479	−1.7065 ± 1.0033	0.67	−0.37	1.72
Glycolate	−0.7410±1.1241	0.5077±0.8685*	**−1.24**	**−2.26**	**−0.23**						
Histamine[LT]	−0.7948±0.2054	−1.2963±0.5869	**1.14**	**0.14**	**2.14**						
Histidine[LT]	−0.9803±0.1740	−1.3268±0.6772	0.70	−0.25	1.66						
Isobutyrate[LT]	−0.8888±0.0540	−1.2206±0.3610	**1.29**	**0.27**	**2.30**						
Isocitrate	0.0247±0.2148	−0.0472±0.3212	0.26	−0.67	1.19						
Isoleucine	−0.0011±0.0804	−0.1332±0.2648	0.67	−0.28	1.63						
Lactate	0.2268±2.4944	−1.8445±3.5699	0.67	−0.28	1.63						
Leucine	−0.0388±0.1024	−0.1232±0.3741	0.31	−0.62	1.24						
Malonate[LT]	−1.2491±0.4161	−1.5383±0.7521	0.48	−0.46	1.42						
Methylamine[LT]	−0.9867±0.0690	−1.0720±0.2065	0.55	−0.39	1.50						
NADP+[LT]	−1.0010±0.0193	−1.0132±0.0625	0.26	−0.67	1.19						
Niacinamide	0.0049±0.0600	−0.1276±0.2090	0.86	−0.11	1.83						
Nicotinate	−0.0240±0.0269	0.0090±0.0472	−0.86	−1.83	0.11						
N–Nitrosodimethylamine	−0.0007±0.0346	−0.0263±0.0662	0.48	−0.46	1.42						
O–Acetylcarnitine[LT]	−1.0525±0.6012	−1.2634±1.1183	0.23	−0.70	1.16						
Ornithine	0.0163±0.0762	−0.1099±0.2270	0.74	−0.21	1.70						

(Continued)

TABLE 4 | Continued

Metabolites (mM.g^{-1})	ET		ES			Metabolites (mM.g^{-1})	HIIT		ES		
	LRE (n = 10)	HRE (n = 10)		95%	CI		LRE (n = 9)	HRE (n = 8)		95%	CI
Phenylalanine	−0.0098±0.0241	−0.0586±0.1284	0.53	−0.41	1.47						
Proline^{LT}	−0.9875±0.2060	−1.5528±1.2327	0.64	−0.31	1.59						
Pyrimidine	0.0066±0.0182	−0.0014±0.0423	0.24	−0.69	1.17						
Pyruvate	0.0499±0.1795	−0.1651±0.1892*	**1.17**	**0.16**	**2.17**						
Theophylline	−0.0069±0.1430	−0.1247±0.2248	0.63	−0.32	1.57						
Threonine^{LT}	−0.9830±0.2012	−1.3190±0.5785	0.78	−0.19	1.74						
Trimethylamine^{LT}	−1.0079±0.0484	−1.0532±0.1455	0.42	−0.52	1.36						
Trimethylamine N–oxide^{LT}	−1.0195±0.1255	−1.2582±0.4373	0.74	−0.22	1.70						
Tyrosine^{LT}	−0.9753±0.0480	−1.0891±0.1656	0.93	−0.04	1.91						
Valine	−0.2042±0.0899	0.0741±0.1140**	**−2.71**	**−4.00**	**−1.43**						

The data are presented as mean ± standard deviation.

*ET, Endurance training; HIIT, High–intensity interval training; LRE and HRE were stratified from the 1st and 3rd terciles, respectively, of the gains in maximal power output (MPO) in response to the ET and HIIT programs. ES, effect size (Cohen's d); # Metabolites with correlation coefficient (r) ≥ |0.2|for the association between MPO gains and pretraining to post–training changes in skeletal muscle metabolites' concentration levels. **P < **0.01** for independent t–test. *P < **0.05** for independent t–test. "Values in bold are ES and 95% CI that did not cross zero.*

differences between LRE and HRE; and (3) contribution in significant altered pathways related to MPO gains.

The metabolites supported by the three levels of evidence in the ET program were: asparagine, glutamine, succinate, glycine, histidine, methionine, threonine, creatine phosphate, guanidinoacetate, ornithine, citrate, 3-hydroxybutyrate, betaine, choline and glycolate in blood serum; and pyruvate, glycolate, valine and histamine in the skeletal muscle. On the other hand, in the HIIT program, they were: succinate, valine, creatine, creatine phosphate, and guanidinoacetate in blood serum; and glycolate in the skeletal muscle (**Table 5**).

From these metabolites, multiple linear regression models were conducted in order to determine the true interindividual response variance of the MPO gains (changes free from the effects caused by the intervention and the changes that would have occurred in the absence of intervention) explained by each metabolite supported by the three levels of evidence (**Table 6**). For ET, the variance in MPO gains was explained: 77.4% by the intervention effects; 6.9, 2.3, 3.2, and 2.2% by changes in skeletal muscle pyruvate and valine, serum glutamine and creatine phosphate, respectively. For HIIT, the variance in MPO gains was explained: 80.9% by the intervention effects; 7.2, 2.2, and 1.2% by changes in skeletal muscle glycolate, serum creatine and creatine phosphate, respectively.

DISCUSSION

This study investigated whether changes in the metabolic profile and metabolic pathways of blood serum and the skeletal muscle are associated with the trainability of CRF, based on MPO, in response to ET and HIIT programs. The results were based on the commonality of three levels of evidence and the main findings were: (i) differences in the metabolic changes associated with the MPO gains between training programs, as well as between LRE and HRE; (ii) associations between changes in the metabolic profile and MPO gains, which were: negative for serum asparagine, glutamine, succinate, glycine, histidine, methionine, threonine, creatine phosphate, guanidinoacetate, ornithine, citrate, 3-hydroxybutyrate, betaine, choline, glycolate, and skeletal muscle pyruvate and histamine in the ET program; negative for serum succinate, valine, creatine, creatine phosphate, and guanidinoacetate in the HIIT program; and positive for skeletal muscle valine in ET and glycolate in both ET and HIIT programs; (iii) identification of key altered metabolites that were able to explain the interindividual response variance of the MPO gains, adjusted by random errors and intervention effects: 14.7%, based on changes of skeletal muscle pyruvate and valine, serum glutamine and creatine phosphate in the ET program; 10.5%, based on changes of skeletal muscle glycolate, serum creatine and creatine phosphate in the HIIT program (**Table 6**); (iv) the most impacted pathways (impact > 0) by these key altered metabolites were: arginine and proline metabolism, glycine, serine and threonine metabolism, and glyoxylate and dicarboxylate metabolism for both ET and HIIT programs; alanine, aspartate and glutamate metabolism, arginine biosynthesis, glycolysis/gluconeogenesis, and pyruvate metabolism for ET (**Table 5**).

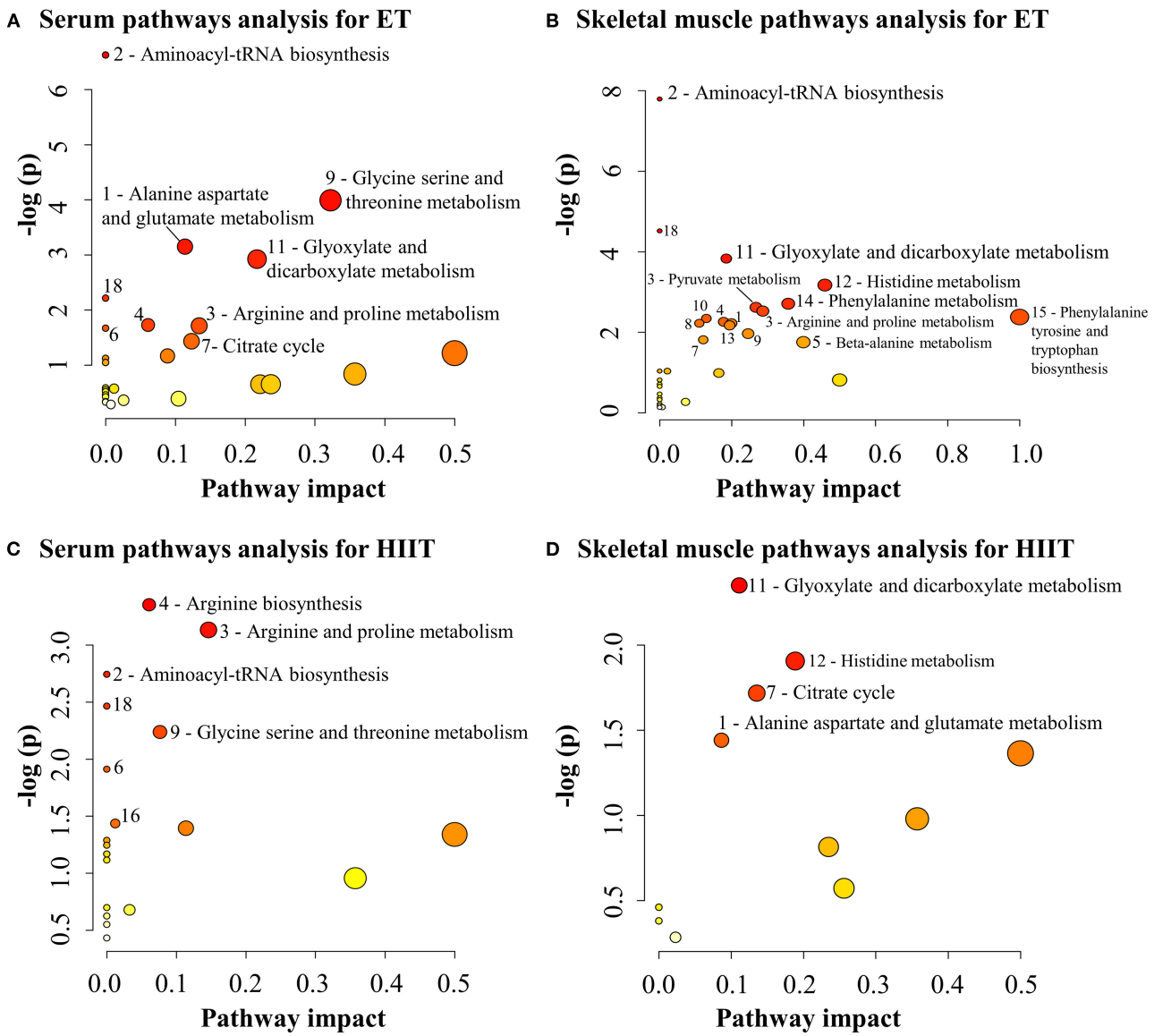

FIGURE 1 | Summary of altered metabolic pathways and their metabolites associated with maximal power output gains after endurance training (ET) and high intensity interval training (HIIT). The numbers and labels in the figures represent the most enriched and impacted pathways. All numbered pathways have significance for a false discovery rate of 0.1 (vertical axis). The pathway's impact on the horizontal axis represents the relative contribution of all identified metabolites in relation to those that compose it. (1) Alanine, aspartate and glutamate metabolism (**A**: asparagine, glutamine, citrate and succinate; **B**: alanine, pyruvate, glutamine and fumarate; **C**: glutamine and succinate; **D**: N-acetylaspartate and citrate); (2) Aminocyl-tRNA biosynthesis (**A**: asparagine, histidine, phenylalanine, glutamine, glycine, methionine, valine and threonine; **B**: histidine, phenylalanine, glycine, alanine, isoleucine, leucine, threonine, tyrosine, proline, valine and glutamate; **C**: phenylalanine, glutamine, valine and isoleucine); (3) Arginine and proline metabolism (**A**: ornithine, guanidinoacetate, creatine phosphate; **B**: ornithine, glutamate, proline, creatine, and pyruvate; **C**: ornithine, guanidinoacetate, creatine, and creatine phosphate); (4) Arginine biosynthesis (**A**: ornithine and glutamine; **B**: glutamate, ornithine, and fumarate; **C**: ornithine, glutamine, and urea); (5) Beta-alanine metabolism (**B**: beta-alanine, anserine and histidine); (6) Butanoate metabolism (**B**, **C**: 3-hydroxybutyrate and succinate); (7) Citric acid cycle (**A**: succinate and citrate; **B**: isocitrate, pyruvate and fumarate; **D**: isocitrate and citrate); (8) Glutathione metabolism (**B**: glycine, glutamate, NADP+ and ornithine); (9) Glycine, serine and threonine metabolism (**A**: choline, betaine, guanidinoacetate, glycine and threonine; **B**: glycine, threonine, creatine and pyruvate; **C**: betaine, guanidinoacetate and creatine); (10) Glycolysis or gluconeogenesis (**B**: pyruvate, lactate, glucose and acetate); (11) Glyoxalate and dicarboxylate metabolism (**A**: glycolate, citrate, glycine, and glutamine; **B**: glycolate, glycine, glutamate, acetate, isocitrate, and pyruvate; **C**: isocitrate, glycolate and citrate); (12) Histidine metabolism (**B**: glutamine, histidine, anserine and histamine; **D**: anserine and histamine); (13) Nicotinate and nicotinamide metabolism (**B**: nicotinamide, NADP+, and nicotinate); (14) Phenylalanine metabolism (**B**: phenylalanine, 2-hydroxyphenylacetate and tyrosine); (15) Phenylalanine tyrosine and tryptophan biosynthesis (**B**: phenylalanine and tyrosine); (16) Purine metabolism (**C**: xanthine and glutamine); (17) Pyruvate metabolism (**B**: pyruvate, lactate, fumarate and acetate); (18) Valine, leucine and isoleucine biosynthesis (**A**: threonine and valine; **B**: threonine, leucine, isoleucine, and valine; **C**: valine and isoleucine).

TABLE 5 | Summary of altered metabolites and their metabolic pathways associated with MPO gains in response to ET and HIIT, supported by the three levels of evidence in the TIMES study.

Metabolic pathways[#]	ET		HIIT		Reference Metabolism
	Serum	Muscle	Serum	Muscle	
Alanine, aspartate and glutamate metabolism	Asparagine, glutamine, citrate, and succinate	Pyruvate	Succinate		Amino acid metabolism
Aminoacyl–tRNA biosynthesis	Asparagine, histidine, glutamine, glycine, methionine, threonine, and valine	Valine	Valine		Translational process
Arginine and proline metabolism	Guanidinoacetate, ornithine, and creatine phosphate	Pyruvate	Guanidinoacetate, creatine phosphate and creatine		Amino acid metabolism
Arginine biosynthesis	Ornithine and glutamine				Amino acid metabolism
Butanoate metabolism	3–hydroxybutyrate and succinate		Succinate		Carbohydrate metabolism
Citrate cycle	Succinate and citrate	Pyruvate			Carbohydrate metabolism
Glycine, serine and threonine metabolism	Choline, betaine, guanidinoacetate, glycine, and threonine	Pyruvate	Guanidinoacetate and creatine		Amino acid metabolism
Glycolysis/Gluconeogenesis		Pyruvate			Carbohydrate metabolism
Glyoxylate and dicarboxylate metabolism	Glycolate, citrate, glycine, and glutamine	Glycolate and pyruvate		Glycolate	Carbohydrate metabolism
Histidine metabolism		Histamine			Amino acid metabolism
Pyruvate metabolism		Pyruvate			Carbohydrate metabolism
Valine, leucine and isoleucine biosynthesis	Threonine	Valine	Valine		Amino acid metabolism

MPO, Maximal power output; ET, Endurance training; HIIT, High–intensity interval training. [#]Based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Database.

TABLE 6 | Results of the multivariate linear regression model with stepwise selection for the MPO gains in response to training in the TIMES Study.

Models	β	B (95% CL)		F–value	Probability > F	r^2 Change	r^2 Model	VIF
Model 1								
Control		Reference		———-	———-	———-	———-	———-
ET	0.89	59.6	(52.4; 66.8)	126.5	< 0.001	0.774	0.774	1.17
Skeletal muscle pyruvate	−0.19	−30.9	(−48.8; −13.1)	96.2	< 0.001	0.069	0.842	1.22
Serum glutamine	−0.16	−26.1	(−43.4; −8.7)	81.5	< 0.001	0.032	0.875	1.08
Serum creatine phosphate	−0.17	−1,132.1	(−1,851.3; −412.9)	74.1	< 0.001	0.022	0.897	1.10
Skeletal muscle valine	0.16	16.3	(5.7; 27.0)	76.3	< 0.001	0.023	0.920	1.09
Model 2								
Control		Reference		———-	———-	———-	———-	———-
HIIT	0.83	60.3	(52.1; 68.5)	144.3	< 0.001	0.809	0.809	1.12
Skeletal muscle glycolate	0.21	10.0	(4.4; 15.6)	122.1	< 0.001	0.072	0.881	1.25
Serum creatine	−0.12	−217.4	(−414.4; −20.3)	98.8	< 0.001	0.022	0.903	1.10
Serum creatine phosphate	−0.12	−870.7	(−1720.3; −21.2)	83.0	< 0.001	0.012	0.915	1.19

MPO, Maximum power output; ET, Endurance training; HIIT, High–intensity interval training; β, Standardized coefficient; B, Unstandardized coefficient; VIF, Variance inflation fator.

In order to summarize the results, only the key metabolites, supported by the three levels of evidence, will be discussed. In the case of ET, these metabolites were serum glutamine and creatine phosphate, skeletal muscle pyruvate and valine. Glutamine is produced from the reaction of ammonia with glutamate, being responsible for the transfer of nitrogen between organs or for the synthesis of nucleotides, detoxification of ammonia and maintenance of the acid-base balance in the kidneys, in addition to serving as fuel for immune cells and signaling the regulation of protein synthesis and degradation (Pérez-Sala et al., 1987; Hood and Terjung, 1990; Newsholme et al., 2003). Previous studies have shown higher circulating levels of glutamine in athletes compared to sedentary people and after endurance training (Kargotich et al., 2007), which were however lower in athletes with *overtraining* (Rowbottom et al., 1995), pointing to glutamine reduction as an indicator of training overload (Rowbottom et al., 1995, 1996). Conversely, in the present study, there was a negative association of changes in serum glutamine with MPO gains. This

can be attributed to the greater degradation of amino acids during prolonged fasting in LRE individuals, increasing the supply of amine groups and the production of ammonia, precursors of glutamine production. This hypothesis can be supported in part by the inverse relationship observed also for essential amino acids such as valine, threonine and histidine, and for the enrichment of the metabolic pathway of alanine, glutamate and aspartate.

Creatine phosphate, on the other hand, is a molecule that stores energy within the muscle and promotes the immediate replenishment of ATP during intense exercise. Although circulating creatine phosphate levels have rarely been reported (Harris et al., 2004; Kalim et al., 2015), the present study identified low concentrations (4–5 μM) of this metabolite in the circulation, which, when changed by training, were negatively associated with the trainability of CRF in both ET and HIIT programs. Although the reasons for this result are not clear, it is possible to speculate on the occurrence of cell damage promoted by the sum of consecutive sessions performed in the last week of training (Baird et al., 2012; Sureda et al., 2015) as a mechanism related to the extravasation of the circulating creatine phosphate. This hypothesis is based on previous studies demonstrating evidence of cell damage due to the increase in creatine kinase observed after up to eight wk of aerobic training (De Araujo et al., 2013), as well as 24–48 h after successive sessions of acute aerobic exercise (Baird et al., 2012), which corroborate with the adopted timing of blood collection in the present study. Unfortunately, the creatine kinase or creatine phosphate levels (quantified by other methods) were not measured in this study, otherwise it would be possible to confirm or refute this hypothesis.

Similarly, in the skeletal muscle, changes in pyruvate were negatively associated with MPO gains. This result corroborates findings from other studies that demonstrated a reduction in pyruvate levels concomitantly with an increase in CRF after moderate to vigorous aerobic training (Henderson et al., 2004; LeBlanc et al., 2004; Huffman et al., 2014). The attenuated production of pyruvate at rest after training has been attributed to the improvement in the cellular energy supply (availability of free ADP and AMP, and inorganic phosphate) and decrease in the glycogenolysis rate, mediated by the decrease in the activity of the pyruvate dehydrogenase complex, which is responsible for decarboxylating pyruvate and supplying the citric acid cycle with Acetyl-CoA (LeBlanc et al., 2004; Han et al., 2020). Additionally, it is likely that the reduction in the amount of Acetyl-CoA supplied by pyruvate and via glycogenolysis is being offset by the amount derived from the oxidation of fatty acids (Nelson and Cox, 2013). In accordance with these results, there was also a positive association with changes in valine levels, an essential branched-chain amino acid (BCAA) which is required for protein synthesis in the skeletal muscle (Harper et al., 1984) and MPO gains. The results found for both pyruvate and valine in the skeletal muscle suggest that HRE individuals may benefit from a more efficient mechanism of fatty acid oxidation and muscle protein synthesis with aerobic training (Overmyer et al., 2015; Li et al., 2018).

For HIIT, the key metabolites were serum creatine and creatine phosphate, and skeletal muscle glycolate. Creatine is synthesized in the liver and kidneys from guanidinoacetate, derived from glycine and arginine, then it is then released into the circulation and transported to the skeletal muscle, where it will be stored as creatine phosphate serve as a source of rapid ATP production during high-intensity exercise (Walker, 1979). In this sense, there is evidence that the increased availability of circulating creatine is associated with an improvement in CRF indicators after HIIT programs (Graef et al., 2009; Kendall et al., 2009). However, in the present study, the increase in creatine levels was demonstrated concomitantly with the increase in its guanidinoacetate precursor, suggesting an imbalance in creatine metabolism (Walker, 1979) and possibly partially explaining the negative association with changes in CRF shown by both. Another point that should be highlighted is that high-intensity exercise can promote changes in renal functions (Bellinghieri et al., 2008). Thus, given that the kidneys are the main producers of guanidinoacetate (Edison et al., 2007), monitoring renal function markers may prove to be useful for understanding the trainability of CRF in future studies.

Additionally, changes in the skeletal muscle glycolate, involved in glyoxalate and dicarboxylate metabolism, were positively associated with MPO gains. Glycolate is a glyoxalate precursor that produces oxaloacetate, an intermediate in the citric acid cycle (Miao et al., 2018). Although the relationship between muscle glycolate and the adaptations induced by exercise or aerobic training in humans is not widely known (Castro A., et al., 2019; Danaher et al., 2020), previous studies with animal models corroborate the results obtained here, showing greater activation of glyoxalate and dicarboxylate metabolism in trained rats compared to sedentary ones (Starnes et al., 2017), as well as in rats with high CRF compared to those with low CRF (Falegan et al., 2017), in addition to a positive association with increased fatigue resistance in rats submitted to exhaustive aerobic exercise (Miao et al., 2018). It is tempting to speculate that increased levels of glycolate may be associated with an improved citrate cycle activity, via oxaloacetate production derived from glyoxalate and dicarboxylate metabolism, perhaps contributing to MPO gains regulation. In this sense, recently studies have shown associations between baseline glyoxalate and dicarboxylate metabolism activity with intrinsic (Castro et al., 2021) and acquired MPO levels (Castro A., et al., 2019).

In summary, our results demonstrated that the inter-individual variability of CRF in responses to ET and HIIT programs seems to be primarily associated with the individual's potential to regulate fasting energy supply through amino acid and carbohydrate metabolism. As we observed, there was a decrease in metabolites indicatives of pyruvate metabolism and glycolysis metabolism pretraining to post-training, as well as of amino acid metabolism (arginine and proline metabolism, glycine, serine and threonine metabolism, alanine, aspartate and glutamate metabolism, and arginine biosynthesis), while an increase in metabolites precursor of intermediates of the citric acid cycle via glyoxylate and dicarboxylate metabolism was found.

Some important limitations to present study should be highlighted. Both training programs tested consisted of eight wk of training, which is generally not sufficient to achieve the maximum response to a given dose of exercise (Ross et al.,

2015b). Thus, it is possible that the specific time needed to achieve the physiological adaptations in each training program (Astorino et al., 2013; Ross et al., 2015b; MacInnis and Gibala, 2017; O'Connor and Malone, 2019) contributed to the differences observed in the association between mechanisms related to the trainability of CRF between ET and HIIT. Diet was not controlled during the entire experimental period. Since the participants were not hospitalized, this type of control would be almost impossible; however, all participants were constantly asked to avoid major changes in nutritional habits, such as, changes that would lead to substantial fluctuations in body weight. Despite this, it is important to highlight that the variability of the interindividual response of CRF or other biochemical health markers is expected to happen regardless of diet (Bouchard et al., 2012; Ross et al., 2015a,b). In addition, the results of the present study are limited to two types of aerobic training, so generalizations to other programs or different intensities should be avoided, since variations in individual responses can be protocol or dose-dependent (Huffman et al., 2014; Bonafiglia et al., 2016; Joyner and Lundby, 2018; Williams et al., 2019). It is important to consider that the moment of biopsy and blood collection, 48 h after the last training session, may not represent the optimal moment to investigate the chronic changes induced by training in all identified metabolites. We also recognize that our tertile-based classification of exercise responders will by default result in 33% low and 33% high responders. In this sense, these classification terminologies must be taken with caution when comparing studies, as they reflect the distribution of MPO gains values and context in the population of the TIMES study. Most of previous studies have investigated the integrative mechanisms of variability of individual CRF responses measured by maximal oxygen uptake, but not necessarily referencing MPO, which makes it difficult to compare with our findings. However, MPO as a surrogate of CRF is known to present low technical error and high test-retest reproducibility (Skinner et al., 1999; Montero and Lundby, 2017). The TIMES study cohort consists of healthy and sedentary young Caucasian men which potentially limiting the generalizability of our results. Replication studies are warranted. Lastly, some strengths of the present study are that the results were based essentially on the commonality among three levels of evidence minimizing the occurrence of metabolites occasionally associated to the MPO gains. Blood and muscle tissue were collected at fasting state after a prior 12 h diet control. The explained variance of the MPO gains was interpreted under the changes in key metabolites adjusted by the effects caused by the intervention and absence of intervention (random error) as previously recommended (Ross et al., 2019).

CONCLUSION

This study has demonstrated distinct serum and skeletal muscle metabolites between ET and HIIT programs, who's pretraining to post-training changes associated with the inter-individual variability of CRF responses. Additionally, the panel of pretraining to post-training changed metabolites also indicated some similar pathways between ET and HIIT associated with variability of CRF responses, suggesting the involvement of amino acid and carbohydrate metabolism. These results provide new insights to investigate the underlying changes in metabolism that are determinant for inter-individual variability of CRF in responses to ET and HIIT programs.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Campinas' Research Ethics Committee (Number: 2.717.688; CAAE: 52997216.8.0000.5404). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AC and MC-M conceptualized and designed the study. AC, RD, and SO-N conducted training program, experimental data collection, and metabolomics analysis. AA performed the muscle biopsies. MC-M and CC provided technical assistance and/or conceptual advice. AC performed statistical analysis and wrote the first draft of the manuscript. All authors have read, edited and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.756618/full#supplementary-material

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SYSTEMATIC REVIEW
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A Systematic Review Examining the Approaches Used to Estimate Interindividual Differences in Trainability and Classify Individual Responses to Exercise Training

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Background: Many reports describe statistical approaches for estimating interindividual differences in trainability and classifying individuals as "responders" or "non-responders." The extent to which studies in the exercise training literature have adopted these statistical approaches remains unclear.

Objectives: This systematic review primarily sought to determine the extent to which studies in the exercise training literature have adopted sound statistical approaches for examining individual responses to exercise training. We also (1) investigated the existence of interindividual differences in trainability, and (2) tested the hypothesis that less conservative thresholds inflate response rates compared with thresholds that consider error and a smallest worthwhile change (SWC)/minimum clinically important difference (MCID).

Methods: We searched six databases: AMED, CINAHL, EMBASE, Medline, PubMed, and SportDiscus. Our search spanned the aerobic, resistance, and clinical or rehabilitation training literature. Studies were included if they used human participants, employed standardized and supervised exercise training, and either: (1) stated that their exercise training intervention resulted in heterogenous responses, (2) statistically estimated interindividual differences in trainability, and/or (3) classified individual responses. We calculated effect sizes (ES_{IR}) to examine the presence of interindividual differences in trainability. We also compared response rates (n = 614) across classification approaches that considered neither, one of, or both errors and an SWC or MCID. We then sorted response rates from studies that also reported mean changes and response thresholds (n = 435 response rates) into four quartiles to confirm our ancillary hypothesis that larger mean changes produce larger response rates.

Results: Our search revealed 3,404 studies, and 149 were included in our systematic review. Few studies (n = 9) statistically estimated interindividual differences in trainability. The results from these few studies present a mixture of evidence for the presence of interindividual differences in trainability because several ES_{IR} values lay above, below,

or crossed zero. Zero-based thresholds and larger mean changes significantly (both $p < 0.01$) inflated response rates.

Conclusion: Our findings provide evidence demonstrating why future studies should statistically estimate interindividual differences in trainability and consider error and an SWC or MCID when classifying individual responses to exercise training.

Keywords: individual response, interindividual variability, trainability, exercise training, responders, non-responder analysis

INTRODUCTION

In 1999, the seminal Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) Family Study reported individual cardiorespiratory fitness responses ranging from approximately −100 to +1,000 ml/min following 20 weeks of supervised and standardized aerobic exercise training (Bouchard et al., 1999). Early studies following HERITAGE continued to examine individual responses to exercise training by (1) interpreting a wide range of observed responses as evidence that exercise training causes interindividual variability and (2) classifying individuals as "responders" or "non-responders" if their observed response was above or below zero, respectively [reviewed in Williamson et al. (2017), Ross R. et al. (2019), Bonafiglia et al. (2020)]. In 2015, biostatisticians raised concerns with these approaches and have advocated for more rigorous statistical approaches when estimating interindividual variability and classifying individual responses (Atkinson and Batterham, 2015; Hecksteden et al., 2015; Hopkins, 2015). Despite many subsequent reviews echoing these concerns (reviews listed in **Supplementary Table S1**), we are aware of several studies published in the past year that did not adopt these statistical approaches (Marsh et al., 2020; Thomas et al., 2020; Vellers et al., 2020; Wang et al., 2020). Because no study has systematically reviewed the approaches used to examine individual responses, it is unclear whether these recent studies are representative of the exercise training literature.

Estimating interindividual variability requires partitioning the variability in outcome measurements caused by exercise training *per se*, herein referred to as interindividual differences in trainability, from the variability caused by random measurement error and within-subject variability (Bonafiglia et al., 2019a). Within-subject variability refers to real physiological responses resulting from changes in behavioral or environmental factors such as diet, sleep, and physical activity outside of a standardized exercise intervention (Bonafiglia et al., 2019a). In 2015, biostatisticians recommended statistical approaches that estimate interindividual differences in trainability by partitioning error or within-subject variability (Atkinson and Batterham, 2015; Hecksteden et al., 2015; Hopkins, 2015). Since then, many reviews have used data simulations or theoretical arguments to emphasize the importance of partitioning error or within-subject variability to encourage researchers to adopt these statistical approaches (Williamson et al., 2017; Swinton et al., 2018; Atkinson et al., 2019; Bonafiglia et al., 2019a; Ross R. et al., 2019; Voisin et al., 2019; Chrzanowski-Smith et al., 2020; Dankel and Loenneke, 2020). What remains unclear is whether the exercise training literature has adopted the recommended statistical approaches of biostatisticians. If the literature has not adopted these approaches, additional lines of evidence (i.e., beyond data simulations and theoretical arguments) may be required to persuade researchers to adopt a statistical approach that partitions error or within-subject variability when attempting to examine individual response heterogeneity.

With respect to classifying individual responses, labeling someone as a "non-responder" to exercise should be avoided because individuals can: (1) demonstrate individual patterns of response across a range of outcomes [e.g., the VO_2max of an individual may "respond" positively while their body fat percentage may "not respond" (Barber et al., 2021)], (2) respond differently following different exercise doses (Bonafiglia et al., 2016; Montero and Lundby, 2017; Marsh et al., 2020), or (3) respond differently to repeated exposure to the same training intervention (Del Giudice et al., 2020). Further, given the difficulty in delineating changes caused by exercise *vs.* behavioral or environmental factors, "responders" and "non-responders" should not be interpreted as individuals who responded or did not respond to exercise *per se* (Swinton et al., 2018). Instead, "responders" and "non-responders" should be interpreted as individuals who did or did not experience benefit following the completion of an exercise training intervention (Swinton et al., 2018). To reduce the risk of misclassifying individuals who did not benefit as "responders," many reports have recommended that "responders" should be classified as individuals whose observed change in a given outcome exceeds the smallest worthwhile change (SWC) or a minimum clinically important difference (MCID) after accounting for random measurement error (Bonafiglia et al., 2018, 2019b; Hecksteden et al., 2018; Swinton et al., 2018; Ross R. et al., 2019). In support of these recommendations, we (Schulhauser et al., 2020) and others (Hecksteden et al., 2018) found that thresholds not considering error and/or a SWC or MCID inflate "response rates" compared with more conservative thresholds that consider both error and a SWC or MCID. However, recent studies have classified "responders" and "non-responders" using non-conservative thresholds (Marsh et al., 2020; Thomas et al., 2020). Whether this observation is representative of the classification approaches used in the exercise training literature is unknown. Further, given that previous reports (Hecksteden et al., 2018; Schulhauser et al., 2020) included few outcomes (n = 6 and

1) and small sample sizes (n = 40 and 84), corroborating the inverse relationship between threshold conservativeness and response rates with a larger dataset may provide more compelling evidence to convince researchers to consider error and a SWC or MCID when classifying individual responses to exercise training.

The primary purpose of the present review was to determine the extent to which studies in the exercise training literature have adopted approaches to statistically estimate interindividual differences in trainability and consider error and an SWC or MCID when classifying individual responses. We performed a systematic review that spanned the aerobic, resistance, and clinical/rehabilitation training literature. We developed our search criteria to only include studies that examined individual responses (i.e., analyzed individual responses or commented on response heterogeneity), and our analyses, therefore, do not contain data from studies that did not consider individual responses.

MATERIALS AND METHODS

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist (see **Supplementary Table S2**). The study selection process was conducted using Covidence's systematic review software (Veritas Health Innovation Ltd., Australia). **Table 1** includes a list of definitions of individual response terms that we use throughout this review.

Eligibility Criteria

Studies were included in the systematic review if they met all of the following inclusion criteria: (1) were an original, published research study (including novel re-analyses, which refer to secondary analyses of datasets where individual responses were not examined in primary reports), (2) used human participants, (3) employed a minimum of 2 weeks (or six sessions) of standardized and supervised exercise training, and (4) examined individual responses to exercise training by (i) stating that exercise training resulted in heterogeneous responses without using a statistical approach to estimate interindividual differences in trainability (e.g., commenting on response heterogeneity or a wide range of individual responses in their results, discussion, or conclusions); (ii) adopting a statistical approach to estimate interindividual differences in trainability (approaches listed in our **Supplementary Table S3**) (Atkinson and Batterham, 2015; Hecksteden et al., 2015, 2018; Hopkins, 2015; Dankel and Loenneke, 2020); and/or (iii) classifying individual responses. The latter criteria are not necessarily mutually exclusive. For example, Hecksteden et al. (2018) adopted the standard deviation of individual response (SD_{IR}) to estimate interindividual differences in trainability (criteria ii) and classified individual responses (criteria iii). Conversely, Yu et al. (2020) adopted the SD_{IR} but did not classify (criteria ii only), and Bonafiglia et al. (2018) did not adopt the SD_{IR} but did classify (criteria iii only). Studies were excluded if they did not meet all of the inclusion criteria, or if the manuscript was not available (i.e., conference abstract only) or not in English.

Literature Search and Study Selection

We conducted a literature search in AMED, CINAHL, EMBASE, Medline, PubMed, and SportDiscus on March 28, 2020. A second, identical up-to-date search took place on January 6, 2021. The search strategy incorporated two main concepts: exercise training and individual response. A complete list of synonyms or related terms for these two main concepts was combined with "OR" (see **Supplementary Table S4** for full list), and the search strategy combined the two separate synonym lists with "AND." Titles and abstracts were extracted from the database searches, and Covidence automatically removed duplicates.

Study selection followed a two-step process and was independently completed by JTB and NP. BJG resolved disagreements (n = 2 total). First, titles and abstracts were screened to identify studies that appeared to meet eligibility criteria. Second, full texts were downloaded for articles that passed title and abstract screening to determine their eligibility. Studies removed during full-text screening were assigned a reason for exclusion. Final analyses included studies that passed both levels of study selection. We used the Cochrane Collaboration Risk of Bias Assessment Tool (Higgins et al., 2011) to assess the risk of bias. We reviewed the protocol and/or primary publications to evaluate the risk of bias for studies that included re-analyses of previously published data.

Data Extraction

JTB and NP performed data extraction using a predetermined data collection template, which included the variables provided in our **Supplementary Table S5** along with response rates, mean changes, and response thresholds. These two reviewers met to compare extracted data and resolve discrepancies. JTB and NP dichotomously categorized all included studies based on whether they did or did report using a statistical approach (Atkinson and Batterham, 2015; Hecksteden et al., 2015, 2018; Hopkins, 2015; Dankel and Loenneke, 2020) to estimate interindividual differences in trainability. Because adopting a statistical approach is needed to partition the sources of variation, this dichotomous categorization allowed us to determine how many studies may have overlooked the confounding influence of random measurement error and within-subject variability on response variability. This dichotomous categorization is further warranted because studies not adopting a statistical approach, and thus not accounting for the confounding sources of variation, risk erroneously interpreting variability in observed responses as evidence of interindividual differences in trainability. After this initial categorization, JTB and NP then sorted studies that classified individual responses into three categories based on whether they considered an error and/or an SWC or MCID (see **Table 2** for details).

We extracted basic study characteristics such as training protocols, participant characteristics, and measured outcomes from all included studies (see **Supplementary Table S5** for

TABLE 1 | Definitions of individual response terms used throughout the present review.

Term	Definition
Terms related to interindividual differences in trainability	
Random measurement error	Source of random variation caused by technical error and day-to-day biological variability
Within-subject variability	Source of random variation caused by real physiological changes that occur due to changes in behavioral and/or environmental factors external to the prescribed intervention
Interindividual differences in trainability	Variability in outcome measurements caused by exercise training; also referred to as "true response variability" or "the subject-by-training interaction" (Atkinson and Batterham, 2015; Hecksteden et al., 2015)
SD_{IR}	A statistical estimate of interindividual differences in trainability calculated by subtracting observed variability in control from exercise groups
ES_{IR}	An effect size of the SD_{IR} estimate
Terms related to classifying individual response	
"Responder"	An observed response that exceeds a given response threshold; importantly, this term should be applied in an outcome and training-specific manner (e.g., "VO_2max responder to aerobic training")
Response threshold	The threshold used to classify "responders"
Zero-based thresholds	Classification method that uses zero as the response threshold whereby "responders" have observed responses exceeding zero
Quantiles	An arbitrary classification approach that guarantees a fixed percentage of "responders" (e.g., using quartiles to identify the top 25% of participants as "responders")
TE	Typical error; calculated to account for random measurement error when classifying individual responses (Hopkins, 2000)
MCID	Minimum clinically important difference; a non-arbitrary threshold for classifying individual responses based on evidence of clinically relevant changes [e.g., 1 MET improvements for cardiorespiratory fitness because this change is associated with risk reduction of all-cause mortality (Ross et al., 2016)]
SWC	Smallest worthwhile change; a response threshold calculated as 0.2 multiplied by baseline standard deviation and is recommended when an evidence-based MCID is not available
Response rate	The proportion of "responders" within a given group under specific classification parameters and training conditions

TABLE 2 | Categorization details for the primary analysis.

Category	Description
Did studies use a statistical approach to estimate interindividual differences in trainability?	
Yes	**Used a statistical approach** (Atkinson and Batterham, 2015; Hecksteden et al., 2015, 2018; Hopkins, 2015; Bonafiglia et al., 2019a) that accounts for random measurement error or within-subject variability to estimate interindividual differences in trainability
No	This category included all studies that commented on variability (e.g., "we observed a wide range of individual responses") or classified individual responses **without performing statistical analysis** to estimate interindividual differences in trainability
How did studies classify individual responses?	
Did not consider error or a SWC or MCID	Classified "responders" and "non-responders" using quantiles or zero-based thresholds (e.g., "responders" identified as individuals with observed responses exceeding zero). These approaches **do not** consider error or a SWC or MCID
Considered error or a SWC or MCID*	Used a response threshold that **either** considered error **or** a SWC or MCID
Considered error and a MCID/SWC	Used a response threshold that considered **both** error and a SWC or MCID

**Studies that only considered error were combined with studies that only considered an SWC or MCID based on our observation that these approaches generally result in similar response thresholds (e.g., Johannsen et al., 2013; Hecksteden et al., 2018; Bonafiglia et al., 2019b; Schulhauser et al., 2020). Bold indicates to visually emphasize and differentiate descriptions of categories.*

study characteristics). For studies that statistically estimated interindividual differences in trainability, we extracted sample sizes and standard deviations (SD) of baseline values and change scores. We used WebPlotDigitizer (Rohatgi, 2018) to calculate SDs of change scores from two studies (Steele et al., 2017; Hammond et al., 2019) that did not report these values but presented individual data in their figures. For studies that classified individual responses, we extracted mean changes, thresholds used to classify "responders," and response rates. For the purposes of this review, response rate refers to the proportion of "responders" for a given outcome following a specific exercise or control condition (**Table 1**).

Data Analysis

In addition to our primary analysis, we conducted two analyses to demonstrate limitations with not statistically estimating interindividual differences in trainability or considering error and an SWC or MCID. First, we calculated estimates of interindividual differences in trainability from included studies that have adopted a statistical approach

(**Supplementary Table S3**) to determine how many outcomes provided evidence of variability caused by exercise training *per se*. Second, we examined the impact of threshold conservativeness on response rates. Details for these two analyses are provided in the following sections.

Interindividual Differences in Trainability

For studies that statistically estimated interindividual differences in trainability, we calculated the SD_{IR}, a statistic that estimates the presence of interindividual differences in trainability (see Bonafiglia et al., 2019a for a detailed explanation), for each outcome using the following equation (Atkinson and Batterham, 2015; Hopkins, 2015):

$$SD_{IR} = \sqrt{SD_{EX}^2 - SD_{CTRL}^2} \quad (1)$$

where SD_{EX} and SD_{CTRL} represent the SD of change scores from the exercise (EX) and control group (CTRL), respectively. Positive SD_{IR} values (i.e., $SD_{EX} < SD_{CTRL}$) suggest that interindividual differences in trainability exist. An SD_{IR} cannot be calculated if SD_{CTRL} exceeds SD_{EX} because you cannot take the square root of a negative number (Eq. 1). In these instances, researchers can either report not being able to calculate an SD_{IR}, or they can switch SD_{CTRL} with SD_{EX} in Eq. 1 and report the value as a negative SD_{IR}. Both incalculable and negative SD_{IR} values should be interpreted as a lack of evidence for interindividual differences in trainability (Bonafiglia et al., 2019a). For studies that had multiple exercise groups, we amalgamated the data of the exercise groups according to chapter 7.7.3.8 in the Cochrane Handbook (Higgins et al., 2019) to calculate one SD_{EX} value and thus one SD_{IR} value for each outcome in each study. The standard error (SE) for each SD_{IR} value was calculated to construct 90% confidence intervals (CIs) using the following equations (Hopkins, 2015; Hecksteden et al., 2018):

$$SE = \sqrt{2\left(\frac{SD_{EX}^4}{(n_{EX}-1)} + \frac{SD_{CTRL}^4}{(n_{CTRL}-1)}\right)} \quad (2)$$

$$90\%\ CI\ Limits = \sqrt{SD_{IR}^2} \pm 1.65 \times SE \quad (3)$$

where n_{EX} and n_{CTRL} represent sample sizes for the EX and CTRL groups, respectively. To visually present these data from different outcomes in one figure, we standardized SD_{IR} values by calculating unitless effect sizes (denoted as ES_{IR}) using the following equation (Hopkins, 2015):

$$ES_{IR} = \frac{SD_{IR}}{SD_{BSL.Pooled}} \quad (4)$$

where $SD_{BSL.Pooled}$ represents the pooled SD of baseline values from the EX and CTRL groups. Upper and lower CI limits were also divided by $SD_{BSL.Pooled}$ to construct 90% CIs for ES_{IR} values (Hopkins, 2015).

The interpretation of interindividual differences in trainability for each outcome was based on the positions of 90% CIs: CIs laying fully above zero suggested that interindividual differences in trainability were present, whereas CIs crossing or laying fully below zero indicated a lack of evidence for interindividual differences in trainability.

Analysis of Variances on Response Rates

We performed one-way analysis of variance (ANOVA) comparing response rates across the three response classification categories outlined in **Table 2**. We performed an ANOVA that compared response rates across every outcome and another ANOVA on the most commonly reported outcome. The ANOVA on the most commonly reported outcome was performed to determine whether the variance introduced by comparing response rates across different outcomes impacted our ability to detect significance. We excluded studies that used quantiles because these approaches guarantee a fixed percentage of responders or non-responders and therefore do not provide estimates of response rates. To determine whether standardized mean changes impacted our ANOVA on all outcomes, we also performed an ANCOVA with a standardized mean ($\bar{x}$) changes inputted as a covariate. Specifically, we first extracted available pre and post-training means and SDs for each outcome to calculate Cohen's *d* values using the following equation:

$$d_{av} = \frac{\bar{x}_{POST} - \bar{x}_{PRE}}{Pooled\ SD} \quad (5)$$

where d_{av} refers to a Cohen's *d* value for a within-subject standardized mean change (Lakens, 2013), and pooled SD was calculated as the standard deviation of pre values plus the standard deviation of post values divided by two. Because many (130 out of 298) of the sample sizes included in our ANCOVA were less than 20, we then converted d_{av} values to Hedge's *g* values (g_{av}) using the following equation:

$$\text{Hedge}'\text{s}\ g_{av} = d_{av} \times \left(1 - \frac{3}{4 \times (n_{POST} + n_{PRE}) - 9}\right) \quad (6)$$

Additionally, our ancillary hypothesis investigated whether response rates were a function of mean change relative to the response threshold. We based this hypothesis on findings from two of our recent studies: (1) larger mean changes produce larger response rates at a given response threshold (Bonafiglia et al., 2021b), and (2) smaller response thresholds produce larger response rates at a given mean change (Schulhauser et al., 2020). We tested this hypothesis using outcomes with reported response rates, mean changes, and response thresholds; outcomes could not be included if any of these three parameters were not reported. Data from both exercise and non-exercising control groups were included in this analysis. Our dependent variable was response rates (in percentage), and our independent variable was calculated as the mean change divided by the response threshold. This approach standardized our independent variable and thus allowed us to compare response rates across different outcomes. We could not include response rates from studies using zero-based thresholds because mean changes cannot be divided by a response threshold of zero. We then sorted outcomes into four response rate quartiles: response rates less than or equal to (1) 100%, (2) 75%, (3) 50%, or (4) 25%. Following this, we used a one-way ANOVA to compare standardized mean changes across

these response rate quartiles. We identified outliers within each quartile as *z*-scores that were either >2.58 or <−2.58, and we removed these data before running our ANOVA.

For all ANOVAs, significant main effects were followed by Bonferroni *post hoc* tests. These statistical tests were performed on GraphPad Prism version 9 with the ANCOVA performed on SPSS version 26. All data are presented as means ± standard deviation.

RESULTS

Study Selection

Figure 1 presents a flow diagram of the study selection process. The literature search retrieved 3,404 studies, and Covidence removed 1,120 duplicates. Two thousand two hundred eighty-four studies entered title and abstract screening, and 2,036 were deemed irrelevant and were subsequently excluded. Full texts were then downloaded for 248 studies, and 99 were excluded (reasons provided in **Figure 1**), leaving a total of 149 included studies. Study details, measured outcomes, analytical approaches, and participant characteristics for these 149 studies can be found in the **Supplementary Table S5**. Most included studies had an unclear-high risk of bias (results presented in **Supplementary Table S6**). For ease of viewing, in-text references for all 149 included studies are provided at the end of this manuscript (see **Appendix**). **Table 3** presents study characteristics and analysis categories (categories outlined in **Table 2**) for these 149 studies.

Timeline of Studies Examining Individual Responses to Exercise Training

We created two timelines of the studies included in our analysis (**Figure 2**). **Figure 2A** includes all studies and is sorted based on whether studies used a statistical approach to estimate interindividual differences in trainability. The number of studies examining individual responses to exercise training has increased substantially since 1999, when the findings from the HERITAGE Family Study were published, with the majority (72.5%; 108/149) being published within the last 5 years (i.e., 2015 onward). The paper by Atkinson and Batterham (2015) is highlighted in **Figure 2A** because it describes the SD_{IR} approach and, to our knowledge, is the first article in the exercise literature outlining how to statistically estimate the presence of interindividual differences in trainability. It was therefore unsurprising that no study before 2015 statistically estimated interindividual differences in trainability (**Figure 2A**). However, strikingly few studies (∼9.5%; 8/84 studies) published in 2017 onward [i.e., after Atkinson et al.'s (2019) SD_{IR} paper] statistically estimated interindividual differences in trainability. Seven of these studies (Steele et al., 2017; Williamson et al., 2017, 2018; Bonafiglia et al., 2019a; Hammond et al., 2019; Walsh et al., 2020; Yu et al., 2020) used the SD_{IR}, and one study explored different SD_{IR} approaches using a variety of statistical parameters (Hecksteden et al., 2018). The 2016 study by Leifer et al. (2016) used Levene's tests to compare variability in observed responses between control and exercise groups – an approach that follows the same principles as the SD_{IR} (Bonafiglia et al., 2019a).

A percentage of 77.9% (116/149) of all included studies classified individual responses, and a timeline of these studies sorted by the three categories outlined in **Table 2** is presented in **Figure 2B**. 31.9% of studies (37/116) classified individuals using an approach that did not consider error or an SWC or MCID (e.g., zero-based thresholds or quantiles). 56.9% of studies (66/116) considered error (66.7%; 44/66) or an SWC or MCID (33.3%; 22/66), and the most common approach in this category (37.9%; 25/66) was classifying responders as individuals whose observed response exceeded a threshold of two times the typical error. Only 8.6% of studies (10/116) considered both error and an SWC or MCID. The most common approach (90%; 9/10) in this last category was classifying responders as individuals with confidence intervals, built using the typical error of measurement and constructed around observed responses, that lay fully above an SWC or MCID. **Supplementary Table S5** provides more information on the specific classification approaches used in each study. Three studies (Hubal et al., 2005; Hagstrom and Denham, 2018; Peltonen et al., 2018) were not categorized because they did not report enough information on how they classified individual responses.

Studies Adopting Approaches for Estimating Interindividual Differences in Trainability

We were unable to calculate ES_{IR} values from three of the nine studies that statically estimated interindividual differences in trainability because they did not report SD of baseline measures and/or change scores (Williamson et al., 2017, 2018; Bonafiglia et al., 2019a). All three of these studies included novel re-analyses of previously published data. Williamson et al. (2017) reported greater variability in VO_2max responses in a control group compared with an exercise group, the 2018 meta-analysis of Williamson et al. (2018) demonstrated trivial interindividual differences in trainability for body weight, and we reported moderate-large variability in behavioral factors (e.g., dietary habits and sedentary time) following a controlled exercise intervention (Bonafiglia et al., 2019a).

We calculated ES_{IR} values in the remaining six studies. These values with 90% CIs along with basic details regarding participant characteristics and training modes are presented in **Figure 3**. Combining data across the three aerobic training groups from Hammond et al. (2019) resulted in positive ES_{IR} values for body mass and waist circumference. However, we can only conclude that interindividual differences in trainability were present for body mass because the 90% CI for waist circumference crossed zero. The aerobic, resistance, and aerobic plus resistance groups were also combined in the study Walsh et al. (2020). Responses in body composition and cardiometabolic health from Walsh et al. (2020) revealed mixed evidence of interindividual differences in trainability because ES_{IR} 90% CIs lay above, below, or crossed zero. ES_{IR} estimates from the Leifer et al. (2016). The Leifer et al. (2016) study also presented a mixture of 90% CI positions for indices of cardiometabolic health. The ES_{IR} values from Hecksteden et al. (2018) and Yu et al. (2020), who measured fitness parameters following aerobic training, were all positive

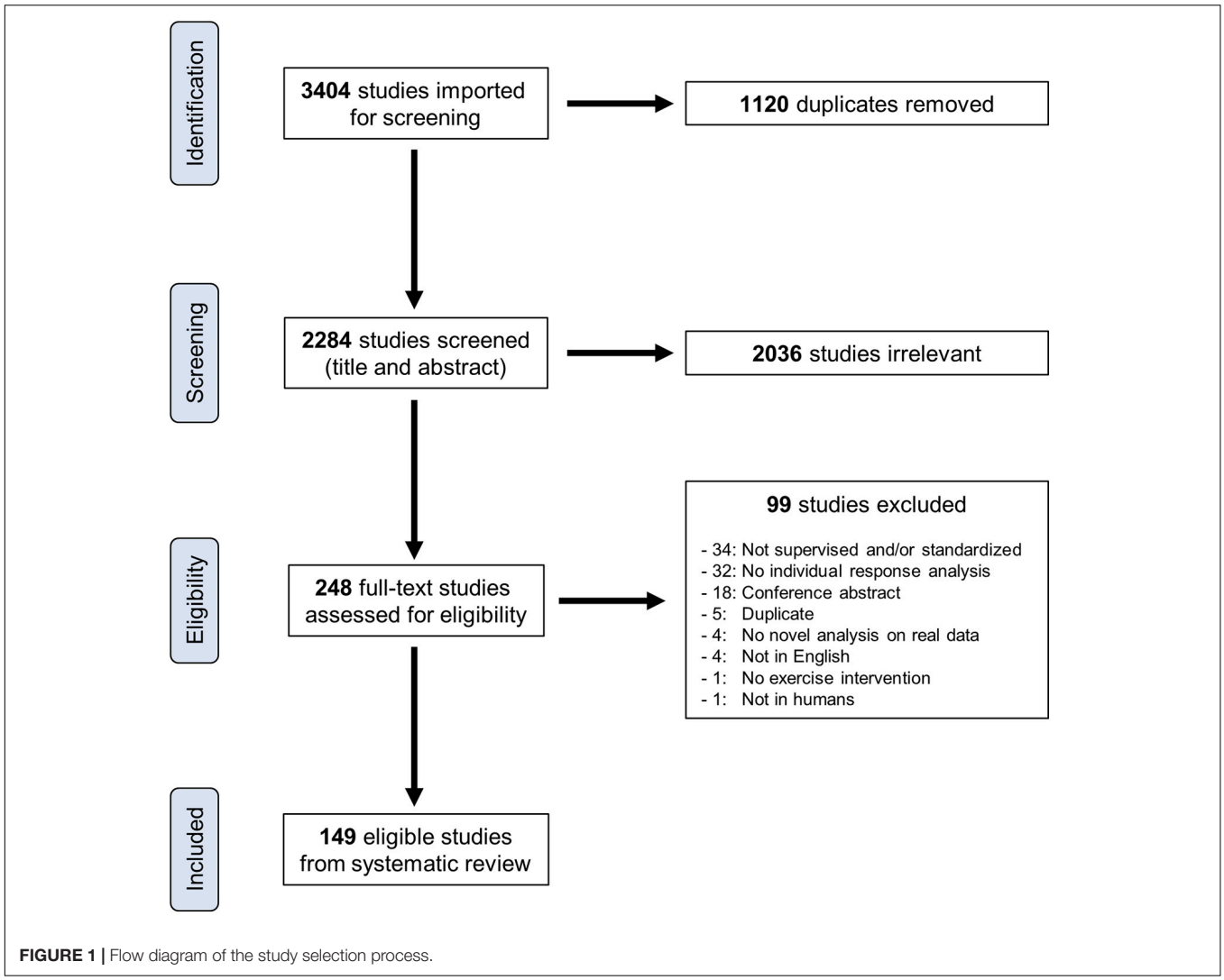

FIGURE 1 | Flow diagram of the study selection process.

but had large 90% CIs that crossed zero. These large CIs were likely attributable to the small sample sizes of these two studies. Interestingly, only the study by Steele et al. (2017), which examined strength responses to resistance training, revealed consistent evidence of interindividual differences in trainability as all ES_{IR} 90% CIs lay above zero.

Analysis of Variances on Response Rates

We obtained response rates for 614 outcomes from the 116 studies that classified individual responses. 71, 491, and 52 response rates were obtained from zero-based thresholds, approaches that considered error or an SWC or MCID, or both error and an SWC or MCID, respectively. Our one-way ANOVA with all 614 response rates (**Figure 4A**; left panel) was significant ($p < 0.01$) with zero-based thresholds producing a significantly ($p < 0.01$) higher mean response rate (71.22 ± 18.09%) compared with approaches that considered one of (50.53 ± 31.08%) or both error and an SWC or MCID (45.49 ± 20.52%). Our second ANOVA was performed on VO_2max because this outcome had the most response rates ($n = 75$) compared with other outcomes (next three outcomes with the most response rates: various strength measures, $n = 51$; waist circumference, $n = 20$; body weight, $n = 17$). The one-way ANOVA on VO_2max response rates (**Figure 4A**; right panel) was also significant ($p < 0.05$) with zero-based thresholds resulting in a significantly ($p < 0.05$) higher mean response rate (78.42 ± 12.98%) compared with approaches that considered both error and an SWC or MCID (46.50 ± 20.85%). We extracted 298 Hedge's g values, and our ANCOVA and associated *post hoc* tests remained significant indicating that standardized mean changes did not confound the relationship between classification category and response rate (**Figure 4A**).

Response rates, mean changes, and response thresholds were reported for 435 of the 614 outcomes. Our ancillary analysis (**Figure 4B**) on these 435 outcomes revealed significant differences in standardized mean changes (mean change divided by response threshold) across each quartile: ≤25% = 0.09 ± 0.46; ≤50% = 0.83 ± 0.41; ≤75% = 1.58 ± 0.69; ≤100% = 3.62 ± 2.19 (ANOVA and all *post hoc* p values < 0.01). We identified ten outlying data points, and only one lay below the mean (blue

TABLE 3 | Overview of study details for the 149 studies included in our systematic review.

Type of training	# of studies (% all studies included)	Avg. duration in weeks (SD)	Avg. frequency (SD)	# in males only	# in females only	# in both sexes	Variability categories[a]		Response classification categories[b]		
							# "Yes"	# "No"	# No TE or SWC or MCID	# TE or SWC or MCID	# TE and SWC or MCID
Aerobic	74 (50%)	13.1 (10.1)	3.9 (1.7)	16 (22%)	9 (12%)	48[c] (65%)	68 (92%)	6 (8%)	14 (27%)	30 (58%)	8 (15%)
Resistance	32 (22%)	14.3 (9.3)	2.7 (0.8)	13 (41%)	8 (25%)	11 (34%)	31 (97%)	1 (3%)	12 (44%)	15 (56%)	0 (0%)
Aerobic and Resistance	35 (23%)	17.1 (8.9)	3.3 (0.7)	4 (11%)	5 (14%)	26 (74%)	33 (94%)	2 (6%)	10 (37%)	16 (59%)	1 (2%)
Other	8 (5%)	11.4 (6.2)	4.1 (2.9)	1 (13%)	0 (0%)	7 (87%)	8 (100%)	0 (0%)	1 (14%)	5 (72%)	1 (14%)

[a]Variability categories outlined in ***Table 2*** *("Did studies use a statistical approach to estimate interindividual differences in trainability?"); [b]Classification categories outlined in* ***Table 2*** *("How did studies classify individual responses?"); [c]One group did not provide sex composition. Note that percentages for the variability categories are based on the total number of studies within each type of training (aerobic = 74; resistance = 32; aerobic and resistance = 35; other = 8), whereas the percentages for response classification categories are based on the number of studies that classified individual responses within each type of training (aerobic = 52; resistance = 27; aerobic and resistance = 27; other = 7).*

data point; all other outliers in red; **Figure 4B**). The two outliers in the upper quartile (response rates ≤100%) had very large standardized mean changes, and both data points represent strength gains (leg press one-repetition maximum) from two resistance training groups in the same study (Barbalho et al., 2017). The remaining eight outlying data points came from six studies (Alvarez et al., 2017b,c; Álvarez et al., 2018a,d; Delgado-Floody et al., 2019; Ramírez-Vélez et al., 2019) produced by the same research group, and homeostasis model assessment-estimated insulin resistance measurements accounted for four of these outliers.

DISCUSSION

This is the first systematic review to investigate the approaches used to examine individual responses to exercise training. Our search revealed a large number of eligible studies (n = 149; **Figure 1**) that spanned the aerobic, resistance, and clinical/rehabilitation training literature. Our primary analysis revealed that few studies have statistically estimated whether exercise training causes interindividual differences in trainability (**Figure 2A**). This finding indicates that the majority of studies may have inappropriately interpreted variability in observed responses as evidence of interindividual differences in trainability. In support of this speculation, our review highlighted several ES_{IR} values that either fell below zero or had a 90% CI crossing zero (**Figure 3**). Given that many recent studies have not adopted the statistical approaches described in previous reviews (listed in **Supplementary Table S3**), we hope our findings help persuade researchers to adopt these approaches when estimating the existence of interindividual differences in trainability in the future work.

Our additional analyses found that few studies considered an error and an SWC or MCID when classifying individual responses (**Figure 2B**). Our analyses on response rates confirmed the hypotheses that: (1) thresholds not considering error and an SWC or MCID inflate response rates (**Figure 4A**), and (2) larger mean changes produce larger response rates (**Figure 4B**). Given the disconnect between the many reviews (listed in **Supplementary Table S1**), highlighting the importance of considering error and an SWC or MCID and the few studies doing so (**Figure 2B**), our findings hopefully encourage researchers to consider error and an SWC or MCID when classifying individual responses in future studies.

Interindividual Differences in Trainability

Our ES_{IR} calculations highlighted mixed evidence for the presence of interindividual differences in trainability (**Figure 3**). For instance, resistance training in the Steele et al. (2017) study appeared to cause variability in strength responses, whereas aerobic training in the Leifer et al. (2016) re-analysis may not have led to interindividual differences in cardiometabolic responses because the 90% CIs crossed zero. Interestingly, fasting insulin from the Leifer et al. (2016) study and peak strength from the Steele et al. (2017) study had similar ES_{IR} values, but only peak strength revealed evidence of interindividual

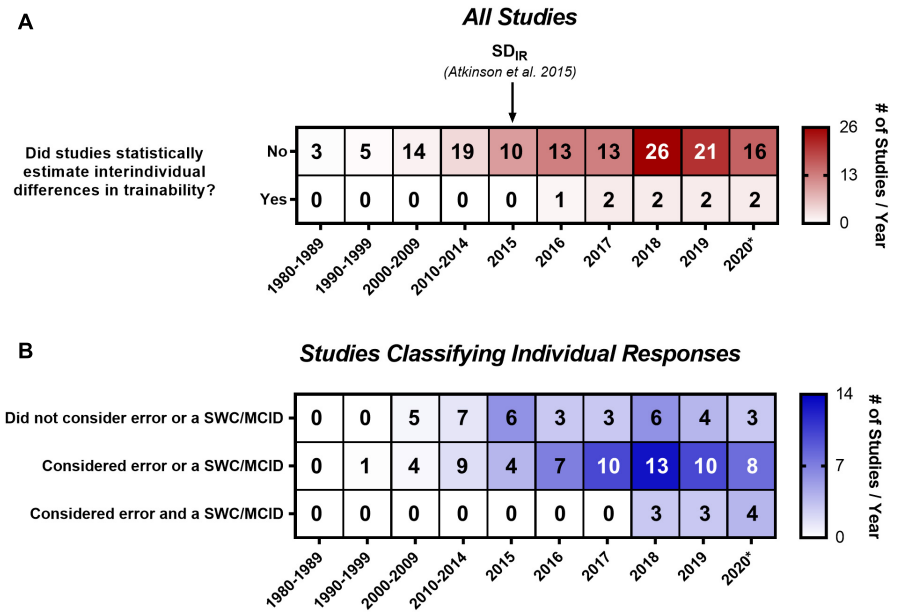

FIGURE 2 | Timeline and heatmap of the 149 studies included in our systematic review. Numbers refer to a total number of studies in each cell, whereas shading depicts the total number of studies divided by the number of years included in that column. **(A)** Includes all studies sorted by whether studies used a statistical approach to estimate interindividual differences in trainability, and **(B)** includes the 116 studies that classified individual responses sorted by the classification categories outlined in **Table 2**. 2020* includes studies published in 2020 and up to the updated literature search (January 6, 2021). MCID, minimum clinically important difference; SD_{IR}, the standard deviation of individual responses; SWC, smallest worthwhile change. **(B)** Only contains 113 of the 116 studies that classified individual responses because three studies did not report how individuals were classified (Hubal et al., 2005; Hagstrom and Denham, 2018; Peltonen et al., 2018).

differences in trainability because its 90% CI lay fully above zero (**Figure 3**). Because fasting insulin had a much larger sample size than peak strength (n = 1188 vs. n = 114), its larger 90% CI may reflect how blood-based physiological outcomes have larger random measurement error compared with strength-based performance outcomes. This observation may also highlight how random measurement error for a given outcome influences interpretations of interindividual differences in trainability. Future work should therefore avoid pooling data across different outcomes when estimating interindividual differences in trainability. Moreover, these discrepancies highlight how reading one of these studies in isolation may lead to either the conclusion that interindividual differences in trainability exist or do not exist (Leifer et al., 2016; Steele et al., 2017). **Figure 3** presents ES_{IR} values across a range of outcomes and studies, highlighting how the existence of interindividual differences in trainability should be interpreted on an outcome, population, and study-specific basis.

The observation that most ES_{IR} values (18/25) lay below or crossed zero (**Figure 3**) adds to a growing body of literature questioning the presence of interindividual differences in trainability following standardized exercise training (Williamson et al., 2017, 2018; Del Giudice et al., 2020; Kelley et al., 2020, 2021; Bonafiglia et al., 2021a; Islam et al., 2021). For example, we recently reported wide-ranging changes in energy intake, diet composition, and sedentary time following a controlled exercise intervention (Bonafiglia et al., 2019a). This apparent variability in behavioral changes suggests that within-subject variability contributes substantially to the variability in observed responses. Recent studies have also demonstrated that individual cardiorespiratory fitness and skeletal muscle responses appear non-reproducible following repeated exposure to an identical exercise training intervention (Lindholm et al., 2016; Del Giudice et al., 2020; Islam et al., 2021). Assuming trainability of an individual is a stable and reproducible trait, this non-reproducibility provides further evidence suggesting that within-subject variability largely comprises the variability in observed responses (Hecksteden et al., 2015). Taken together, these findings indicate that it may be erroneous to assume that variability in observed responses reflects interindividual differences in trainability. Despite this indication, also highlighted in many previous reviews (listed in **Supplementary Table S1**), the majority of recent studies have not adopted a statistical approach to investigate the existence of interindividual differences in trainability (**Figure 2A**). Future work should therefore use a statistical approach to determine whether interindividual differences in trainability are present following exercise training (Atkinson and Batterham, 2015; Hecksteden et al., 2015, 2018; Hopkins, 2015; Dankel and Loenneke, 2020).

Although we focused on interindividual differences in trainability, other disciplines have reported a similar lack of evidence for "true response variability" following non-exercise interventions (Mills et al., 2020). For example, a recent meta-analysis of psychiatric assessment responses in patients with schizophrenia reported greater variability in control groups

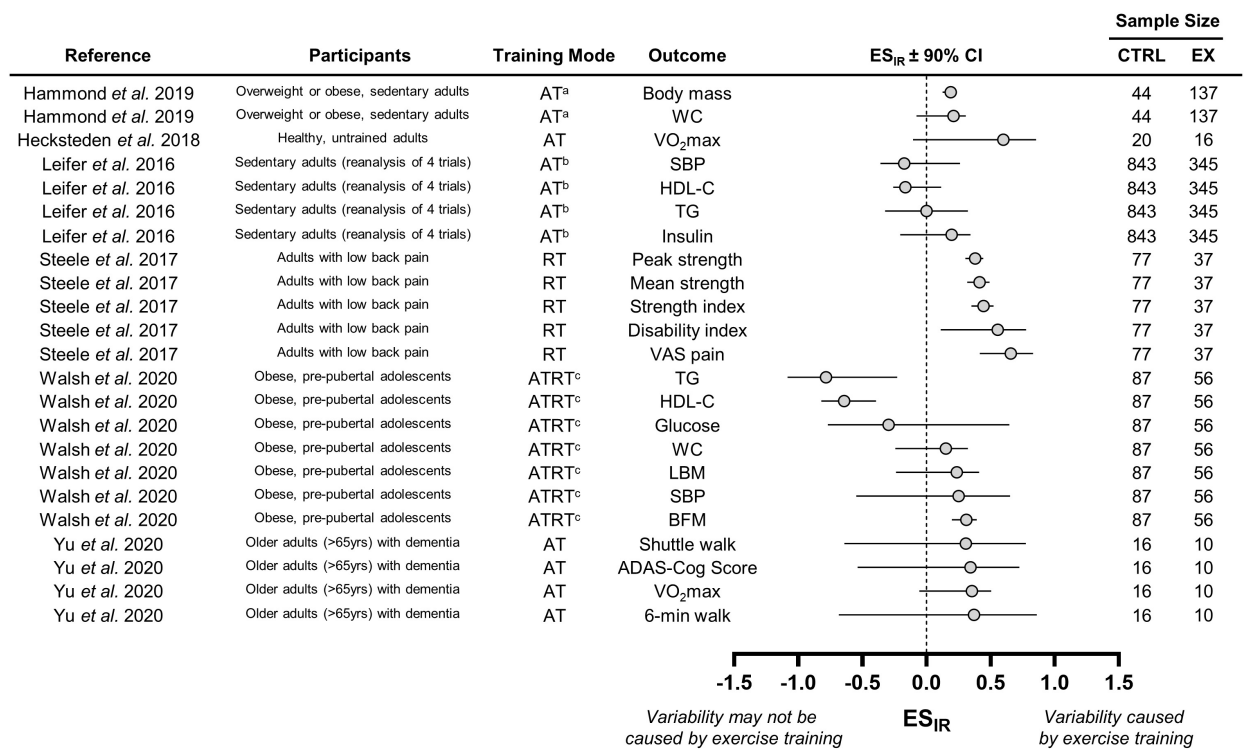

FIGURE 3 | Forest plot of the studies that statistically estimated interindividual differences in trainability. CTRL, control group; EX, exercise training group; WC, waist circumference; VO_2max; maximal oxygen uptake; SBP, systolic blood pressure; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; VAS, visual analog scale; LBM, lean body mass; BFM, body fat mass; ADAS-cog, Alzheimer's disease assessment scale-cognitive subscale. [a]We combined data from three aerobic training groups (see section "Interindividual Differences in Trainability"); [b]Data from four trials combined by Leifer et al. (2016); [c]We combined data from the three training groups (see section "Interindividual Differences in Trainability"), which included an aerobic, resistance, and combined training group.

compared with treatment groups (Winkelbeiner et al., 2019). Rather than using the SD_{IR}, these non-exercise studies utilized the "variability ratio" (Winkelbeiner et al., 2019; Mills et al., 2020), an approach that is similar to the SD_{IR} because it relies on the assumption of independence to estimate true response variability. In this context, the assumption of independence refers to the assumption that random measurement error and within-subject variability are equal between groups in a randomized controlled trial (Bonafiglia et al., 2019a). However, the variability ratio divides, instead of subtracts, the variability of observed responses in the treatment group by the variability of observed responses in the control group. The variability ratio was used in recent meta-analyses (reviewed in Mills et al., 2020) across scientific disciplines other than exercise and sport science to empirically test for the existence of "true response variability."

Classifying Individual Responses

We found that zero-based thresholds inflate response rates compared with classification approaches that consider error and an SWC or MCID (**Figure 4A**). These findings add to previous results derived from fewer outcomes and smaller datasets demonstrating that non-conservative thresholds increase the proportions of participants classified as "responders" (Hecksteden et al., 2018; Schulhauser et al., 2020). We hope our large dataset (i.e., 614 response rates from 116 studies) demonstrating inflated response rates with zero-based thresholds (**Figure 4A**) better convinces researchers to consider error and an SWC or MCID when classifying individual responses. Although considering error and an SWC or MCID will help conservatively identify individuals who experienced meaningful benefit, it is also possible these conservative thresholds increase the risk of failing to classify individuals who responded to exercise *per se* as "responders" (i.e., type II error). Conversely, less conservative thresholds (e.g., zero-based or considering one of error or an SWC or MCID only) increase the risk of classifying individuals who did not experience meaningful benefit as "responders" (i.e., type I error). While researchers can choose which risk of misclassification is more acceptable for their study, avoiding dichotomous classification and considering uncertainty (e.g., individual confidence intervals) can help reduce the risk of misclassifying "responders" or "non-responders" (Bonafiglia et al., 2018; Swinton et al., 2018).

Controlling for differences in response thresholds supported our ancillary hypothesis that larger mean changes produce larger response rates (**Figure 4B**). This finding corroborates our recent demonstration that larger mean changes explain why higher doses of exercise produce larger cardiorespiratory fitness response rates (Bonafiglia et al., 2021b). We speculate that the red outliers in the 25, 50, and 75% quartiles had

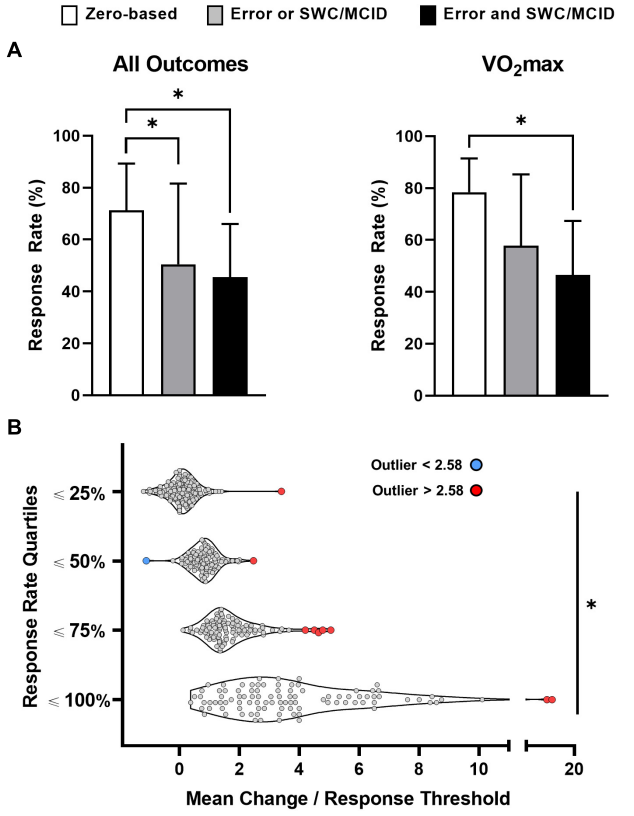

FIGURE 4 | Impact of classification approach [**A**; n = 614 (all outcomes); n = 75 (maximal oxygen uptake, VO_2max)] and mean change **(B)** on response rates. **Table 2** outlines the classification categories outlined in **(A)**. *Significant at $p < 0.01$.

large variability (or a single outlying data point), which resulted in a low response rate despite having a large standardized mean change (and *vice versa* for the blue data point in **Figure 4B**). However, analyzing these raw data is needed to confirm this speculation. Both analyses demonstrate how response rates are group statistics that highly depend on response thresholds (**Figure 4A**) and mean changes (**Figure 4B**; Atkinson et al., 2019). Future research should therefore avoid attributing lower response rates to reduced interindividual variability, an interpretation made in several studies included in our review (Wolpern et al., 2015; Dalleck et al., 2016; Byrd et al., 2019), and should recognize that response rates provide little if any, useful information at the individual level (Atkinson et al., 2019).

Although our results demonstrate *how* we should classify observed changes following exercise training (i.e., consider error and an SWC or MCID; **Figure 4A**), what remains less clear is *why* we should classify individuals in the first place. Although individual classification may help guide exercise prescription decision-making in clinical and applied settings, to our knowledge, only one study (Montero and Lundby, 2017) has utilized initial response classifications to guide subsequent exercise prescription decisions. A key challenge to individualizing exercise prescription is choosing a response threshold that incorporates an equipment-specific error estimate (Weatherwax et al., 2018a) and a change linked to clinical benefit (i.e., MCID). While equipment-specific errors can be easily generated using simple test re-test experiments, MCIDs are not available for many outcomes (Hopkins, 2000). For VO_2max, we recently used an MCID of 1.0 MET because this change confers a ∼8–14% risk reduction in all-cause morbidity and mortality (Dorn et al., 1999; Bonafiglia et al., 2019b). When MCIDs are unavailable, researchers can use SWCs (i.e., 20% of baseline standard deviation) to estimate a threshold representing a small effect size (Swinton et al., 2018). It is important to emphasize that, unlike MCIDs, SWCs are clinically arbitrary and should not be used to gauge whether an individual has clinically benefited following exercise training. Future work is needed to examine the validity of the classification of individual responses for the purpose of optimizing exercise prescriptions and for establishing MCIDs for a wider range of outcomes.

Limitations and Future Directions

The few studies that statistically estimated the presence of interindividual differences in trainability varied substantially in participant characteristics, training modes, and outcomes assessed (**Figure 3**). Recent meta-analyses have reported pooled SD_{IR} estimates indicating a lack of interindividual differences in trainability in body weight and body composition (Williamson et al., 2018; Kelley et al., 2020, 2021). Unlike the present study, these meta-analyses focused on a single outcome and used search criteria that identified all studies reporting standard deviations of change scores and not just those evaluating individual responses as we did in the present review. We did not pool ES_{IR} values because of the heterogeneity in populations (e.g., overweight adolescents, adults with low back pain, or older adults with cognitive impairment) and outcomes assessed (e.g., VO_2max, perceptions of back pain, or an Alzheimer's cognition score) across studies (**Figure 3**). Nevertheless, the observation that most ES_{IR} values (18/25) lay below or crossed zero supports conclusions from pooled estimates of single outcomes demonstrating a lack of interindividual differences in trainability. More studies should adopt the SD_{IR} approach to allow for additional population- and outcome-specific meta-analyses on other clinically relevant outcomes (e.g., VO_2max, waist circumference, grip strength, etc.). Importantly, these meta-analyses can provide robust assessments of the presence or absence of interindividual differences in trainability.

Large variation in our dataset may also explain why response rates were not significantly different between studies that considered error or SWC or MCID *vs.* studies that considered both (**Figure 4A**). Comparing response rates across different thresholds within the same group (and thus mean change) overcomes this limitation by eliminating between-study variation in participant characteristics, training modes, and random measurement error. Although we and others have explored the relationship between response rates and thresholds using VO_2max and exercise performance data (Hecksteden et al., 2018;

Schulhauser et al., 2020), future work should confirm that failing to account for error and an SWC or MCID inflates response rates in different outcomes.

CONCLUSION

The present systematic review found that despite many previous reviews (listed in **Supplementary Table S1**) advocating for more statistically sound approaches when examining individual responses, few studies have statistically estimated interindividual differences in trainability or used response classification thresholds that consider error and an SWC or MCID. We also presented ES_{IR} values that question the presence of interindividual differences in trainability, which demonstrates why it is inappropriate and potentially erroneous to assume variability in observed responses reflects interindividual differences in trainability. Further, we found that zero-based thresholds inflate response rates, which demonstrates why it is important to classify responses using an approach that considers both error and an SWC or MCID. Additionally, our analysis examining mean changes supported the notion that response rates are group statistics that provide little information about an individual's response to exercise training. We hope our findings and novel data presentations better convince researchers to statistically estimate interindividual differences in trainability and consider error and an SWC or MCID in future work.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

JTB and NP wrote the first draft of the manuscript. All authors contributed to conception and design of the study, conducted the systematic review, statistical analysis, and manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.665044/full#supplementary-material

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APPENDIX

In-Text Citations for the 149 Included Studies

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Levels of Adherence of an Exercise Referral Scheme in Primary Health Care: Effects on Clinical and Anthropometric Variables and Depressive Symptoms of Hypertensive Patients

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Among the modifiable health behaviors, physical activity (PA) promotion has been one of the challenges in primary care, particularly how to translate the results of proven interventions and implement them in the real world. This study was aimed to compare whether two programs designed for hypertensive patients achieve changes in clinical and anthropometric variables, quality of life, and depressive symptoms; and if higher levels of adherence to one of the interventions using an exercise referral (ER) approach achieved better health outcomes. Pragmatic cluster randomized trials were carried out in four Primary Health Care Units (PHCUs). Physicians in the PHCUs identified hypertensive patients and assessed whether they were eligible to be part of this trial. Each center was randomized to a brief PA counseling (BC, $n = 2$) or an exercise referral (ER, $n = 2$) intervention to conducted PA programs among hypertensive patients aged 35–70 years, self-reported as physically inactive. Outcome variables included changes in blood pressure levels, triglycerides, HDL cholesterol, fasting glucose, body mass index, waist/hip ratio, abdominal obesity, and metabolic syndrome risk score, health-related quality of life, and depressive symptoms. Longitudinal multilevel analyses assessed the effects of the BC and ER programs and the level of adherence of the ER on clinical, anthropometric, and mental health variables, models were linear for continuous variables, and logistic for dichotomous variables. Differences were observed in triglycerides, BMI, metabolic risk scores variables, and depressive symptoms among ER and BC programs. In addition, differences in the ER group were observed according to the level of adherence in blood pressure levels, waist circumference and waist/hip

ratio, depressive symptoms, and the mental health component of health-related quality of life. An ER program in comparison to a BC intervention is promoting changes in some specific health indicators of hypertensive patients, showing the usefulness of these PA programs in primary health care facilities.

Keywords: health behaviors, physical activity, hypertension, adherence, primary care (MeSH)

INTRODUCTION

In the global health community, one of the greatest challenges is how to translate the results of proven interventions and implement them in the real world. Part of this challenge resides on the individuals themselves who are responsible for making these recommendations.

The efficacy of interventions to reduce high-risk behaviors through healthy lifestyle promotion, including healthy food habits, smoking cessation, and increased physical activity (PA) levels, has been determined in randomized or quasi-randomized controlled trials (Ashenden et al., 1997; Lawlor and Hanratty, 2001; Eden et al., 2002; Stead et al., 2016). However, some studies reveal that interventions aimed to change unhealthy health behaviors under realistic conditions of the health service delivery are still challenging to accomplish (Eakin et al., 2004; Aittasalo, 2008).

Among the modifiable health behaviors, the promotion of PA has been one of the challenges in primary care. Estimations indicated that not meeting PA recommendations is responsible for more than 5 million deaths globally each year (Lee et al., 2012). Although regular PA and health levels have a strong causal relationship, only 47% of the general Mexican population is achieving the PA levels. Among the strategies to increase PA levels exercise referral schemes (ERs) provide a promising alternative to PA promotion, facilitating changes in behavior in the at-risk population (Dugdill et al., 2005; Pavey et al., 2011).

Few studies have evaluated the impact of the levels of adherence of PA interventions carried out in primary health care, and its effects on participants physical health, anthropometry, perception of their quality of life, as well as mental well-being. Buckley et al. (2020), considered participant monthly attendance to a fitness center to assess differences of the exercise referral scheme and usual care in the United Kingdom, participants in the usual care program had lower attendance to the program and less attendance sustainability after 6-month follow-up, however, this level of attendance was not used to determine impact of the ER on PA, clinical or anthropometrics outcomes. Other studies in high-income countries have observed moderate effects of the ERS in self-reported PA levels and cardiovascular health outcomes (Murphy et al., 2012; Onerup et al., 2019), while other approaches to assess the effectiveness of ERS through measuring PA adherence concluded that a higher adherence (a more active stage of chance) of those in the ERs compared to usual care show an association with higher levels of PA (Martín-Borrás et al., 2018).

The aim of this study was to compare if two PA programs designed for increasing the PA levels of hypertensive patients achieve changes in clinical and anthropometric variables, quality of life, and depressive symptoms between baseline, 16 and 24 weeks; and if higher levels of adherence to one of the interventions using an exercise referral (ER) approach achieve better outcomes.

MATERIALS AND METHODS

Study Design

Cluster randomized trial, with Primary Health Care Units(PHCUs) as the unit of randomization and hypertensive patients as the unit of assessment.

The physicians in all PHCUs identified hypertensive patients and assessed that they met the established criteria. Study design and details of the recruitment process are described elsewhere (Gallegos-Carrillo et al., 2014).

Participants

Trained staff in all the PHCUs used the eligibility criteria to warrant both that the practice of PA was safe for all participants, and the conditions to apply the behavioral theory-based intervention: women and men with IMSS affiliation, between 35 and 70 years old, less than 5 years from hypertension diagnosis, and/or without drug treatment (according to JNC 7 criteria) (Chobanian et al., 2003; U.S. Department of Health and Human Services, 2004), self-reported performance of less PA than recommended (<150 min per week of moderate to vigorous intensity PA), and self-reported willingness to practice PA, according to Transtheoretical model of behavioral change (Prochaska and DiClemente, 1983); moderate level of cardiovascular risk, according to the Guidelines for exercise testing and recommendations of the (American College of Sports Medicine, 2009; Thompson et al., 2013), and complemented with a pre-participation screening questionnaire from the American Heart Association (Thomas et al., 1992) and ACSM. Specific information about inclusion criteria has been previously detailed (American College of Sports Medicine, 2009; Thompson et al., 2013; Gallegos-Carrillo et al., 2014).

Hypertensive patients complying with the inclusion criteria were not eligible if they reported a high PA level (>300 min a week of moderate to vigorous intensity), and have previously attended a PA program at IMSS facilities.

Setting

The Mexican Social Security Institute (IMSS) includes facilities for primary, secondary, and tertiary health care services as well as available resources designed to develop PA programs (such as sports fields, gyms, pools, and outdoor spaces for PA) called

Social Security Centers (SCC). The screening assessment and the exercise reference by physicians were carried out in PHCUs, while the PA program exercise program was performed during 16 weeks at SCCs. The functioning and staff of the SCCs were described previously.

Sample Size

The sample size estimation was based on the goal of increasing the percentage of hypertensive patients who are physically active from 17 to 37%, the proportion that has been shown in preliminary assessments. An increase in compliance with PA recommendations of 20% showed whether the intervention was effective. The proportion is consistent with the effectiveness level tested in other studies (Harrison et al., 2005). Thus, to identify a difference of 20% more of the population becoming physically active, with a power of 90, and a level of significance of $\alpha = 0.05$, 68 patients were required in each group (IG and CG). We considered increasing our sample size by 40%, due to the high dropout rates reported in previous studies (Hillsdon et al., 2004). Therefore, the sample size was estimated to be 224 hypertensive patients, 112 for each group. We assumed that a PA program with planned sessions of PA and surveillance as it is the Exercise Referral Scheme (ER), would be a better approach to achieve better health outcomes at the end of the intervention than just brief counseling (BC).

Randomization

To avoid contamination randomization was conducted with IMSS facilities PHCUs located on average 5.5 kilometers (kms) from the place where patients were referred (ER at 5.1 kms, and BC at 6.6 kms), within the urban area of the city, and had public transportation available, average transfer time being 20 to 30 min. The four PHCUs were randomized into an intervention group called exercise referral (ER) (two centers) and a BC (two centers) using sealed envelopes by a health researcher who was not involved in this study.

Recruitment Process

The recruitment process took place from September 2011 to March 2012, in four PHCUs; 506 hypertensive patients were identified and assessed by 108 PHC physicians who took part in the four PHCUs that were randomized as the study groups. The activities of the physicians during the daily consultation to identify potential participants were expressed in a previous publication (Gallegos-Carrillo et al., 2014). **Figure 1** shows the flow diagram detailing the recruitment of participants during the study, according to guidelines of the Consolidated Standard of Reporting Trials (CONSORT) (Schulz et al., 2010). During recruitment, we identified 506 hypertensive patients as potential candidates for the study for both groups, ER and BC; of them, 21.7% were excluded because their levels of blood pressure were >160 mmHg systolic, or >100 mmHg diastolic during screening. In addition, 5.3% declined to participate, and 27.1% were unable to attend or complete assessments. Therefore, at the end of the recruitment process, 117 patients (50.4 years old on average and 67.5 % women) were incorporated into the intervention group and 115 into the control group (51.7 years old on average and 73.04 % women).

Outcome Measures

The main health outcomes were: systolic and diastolic blood pressure levels, triglycerides, HDL cholesterol, fasting glucose, body mass index, weight, waist/hip ratio, and metabolic syndrome risk score, health-related quality of life, and depressive symptoms.

Follow-up measurements were carried out at the end of the intervention (16 weeks) for both ER and BC groups, while the sustainability of intervention effects was assessed 24 weeks after the beginning of the study. In addition, differences in health outcomes were observed among hypertensive patients belonging to the ER group who achieved 50% of adherence to the PA program, which means they attended at least 24 planned PA sessions or more.

(a) Blood pressure levels measured with a digital instrument following criteria established by the JNC 7 (Chobanian et al., 2003; U.S. Department of Health and Human Services, 2004);

(b) Biochemical markers measured according to the procedures of the International Federation of Clinical Chemistry and Laboratory Medicine (Tate et al., 1999): (1) Fasting glucose levels: blood samples were taken following a fasting period of at least 8 h. The serum glucose determination was made using the enzymatic calorimetric method. All blood biochemistry tests, including (2) total cholesterol, (3) high-density lipids (HDL), and (4) triglycerides were conducted with a Selectra XL (Randox).

(c) Anthropometric measures were carried out by trained nurses, following standard procedures: (1) weight, measured with TANITA electronic scale, with participants wearing minimal clothes and without shoes; (2) Body Mass Index (BMI), was calculated dividing kilograms by height in meters squared. The data obtained were categorized according to the following criteria: normal (BMI = 18.5–24.9 kg/m^2), overweight (BMI = 25–29.9 kg/m^2) and obese (BMI = > 30 kg/m^2) (World Health Organization [WHO], 1995); (3) waist circumference, measured at the highest point of the iliac crest at the end of expiration, to the nearest measuring tape point of 0.1 cm. The criteria for abdominal obesity will be: men >100 cm and women >88 cm (Expert panel, 2001); and (4) hip circumference, participants were stood with feet separated about 20 cm and weight distributed evenly on both feet, at the level of the maximum extent of the gluteus in a horizontal plane, verifies that the measuring tape covered at same high the perimeter of the body, near to the skin but without compress (World Health Organization [WHO], 2000) and waist/hip ratio.

(d) Physical and psychological measurements: (1) health-related quality of life (HRQoL), measured through the short form of 12 items (SF-12), considering two components physical (PCS) and mental (MCS) (Ware et al., 1996). The Physical Activity Summary Measures (PCS)

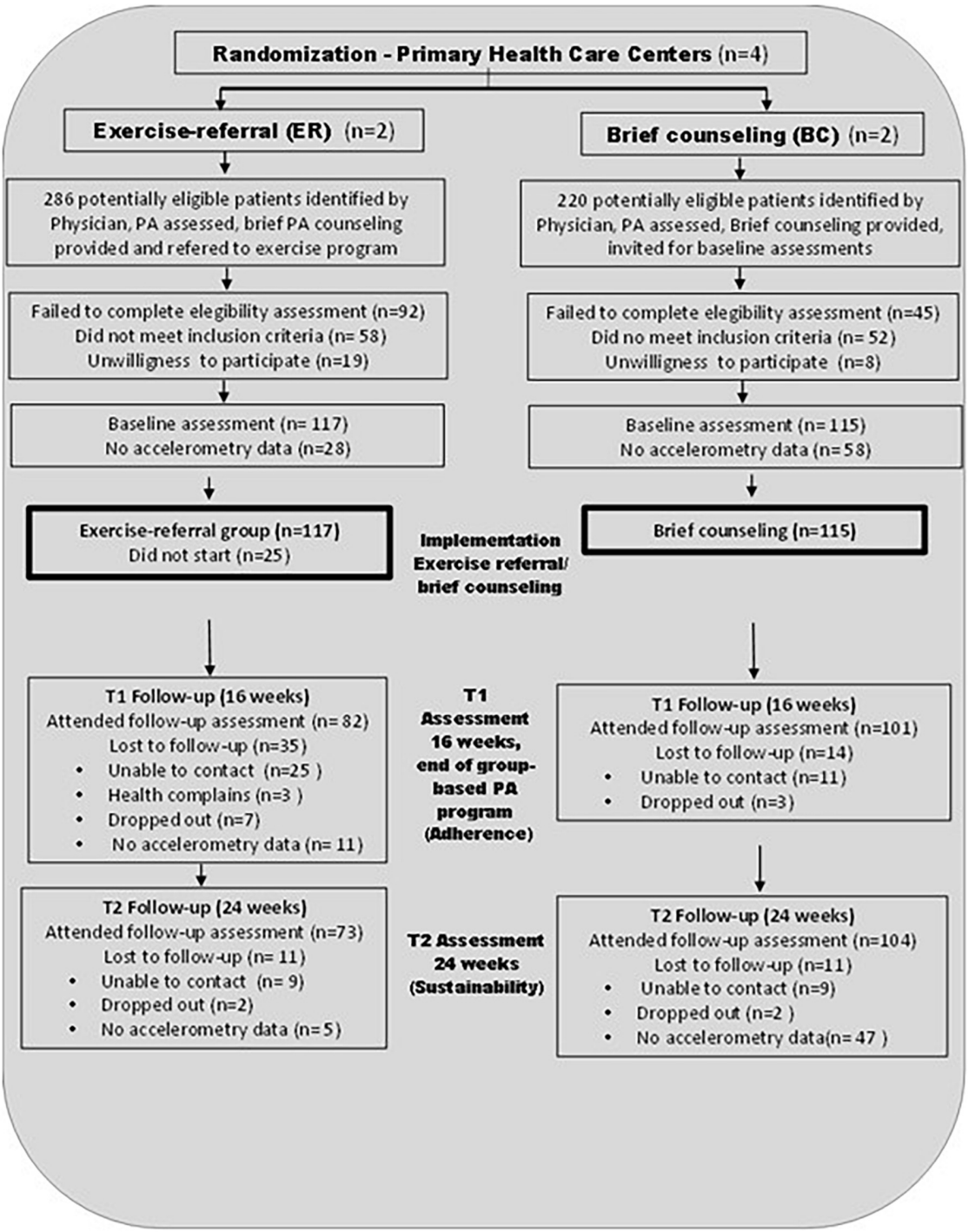

FIGURE 1 | Consort flow diagram.

included four scales, whose scores varied from 0 to 100, which were summarized to generate the PCS, a higher score meaning better PA functioning and fewer limitations. The scales are the following: Physical Functioning (PF), Role-Physical (RP), Bodily Pain (BP), and General Health (GH). While the Mental Health Activity Summary Measure (MCS) included four scales which also varied between 0 and 100 in which a higher score represents better mental health functioning: Vitality (VT), Social Functioning (SF), Role-Emotional (RE), and Mental Health (ME) were the criteria.

(2) Symptoms of depression were measured with the Center for Epidemiologic Studies–Depression Scale, (CESD), this

version included 20 items which are summarized to create the scale, the score range is from 0 to 60, higher scores are indicating a certain level of depressive symptomatology (Radloff, 1977).

Metabolic Syndrome Risk Score (MetSyn): Abdominal obesity; elevated fasting glucose as 100 mg/dL, triglycerides as 150 mg/dL; Cholesterol HDL <40 mg/dL and blood pressure as a resting systolic 130 mm Hg or a resting diastolic 85 mm Hg, which corresponds to a mean arterial blood pressure (MABP) of 100 mm Hg. For each MetSyn component, the clinical cutoff value was subtracted from the individual's value and then divided by the population's standard deviation (SD). The standardized score for each MetSyn component was then averaged to derive a clustered CVD risk score relative to IDF MetSyn definition (CVD risk score). To compile the CVD risk score with units of SD, positively skewed variables (FG and TG) were previously transformed (natural log).

Physical activity: PA levels were assessed at baseline, T1 (16 weeks) and T2 (24 weeks), to determine changes in the following: percentage of participants meeting the PA recommendation, minutes of moderate and vigorous PA performed per week, and minutes per day of sedentary behavior. PA was measured by self-report (indirect methods) and accelerometry (direct methods). Self-reported (subjective) through the short version of the International Physical Activity Questionnaire (IPAQ) (Craig et al., 2003), and objectively by ActiGraph GT3X accelerometers (ActiGraph LLC, Ft. Walton Beach, FL, United States), this device has an internal time clock and extended memory, and it is able to record and store the magnitude of acceleration and deceleration of movements. The recorded data is scored as a "count," which can be summed in a specific time interval called an "epoch." In this study, an epoch is equal to 60 s. Participants were instructed to wear the device for 7 days during all waking hours, removing it only during swimming, bathing, or having other contact with water. The accelerometers were mounted on elastic belts and placed at the right hip. For this study wear time was valid if the patient wore the device for 5 days in 1 week (including at least 1 weekend day) to accumulate at least 600 min daily.

Each patient's accelerometer record was analyzed in terms of time (in minutes) to perform PA of moderate to vigorous intensity per day and then calculate the average time spent (in minutes) of PA during the week. Using this data, patients were categorized as complying or not complying with PA recommendation, 6 (150 min of PA at moderate to vigorous intensity a week) at baseline, T1 and T2, in both IG and CG. Additionally, PA measures were complemented with a PA log that participants completed every week.

Other Variables

Socio-demographic variables included age, age group, gender, marital status, and main activity.

Confounding or Modifier Effects Variables

We defined as confounding and modifier variables the following: met PA recommendation measured by both self-reported and objectively with the accelerometer, weight, and BMI at baseline, age, gender, education, main activity.

Intervention Group: Exercise Referral Scheme

The intervention was based on the assumptions of the Transtheoretical Model and Social Cognitive Theory (Bandura, 1977; Bandura and National Inst of Mental Health, 1986). The information about the procedure of implementation and development of the PA program has already been described and is summarized below.

During the first session, a functional capacity assessment and PA prescription were carried out. The program of 16 weeks had 48 sessions of 60 min, a frequency of three times per week, and an average intensity of moderate to vigorous. Three levels or stages during the 16 weeks of the PA program were developed: Level 1, or Induction; Level 2, or Development; and Level 3, or Maintenance. In addition, each session consisted of three phases of exercise: warm-up and flexibility phase; aerobics phase, and recovery phase. The ER program had 48 sessions of planned PA. We defined a high level of adherence (>50%) to those patients who attended at least half of the sessions (24 sessions or more), while a low level of adherence (<50%) was determined for patients who attended less than 24 sessions of the PA program.

At the end of the 16 weeks of the program, PA specialists encouraged the participants to continue with PA practice at the CSS facilities (at no cost for the following 6 months) or practice regular PA during leisure time in their preferred facilities, but without financial support from IMSS.

Control Group: Brief Counseling

Participants belonging to PHCUs were randomized to the control group after inclusion criteria were confirmed. Additionally, written and verbal information about the health benefits of PA practice, and how to increase PA levels in a safe way was distributed to the participants. All the patients in this group continued receiving their usual care at PHCU facilities but were also informed about the health benefits of PA and a balanced diet, and how to safely increase PA levels during free time, their participation in this PA program did not include planned PA sessions, therefore adherence to PA program was not estimated. In addition, they were invited to undergo two subsequent measurements at the PHCU facilities, at 16 weeks and 24 to the baseline measurement. Monthly contact with participants was carried out during their consultation at PHCUs.

Statistical Analyses

Analyses were carried out according to an intention to treat analysis.

A baseline comparability analysis was carried out among the ER and BC, in relation to the variables studied during the baseline, 16 and 14 weeks. To compare means, a *T*-test or analysis of variance (ANOVA) was conducted, while U of Mann Whitney was used to compare variables with not normal distribution.

Due randomization took place in PHCUs and multilevel analyses were used. Longitudinal multilevel analyses were developed to account for the three measurements between intervention and control groups, thus the three follow-up measurements of the outcome measure concerned were defined as dependent variables. The multilevel model allowed taking into account the clustering of individuals within PHCUs through the next levels: (1) the individual and (2) PHCUs. These models were linear for all outcome variables including blood pressure, biochemical and anthropometric measurements, depressive symptoms, the physical and mental component of quality of life, and metabolic syndrome risk score. The Wald statistic was used to determine the statistical significance of intervention effects and level of compliance on the outcome variables.

For the variables, the models were adjusted for confounding or modifier effects variables following the next steps: (1) crude model, adjusted by group (intervention/control), stage (time), and baseline value of the outcome measure concerned, the latest to avoid a regression bias to the estimated mean (Barnett et al., 2005); (2) Additionally adjusted by age, gender, education, main activity, and meet PA recommendation measured both self-reported and objective with an accelerometer. (3) Additionally, adjusted by weight, BMI, and waist circumference. (a) Weight additionally adjusted by BMI and waist circumference; (b) waist circumference additionally adjusted by weight and BMI.

Ethical Issues

The National Research Commission and Ethics Committee at Mexican Social Security Institution and National Institute of Public Health in Mexico, approved this study and all its procedures, as stated before (Gallegos-Carrillo et al., 2014).

RESULTS

The characteristics of the study population according to meeting PA recommendations at 16 (T1) and 24 (T2) weeks of follow-up in the BC and the ER groups and by the level of adherence for the ER are shown in **Table 1**. The proportion of participants in the ER and BC group who met PA recommendations in both T1 and T2 was higher in the ER group (32.4%) than in the BC (25.4%), Body Mass Index (BMI) values were lower in participants meeting PA recommendations in T1 and T2 in comparison with those meeting PA recommendations in T1 only, BMI = 32.6 vs. 28.2 in the ER and BMI = 28.5 vs. 28.2 for the BC group. While differences in glucose levels were only observed in the ER group 109 vs. 103 mg/dL (**Table 1**), and blood pressure level observed a decrease in the BC, systolic 130 vs. 128 mmHg and diastolic blood pressure 79.9 vs. 76.6 mmHg, respectively (**Table 1**).

Longitudinal Analysis in Biological, Biochemical, and Anthropometric Variables

The percentage change observed in systolic and diastolic blood pressure was not statistically significantly different in longitudinal multilevel analyses, although blood pressure levels decreased in the BC group and remained at the same levels in the ER group. We also observed a statistically significant difference in weight and in metabolic syndrome risk score, whose values in participants in the ER group decreased among basal, T1 (16 weeks), and T2 (24 weeks) of follow-up, however, difference by level of compliance was not observed in biological (systolic and diastolic blood pressure, levels of cholesterol HDL AND LDL, biochemical and anthropometrics) variables (**Table 2**).

Longitudinal changes in depressive symptoms and quality of life showed differences statistically significant among Baseline, T1, and T2 in depressive symptoms among the ER and BC groups, and by level of compliance >50% of the ER group in the mean of the mental health component of quality of life (**Table 3**).

Multilevel longitudinal analyses according to intention to treat analysis showed statistically significant changes in the biochemical variables measured in the study among ER and BC groups: triglycerides β adjusted = −14.1 (95% C.I. −25.2, −3.1) and Metabolic syndrome risk score β adjusted = −3.5 (95% C.I. −5.7, −1.4), (**Table 4**). Anthropometric variables as weight β adjusted −1.0 (95% C.I.−1.56, −0.43), β adjusted BMI −0.28 (95% C.I. −0.54, −0.02) and rate waist/hip β adjusted −13.9 (95% C.I. −20.7, −8.8) and perception of depressive symptoms shown difference statistically significant after adjusting for the variables specified in **Table 4**, including socio-demographic and health status variables, p-value < 0.05.

The results of longitudinal multilevel analysis by the level of compliance in the ER group observed differences statically significant in diastolic blood pressure β adjusted −2.56 mmHg (95% C.I. −0.47, −0.041), waist circumference β adjusted −1.8 (95% C.I. −3.5, −0.01), depressive symptoms β adjusted −1.66 (95% C.I. −3, −0.28) and the score of the Mental Health Component (MCS) of quality of life, $p < 0.05$, after adjusting for group, stage (time), and baseline value of outcome measure (**Table 4**).

DISCUSSION

The results of this study show firstly the feasibility of carrying out an exercise-referral scheme intervention in the context of a social security institution in an upper-middle-income country like Mexico. We observed that both PA programs achieved changes in clinical indicators as triglycerides and anthropometric values Body Mass Index and Metabolic syndrome risk score. While higher levels of adherence of patients who got the ER scheme to increase PA levels accomplished better outcomes in the Mental Health Component in comparison with hypertensive patients who had a lower level of adherence to the ER scheme. In comparison to the BC group, the ER and higher levels of adherence achieved both changes in the perception of social functioning.

Backgrounds in a similar context are lacking, and thus the findings of this cluster-randomized trial would be helpful to encourage the design of programs and strategies to increase PA among patients with chronic diseases in a context such as Mexico.

TABLE 1 | Baseline characteristics among exercise-referral and brief counseling groups according to meeting physical recommendations at the end of the interventions.

Variables	Exercise referral (ER) $n = 117$			Brief counseling (BC) $n = 115$		
	No meet PA recommendation both T1 and T2 $n = 58$ (52.2%)	No meet PA recommendation T1 meet in T2 $n = 17$ (15.3%)	Meet PA recommendation both T1 and T2 $n = 36$ (32.4%)	No meet PA recommendation both T1 and T2 $n = 59$ (53.6%)	No meet PA recommendation T1 meet in T2 $n = 23$ (20.9)	Meet PA recommendation both T1 and T2 $n = 28$ (25.4)
Socio-demographic						
Age, mean (SD)	50.8 (10.9)	48.7 (11.6)	50.3 (11.2)	52.5 (9.9)	51.5 (10.6)	50.3 (11.2)
Age group %						
35–49	23 (46.9)	8 (16.3)	18 (36.7)	19 (43.2)	10 (22.7)	15 (34.1)
50–65	28 (53.8)	8 (15.4)	16 (30.8)	34 (60.7)	11 (19.6)	11 (19.6)
65 and more	7 (70)	1 (10)	2 (20)	6 (60)	2 (20)	2 (20)
Male (%)	17 (47.2)	5 (13.9)	14 (38.9)	15 (48.4)	8 (25.8)	8 (25.8)
Female (%)	41 (54.7)	12 (16)	22 (29.3)	44 (55.7)	15 (19)	20 (25.3)
Educational level %						
Elementary school or less	17 (54.8)	4 (12.9)	10 (32.3)	25 (67.6)	5 (13.5)	7 (18.9)
Middle school	12 (46.1)	3 (11.5)	11 (42.3)	7 (36.8)	4 (21.1)	8 (42.1)
High school/technical	18 (46.1)	7 (17.9)	14 (35.9)	16 (51.6)	7 (22.6)	8 (25.8)
Bachelor or higher	11 (73.3)	3 (20)	1 (6.7)	11 (47.8)	7 (30.4)	5 (21.7)
Marital status % (C.I. 95%)						
Single	4 (40)	1 (10)	5 (50)	8 (66.7)	1 (8.3)	3 (25)
Married	45 (51.7)	13 (14.9)	29 (33.3)	40 (51.3)	16 (20.5)	22 (28.2)
Divorced	5 (83.3)	1 (16.7)	0	7 (63.6)	3 (27.3)	1 (9.1)
Widowed	4 (50)	2 (25)	2 (25)	4 (44.4)	3 (33.3)	2 (22.2)
Main activity % (C.I. 95%)						
Homemaker	24 (53.3)	6 (13.3)	15 (33.3)	17 (50)	6 (17.6)	11 (32.3)
Student	0	0	1 (100)	1 (100)	0	0
Works outside home	24 (48.9)	10 (20.4)	15 (30.6)	35 (56.4)	13 (20.69)	14 (22.6)
Retired or pensioned	5 (50)	1 (10)	4 (40)	5 (50)	3 (30)	2 (20)
Unemployed	4 (80)	0	1 (20)	1 (33.3)	1 (33.3)	1 (33.3)
Permanently disabled	1 (100)	0	0	0	0	0
Blood pressure and biochemical measurements mean (SD)						
Systolic	123.6 (16.7)	126 (27.5)	131 (18.5)	132 (19.3)	130 (15)	128 (17.2)
Diastolic	79 (12.3)	82.1 (14.1)	83.5 (8.6)	77.3 (11.9)	79.9 (12)	76.6 (8.8)
Fasting glucose	108 (35.5)	109 (27.1)	103 (16.8)	106 (23.1)	106.5 (21.6)	106 (28.3)
Cholesterol HDL	47.6 (13.3)	40.6 (8.1)	45.4 (9.3)	46.7 (12.2)	49.7 (12.1)	46.3 (10.9)
Triglycerides	178.9 (71.9)	167.9 (66.1)	162.4 (78.2)	166.5 (62.9)	164.4 (64.4)	151.1 (63.5)
Anthropometric measures mean (SD)						
Weight	75.8 (16.3)	82.8 (20.7)	72.9 (13.5)	73.2 (14.3)	72.4 (16.1)	71.5 (9.4)
Body Mass Index	30.2 (5.4)	32.6 (5.8)	28.2 (4.32)	29.9 (4.9)	28.5 (4.9)	28.2 (3.4)
Waist circumference	101.5 (12.3)	105.1 (14.4)	96.2 (11.6)	98.9 (10)	96.3 (9.6)	93.3 (8.2)
Quality of life and depressive symptoms						
Physical component summary	48.2 (5.3)	45.7 (6)	47.8 (5.7)	46.8 (4.2)	45.8 (5.5)	47.9 (3.66)
Mental component summary	30.5 (5.2)	33.1 (5.4)	31.2 (5.4)	31.1 (5.4)	31.4 (4.5)	30.6 (3.98)
Depressive symptoms (CES-D 20)	18.8 (7.9)	17 (19.1)	15.4 (6.9)	20.1 (7.9)	21.4 (9.5)	22.8 (10.4)
Diabetes self-reported %	9 (56.2)	1 (6.25)	6 (37.5)	11 (61.1)	3 (16.7)	4 (22.2)

Physical activity recommendations measured by accelerometers.

Physical activity promotion through an exercise-referral scheme, compared to the control group with BC to encourage PA, showed findings which are according to previous studies of effectiveness in which the BC has achieved increased the PA levels compared to the exercise-referral scheme (Galaviz et al., 2013); or as has been found in PA promotion studies, in which increased in PA was reported for both the ER and the BC groups (Hillsdon et al., 2005).

The differences observed in clinical variables such as blood pressure levels, triglycerides, and fasting glucose among those

TABLE 2 | Longitudinal changes and multilevel analyses in biological, biochemical and anthropometrics variables among intervention and control groups and by level of compliance.

Variables	Exercise referral	Brief counseling	Crude model[a]	Level of compliance (50% and more)		Crude model (comp. <50% vs. ≥50%)[a]
			P value	<50 (*n* = 45)	≥50 (*n* = 72)	*P* value
Systolic Blood pressure, mm Hg Mean (SD)						
0 weeks (T0)	124.9 (22)	130.1 (17.9)	0.105	121.1 (24.8)	127.4 (19.9)	0.386
16 weeks (T1)	124.7 (19)	124.6 (18.4)		121.5 (21.2)	126.7 (17.2)	
24 weeks (T2)	124.4 (19.1)	125.9 (18.1)		121.1 (20.6)	126.5 (17.8)	
Diastolic Blood pressure, mm Hg Mean (SD)						
0 weeks (T0)	79.9 (13.8)	77.5 (11.2)	0.417	77.4 (17)	81.5 (11.1)	0.905
16 weeks (T1)	76.7 (12.3)	74.2 (12.6)		76.5 (14.6)	76.9 (10.8)	
24 weeks (T2)	76.5 (12.2)	72.1 (12.6)		75.5 (14.3)	77.2 (10.7)	
Cholesterol HDL (mg/dL) Mean (SD)						
0 weeks (T0)	45.8 (12.1)	47.1 (11.6)	0.990	45.4 (14.1)	46.1 (10.7)	0.475
16 weeks (T1)	46 (11.1)	47.5 (10.4)		46.4 (12.4)	45.8 (10.2)	
24 weeks (T2)	46.2 (11)	47.4 (10.5)		46.4 (12.2)	46 (10.3)	
Fasting glucose (mg/dL) Mean (SD)						
0 weeks (T0)	105.4 (31.3)	106.7 (24.2)	0.243	99.1 (23.4)	109.3 (34.8)	0.299
16 weeks (T1)	105.5 (30.3)	108.2 (28.9)		101.6 (24.9)	107.9 (33)	
24 weeks (T2)	104.6 (31.3)	107.3 (29.8)		98.4 (26.7)	108.5 (33.3)	
Triglycerides (mg/dL) Mean (SD)						
0 weeks (T0)	168 (73.3)	161.9 (61.8)	0.016	169.2 (72.7)	167.3 (74.1)	0.829
16 weeks (T1)	179.4 (88.3)	176.6 (82.8)		186.3 (90.2)	170.5 (77.4)	
24 weeks (T2)	178.5 (88)	176.4 (82.5)		182.6 (91.4)	172.6 (76.4)	
Weight (Kgs) Mean (SD)						
0 weeks (T0)	76.2 (16.2)	72.6 (13.3)	0.000	81.5 (20.5)	72.9 (11.7)	0.590
16 weeks (T1)	74.8 (15.9)	72.1 (13.1)		79.9 (19.9)	71.7 (11.7)	
24 weeks (T2)	74.9 (15.7)	72.2 (13.2)		79.9 (19.6)	71.8 (11.8)	
BMI Mean (SD)						
0 weeks (T0)	30.1 (5.2)	29.2 (4.6)	0.031	31.3 (6)	29.3 (4.5)	0.846
16 weeks (T1)	29.7 (5.2)	29.1 (4.7)		30.9 (6.1)	28.9 (4.4)	
24 weeks (T2)	29.6 (5.1)	29.1 (4.6)		30.7 (5.9)	28.8 (4.4)	
Waist circumference (cms) Mean (SD)						
0 weeks (T0)	100.5 (12.5)	97 (9.6)	0.269	103.9 (13.8)	98.3 (11.2)	0.721
16 weeks (T1)	98.1 (11.7)	95.3 (9.4)		101.4 (12.9)	96.1 (10.4)	
24 weeks (T2)	98.2 (11.5)	95.3 (9.4)		100.9 (12.8)	96.5 (10.3)	
Rate waist/hip Mean (SD)						
0 weeks (T0)	0.93 (0.08)	0.91 (0.09)	0.759	0.94 (0.08)	0.92 (0.08)	0.530
16 weeks (T1)	0.92 (0.07)	0.90 (0.06)		0.93 (0.07)	0.91 (0.08)	
24 weeks (T2)	0.91 (0.07)	0.91 (0.06)		0.92 (0.08)	0.91 (0.07)	
Metabolic syndrome Risk Score Mean (SD)						
0 weeks (T0)	97.5 (17.7)	94.6 (14.6)	0.002	96.1 (17.7)	97.6 (17.9)	0.726
16 weeks (T1)	98.1 (18.7)	99.4 (21.9)		97.1 (44.8)	98.7 (17.5)	
24 weeks (T2)	94.5 (16.5)	99.6 (20.7)		93.3 (16.4)	95.2 (16)	

Intention to treat analysis. [a]*Adjusted for group, stage (time), and baseline value of outcome measure.*

who reported higher levels of adherence to the intervention could be supported by findings in previous studies with similar approaches in which a "Hawthorne" effect could have been present. In fact, the findings of improved clinical and anthropometric factors have been reported in studies carried out mostly in high-income countries (Dugdill et al., 2005).

In a way consistent with the evidence generated in studies of exercise-referral schemes, the ability of the intervention to increase physical health outcomes was low (Buckley et al., 2020). The eligibility assessment carried out in PHCU, might have affected the rate of participation, because 78.6% of patients attended the initial session, in which the exercise was prescribed, and the individualized program was developed. In addition, eligibility criteria as being a hypertensive patient with blood pressure values under control parameters, and having reported a stage of change with a willingness to PA practice would influence

TABLE 3 | Longitudinal changes and multilevel analyses in quality of life and depressive symptoms variables among exercise referral and brief counseling groups and by level of compliance.

Variables	Exercise referral	Brief counseling	Crude model[a]	Level of compliance (50% and more)		Crude model (comparing <50% vs. ≥50%)[a]
			P value	<50 (*n* = 45)	≥50 (*n* = 72)	*P* value
Physical Component Summary Mean (SD)						
0 weeks (T0)	46.7 (8.3)	47 (4.6)	0.125	46.9 (5.8)	46.5 (5.2)	0.108
16 weeks (T1)	46.6 (5.2)	45.7 (4.4)		47.5 (5.5)	46.1 (4.9)	
24 weeks (T2)	46.8 (4.9)	45.7 (4.4)		47.6 (5.1)	46.4 (4.8)	
Mental Component Summary Mean (SD)						
0 weeks (T0)	30.7 (6.5)	30.8 (4.9)	0.215	28.1 (8.2)	32.4 (4.6)	0.001
16 weeks (T1)	30.9 (5.1)	31.1 (4.4)		30.2 (5.2)	31.3 (4.9)	
24 weeks (T2)	31.1 (5.0)	31.2 (4.4)		30.8 (5.2)	31.3 (4.9)	
Depressive symptoms (CESD-20) Mean (SD)						
0 weeks (T0)	18 (7.6)	21.3 (9.3)	0.037	19.3 (9.5)	17.1 (5.9)	0.686
16 weeks (T1)	15.6 (6.7)	17.5 (7.8)		16.4 (7.6)	15.1 (6.1)	
24 weeks (T2)	15.6 (6.9)	17.4 (7.9)		17 (7.8)	14.8 (6.2)	

Intention to treat analysis. [a]Adjusted for group, stage (time), and baseline value of outcome measure.

TABLE 4 | Results of longitudinal multilevel analysis.

Outcomes among intervention and control groups	Crude model[a] β or OR (95% CI)	*P* value	Adjusted model[b] β or OR (95% CI)	*P* value	Adjusted model[c] β or OR (95% CI)	*P* value
Triglycerides (mg/dL)	−13.5 (−24.6, −2)	0.016	−14.1 (−25.2 −3.1)	0.012	−11.8 (−25.6, 2.02)	0.094
BMI	−0.27 (−0.52, 0.2)	0.031	−0.29 (−0.52, −0.05)	0.018	0.28 (−0.54, −0.02)	0.034
Weight	−0.76 (−1.16, −0.36)	0.000	−0.79 (−1.18, 0.39)	0.000	−1.0 (−1.56, −0.43)	0.001
Depressive symptoms (CESD-20)	−3.26 (−5.5. −0.57)	0.019	−3.17 (−5.9, −0.43)	0.025	−2.28 (−7.18, 2.6)	0.305
Rate waist/hip	0.01 (0.0, 0.02)	0.031	0.01 (0.0, 0.02)	0.045	0.007 (−0.00, 0.01)	0.200
Metabolic syndrome risk score	−3.4 (−5.5, −1.2)	0.002	−3.5 (−5.7, −1.4)	0.001	−1.8 (−4.2, 0.39)	0.105
Outcomes by level of compliance (50%)						
Mental Component Summary Mean (SD)	−2.0 (−3.2, −0.81)	0.001	−0.6 (−1.57, 0.35)	0.154	−0.56 (−5.1, 4.01)	0.558
Diastolic Blood pressure mm Hg	−2.56 (−4.7, −0.41)	0.019	−1.9 (−4.1, 0.36)	0.100	−1.35 (−3.6, 0.9)	0.246
Waist circumference (cms.)	−1.8 (-3.5, −0.01)	0.048	−1.4 (−3.2, 0.43)	0.136	−0.41 (−2.2, 1.4)	0.650
Rate waist/hip	−0.02 (−0.03 −0.005)	0.006	−0.01 (−0.3, −0.001)	0.029	−0.01 (−0.03, 0.006)	0.190
Depressive symptoms (CESD-20)	−1.66 (−3, −0.28)	0.018	1–56 (−3.2, 0.09)	0.064	−0.88 (-2.4, 0.66)	0.263

[a]Adjusted for group, stage (time), and baseline value of outcome measure.
[b]Additionally adjusted by age, gender, education, employment, acute morbidity and meet physical activity recommendation measured both self-reported and objective with accelerometer.
[c]Additionally adjusted by age, gender, education, employment, acute morbidity, meet physical activity recommendation measured both self-reported and objective with accelerometer, weight, BMI and waist circumference (1) Weight additionally adjusted by BMI and waist circumference; (2) waist circumference additionally adjusted by weight and BMI.
P value of the interaction term (assessment time × intervention group) and the interaction between (assessment time × adherence).

in rates of participation in ER programs, which has not been applied for participants in the BC group.

Likewise, in the follow-up, the rates of participation among the ER and BC were different, while the control group observed a follow-up rate at 24 weeks of 90%, the intervention group saw a decrease to 62%. Independent of changes of behavior and contrary to expectations, a behavioral theory-based intervention carried out with an exercise-referral scheme had less impact hooking the subjects as participants in the study. Studies in primary health care settings have suggested that motivational activities including the provision of exercise are more effective than interventions with only exercise aims (Lee et al., 2012). However, in this cluster-randomized trial provision of BC and health promotion materials combined with monthly monitoring attained a higher rate of participation than closer supervision and prescription. Therefore, as was shown in this study, unless a certain level of adherence to an exercise-referral scheme can be guaranteed; BC would be a good alternative to promote PA and achieve the recommended levels (Pate et al., 1995).

The modest effect of an exercise-referral scheme using a behavioral theory-based approach to achieve a recommended level, as stated before in National Exercise-Referral Schemes depends on the level of adherence; one of the most questionable issues of exercise-referral schemes. In this cluster trial, we set a goal of 50% of compliance (attendance of at least 24 sessions) which resulted in 78.2% of participants who began the exercise

program achieving this level of adherence; a top-level compared to the levels observed in other trials. In Taylor et al. (1998) observed a 28% of adherence and 33% Lamb et al. (2002). However, the changes observed in the intervention group along time in reducing high blood pressure along the follow-up period keep no consistency whether among ER and BC, nor by level of adherence or compliance; therefore a larger sample size that allows a longitudinal stratified analysis and determination of the factors associated with adherence to these kinds of initiatives meant to increase physical health outcomes, i.e., blood pressure and fasting glucose of hypertensive populations, is necessary.

Strengths and Limitations

This cluster-randomized trial has several strengths. To our knowledge, this is perhaps the first study to carry out an intervention to increase PA through an exercise-referral scheme among sedentary patients with a recent diagnosis of hypertension; in an upper-middle-income country.

This study used a control group with the same measurements throughout the study, which allowed for the assessment of the intervention effects from several dimensions during the three stages of the study. Particularly it had the strength of assessing PA levels through an objective method, thereby reducing the bias of self-reported PA measures.

It measured the sustainability effects and the level of adherence to the intervention, reinforcing the capacity of measuring the effects carefully. The trial was clustered by practice to reduce the risk of the intervention being contaminated; however, this mechanism of allocation was not enough to avoid changes in lifestyles in the control group, thus changes in outcome variables were observed in the BC group, which as it has been mentioned received a less intensive PA program under a BC approach, influencing not only in PA variables but in other lifestyles, findings that have been previously reported (Gallegos-Carrillo et al., 2017). Finally, and according to the protocol study, the intervention was extended to the control group, after the follow-up of 24 weeks ended.

CONCLUSION

This intervention achieved changes among the hypertensive patients who participated in the exercise referral program in comparison with those in the BC group. Changes observed among PA programs at time 0, 16, and 24 weeks pointed out a better performance of the ER in comparison to the BC program in clinical indicators and anthropometric variables, such as triglycerides and the Metabolic syndrome risk score, BMI, and also depression symptoms. About the objective aimed to determine differences in the outcome variables according to level of adherence to the exercise referral scheme, we observed that whether in clinical outcomes as blood pressure levels, waist circumference, and the rate waist/hip and in depressive symptoms and health-related quality of life perception of hypertensive patients who achieved at least 50% adherence to the ER have a better outcome than those with a lower level of adherence. However, although these results did not keep statistical significance in the adjusted models, they at least allow us to glimpse the benefits of PA when programs achieved higher attachment, which could be an important finding to promote a higher engagement in PA programs in the population with chronic conditions.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The National Research Commission and Ethics Committee at Mexican Social Security Institution, and National Institute of Public Health in Mexico, approved this study and all its procedures. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KG-C wrote the original protocol and manuscript, collected the data, designed statistical models, and performed analyses. CG-P contributed to the original protocol and wrote the first and final draft. NS-D-S wrote the first draft with contributions from KG-C. JS contributed to the original protocol and wrote the first draft with contributions from KG-C. FL contributed to the original protocol, supported in developing the strategy to analyze the data, and wrote the first draft with contributions from KG-C. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.712135/full#supplementary-material

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