

**Effect of Moderate Versus High Intensity Interval Exercise  
Training on Vascular Function in Inactive Latin-American  
Adults: A Randomized Clinical Trial**

By

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A thesis submitted to the School of Medicine and Health Science of Rosario University  
in partial fulfillment of the requirements for the degree of Master of Physical Activity  
2016

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## Abstract

**Background.** Exercise training is effective for improving cardiometabolic health and physical fitness in inactive adults. However, limited research has been conducted on the optimal exercise training intensity for this population. We investigate the effect of moderate versus high intensity interval exercise training on vascular function and physical fitness in physically inactive adults.

**Methods.** Twenty inactive adults were randomly allocated to receive either moderate intensity training (MCT group) or high intensity interval training (HIT group). The MCT group performed aerobic training at an intensity of 55-75% of the walking on a treadmill at 60-80% heart rate max (HRmax) until expenditure of 300 kcal until the end of training. The HIT group performed running on a treadmill during 4 minutes at 85-95% peak HRmax and had a recovery of 4 minutes at 65% peak HRmax until expenditure of 300 kcal until the end of training. Vascular function (flow-mediated vasodilation, FMD [%], aortic pulse wave velocity, PWV [ $\text{m}\cdot\text{s}^{-1}$ ]), blood lipids [fasting glucose, triacylglycerol, total cholesterol, LDL-cholesterol, HDL-cholesterol], blood pressure, and physical fitness (Muscle strength (handgrip [kg]), exercise capacity ( $\dot{V}\text{O}_2\text{peak}$  and graded exercise test duration [minutes])), were measured at baseline and 12-weeks thereafter.

**Results.** FMD changes were 2.2 (4.9) % in the MCT group, 7.7 (5.3) % in the HIT group (difference between groups  $-5.4$  [95% CI,  $-10.3$  to  $-0.6$  ( $P < 0.001$ )]. PWV changed 0.1 in the MCT group but decreased  $-0.3$  in the HIT group, (not significantly different from the MCT or HIT group,  $P$  between groups = 0.91). Percentage body fat did not change in the MCT group 0.0 (0.8) but decreased in HIT group,  $-1.1$  (difference between groups  $1.2$  [95% CI,  $0.1$  to  $2.4$   $P$  between groups = 0.04]). No significant group differences were observed in physical fitness, blood lipids or blood pressure.

**Conclusions.** HIT is more effective in improving endothelial function and reducing body fat than MCT in inactive Latin-American adults.

**Trial registration.** ClinicalTrials.gov NCT02738385, registered on 23 March 2016.

**Keywords.** Randomized controlled trial; Exercise training; Endothelial function; Cardiovascular risk factors; Physical fitness

## Introduction

Strong evidence shows that physical inactivity (<150 min/wk of moderate-intensity activity or 75 min/wk of vigorous-intensity activity) increases the risk of many adverse health conditions, including major non-communicable diseases such as cardiovascular disease (CVD), metabolic syndrome, and breast and colon cancers, and shortens life expectancy [1,2,3]. Physical inactivity has a deleterious effect that is comparable to smoking and obesity and is now recognised as the fourth leading risk factor for global mortality, accounting for 6% of all deaths [2]. The results of a recent meta-analysis revealed that the effect of insufficient physical activity (PA) on all-cause mortality was greater among those with low levels of PA compared with those with high levels of PA [4].

Another effect of physical inactivity is loss of physical fitness. Individuals physical inactive also have lower levels of physical fitness, specifically muscle strength [5] and exercise capacity [6,7]. The findings of Lee et al. [8] suggest that *fit* individuals had more than 35% lower odds of mortality after adjusting for physical inactivity and other major risk factors. Studies with predominantly middle-aged and elderly individuals show that low hand-grip is inversely associated with CVD [9,10], generally expressing muscle strength in relative terms [11]. In addition, muscular weakness has been associated with an increased risk of mortality in many cross-sectional and longitudinal studies [12,13].

Previous trials have found that inactive people who participate in supervised physical training can experience improvement in physical fitness [14] and reduce the risk of several major non-communicable diseases such as type 2 diabetes [15]. The current US guidelines for aerobic PA suggest that all individuals should perform at least 150 min/wk of moderate PA, 75 min/wk of vigorous PA, or an equivalent of a combination of both [16]. However, recommendations on dosing variables, such as intensities, of these exercise training programs have varied

[17,18,19]. A growing body of evidence has demonstrated comparable or superior improvements in cardiovascular function using low-volume, high-intensity training (HIT) compared to traditional moderate continuous training (MCT) [19,20]. HIT provides rapid physiological adaptations, as indicated by improvements in maximal oxygen uptake, anaerobic threshold, and stroke volume [21]. Furthermore, it was suggested that the ability of HIT to restore vascular homeostasis through enhancement in shear stress-induced nitric oxide bioavailability may be another important mechanism that explains the protective role of exercise against CVD development [17-22]. Interestingly, despite this evidence, there are few randomised trials that have directly evaluated the effects of sustained MCT, HIT, or a combination of the two (MCT/HIT) on cardiometabolic health of inactive adults [23,24,25,26].

In Latin-American population, a region which has undergone a well-documented epidemiologic transition and epidemic of CVD [27,28,29], relatively little research on physical activity [30] and physical fitness exists [31,32,33]. A randomised clinical trial comparing different intensities of exercise training in adults with insufficient PA is clinically relevant because it could provide evidence for a precise, prescribed intensity of exercise training for optimal outcomes in this population [34,35,36]. Thus, the purpose of this randomised clinical trial was to compare the effect of MCT versus HIT on vascular function and physical fitness in Latin-American adults physically inactive.

## **Methods**

### ***Study design and setting***

The HIIT-Heart Study was a randomised clinical trial (ClinicalTrials.gov ID: NCT02738385) including physically inactive patients randomly allocated to either MCT or HIT. The study was performed in accordance with the Declaration of Helsinki (2000) and was

approved by the local office for Medical Research Ethics Committee of The University of Santo Tomás (ID 27-0500-2015). Cardiometabolic health parameters and physical fitness outcomes were assessed at baseline and 12 weeks thereafter. We provide an overview of the methods as per the Consolidated Standards of Reporting Trials (CONSORT) checklist [37].

### ***Participants and recruitment***

This randomised clinical trial was conducted at the University of Rosario in Bogota and Santo Tomás, Colombia, from February 2015 to May 2016. Participants aged 18–45, inactive (<150 min/wk of moderate-intensity activity or 75 min/wk of vigorous-intensity activity), with body mass index  $\geq 18$  and  $\leq 30$  kg/m<sup>2</sup>; identified as being willing and with almost immediate availability were recruited from the Centre of Studies in Physical Activity Measurements (*in Spanish*, CEMA), by posting study recruitment flyers at community centers, study recruitment announcements at CEMA, and word-of-mouth.

Risks was minimised by ruling out contraindications to the testing and training protocols via a health history and a thorough physical examination prior to the testing sessions. Individuals with a history of a medical condition identified by the American Heart Association (AHA) as an absolute contraindication to exercise testing were excluded from this study [38]. Furthermore, individuals were also excluded if they presented any of the following: systemic infections, weight loss or gain of >10% of body weight in the past 6 months for any reason, currently taking medication that suppresses or stimulates appetite, uncontrolled hypertension (systolic blood pressure 160 mm Hg or diastolic blood pressure 95 mm Hg on treatment), gastrointestinal disease (including self-reported chronic hepatitis or cirrhosis, any episode of alcoholic hepatitis or alcoholic pancreatitis within past year, inflammatory bowel disease requiring treatment in the past year, recent or significant abdominal surgery e.g., gastrectomy), asthma, diagnosed diabetes (type 1 or 2), fasting impaired glucose tolerance (blood glucose  $\geq 118$  mg/dL), or use of any prescribed

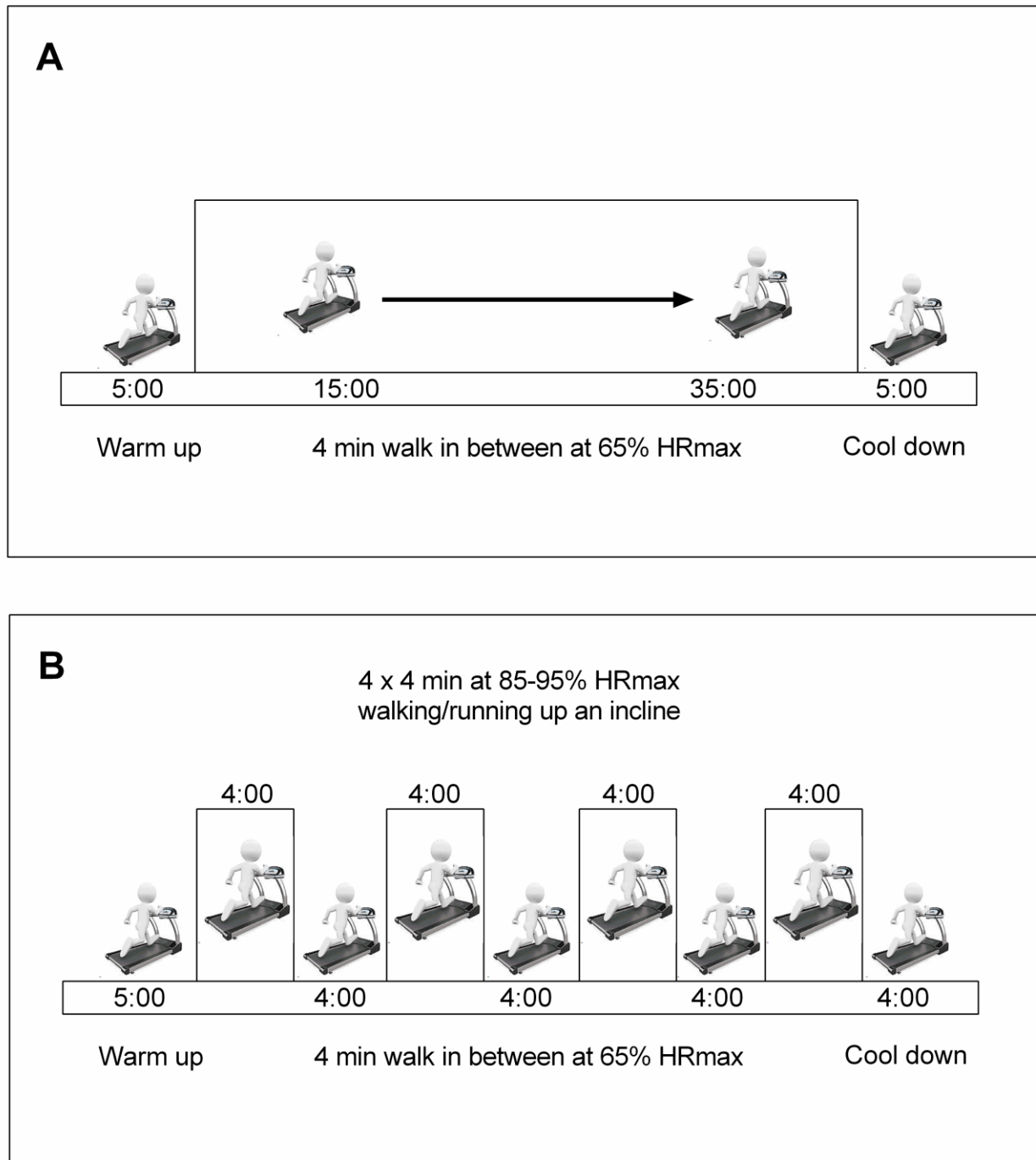
drugs, any active use of illegal or illicit drugs, or being unable to participate due to a physical impairment. Participants were required to sign a written, informed consent form.

### ***Blinding and randomisation***

The randomisation into the two study arms was performed by the CEMA at University of Rosario, Bogotá, Colombia, using block randomisation with a block size of four. As each participant consecutively entered this randomized clinical trial, he/she was randomly allocated to either the MCT versus HIT according to the computer-generated sequence of group allocation. The randomization sequence was not concealed from the investigator who was responsible for assigning participants to groups. All participants and study personnel (including investigators, and statisticians) were blinded to treatment allocation throughout the trial protocol. Further, the investigators who performed the statistical analyses were masked from group assignment. The importance of maintaining the blinding and allocation concealment was reinforced by regularly scheduled conference calls at the sites and daily meetings with the field investigators.

### ***Interventions***

The participants randomly assigned to the intervention group participated in the cardiometabolic programme as recommended by the Colombian guidelines COLDEPORTES (*in Spanish*, Departamento Administrativo del Deporte, la Recreación, la Actividad Física y el Aprovechamiento del Tiempo Libre) [39], and AHA [40] for cardiovascular health promotion and disease reduction. At the beginning of the training protocol, we obtained the participants' weight to determine weekly energy expenditure necessary to achieve their target of 12-kcal•kg<sup>-1</sup>•week<sup>-1</sup> (iso-energetic). It is expected that the gradual increase in total energy expenditure would minimise fatigue, soreness, injuries, and attrition, Figure 1.



**Figure 1.** Example of a 30–40 min MCT session (A) and 4 × 4 HIIT session (B)

*Moderate-continuous training (MCT) group*

The MCT protocol was completed with fast walking or running on a treadmill with the deck inclined to reach the desired intensity. Each preparatory period started with an exercise dose of 6-

$\text{kcal}\cdot\text{kg}^{-1}\cdot\text{week}^{-1}$ , which was increased progressively  $2\text{-kcal}\cdot\text{kg}^{-1}\cdot\text{week}^{-1}$  until week 4, and was then maintained at  $12\text{-kcal}\cdot\text{kg}^{-1}\cdot\text{week}^{-1}$  for weeks 5 to 12.

#### *Preparatory training phase: Weeks 1–2*

An initial 2-week preparatory phase of training to bring all participants up to a 150 kcal goal per session was performed. Exercise training sessions were designed to elicit a response in the acceptable moderate-to-vigorous range, i.e., 55–75% HR<sub>max</sub>, and was adjusted according to ratings on the Borg scale [28]. Sessions consisted of a warm-up walk (5 min) followed by an aerobic exercise session (15–35 min), and a final relaxation/cool-down period (10 min). Figure 1A.

#### *Protocol of interval training: Weeks 3–12*

The overall goal for the MCT group was to perform exercise sessions at 55–75% HR<sub>max</sub>. During each exercise session, participants adhered to the  $12\text{-kcal}\cdot\text{kg}^{-1}\cdot\text{week}^{-1}$  energy expenditure format, equivalent to 300 kcal of expended energy at the end of training and cool-down (5 min), with a range total exercise time of 35 to 55 min, and a final relaxation/cool-down period (10 min). Exercise was performed for three sessions per week. During the supervised intervention, HR was recorded using a HR monitor (Polar Pacer, USA) to ensure compliance with the exercise stimulus at the predetermined target HR zone. In addition, Borg ratings [28] were also measured in each exercise session.

#### *High-intensity training (HIT) group*

The HIT protocol was completed with fast walking or running on a treadmill with the deck inclined to reach the desired intensity. We calculated the training energy expenditure for participants' age ranges associated with meeting the consensus public health recommendations from the Cardiometabolic HIIT-RT Study [17]. A complete description of the design and methods has been published elsewhere [17]. Each preparatory period started with an exercise dose of 6-



$\text{kcal}\cdot\text{kg}^{-1}\cdot\text{week}^{-1}$ , which was increased progressively  $2\text{-kcal}\cdot\text{kg}^{-1}\cdot\text{week}^{-1}$  until week 4, and was then maintained at  $12\text{-kcal}\cdot\text{kg}^{-1}\cdot\text{week}^{-1}$  for weeks 5 to 12. Figure 1B.

*Preparatory training phase: Weeks 1–2*

An initial 2-week preparatory phase of training was performed to bring all participants up to a 150 kcal goal per session. To accomplish this, subjects warmed up at 65% of maximum heart rate (HR<sub>max</sub>) (5 min);  $4 \times 4$  min intervals at 60–80% HR<sub>max</sub>, interspersed with 4 min of recovery at 55% HR<sub>max</sub>. These sessions were performed at a frequency of 3 times per week.

*Protocol of interval training: Weeks 3–12*

The overall goal for the HIT group was to perform exercise sessions in  $4 \times 4$  min intervals at 85–95% HR<sub>max</sub> (with the target zone maintained for at least two minutes), interspersed with 4 min recovery at 65% HR<sub>max</sub>. During each exercise session, participants adhered to the  $12\text{-kcal}\cdot\text{kg}^{-1}\cdot\text{week}^{-1}$  energy expenditure format, equivalent to 300 kcal of expended energy at the end of training and cool-down (5 min), with a range total exercise time of 35 to 55 min. Exercise was performed for three sessions per week. During the supervised intervention, HR and Borg ratings were measured as described for the MCT group.

Participants in both groups were supervised during each exercise training session by an investigator or research assistant. Exercise training was conducted at a fitness center “CEMA” on the campus of the University of Rosario, which contained the resistance training equipment and treadmills needed to complete the prescribed exercise program. Each participant was instructed to immediately inform the supervisor if he or she experienced any unusual symptoms while exercise training and to consult a physician if needed. Participants were instructed to refrain from exercise training and to avoid changing their physical activity levels, outside of this study, and all participants reported adhering to these instructions.

We estimated the energy expenditure during the exercise sessions by calibrating the energy expenditure to the HR during the maximal oxygen uptake tests performed at the baseline and post-intervention time-points. The regression of the energy expenditure was calculated for each participant according to HR and minutes spent exercising during the training sessions. Trainers were physical therapists and physical educators with experience developing and monitoring exercise programmes among clinical populations [17]. Adherence to the exercise programme was encouraged by the exercise professional who supervised each of the group sessions. To maximise adherence to the training programme only a maximum of 3–5 participants were trained simultaneously. Each participant met with the study dietician for nutrition assessment and counselling, and an individualised nutrition intervention plan was developed from the baseline food intake assessment according to participant preferences [17]. This plan was a standardised meal consisting of 1300 to 1500 kcal (50–55% carbohydrates, 30–35% total fat, <7% saturated fat and 15–22% protein).

### ***Data collection and outcome measures***

Outcome measures were assessed at baseline and at 12 weeks follow-up by personnel blinded to the treatment allocation. Data was recorded on standardised forms and entered into a secured Access database that contained quality control checks (e.g., range checks, notification of missing data).

### ***Primary outcome measures***

The primary outcomes measure was endothelial function as measured by FMD and aortic pulse wave velocity (PWV). FMD was measured as described in previous studies from our group [17,41] in the Colombian population using the guidelines reported by Corretti et al. [42]. The same operator performed Doppler ultrasound (Mindray® DS USA; Mahwah, NJ) examinations, with a 7.5-MHz linear array probe being used to locate and interrogate flow velocity profiles in the right brachial

artery. Ultrasound images were obtained after 20 min of supine rest, in a dark, climate-controlled, quiet room (22–24°C) with the participants arm immobilized and slightly supinated and elevated. An additional 10 minute of rest was given prior to imaging the opposite arm. The right arm was imaged first in each case. After a resting period of at least 20 minutes, 1 minute of baseline recording of the brachial artery diameter was performed. Subsequently, the occlusion cuff was inflated to >200 mmHg for 5 minutes. Brachial artery diameter recording was restarted at least 30 seconds before cuff deflation and continued for 3 minutes thereafter. Peak artery diameter and the time to reach this peak after cuff deflation were recorded. The intra-session coefficient of variations was  $\leq 1\%$  for baseline diameter. Reliability, estimated by intra-class correlation coefficients (ICC) based on four baseline measurements (n=8 subjects), showed an ICC of 0.91 for the baseline diameter and 0.83 for FMD (own data). The percentage technical error of measurement was 1.23% for baseline diameter measurement, 1.77% for maximum diameter measurement and 20% for %FMD measurement. Images were recorded on a DVD player for subsequent measurements by one observer blinded to the study. FMD was calculated as the percent rise of peak diameter from the preceding baseline diameter and measured at every 1 minute after deflation for 3 minutes. PWV was measured with the oscillometric method using the occlusion technique. Patient data and the measured distance between the jugulum and the symphysis were registered in the arteriography programmed computer (TensioMed Software v.1.9.9.2; TensioMed, Budapest, Hungary). A tape measure was used for measuring the jugulum and the symphysis distance, namely the aortic distance. The cuff was placed on the patient's upper arm and connected to the device. The algorithm measuring blood pressure in the arteriography device has been validated [43]. PWV was calculated as the jugulum and the symphysis distance (m) divided by return time (return time/2) (s). For PWV, two recordings with the lowest standard deviation were chosen. The standard deviation was calculated from every heartbeat during a period of 8 s.

### *Secondary outcomes*

*Resting blood pressure:* Blood pressure was measured using an electronic oscillometric device (Riester Ri-Champion model, Jungingen, Germany) after being seated in a quiet room for 10 min with their back supported and feet on the ground according to the International Protocol of the European Society of Hypertension [44]. Two blood pressure readings were taken with a 10 min interval. Before blood pressure monitoring, the accuracy of the device was tested against a standard mercury sphygmomanometer in a random sub-sample (n=25) to ensure that there was no consistent difference of >10 mm Hg in measured blood pressure; and inter-observer variability was R=0.96.

*Metabolic biomarkers:* Blood samples were collected between 5:30 and 7:00 am by two experienced phlebotomists after at least 12 hours of fasting. Blood samples were obtained from an antecubital vein, and analyses were subsequently completed within one day from collection. The biochemical profile included: 1) the plasma lipid triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c) (by enzymatic colorimetric methods). Inter-assay reproducibility (coefficient of variation) was determined from 80 replicate analyses of 8 plasma pools over 15 days, and shown to be 2.6%, 2.0%, 3.2%, 3.6% for triglycerides, total cholesterol, HDL-c and LDL-c, respectively and 1.5% for serum fasting glucose.

*Physical fitness:* Physical fitness was measured using tests that have previously shown high validity and reliability levels [17]. Cardiorespiratory fitness was determined using a maximum treadmill exercise test (Precor TRM 885, Italy) following the modified Balke protocol [45], which has been extensively used [46,47] and validated [48] in people inactive. The treadmill test used a ramp protocol where the inclination is constant (5.5%) and the speed increases by 0.5 km/h every minute, starting at 4 km/h. Each session began with a 5 to 10 min warm-up at 50 W. We asked participants to refrain from smoking two hours before the test, and from drinking alcohol or doing any vigorous or moderate intensity activities 48 h before the test. HRmax was used to determine

the training intensity for each participant. We measured blood pressure prior to and during the test. Exercise was terminated if participants were fatigued, or earlier if they fulfilled the American College of Sports Medicine's guidelines for 'Indications for Terminating Exercise Testing' [49]. Maximal oxygen uptake was defined as the highest recorded  $\dot{V}O_{2peak}$  after two of three criteria were met: (1) a plateau in  $\dot{V}O_2$  after increase in workload; (2) a respiratory exchange ratio  $>1.10$ , and (3) a maximal heart rate within 10 bpm of their age-predicted maximum. Exercise capacity was defined as the total duration (minutes) of the graded exercise test. The findings of previous research suggest that graded exercise testing as described in this study is reliable and is a standard for measuring exercise capacity [37]. Muscular strength was measured using a standard adjustable hand held dynamometer (Takei Digital Grip Strength Dynamometer Model T.K.K.540®, Takei Scientific Instruments Co., Ltd, Niigata, Japan). Participants were given a brief demonstration and verbal instructions for the test, and if necessary, the dynamometer was adjusted to the participant's hand size according to predetermined protocols [50]. Handgrip strength was measured with the subject in a standing position, with the shoulder adducted and neutrally rotated, and arms parallel but not in contact with the body. The participants were asked to squeeze the handle as hard as possible for a maximum of 3-5 seconds, and no verbal encouragement was given during the test. Handgrip strength performance was recorded as the best score from either hand, without consideration for hand dominance. The reproducibility of our data was  $R=0.96$ . Intra-rater reliability was assessed by determining the intraclass correlation coefficient (0.98, CI 95% 0.97 to 0.99). The results of previous research indicate that isokinetic dynamometry testing is reliable and is a standard for measuring muscle strength [51,52].

*Anthropometric and body composition measurements:* Body weight was measured using electronic scales (Tanita® BC544, Tokyo, Japan) with a low technical error of measurement (Technical error of measurement = 0.510%). Height was measured using a mechanical stadiometer

platform (Seca® 274, Hamburg, Germany; Technical error of measurement = 0.01%). Body mass index (BMI) was calculated as the body weight in kilograms divided by the square of height in meters ( $\text{kg/m}^2$ ). The waist circumference (WC) was measured as the narrowest point between the lower costal border and the iliac crest; and in cases where it was not evident, it was measured at the midpoint between the last rib and the iliac crest, using a tape measure (Ohaus® 8004-MA, New Jersey, USA). We measured each variable twice and used the average measure obtained, unless the first and second measures varied by more than 1%, in which case we used the median of three measurements. Percentage of body fat mass was obtained by Tetrapolar Bioelectrical Impedance Analysis (BIA) system (BF-350, Tanita Corp, Tokyo, Japan). BIA measurements were carried out at 50 kHz with a 0.8 mA since wave constant current under standard conditions. Whole-body composition was estimated using equations provided by the BIA manufacturer for all participants [53]. Subjects stood on the metal contacts in bare feet, and body fat mass was determined. This measurement was repeated twice, and the average value was obtained.

*Recent Physical Activity Questionnaire (RPAQ):* Self-reported physical activity was measured using the RPAQ. This questionnaire assesses physical activity across four domains (domestic, recreational, work, and commuting) over the previous 7 days. It has shown moderate-to-high reliability for physical activity energy expenditure and good validity for ranking individuals according to their time spent in vigorous intensity physical activity and overall physical activity energy expenditure [54]. The outcome was assessed in METs (units of metabolic equivalence) per week. This questionnaire was administered immediately before and after the training period, as well as 12-weeks following the completion of the exercise intervention.

Additional outcomes of this study were participant adherence and adverse events. The investigator or research assistant, who supervised each group, recorded the date of each

completed exercise training session and the length of time spent during each exercise training session, which were used to assess each group's adherence to the exercise program. Total exercise time was defined as total time spent on exercise training during the study. Interim monitoring focused on patient intake, adherence to the protocol, baseline comparability of treatment groups, completeness of data retrieval, and adverse events. Data about participant adherence to the prescribed exercise training variables are expressed in the Interventions section.

### *Sample size*

The measurement of FMD, validated in several population studies, was selected as the critical variable to calculate the sample size [55,56]. A randomised clinical trial of the effect of aerobic training on FMD calculated a standardized effect size of 2.0 for improvement in endothelial function [43]. A priori power analysis estimated a total sample size of 10 participants in each group would detect a 0.5 standardized effect size for a between-group difference in improvements in FMD with a statistical power of at least 0.80 at an alpha level of 0.05.

### *Statistical Analysis*

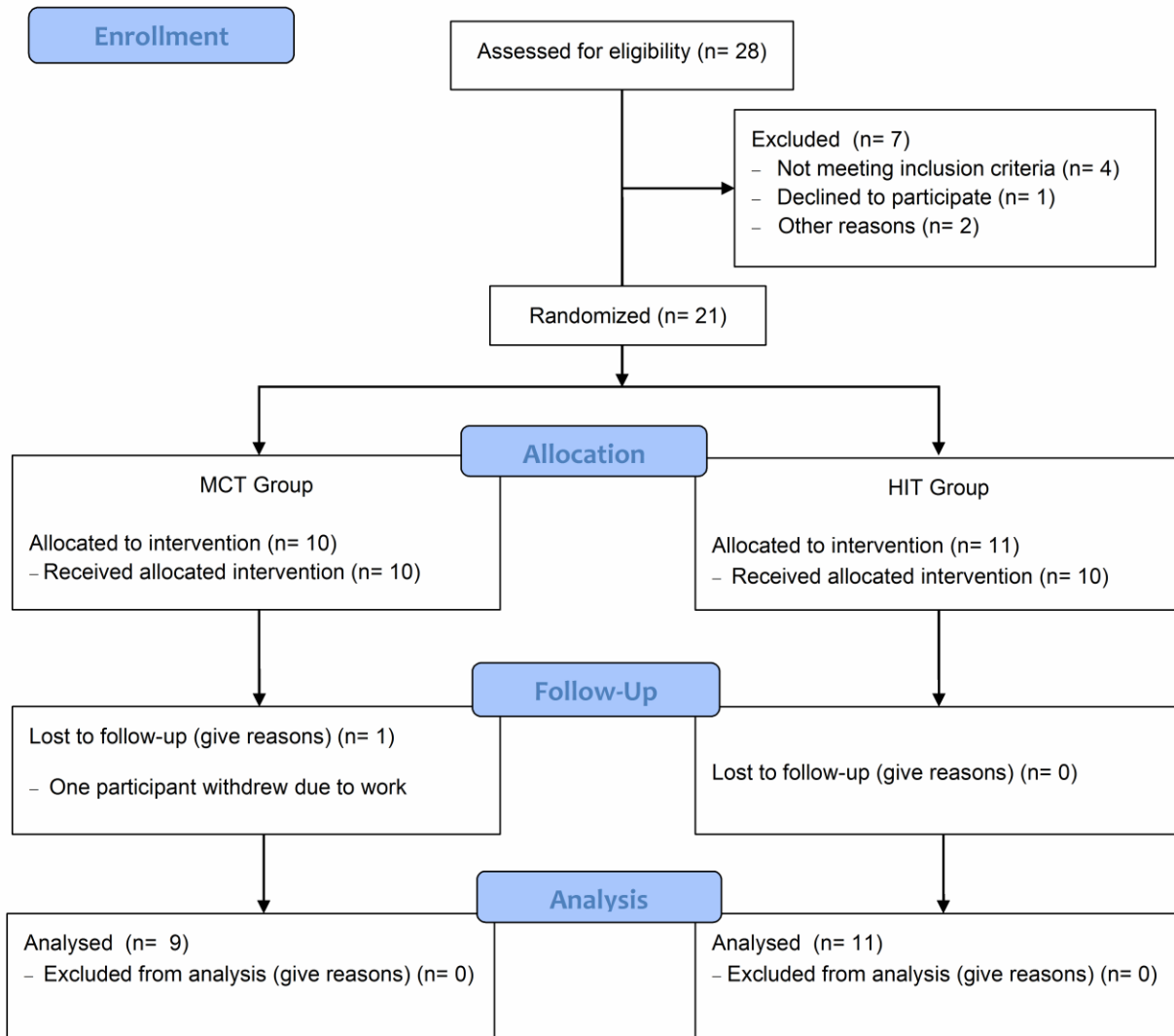
In order to retain data of all randomly allocated participants, an intention-to-treat analysis population (all randomly assigned patients) was performed. Prior to the planned statistical analyses, preliminary analysis was conducted (*Kolmogorov-Smirnov* test) to confirm data distribution normality. Adherence to the exercise program for both groups was expressed as the total number of training days that each participant completed out of the prescribed number of training days and total exercise time during the 12-weeks supervised exercise program. Once it was confirmed that the sample data satisfied the normality assumption, statistical analyses relevant for our main research interest were conducted. A *t*-test was used to investigate any possible differences in baseline characteristics and adherence between the groups. A general

linear model (GLM) was used to analyze between-group, within-group, and interaction effects. Change between the pre- and post-measures was calculated for each outcome variable of interest. An alpha level of 0.05 was used for all statistical analyses. Statistical analyses were conducted using PASW Statistics 17 for Windows (SPSS, Inc., Chicago, Illinois).

## **Results**

Figure 2 shows the flowchart of this randomised clinical trial. A total of 28 potential physical inactive subjects were assessed for eligibility, of which seven persons were excluded for not meeting inclusion criteria. Ten participants were randomly allocated to the MCT group and 11 participants were randomly allocated to the HIT group. After allocation, one participant in the MCT group withdrew from this investigation for reasons unrelated to this study (lack of time due to work schedule).





**Figure 2.** CONSORT guidelines flow diagram for enrolment and randomisation HIIT-Heart Study.

Baseline characteristics of the MCT group, HIT group and total sample are outlined in Table 1. The t-test indicated no statistically significant differences ( $p > 0.05$ ) in baseline characteristics between groups.

**Table 1.** Baseline participant characteristics

	Total sample (n = 20)	MCT (n = 9)	HIT (n = 11)
Sex, N (%)			
Male	8 (40.0)	5 (55.6)	3 (27.3)
Female	12 (60.0)	4 (44.4)	8 (72.7)
Age, mean (sd), y	31.8 (7.8)	31.4 (6.4)	32.1 (9.0)
Race/ethnicity, N (%)			
Black or Afro-Colombian	18 (90.0)	7 (77.7)	11 (100)
Others (Indigenous)	2 (10.0)	2 (22.3)	0 (0.0)
Socioeconomic level, N (%)			
Low-mid	11 (55.0)	5 (55.5)	6 (54.5)
Mid-high	9 (45.0)	4 (45.5)	5 (45.4)
Education, N (%)			
Secondary	1 (5.0)	0 (0.0)	1 (9.1)
Technical	1 (5.0)	1 (11.1)	0 (0.0)
University	18 (90.0)	8 (88.9)	10 (90.9)
Occupation, N (%)			
Student/work	20 (80.0)	7 (77.7)	9 (81.8)
Housewife	5 (20.0)	2 (22.3)	3 (18.2)
Marital status			
Single	4 (20.0)	3 (33.3)	1 (10.0)
Married/de facto	16 (80.0)	6 (66.3)	10 (90.0)
Weight, mean (sd), kg	68.2 (11.3)	69.3 (15.3)	66.8 (10.9)
Height, mean (sd), m	1.67 (0.06)	1.69 (0.05)	1.68 (0.09)
BMI, mean (sd), kg/m <sup>2</sup>	24.9 (3.7)	23.6 (3.6)	25.5 (4.2)

Table 2, lists the effects of the exercise interventions on endothelial function and metabolic biomarker outcomes. FMD changes were 2.2 (4.9) % in the MCT group and 7.7 (5.3) % in the HIT group (difference between groups  $-5.4$  [95% CI,  $-10.3$  to  $-0.6$  ( $P < 0.001$ )]. PWV slightly increased in the MCT group 0.1 (0.7) and decreased in HIT group  $-0.3$  (0.7) (not significantly different between groups,  $P = 0.91$ ). No significant intergroup differences were observed in the levels of blood pressure, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, glucose fasting, TC/HDL-c or TG/HDL-c ratio.

**Table 2.** Intent-to-Treat Analysis of Endothelial Function and Metabolic Biomarkers at baseline and changes after 12 weeks

	Groups				From Baseline to 12-week, Mean (95% CI)			MCT effect (p value)	HIT effect (p value)	Time x group (p value)
	Baseline		Follow-up		Whiting-Group Changed		Between-Group Difference in Change			
	MCT	HIT	MCT	HIT	MCT	HIT				
	(n = 10)	(n = 11)	(n = 9)	(n = 11)	(n = 9)	(n = 11)				
Endothelial function										
FMD, %	6.7 (5.7)	5.9 (5.0)	9.0 (2.7)	13.6 (4.7)	2.2 (4.9)	7.7 (5.3)	-5.4 (-10.3 to -0.6)	0.10	<0.001	<0.001
PWV, m·s <sup>-1</sup>	6.6 (0.7)	7.0 (1.1)	6.7 (0.9)	6.5 (1.5)	0.1 (0.7)	-0.3 (0.7)	-0.4 (-0.3 to 1.2)	0.40	0.05	0.91
Systolic blood pressure, mmHg	116.8 (5.1)	116.2 (6.5)	113.0 (7.6)	112.5 (9.1)	-3.8 (7.6)	-3.7 (6.5)	-0.2 (-6.8 to 6.5)	<0.001	0.004	0.67
Diastolic blood pressure, mmHg	72.3 (7.0)	71.0 (8.7)	67.8 (9.4)	67.0 (10.3)	-4.4 (8.5)	-4.0 (6.8)	-0.4 (-7.7 to 6.8)	<0.001	<0.001	0.71
Metabolic biomarkers										
Total cholesterol, mg/dL	170.1 (41.8)	159.4 (47.4)	153.1 (29.0)	146.6 (26.1)	-17 (37.4)	-12.7 (33.9)	- 4.3 (-38.8 to 30.2)	0.10	0.21	0.45
High-density lipoprotein, mg/dL	43.0 (14.1)	46.9 (9.6)	42.1 (9.5)	46.1 (14.1)	-0.8 (6.8)	-0.8 (10.4)	-0.1 (-8.6 to 8.5)	0.35	0.40	0.16
Low-density lipoprotein, mg/dL	100.3 (33.8)	92.2 (44.0)	88.9 (28.0)	81.6 (21.4)	-11.4 (36.2)	-10.5 (37.0)	-0.9 (-35.5 to 33.7)	0.17	0.18	0.93
Triglycerides, mg/dL	134.1 (82.2)	100.4 (36.8)	110.2 (40.1)	92.3 (45.0)	-23.8 (65.1)	-8.0 (30.9)	-15.8 (-70.5 to 38.9 )	0.12	0.20	0.50
TC/HDL-c ratio	4.3 (1.8)	3.5 (1.2)	3.8 (1.1)	3.3 (1.0)	-0.5 (1.2)	-0.1 (1.1)	-0.5 (-1.6 to 0.7)	0.11	0.38	0.46
TG/HDL-c ratio	3.7 (3.1)	2.2 (0.9)	2.8 (1.4)	2.2 (1.3)	-0.9 (2.2)	0.0 (1.0)	-1.0 (-2.5 to 0.6)	0.12	0.43	0.58
Glucose fasting, mg/dL	82.3 (13.7)	78.3 (5.6)	85.9 (6.4)	80.2 (7.6)	3.5 (14.7)	1.9 (5.8)	1.6 (-8.5 to 11.8)	0.24	0.15	0.63

Anthropometric, body composition and physical fitness analysis results are listed in Table 3. Percentage body fat did not change in the MCT group 0.0 (0.8) and decreased in HIT group -1.1 (difference between groups 1.2 [95% CI, 0.1 to 2.4 P = 0.04]). In addition,  $\dot{V}O_{2peak}$  had a similar increase in in both groups ( $\approx 12 \text{ ml} \cdot \text{kg} \cdot \text{min}^{-1}$ ), but this change was not significantly different between groups. Although within-group  $\dot{V}O_{2peak}$  and treadmill time increased in the combined group, these changes were not significantly between groups. Treadmill time increased in the MCT group vs. the HIT. There were no significant treatment effects in other parameters.

**Table 3.** Intent-to-Treat Analysis of Anthropometric, Body Composition and Physical Fitness at baseline and changes after 12 weeks

	Groups				From Baseline to 12-week, Mean (95% CI)			MCT effect (p value)	HIT effect (p value)	P
	Baseline		Follow-up		Whiting-Group Changed		Between-Group Difference in Change			
	MCT	HIT	MCT	HIT	MCT	HIT				
	(n = 9)	(n = 11)	(n = 9)	(n = 11)	(n = 9)	(n = 11)				
Anthropometric/Body composition										
Weight, kg	69.3 (15.3)	66.8 (10.9)	68.6 (13.5)	66.7 (10.5)	-0.6 (1.9)	-0.1 (1.6)	-0.5 (-2.2 to 1.2)	0.17	0.35	0.45
BMI, kg/m <sup>2</sup>	23.6 (3.6)	25.5 (4.2)	23.4 (3.0)	24.4 (4.2)	0.2 (0.7)	1.1 (3.2)	-0.9 (-1.4 to 3.3)	0.19	0.13	0.87
Waist circumference, cm	81.9 (12.2)	75.4 (7.6)	79.5 (10.6)	75.7 (8.3)	-1.7 (3.0)	0.3 (2.6)	-2.1 (-4.7 to 0.5)	0.07	0.35	0.67
Body fat, %	27.4 ( 7.2)	31.2 (12.1)	27.4 (6.5)	30.0 (11.5)	0.0 (0.8)	-1.1 (1.5)	1.2 (0.1 to 2.4)	0.50	0.01	0.04
Physical Fitness										
Handgrip, kg	31.0 (5.6)	31.6 (6.8)	34.6 (10.3)	37.2 (10.1)	3.6 (9.8)	5.6 (8.3)	2.0 (-6.5 to 2.5)	0.01	0.49	0.55
VO <sub>2</sub> peak, ml·kg·min <sup>-1</sup>	52.0 (17.1)	51.1 (11.0)	64.3 (16.6)	62.8 (14.4)	12.2 (18.0)	12.0 (7.8)	0.2 (-12.5 to 12.9)	0.03	<0.001	0.96
Test duration, min	20.0 (3.7)	22.4 (4.6)	22.7 (5.1)	24.6 (4.5)	2.7 (4.8)	2.8 (1.6)	0.1 (-3.4 to 3.2)	0.06	<0.001	0.24

No adverse events were reported over the course of this investigation. All data related to adherence and self-reported physical activity levels are presented in Table 4. The average exercise-training days and total exercise time during the programme were 35.5 days (standard deviation 1.3) and 1100.0 minutes (standard deviation 258.1) in HIT group; and 35.4 exercise

training days (standard deviation 0.9) and spent a mean total exercise time of 1031.3 minutes (standard deviation = 147.2) with no difference between groups. As expected self-reported physical activity increased as a result of training ( $F(1.65, 135.03) = 4.37$ ;  $p < 0.001$ ). Pairwise comparison analyses showed that the participants sustained these levels of vigorous or moderate physical activity at the 12-weeks follow-up. Walking increased over time in both groups (MCT group 945 min vs HIT group 514 min,  $p < 0.001$ ), but this increase was evident from levels of vigorous physical activity (MCT group 885 min vs HIT group 1168 min,  $p < 0.001$ ).

**Table 4.** Attendance to prescribed exercise sessions and self-reported physical activity

Variable	MCT (n = 9)	HIT (n = 11)	Group effect (P value)
Adherence (% of prescribed sessions completed), mean (sd)	98.7 (3.7)	98.4 (2.8)	0.96
Total number of sessions completed, mean (sd)	32.5 (1.3)	32.5 (0.9)	0.99
Total time spent training (min) per week, mean (sd)	1100 (258)	1031 (147)	0.04
<i>Recent Physical Activity Questionnaire</i>			
Walking MET-minutes/week, mean (sd)	945 (1890)	514 (1014)	<0.001
Moderate MET-minutes/week, mean (sd)	200 (276)	128 (260)	<0.001
Vigorous MET-minutes/week, mean (sd)	885 (712)	1168 (588)	<0.001

Sd, standard deviation

## Discussion

To our knowledge, this is the first randomised clinical trial of the effect of exercise training intensity on vascular function and physical fitness in physical inactive adults from Latin-American population. The present study demonstrates that HIT was a more potent stimulus for improving endothelial function (i.e., FMD) than MCT. The secondary findings showed modest but clinically significant reductions in percentage body fat can be achieved through HIT in the current population. Collectively, the magnitude of the change for both training groups were not different from each other in the rest of parameters, suggesting either training may provide similar benefits at medium term in cardiovascular health.

HIT and MCT on a treadmill have previously shown to be highly effective in patient population [57]. Also, exercise training has been shown to be a therapeutic strategy towards vascular function improvement in different clinical populations [58]. A recent meta-analysis shows that HIT is a more potent stimulus than MIT in enhancing FMD, with a mean difference of 2.26%. Specifically, this review suggests that 4 x 4 HIT, three times per week for at least 12 weeks, is a powerful form of exercise to enhance vascular function. Our study confirms these findings with a similar training programme protocol, showing a mean difference between groups of 5.4%. Another meta-analysis of prospective studies reported a 13% reduction in risk of cardiovascular events with a 1% increase in FMD; therefore, the magnitude of FMD improvement found following both types of exercise in our study (pre vs. post; HIT 7.7%; MCT 2.2%) are deemed to be clinically significant. Siasos et al. [59] suggest that both acute HIT and MCT can favorably affect endothelial function in young healthy adults, suggesting another cardioprotective effect of exercise on the progression of atherosclerosis. The effects of these acute exercises on the FMD could reflect a combination of haemodynamic changes and nitric oxide endothelium-dependent mechanisms [60]. Exercise induces increase in blood flow and the augmented blood flow causes vasodilation, which directly impacts the magnitude of FMD [61]. Difference between exercise programmes could be due to its ability to generate a greater blood flow through the vessels supplying oxygen to the working muscles, which could in turn promote greater shear stress-induced nitric oxide bioavailability [45,62] and may induce favourable endothelial adaptations [63]. In this context, several biologically plausible mechanisms could explain the effects of exercise in modulating the endothelial function and arterial stiffness. It is widely known that exercise has the potential to reduce oxidative stress by increasing the efficiency of the antioxidant system, finally improving endothelial dysfunction [64]. The main physiological mechanisms involved up-regulate

endothelial nitric oxide synthase activity in cell culture, animal or human studies, with subsequent reduction in the expression of nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) oxidase and stimulating radical scavenging systems that include copper/zinc-containing superoxide dismutase, extracellular superoxide dismutase, glutathione peroxidase and glutathione levels [63,65]. However, further research is needed to confirm these mechanisms, especially in childhood obesity during and after weight loss exercise regimens.

On the other hand, results for  $\dot{V}O_{2peak}$  did support our results, as both MCT and HIT groups had similar increases in  $\dot{V}O_{2peak}$  after 12-week of training. Although most previous studies [47-51] indicate that HIT is superior to MCT for improving  $\dot{V}O_{2peak}$ , there are several exceptions [45,47,49]. In addition, the improvement in  $\dot{V}O_{2peak}$  in both groups despite only the HIT group improving FMD suggests that the association between  $\dot{V}O_{2peak}$  and FMD reported in clinical trials studies is not causal [45,49].

Regarding arterial stiffness, aerobic exercise seems to improve arterial stiffness significantly and that effect seems enhanced with higher aerobic exercise intensity and in participants with greater arterial stiffness at baseline [66]. Pulse wave reflection characteristics, including blood pressure and PWV have been suggested to be more sensitive predictors of cardiovascular events than the traditional measure of cardiovascular function. In addition, an increase in the PWV is linked with increased cardiovascular incidences [67] related to increases in left ventricular afterload and wasted left ventricular energy [68]. To our knowledge this was the first study to investigate alterations in PWV after a HIT intervention. According to Siasos et al. [47] study, different intensity of aerobic exercise has different effects on the central and peripheral arterial stiffness. In our study, despite no difference between groups in PWV, there was a greater reduction trend in this parameter by the HIT intervention. It could be hypothesized that the lack of

effect in PWV may be due to the normal PWV at baseline for most of the subjects (85%) (those with  $PWV \geq 8$  m/s at baseline) [51]. Also, aerobic exercise training seems to have a greater effect in peripheral rather than in central indices of arterial stiffness [51], which could justify our findings.

Finally, the impact of HIT on body composition compared to MCT is controversial. Cycling protocols showed that HIT interventions are superior to MCT in inducing FM loss [69], or generate similar improvements [70]. Contrasting our results, studies using treadmill protocols have not shown any difference in body weight and composition between these isocaloric programmes [45,49,71]. Our results support that HIT interventions are superior in terms of enhancing fat oxidation than MCT [72]. Therefore, difference between fat reductions following HIT compared with MCT could suggest that obesity is a key contributing factor to vascular dysfunction; which has been corroborated in obese [73] and type 2 diabetic subjects [74]. Discrepancies between findings could be due to exercise mode or HIT duration intervals; Schjerve et al. [72] and Gibala et al. [21] suggest that the metabolic responses to HIT vary depending on the duration of the work: rest periods.

Strengths of this study included state of the art measures of vascular function, physical fitness, metabolic biomarkers and supervised exercise training in a non-clinical setting. In addition, adherence to the intervention was  $\approx 98\%$ . All subjects completed 32 of 36 exercise sessions, and research technicians supervised each session while HR was being monitored. A primary limitation in this study was the lack of a true no-exercise control group. Thus, we are unable to determine causality in our interpretation of the observed exercise-induced improvements in cardiovascular health parameters. Furthermore, in studies comparing HIT and MCT that have included a control group, no changes in FMD were observed in the control group [45,49,75]. Second, as a common



tool to assess body weight and the relevant parameters of body composition, BIA was used in the present study. However it is not the “gold standard” in body composition measurement. Finally, another limitation of this study is the lack of dietary control during the course of the intervention. To minimize the influence of diet, we continually reminded subjects of their commitment to maintain their current dietary habits. Future studies may consider tighter control of these factors such that the effects of these different factors could be isolated and identified in a relatively longer intervention.

## **Conclusion**

In summary, HIT is more effective at improving brachial artery FMD and reducing body fat than MCT, but seem to offer a similar metabolic and cardiovascular protection in most of the parameters. This research demonstrates the efficacy of high-intensity exercise to improve cardioprotective effect of exercise on the progression of atherosclerosis in the sedentary population.

## **Abbreviations**

MCT: Moderate intensity training

HIT: High Intensity interval training

HRmax: Heart rate max

FMD: Flow-mediated vasodilation

PWV: Aortic pulse wave velocity

CVD: Cardiovascular disease

PA: Physical activity

CEMA: Centre of studies in physical activity measurements (in Spanish)

AHA: American heart association

COLDEPORTES: in Spanish, Departamento Administrativo del Deporte, la Recreacion, la Actividad Fisica y el Aprovechamiento del Tiempo Libre.

ICC: Intra-class correlation coefficients

HDL-c: High-density lipoprotein cholesterol

LDL-c: Low-density lipoprotein cholesterol

BMI: Body mass index

WC: Waist circumference

RPAQ: Recent physical activity questionnaire

METs: Units of metabolic equivalence

## **Declarations**

## **Ethics and Consent to Participate statement**

The Review Committee for Research on Human Subjects at the University of Santo Tomás (ID 27-0500-2015) approved all study procedures. A comprehensive verbal description of the nature and purpose of the study and its experimental risks was given to the participants. Written informed consent was obtained from the subjects before they participated in the study. The protocol was in accordance with the latest revision of the Declaration of Helsinki and current Colombian laws governing clinical research on human subjects (Resolution 008430/1993 Ministry of health). HIIT-Heart was conducted in accordance with the principles of Good Clinical Practice following the tri-council guidelines.

## **Consent for publication**

I have obtained consent to publish and to report individual patient data from the participants.

**Competing interests**

The authors declare that they have no competing interests.

**Funding**

This study was part of the project entitled “Body Adiposity Index and Biomarkers of Endothelial and Cardiovascular Health in Adults: Effect of Physical Training”, which was funded by Centre for Studies on Measurement of Physical Activity, School of Medicine and Health Sciences, Universidad del Rosario (Code N° FIUR DN-BG001). The funder had no role in the study design, data collection, data analysis and interpretation, preparation of the manuscript, or decision to publish.

**Acknowledgements**

We thank our community collaborators and research participants for their efforts in coordination, implementation, and participation in the study. We are also grateful for the substantial contributions of our research team (Dr. Katherine Gonzalez-Ruiz, Mr. David Chaparro and Dr. Diana Diaz-Vidal).

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