Public University of Navarra,
Department of Health Sciences

Intensity Training and Cardiovascular Health in Colombian Adults: Results from HIIT-Heart Study and Cardiometabolic HIIT-RT Study

ClinicalTrials.gov NCT02738385 registered on March 23, 2016 and ClinicalTrials.gov identifier (NCT number): NCT02715063 First registered on March 22, 2016

DOCTORAL THESIS
Robinson Ramírez Vélez

October 2018

Supervisors
Mikel Izquierdo, Ph.D
Jorge Enrique Correa Bautista, Ph.D
Intensity Training and Cardiovascular Health in Colombian Adults: Results from HIIT-Heart Study and Cardiometabolic HIIT-RT Study

ClinicalTrials.gov NCT02738385 registered on March 23, 2016 and ClinicalTrials.gov identifier (NCT number): NCT02715063 First registered on March 22, 2016

Ph.D. Thesis
Robinson Ramírez Vélez

Department of Health Sciences
Public University of Navarre
Pamplona
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table of context</td>
<td>3</td>
</tr>
<tr>
<td>Table Index</td>
<td>4</td>
</tr>
<tr>
<td>Figure Index</td>
<td>5</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td>6</td>
</tr>
<tr>
<td>Summary/resumen</td>
<td>9</td>
</tr>
<tr>
<td>Declaration</td>
<td>16</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>17</td>
</tr>
<tr>
<td>Financial Support, List of Publications and Conference Papers</td>
<td>18</td>
</tr>
<tr>
<td>Overview</td>
<td>20</td>
</tr>
<tr>
<td>General Introduction</td>
<td>16</td>
</tr>
<tr>
<td>Chapter 1: Theoretical Background: A brief historical remark on endothelial function, arterial stiffness, autonomic function and cardiovascular health</td>
<td>24</td>
</tr>
<tr>
<td>Aims and layouts of the thesis</td>
<td>38</td>
</tr>
<tr>
<td>Chapter 2: Effectiveness of HIIT Compared to Moderate Continuous Training in Improving Vascular Parameters in Inactive Adults</td>
<td>40</td>
</tr>
<tr>
<td>Chapter 3: Similar Cardiometabolic Effects of High- and Moderate-Intensity Training Among Apparently Healthy Inactive Adults</td>
<td>60</td>
</tr>
<tr>
<td>Chapter 4: Effect of Moderate Versus High-Intensity Interval Exercise Training on Heart Rate Variability Parameters in Inactive Latin-American Adults</td>
<td>77</td>
</tr>
<tr>
<td>Chapter 5: Exercise and Postprandial Lipaemia: Effects on Vascular Health in Inactive Adults</td>
<td>101</td>
</tr>
<tr>
<td>Chapter 6: Effects of Exercise Training Type and Intensity or Nutritional Guidance on Metabolic Syndrome Risk Factors, Ideal Cardiovascular Health Parameters, Endothelial Function and Arterial Stiffness in Overweight Adults: Cardiometabolic HIIT-RT Study, A Randomized Controlled Trial</td>
<td>117</td>
</tr>
<tr>
<td>Chapter 7: General Discussion</td>
<td>142</td>
</tr>
<tr>
<td>Chapter 8: Conclusions, practical applications and future perspectives/Discusión, principales resultados, conclusiones, aplicaciones prácticas y perspectivas futuras</td>
<td>153</td>
</tr>
<tr>
<td>Chapter 9: Relevant Papers</td>
<td>158</td>
</tr>
</tbody>
</table>
Table Index

Chapter 2
Table 1. Intent-to-Treat Analysis of anthropometric and vascular function parameters at baseline and changes after 12 weeks 49
Table 2. Attendance to prescribed exercise sessions and self-reported physical activity 51

Chapter 3
Table 1. Baseline participant characteristics 67
Table 2. Intent-to-treat analysis of IDF criteria for MetS characteristics and body composition at baseline and changes after 12 weeks 69
Table 3. Partial correlation between MetS Z-score and anthropometric/body composition characteristics after 12 weeks of program training 70

Chapter 4
Table 1. Intent-to-Treat Analysis of indices of HRV and physiologic characteristics at baseline and changes after 12 weeks 88
Table 2. Partial correlation between physiologic characteristics and indices of heart rate variability after 12 weeks of exercise training 89

Chapter 5
Table 1. Intent-to-treat analysis of the effect of 12 weeks of HIT or MCR on postprandial lipemia biochemical and vascular function response after HFM ingestion. 108

Chapter 6
Supplement Table 1. Definition of the Ideal Cardiovascular Health Metrics (>20 Years of Age) as Defined by the American Heart Association and the Criteria Used in this Study 122
Table 2. Characteristics of the subjects 126
### Figure Index

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 1</td>
<td>Figure 1</td>
<td>Endothelial cells have both metabolic and synthetic functions</td>
</tr>
<tr>
<td>Chapter 1</td>
<td>Figure 2</td>
<td>Progression from risk factors to atherosclerosis and cardiovascular disease mediated by oxidative stress and endothelial dysfunction</td>
</tr>
<tr>
<td>Chapter 1</td>
<td>Figure 3</td>
<td>Mechanisms for the contribution of genetics, environment and behavior to atherosclerosis</td>
</tr>
<tr>
<td>Chapter 2</td>
<td>Figure 1</td>
<td>Schematic representation of a 4 × 4 HIT session or 30–40 min MCT session</td>
</tr>
<tr>
<td>Chapter 2</td>
<td>Figure 2</td>
<td>CONSORT guidelines flow diagram for enrolment and randomization HIIT-Heart Study</td>
</tr>
<tr>
<td>Chapter 2</td>
<td>Figure 3</td>
<td>Differences in the prevalence of non-responders in vascular parameters after 12 weeks training</td>
</tr>
<tr>
<td>Chapter 4</td>
<td>Figure 1</td>
<td>A and B, within-subject effect sizes (Cohen’s $d \pm 95%$ CI) following 12 weeks of program training by groups</td>
</tr>
<tr>
<td>Chapter 4</td>
<td>Figure 2</td>
<td>The relationship between the R-R interval length and the natural logarithm of the square of the mean sum of the squared differences between R-R intervals (rMSSD) after 12 weeks of program training by groups</td>
</tr>
<tr>
<td>Chapter 4</td>
<td>Figure 3</td>
<td>Individual patterns of response following 12 weeks of program training by groups.</td>
</tr>
<tr>
<td>Chapter 5</td>
<td>Figure 1</td>
<td>Schedule of experimental events for each subject</td>
</tr>
<tr>
<td>Chapter 5</td>
<td>Figure 2</td>
<td>Total cholesterol, TG and glucose responses to PPL (left) and incremental AUC (right) after 12 weeks of HIT and MCT</td>
</tr>
<tr>
<td>Chapter 5</td>
<td>Figure 3</td>
<td>FMD (%), FMDn (%) and PWV responses to PPL (left) and incremental AUC (right) after 12 weeks of HIT and MCT</td>
</tr>
<tr>
<td>Chapter 6</td>
<td>Figure 1</td>
<td>Consolidated Standards of Reporting Trials (CONSORT) flow diagram.</td>
</tr>
<tr>
<td>Chapter 6</td>
<td>Figure 2</td>
<td>Training response in body composition parameters between intervention groups</td>
</tr>
<tr>
<td>Chapter 6</td>
<td>Figure 3</td>
<td>Training response in metabolic parameters between intervention groups.</td>
</tr>
<tr>
<td>Chapter 6</td>
<td>Figure 4</td>
<td>Training response in vascular function parameters between intervention groups</td>
</tr>
<tr>
<td>Chapter 6</td>
<td>Figure 5</td>
<td>Training response in exercise parameters between intervention groups</td>
</tr>
<tr>
<td>Chapter 6</td>
<td>Figure 6</td>
<td>Changes after the 12-wk follow-up in ideal cardiovascular health metrics criteria according to AHA by intervention groups</td>
</tr>
<tr>
<td>Chapter 6</td>
<td>Figure 7</td>
<td>Changes after the 12-wk follow-up in categorical ideal cardiovascular health metrics criteria according to AHA by intervention groups</td>
</tr>
<tr>
<td>Chapter 8</td>
<td>Figure 1</td>
<td>Shear stress signals derived from endothelial cells by exercise training</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>ACH</td>
<td>Acetylcholine</td>
<td></td>
</tr>
<tr>
<td>ACSM</td>
<td>American College of Sports Medicine</td>
<td></td>
</tr>
<tr>
<td>AHA</td>
<td>American Heart Association</td>
<td></td>
</tr>
<tr>
<td>AngII</td>
<td>Angiotensin II</td>
<td></td>
</tr>
<tr>
<td>ANS</td>
<td>Autonomic nervous system</td>
<td></td>
</tr>
<tr>
<td>BF</td>
<td>Blood flow</td>
<td></td>
</tr>
<tr>
<td>BIA</td>
<td>Bioelectrical Impedance Analysis</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
<td></td>
</tr>
<tr>
<td>CEMA</td>
<td>in Spanish, Centro de Estudios para la Medición de la Actividad Física</td>
<td></td>
</tr>
<tr>
<td>CHF</td>
<td>Congestive heart failure</td>
<td></td>
</tr>
<tr>
<td>CONSORT</td>
<td>Consolidated standards of randomized clinical trials</td>
<td></td>
</tr>
<tr>
<td>CRF</td>
<td>Cardiorespiratory fitness</td>
<td></td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
<td></td>
</tr>
<tr>
<td>CVRF</td>
<td>Cardiovascular risk factors</td>
<td></td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
<td></td>
</tr>
<tr>
<td>ED</td>
<td>Endothelial dysfunction</td>
<td></td>
</tr>
<tr>
<td>EF</td>
<td>Endothelial function</td>
<td></td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide synthase</td>
<td></td>
</tr>
<tr>
<td>ES</td>
<td>effect sizes</td>
<td></td>
</tr>
<tr>
<td>ET-1</td>
<td>Endothelin-1</td>
<td></td>
</tr>
<tr>
<td>FMD</td>
<td>Flow-mediated dilation</td>
<td></td>
</tr>
<tr>
<td>GLM</td>
<td>Generalized linear model</td>
<td></td>
</tr>
<tr>
<td>HDL-c</td>
<td>High-density lipoprotein cholesterol</td>
<td></td>
</tr>
<tr>
<td>HF</td>
<td>High-frequency</td>
<td></td>
</tr>
<tr>
<td>HIIT-Heart Study</td>
<td>High Interval Intensity Training and ideal cardiovascular Heart Study</td>
<td></td>
</tr>
</tbody>
</table>
HIT: High Intensity interval training
HR: Heart rate
HRR: Heart rate reserve
HRV: Heart rate variability
ICAM-1: Intercellular cell adhesion molecule-1
ICC: Intra-class correlation
IDF: International Diabetes Federation
IKK-β: Inhibitor of nuclear factor kappa-B kinase subunit beta
IL-6: Interleukin-6
LDL-c: Low-density lipoprotein cholesterol
LF: Low-frequency
MCT: Moderate-intensity continuous training
MET: Metabolic equivalents
MetS: Metabolic syndrome
METs: Units of metabolic equivalence
MHC II: Major histocompatibility complex class II
MVPA: Moderate-to-vigorous physical activity
NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells
NO: Nitric oxide
PA: Physical activity
PAI-1: Plasminogen activator inhibitor-1
PNS: Parasympathetic nervous system
PWV: Aortic pulse wave velocity
rMSSD: Root mean square successive difference of RR intervals and frequency domain
RCT: Randomized clinical trial
ROS: reactive oxygen species
Rs: Responders
rs: Spearman correlation
SNS: Sympathetic nervous system
SDNN: Standard deviation of normal-to-normal intervals
TNFα: Tumor necrosis factor alpha
ULF: Ultra-low-frequency
VC: Vasoconstriction
VCAM-1: Vascular cell adhesion molecule-1
VD: Vasodilation
VLF: Very-low-frequency
VO2max: Maximal oxygen consumption
VR: Vascular reactivity
VSM: Vascular smooth muscle
VSMC: Vascular smooth muscle cell
WC: Waist circumference
WHO: World Health Organization
Summary
(Ingles-Español)
Summary

The current Ph.D. dissertation revolves around the relationship between exercise intensity and improvement cardiometabolic health. It has been suggested that high intensity interval training and also moderate or resistance training generate positive effects on metabolic risk factors. For these reasons, it is necessary to clarify which type of training, is more effective to improve cardiometabolic health in Latin American population. This doctoral thesis is based on 6 scientific studies that have been published or submitted for publication in scientific international journals. The first study (Chapter 2), we aimed to determine the effects of moderate- versus high-intensity interval exercise training on vascular function parameters in physically inactive adults. The second study (Chapter 3) to compare the effects of high-intensity interval training and steady-state moderate-intensity training on clinical components of metabolic syndrome (MetS) in healthy physically inactive adults. The third study (Chapter 4), we investigated the effect of moderate versus high-intensity interval exercise training on the HRV indices in physically inactive adults. Study five (Chapter 6) are Lab-based studies to evaluate the chronic impact of MCT or HIT on biomarkers of endothelial function, arterial stiffness and heart rate variability parameters postprandially after a high-fat meal. The last study of the current Ph.D. dissertation we aimed to investigate whether 12 weeks of high-intensity interval training (HIIT), resistance training (RT), concurrent training (CT=HIIT+RT) or nutritional guidance (NG) induced improvements in metabolic syndrome (MetS) risk factors, vascular function parameters and ideal cardiovascular health (CVH) in sedentary and overweight adults, and to compare the training adaptations between intervention groups. ClinicalTrials.gov NCT02738385 registered on March 23, 2016 and ClinicalTrials.gov identifier (NCT number): NCT02715063 First registered on March 22, 2016.

Study 1 (Chapter 2)

The this first study we aimed to determine the effects of moderate- versus high-intensity interval exercise training on vascular function parameters in physically inactive adults. Twenty adults were randomly allocated to receive either moderate-intensity continuous training (MCT group; 60-80% heart rate reserve [HRR]) or high-intensity interval training (HIT group; 4 × 4 min at 85–95% peak HRR interspersed with 4 min of active rest at 65% peak HRR). Vascular function (Normalization of brachial artery flow-mediated dilation, FMDn [%], aortic pulse wave velocity, PWV [m·s\(^{-1}\)], Alx, augmentation index) was measured at baseline and over 12 weeks of training. FMD changed by -1.0 (6.3) % in the MCT group and 1.9 (6.2) % in the HIT group (no significant difference between groups: 2.9 [95% CI, -3.0 to 8.8; \(\eta^2 = 0.15, p=0.131\)]. PWV changed by 0.1 in the MCT group but decreased by -0.4 in the HIT group (\(\eta^2 = 0.39, p < 0.01\) interaction), and there was not a significant difference in the prevalence of NR for PWV between the MCT and HIT groups (66% versus 33%, \(P = 0.051\)). Regarding FMDn (%), an analysis showed that the prevalence of NR was 66% (6 cases) in the MCT group and 18% (2 cases) in the HIT group (\(P = 0.013\)). Under the conditions of the present study, physically
inactive adults in both groups experienced changed in FMD. The rate of response was significant different between exercise groups for FMDn measure but not PWV outcome. The sustained change in PWV in the HIT group may represent a signal of vascular adaptation or endothelial fatigue.

**Study 2 (Chapter 3)**

The aim of the second study was to investigate the effects of high-intensity interval training and steady-state moderate-intensity training on clinical components of MetS in healthy physically inactive adults. We used the revised International Diabetes Federation criteria for MetS. A MetS Z-score was calculated for each individual and each component of the MetS. We found changes in MetS Z-score were 1.546 (1.575) in the MCT group and −1.249 (1.629) in the HIT group (between-groups difference, P= 0.001). The average number of cardiometabolic risk factors changed in the MCT group (−0.133, P=0.040) but not in the HIT group (0.018, P=0.294), with no difference between groups (P=0.277). These findings among apparently healthy physically inactive adults, shown that HIT and MCT offer similar cardiometabolic protection against single MetS risk factors but differ in their effect on average risk factors per subject.

**Study 3 (Chapter 4)**

In the third study we proposed the effect of moderate versus high-intensity interval exercise training on the HRV indices in physically inactive adults. Supine resting HRV indices (time domain: SDNN, standard deviation of normal-to-normal intervals; rMSSD, Root mean square successive difference of RR intervals and frequency domain: HFLn, high-frequency spectral power; LF, low-frequency spectral power and HF/LF ratio) were measured at baseline and 12 weeks thereafter. The SDNN changes were 3.4 (8.9) ms in the MCT group and 29.1 (7.6) ms in the HIT group (difference between groups 32.6 [95% CI, 24.9 to 40.4 (P = 0.01)]. The LF/HFLn ratio change 0.19 (0.03) ms in the MCT group and 0.13 (0.01) ms in the HIT group (P between groups = 0.016). No significant group differences were observed for the rMSSD, HF and LF parameters. In inactive adults, this study showed that a 12-week HIT training program could increase short-term HRV, mostly in vagally mediated indices such as SDNN and HF/LFLn ratio power.

**Study 4 (Chapter 5)**

In the fourth study we determined the effect of 12-weeks of high-intensity training (HIT) and moderate continuous training (MCT) on postprandial lipemia, vascular function and arterial stiffness after high-fat meal (HFM) ingestion in inactive adults. Participants completed two exercise protocols, namely, HIT and MCT, for 12 weeks. To induce a state of postprandial lipemia (PPL), all subjects received an HFM containing 1049 kcal, 31 g of protein, 79 g of fat (31 g of saturated fat), 666 mg of cholesterol and 69 g of carbohydrates. Endothelial function was measured using flow-mediated vasodilation (FMD), normalized brachial artery FMD (nFMD), aortic
pulse wave velocity (PWV) and augmentation index (Alx). Plasma total cholesterol, high-density lipoprotein cholesterol (HDL-c), triglycerides, and glucose were also measured. The effects of the HFM were measured in a fasted state and 60, 120, 180, and 240 minutes postprandially. The area under the curve from 0 to 240 minutes [AUC (0-240)] for glucose was lower after HIT than after MCT (10%, P=0.008). FMD and nFMD AUC (0-240) were increased in HIT compared with MCT (46.9%, P=0.021 and 67.3%, P=0.009, respectively). Regarding between-group differences, the results showed for glucose, and nFMD. In addition, the average delta of nFMD value was significantly higher in HIT than MCT (P = 0.03). In conclusion, supervised exercise-training mitigate endothelial dysfunction and glucose response induced by PPL. Exercise intensity plays an important role in these protective effects, suggesting that HIT might be more effective than MCT in reducing postprandial glucose levels and attenuating vascular impairments.

**Study 5 (Chapter 6)**

The last study of the current Ph.D. dissertation we aimed to investigate whether 12 weeks of high-intensity interval training (HIIT), resistance training (RT), concurrent training (CT=HIIT+RT) or nutritional guidance (NG) induced improvements in metabolic syndrome (MetS) risk factors, vascular function parameters and ideal cardiovascular health (CVH) in sedentary and overweight adults, and to compare the training adaptations between intervention groups. Our results shown that 12-week HIIT training program resulted in greater cardiorespiratory fitness than nutritional guidance alone, whereas an RT program improved arterial stiffness over CT. Both HIIT and RT programs increase ideal CVH metrics, supporting the positive effect of both exercise training programs on CVH in sedentary and overweight adults.
Resumen

La actual disertación doctoral gira en torno a la relación entre la intensidad del ejercicio y la mejora de la salud cardiometabólica. Se ha sugerido que el entrenamiento de alta intensidad por intervalos y que el entrenamiento moderado genera efectos positivos sobre varios de los factores de riesgo cardiometabólicos. Por estos motivos, es necesario aclarar qué tipo de entrenamiento es más efectivo para mejorar la salud cardiometabólica en población latinoamericana. Esta tesis doctoral se basa en cinco estudios científicos que han sido publicados o enviados para su publicación en revistas científicas internacionales. En el primer estudio (Capítulo 2), nuestro objetivo fue determinar los efectos del entrenamiento con ejercicios de intervalo de intensidad moderada versus alta intensidad sobre los parámetros de función vascular en adultos físicamente inactivos. El segundo estudio (Capítulo 3) compara los efectos del entrenamiento por intervalos de alta intensidad y entrenamiento de intensidad moderada en estado estable sobre los componentes clínicos del síndrome metabólico (MetS) en adultos saludables físicamente inactivos. En el tercer estudio (Capítulo 4), en el cuarto estudio determinamos de 12 semanas de programa de ECIM versus un programa de EIIA sobre la función vascular y la rigidez arterial después de ingerir una comida alta en grasas (CAG) en adultos inactivos. En el cuarto estudio (Capítulo 5), investigamos el efecto del entrenamiento con ejercicios por intervalos de intensidad moderada versus alta intensidad sobre los índices VFC en adultos físicamente inactivos. El estudio 5 (Capítulo 6) El último trabajo, demuestra que un programa de entrenamiento de 12 semanas de HIT incrementa en mayor medida la capacidad cardiopulmonar que un protocolo de orientación nutricional o combinado (HIT+fuerza), mientras que un programa de entrenamiento de fuerza muscular mejoró en mayor medida la rigidez arterial, apoyando la noción de que diferentes regímenes de entrenamiento podrían producir diferentes adaptaciones en diversos marcadores metabólicos en sujetos con exceso de peso y sedentarios. ClinicalTrials.gov NCT02738385 registrado marzo 23, 2016.

Estudio 1 (Capítulo 2)

En este primer estudio buscamos determinar los efectos del entrenamiento con ejercicio continuo de intensidad moderada (ECIM) versus ejercicio de intervalos de intensidad alta (EIIA) en adultos físicamente inactivos. Veinte adultos fueron asignados aleatoriamente para recibir entrenamiento continuo de intensidad moderada (grupo ECIM; 60 % - 80 % frecuencia cardíaca de reserva [FCR]) o entrenamiento por intervalos de alta intensidad (grupo EIIA; 4 × 4 min a 85 % – 95 % FCR máximo intercalado con 4 min de descanso activo a 65 % FCR máximo). Función vascular (Normalización de la dilatación mediada por flujo, DMFn [%], de la arteria braquial, velocidad de onda pulso aórtica, VOP [m·s¹], índice de aumento aórtico [AIx]) fueron medidos al inicio y luego de 12 semanas de entrenamiento. La DMF cambió en -1,0 (6,3) % en el grupo ECM y 1,9 (6,2) % en el grupo EAI (sin diferencia significativa entre grupos: 2,9 [95 % IC, −3,0 a 8,8; n² = 0,15; p = 0,131]. La VOP cambió en 0,1 en el grupo ECM, pero disminuyó en −0,4 en el grupo EAI (n² = 0,39; p <0,01 interacción), y no hubo diferencia significativa en la prevalencia de no responder “NR” para VOP entre los grupos ECM y EAI (66 % versus 33 %, p = 0,051). En cuanto a DMFn (%), un análisis demostró que la prevalencia de NR fue de 66 % (6 casos) en el grupo ECIM y 18 % (2 casos) en el grupo EIIA (P = 0,013).
La tasa de respuesta fue significativamente diferente entre los grupos de ejercicio para medición de DMFn pero no para el resultado de VOP. El cambio sostenido en la VOP en el grupo EAI puede representar una señal de la adaptación vascular o de fatiga endotelial.

**Estudio 2 (Capítulo 3)**

El propósito del segundo estudio fue de investigar los efectos del EIIA versus un programa de EIIA sobre los componentes del MetS en adultos saludables físicamente inactivos. Utilizamos los criterios de la Federación Internacional de la Diabetes para el diagnóstico de MetS. Una puntuación Z del MetS se calculó para cada individuo y cada componente del MetS. Hallamos cambios en la puntuación Z del MetS de 1,546 (1,575) en el grupo ECIM y −1,249 (1,629) en el grupo EIIA (diferencia entre grupos, p = 0,001). El número promedio de factores de riesgo cardiometabólico cambió en el grupo ECIM (−0,133, p = 0,040), pero no en el grupo EIIA (0,018, p = 0,294), sin diferencia entre grupos (p = 0,277). Estos hallazgos entre adultos aparentemente saludables y físicamente inactivos demuestran que EIIA y ECIM ofrecen similar protección cardiometabólica contra factores de riesgo únicos del MetS, pero difieren en su efecto sobre los factores promedio por sujeto.

**Estudio 3 (Capítulo 4)**

En el tercer estudio, planteamos el efecto de un programa de ECIM versus un programa de EIIA sobre algunos parámetros de la variabilidad de la frecuencia cardiaca (VFC) en adultos físicamente inactivos. En posición supina, estimamos los Índices (dominio del tiempo: SDNN: desviación estandar de los intervalos RR; rMSSD, cuadrado de la raíz media de la unión de los intervalos R-R adyacentes y dominio de frecuencia: HFLn, Log potencia espectral de alta frecuencia; LF, potencia espectral de baja frecuencia y la razón HF/LF) fueron medidos al inicio y luego de 12 semanas de entrenamiento. Cambios en el SDNN fueron 3,4 (8,9) ms en el grupo ECIM y 29,1 (7,6) ms en el grupo EIIA (diferencia entre grupos de 32,6 [95 % IC; 24,9 a 40,4 (p = 0,01)]. El cambio en la razón LF/HFLn fue 0,19 (0,03) ms en el grupo semanas y 0,13 (0,01) ms en el grupo EIIA (p entre grupos = 0,016). No se observaron diferencias entre grupos para los parámetros rMSSD, HF y LF. En adultos inactivos, este estudio demostró que un programa de entrenamiento de EIIA de 12 semanas podría incrementar la VFC a corto plazo, principalmente en índices mediados vagalmente, como SDNN y la razón HF/LFLn.

**Estudio 4 (Capítulo 5)**

En el cuarto estudio determinamos de 12 semanas de programa de ECIM versus un programa de EIIA sobre la función vascular y la rigidez arterial después de ingerir una comida alta en grasas (CAG) en adultos inactivos. Para inducir un estado de lipemia postprandial (LPP), todos los sujetos recibieron una CAG que contenía 1049 kcal, 31 g de proteína, 79 g de grasa (31 g de grasa saturada), 666 mg de colesterol y 69 g de carbohidratos. La función endotelial se midió mediante el uso de la vasodilatación mediada por flujo (DMF), DMF de arteria braquial...
normalizada (nDMF), la velocidad de onda de pulso aórtica (VOP) e índice de aumento aórtico [Alx], además del colesterol total, el colesterol de lipoproteínas de alta densidad (HDL-c), triglicéridos y glucosa. Los efectos de la CAG se midieron en un estado de ayuno y 60, 120, 180 y 240 min pos El área bajo la curva de 0 a 240 min [AUC (0-240)] para glucosa fue más baja luego del EAI que después del ECM (10 %, p = 0,008). La DMF y la nDMF AUC (0-240) se incrementaron en el EAI en comparación con el ECM (46,9 %, p = 0,021 y 67,3 %, p = 0,009, respectivamente). En cuanto a las diferencias entre grupos, los resultados se mostraron para glucosa, y nDMF. Además, el delta promedio del valor de nDMF fue significativamente mayor en el EAI que en ECM (p = 0,03). En conclusión, el entrenamiento con ejercicio supervisado mitiga la disfunción endotelial y la respuesta a la glucosa inducida por LPP. La intensidad del ejercicio juega un papel importante en estos efectos protectores, sugiriendo que el EAI podría ser más efectivo que el ECM en la reducción de los niveles de glucosa postprandial y atenuación del deterioro vascular.

Estudio 5 (Capítulo 6)

El último trabajo, demuestra que un programa de entrenamiento de 12 semanas de HIT incrementa en mayor medida la capacidad cardiorrespiratoria que un protocolo de orientación nutricional o combinado (HIT+fuerza), mientras que un programa de entrenamiento de fuerza muscular mejoró en mayor medida la rigidez arterial, apoyando la noción de que diferentes regímenes de entrenamiento podrían producir diferentes adaptaciones en diversos marcadores metabólicos en sujetos con exceso de peso y sedentarios. Nuestros resultados muestran evidencias de que el entrenamiento HIT o fuerza muscular y sin cambios en la alimentación, disminuye la adiposidad corporal/abdominal y mejoran la rigidez vascular, al mismo tiempo que incrementa el fitness cardiorrespiratorio (VO2max) en adultos colombianos sedentarios con exceso de peso corporal. Se ha reportado que el VO2max es un predictor independiente de todas las causas de mortalidad y el riesgo de muerte se reduce un 13% por cada 3.5 ml/kg*min de VO2 o METs que se incrementa. De acuerdo a esto, consideramos que estos participantes redujeron su riesgo de muerte por ECV pues encontramos una mejoría de 2 METs
Declaration

I, Robinson Ramírez Vélez, do hereby declare that the research presented in this dissertation is based on 7 articles (chapters 2 to 7) that have been published or submitted for publication in international peer-reviewed journals. To meet the stylistic requirements of a thesis, the formats of the papers have been adjusted accordingly throughout. These edits did not substantially change the content of the published articles. The role which I fulfilled within each of the publications is presented below.

This thesis describes the rationale, design, methodologies and results used in a parallel and factorial randomised controlled trial (HIIT-Heart Study). We hypothesised that combined training (resistance training + high-intensity interval exercise training) would result in greater improvements in vascular function, arterial stiffness and cardiometabolic parameters compared to moderate or high-intensity interval exercise training protocol and the usual clinical care. ClinicalTrials.gov NCT02738385 registered on March 23, 2016 and ClinicalTrials.gov identifier (NCT number): NCT02715063 First registered on March 22, 2016.

PhD student involvement:

- Elaboration in the study design.
- Data analysis and results interpretation.
- Writing of the papers included in the present thesis.
Acknowledgments

Gratitude is expressed to the Center of Studies for Physical Activity Measurement (CEMA) for the technical and scientific support to conduct this thesis project.

Gratitude is also expressed to the researchers, students, professors, and colleagues from the Master’s program on Physical Activity and Health and the Specialization program on Physical Exercise for Health at the School of Medicine and Health Sciences from Colegio Mayor Nuestra Señora del Rosario for their unconditional support.

In addition, we thank the participants in the “HIIT-Heart Study” project, who by running and pedaling have contributed to improve my: Capacity to understand the relationship between the mechanical and the biological; Strength to challenge the difficulties appertaining the investigation; Flexibility to understand other people, other ways of life, other ways of working and thinking; Speed, above all mental, to meet administrative and financial challenges; Coordination, of my life, to keep my friends, colleagues, and family; and Balance to harmonize professional and personal aspects.
Financial Support, List of Publications and Conference Papers

Financial Support

This study was part of the project entitled “Body Adiposity Index and Biomarkers of Endothelial and Cardiovascular Health in Adults” and “High Interval Intensity Training and ideal cardiovascular Heart Study (HIIT-Heart Study, ClinicalTrials.gov NCT02738385)”, which was funded by Centre for Studies on Measurement of Physical Activity, School of Medicine and Health Sciences, Universidad del Rosario (Code No 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.

Scholarships

Educational Travel Grant for the Formation in Research Personnel. University of Rosario January to February 2017 and September 2108 to December 2018

List of Publications


Conference Papers

Poster presentation by Ramírez-Vélez R.

30 May to 5 Jun /2017. ACSM's 64st Annual Meeting World Congress in Exercise, Denver, CO, Estados Unidos.


Overview

Lack of engagement in sufficient weekly physical activity (PA) is a major risk factor for morbidity and premature mortality [1]. Estimates from 2012 indicated that not meeting PA public health recommendations is responsible for more than 5 million deaths globally each year [2]. Furthermore, insufficient PA was estimated to account for more than 11% of the US aggregated health care expenditures in 2014, which translates to approximately $120 billion per year or an excess $1400 in per capita costs [3]. Although low- and middle-income countries bear 75% of the disease burden associated with physical inactivity, more than 80% of health care costs and 60% of indirect costs occur in high-income countries [4].

Despite numerous trials and programs, PA levels in the United States have remained flat for decades, [5] with most adults not meeting aerobic (48%) and muscle-strengthening (70%) guidelines [6]. In fact, when assessed objectively, noncompliance with PA guidelines constitutes the most prevalent of the “hard” risk factors for major chronic diseases [7]. Evidence shows that PA has a significant role, in many cases comparable or superior to drug interventions, [8] in the prevention and treatment of more than 40 noncommunicable chronic diseases, such as obesity, heart disease, diabetes, hypertension, cancer, depression, Alzheimer disease, arthritis, and osteoporosis. However, despite the substantial health and economic burden associated with physical inactivity and the fact that ample evidence supports the effectiveness of PA promotion by the health care sector [9,10] PA promotion is not yet a standard of care for disease prevention and management in health care settings [11].

Precision/personalized medicine is an emerging approach for prevention as well as more effective diagnosis and disease treatment that takes into account variability in genes, environment, and lifestyle for each individual. Precision medicine will enable health care providers to more accurately define the optimal treatment and prevention strategies for a particular disease phenotype and if widely implemented may lead to large clinical and public health improvements both in the United States and globally. Despite an initial focus on “genes, drugs, and disease” aspects related to social, environmental, and behavioral disease determinants are increasingly being emphasized [12]. This viewpoint emphasizes the importance of integrating personalized PA and exercise prescriptions as part of precision lifestyle and behavioral medicine.

Precision PA and exercise prescriptions can help address the substantial variability in individual patient response to health-related fitness outcomes and tailoring of exercise programs to the individual phenotype of each patient [13]. In this context, there are 2 main components: a short-term focus on improving chronic disease related declines in functional capacity and a longer-term aim to generate knowledge applicable to the whole range of maintaining optimal health and preventing diseases.
Physical inactivity is a key factor contributing to the onset of muscle mass and function decline (i.e., sarcopenia), which in turn appears to be a vital aspect related to frailty [14]. Poor health, disability, and dependency do not need to be the inevitable consequences of aging. PA, as an intervention, is one of the most important components in improving the functional capacity of frail seniors [15]. Accordingly, an important conceptual idea for frailty is that the focus should be on functionality and not on the diagnosis of disease for older patients. Furthermore, provision of PA interventions is relatively free of potential unwanted side effects caused by common medications that are prescribed in patients with multiple comorbidities [15]. In this line, PA is a very promising intervention for the modulation of both health span and life span in a number of species [16].

Substantial evidence already exists in support of multipronged PA and exercise counseling, prescription, and referral strategies. [9]. Despite the overwhelming scientific evidence that PA improves the health of the population, society in general and health professionals in particular are not yet clear on how to approach this matter [17]. Although an initial good step, PA and exercise prescriptions as a form of medicine are much more than just walking. Unfortunately, exercise programs are typically developed for the average person controlling relative intensity efforts (i.e., “one-size-fits-all” approach), with less consideration for the differences between individuals. This would necessarily involve an individualized prescription according to the functional capacity of the person, with specific recommendations about the dose (intensity, volume, and frequency), similar to those of other medications.

Indeed, researchers have recognized the substantial variability in patient response to physical exercise interventions and have sought to understand these differences [18,19]. For example, in cancer disease there is increasing interest in health-related fitness outcomes by exercise researchers that makes the application of precision medicine (i.e., the focus on genetic and molecular subgroups) much more relevant [20]. Nevertheless, there are some differences between exercise and medical interventions that may have implications for the application of precision medicine to exercise oncology. On the other hand, several reports pertain to “average data,” and there is wide interindividual variability in response to exercise training (IVRET), which has mainly been explored in endurance-based studies.

The IVRET implies that under the same stimulus, some individuals may achieve benefits, who are considered responders, whereas others may exhibit a worsened or unchanged response after training, termed non-responders. In the era of precision medicine, IVRET in the magnitude of response to supervised exercise training (subject-by-training interaction; “individual response”) has received increasing scientific interest [18].

This is an exciting time to help patients combat the increasingly recognized impact of aging as well as noncommunicable diseases on the broad-based benefits of PA while targeting exercise prescriptions and programs using precision behavioral and lifestyle medicine approaches.
Thus, this thesis describes the rationale, design, methodologies and results used in a parallel and factorial randomised controlled trial (HIIT-Heart Study). We hypothesised that combined training (resistance training + high-intensity interval exercise training) would result in greater improvements in vascular function, arterial stiffness and cardiometabolic parameters compared to moderate or high-intensity interval exercise training protocol and the usual clinical care. ClinicalTrials.gov NCT02738385 registered on March 23, 2016 and ClinicalTrials.gov identifier (NCT number): NCT02715063 First registered on March 22, 2016.

References


1. Endothelial function: Basic, clinical, and epidemiological aspects

Historically, the endothelium was considered a passive barrier between the intravascular and extravascular compartments. A single layer of endothelial cells lines the entire vascular system. In adults, approximately ten trillion ($10^{13}$) cells form an almost 1 kg “organ” [1]. During the 1980s, evidence pointed to the endothelium having an active role modulating vasodilation (VD)/vasoconstriction (VC) vascular capacity and reacting against different stimuli through synthesis and release of substances that modify the vascular smooth muscle (VSM) tone [2]. It is currently recognized that besides modulating the VSM tone, the endothelium performs other multiple functions, highlighting its anti-atherogenic and anti-thrombogenic roles independently from lipids profile levels [3]. Under normal conditions, the endothelium maintains a vasodilator, antithrombotic and anti-inflammatory state (for example, it inhibits platelet aggregation and adhesion, leukocyte adhesion, VSM proliferation, as well as responds to circulating substances, maintains reduced vascular permeability, and modulates the inflammatory process). Endothelial cells are dynamic and have both metabolic and synthetic functions, Figure 1.

Figure 1. Endothelial cells have both metabolic and synthetic functions.
Note: Through the secretion of a large variety of mediators they are able to influence cellular function throughout the body. LDL: low-density lipoprotein. Modified from Ref [1].
During endothelial dysfunction (ED), the synthesis/bioactivity of vasodilators is reduced and the balance tips in favor of the endothelium-derived vasoconstrictors. The term ED would describe any alteration of endothelial functions described [4]. However, in non-invasive vascular studies, the term ED refers to the reduced endothelial capacity to generate arterial VD or VC and/or microcirculatory (arterioles) against stimuli that would normally generate endothelium-dependent vasomotoricity (for example, increased blood flow [BF]) [5]. In spite of the definition established, upon a reduced endothelial function (EF), it must be interpreted that all endothelial actions can be altered [6]. Figure 2.

Figure 2. Progression from risk factors to atherosclerosis and cardiovascular disease mediated by oxidative stress and endothelial dysfunction.
Note: The early detection of endothelial dysfunction is a critical point in the prevention of atherosclerosis and cardiovascular disease because this dysfunction could be an initial reversible step in the process of atherosclerosis. Modified from Ref [4].

The ED can be explained by at least three basic mechanisms that act in an isolated or joint manner:

1) Reduced capacity to sense stimuli (for example, change in the shear stress applied to the endothelium) and inducers of increased or reduced production and release of vasoactive factors;
2) Reduced synthesis, release, and/or bioavailability of vasoactive substances (for example, reduced endothelial nitric oxide synthase (eNOS) activity, or increased nitric oxide (NO) degradation through reactive oxygen species (ROS);
3) Antagonism of the endothelium’s VD substances by VC substances. It must be indicated that the dysfunction of one of the multiple biochemical pathways does not necessarily cause DE, given that other pathways can increase their activity in a compensatory manner [7].

In addition, without constituting ED, altered vascular reactivity (VR) may exist in case of the VSM’s incapacity to modify its tone upon endothelial stimulation. In addition, decreased production of NO in endothelium mediated by insulin resistance and low fitness level or physical inactivity also contributes to accelerated atherosclerosis by multiple mechanisms, Figure 3.

**Figure 3.** Mechanisms for the contribution of genetics, environment and behavior to atherosclerosis.

Note: VSMC: Vascular smooth muscle cell; CHF: Congestive heart failure; PAI-1: Plasminogen activator inhibitor-1; ET-1: Endothelin-1; AngII: Angiotensin II; IKK-β: Inhibitor of nuclear factor kappa-B kinase subunit beta; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; NO: Oxide nitric; IL-6: Interleukin-6; TNFα: Tumor necrosis factor alpha; VCAM-1: Vascular cell adhesion molecule-1; ICAM-1: Intercellular cell adhesion molecule-1.

Considering the aforementioned, studies evaluating EF and/or VR seek to discriminate endothelial and/or VSM alterations [8]. For such studies, these compare the response to endothelium-dependent and -independent stimuli (for example, VD) [9]. Besides, for an adequate interpretation of the response, it is important to understand that an artery might not dilate, because it may already be dilated to the maximum (structural limit); a fact that should not be considered ED [10].
Finally, it is worth indicating that most studies have investigated the NO pathway, which is why commonly (although formally incorrect) ED is associated with a state of reduced NO production, release, and/or bioavailability.

2. Clinical importance of evaluating endothelial function

The EF and/or VR alteration is recognized as a factor associated with and/or contributing to [11,12]:

1) Increased cardiovascular risk factors (CVRF), independent of other factors;
2) Predicting cardiovascular events in asymptomatic individuals, even with normal coronary arteries, independent of other CVRF;
3) Determining and reclassifying CVRF in asymptomatic patients;
4) Predicting cardiovascular events, cardiovascular disease death, and/or revascularization in patients with cardiovascular disease (for example, coronary disease, congestive heart failure);
5) Predicting intra-stent restenosis and vasculopathy of the arterial substitute (implant);
6) Predicting atheromatous plaque vulnerability;
7) Predicting mortality in patients with orthotrophic heart transplant;
8) Defining the probability of response to cardiac resynchronization;
9) Determining vascular function and CVRF damage associated with systemic disease (for example, autoimmune disease, kidney failure).

In general, the clinical value/importance is independent of the EF and/or VR evaluation method used and of the arterial wall studied [13].

3. Under what conditions could EF and/or VR be altered?

The EF and/or VR is altered by exposure to CVRF factors and/or upon the presence of diverse systemic pathologies. In this regard, CVRF factors (for example, smoking, arterial hypertension, hyperlipidemia, obesity, hyperglycemia, resistance to insulin) are associated with endothelial activation (necessary to induce the atherogenic process) and to reduced ED and/or VR [14]. Systemic diseases/alterations (for example, atherosclerosis, kidney failure, diabetes, erectile dysfunction, peripheral arterial disease, sleep apnea, low birth weight) are associated (as cause or effect) with reduced ED and/or VR. In many of them, changes in EF and/or VR give way to a detrimental vicious circle that favors their progression (for example, congestive heart failure) and/or the secondary repercussion on the cardiovascular system [15].

4. Methodological issues in the assessment of EF

Although we will center our description on non-invasive techniques, it must be kept in mind that invasive approaches exist (less used) to evaluate EF and/or VR, which are clinically useful, considered the “gold standard,” and are used to validate non-invasive methods [16]. These methods can be divided into:
1) Direct study of the response of coronary arteries or of the upper limb to intra-arterial vasoactive stimuli (for example, coronary distension in response to intra-arterial acetylcholine (ACH), assessed through quantitative angiography);

2) Studies of EF indicator biochemical/cellular markers (for example, plasma concentration of oxidized low-density lipoprotein (ox-LDL), circulating endothelial progenitor cells), and others [17].

Non-invasive techniques focus on the study of arteries and/or microcirculation of upper limbs, “window” territories to assess the cardiovascular system (considering that the DE is a systemic process). Additionally, a relationship exists between coronary or cerebrovascular disease and DE presence in limbs; the DE of brachial and/or radial arteries predicts coronary heart disease and cardiovascular events [18]. Furthermore, accessibility and the response level of the cutaneous microcirculation calls for the use of a model to study the microcirculation [19]. Cutaneous microvascular dysfunction is associated with CVD (for example, hypertension) [20], recognized as an independent marker of CVD in specific groups (for example, type 2 diabetes or obesity) [21].

4.1. Non-invasive assessment of vascular function in humans

Approaches exist to assess EF and/or VR: (a) invasive vs. non-invasive, (b) arterial vs. venous evaluation, (c) of territories or the arterial and/or cardiovascular system vs. of specific microcirculatory territories. The different approaches and techniques can evaluate different aspects of EF and/or VR, with the results not being extrapolatable or equivalent (i.e., possibly not correlating with each other) [22]. In this thesis, we consider:

4.1.1. Flow-mediated dilatation (FMD)

It is possible to measure endothelial function by measuring vasodilatation after intra-arterial pharmacologic stimulation with substances that enhance the release of endothelial nitric oxide (such as ACH and bradykinin). The major disadvantage of these methods is their invasive nature, which makes them generally unsuitable for use in asymptomatic young subjects [23]. Flow-mediated dilation (FMD) changes in conduit artery diameter are caused by shear-stress induced generation of endothelial derived vasoactive mediators, technique introduced by Celermajer et al. (1992) for the non-invasive evaluation of the EF in the arteries of upper and lower limbs [24].

Its strengths include non-invasiveness, high sensitivity, and reproducibility. Its limitations involve requiring considerable operator experience and patient collaboration; it is operator-dependent, uses relatively costly equipment/software, and lengthy analysis of the registries [25].
5. Arterial stiffness

The wording “arterial stiffness” is a general term that refers to the loss of arterial compliance and/or changes in vessel wall properties. The predictive value of arterial stiffness for cardiovascular events has been well demonstrated [26]. The largest amount of evidence has been given for aortic stiffness, measured through carotid-femoral pulse wave velocity. Currently, as many as 19 studies consistently showed the predictive value of aortic stiffness for fatal and non-fatal cardiovascular events in various populations having different levels of CVRF: general population, hypertensive patients, elderly subjects, type 2 diabetic patients and patients with end-stage renal disease [27-31].

5.1. Non-invasive assessment of arterial stiffness in humans

5.1.1. Arterial pulse wave velocity (PWV)

Arterial stiffness can be evaluated at different levels: systemic, regional and local. Schematically, the determinants of the pulse wave velocity (PWV), a non-invasive indicator and “gold standard” of regional arterial rigidity [29], are (Moens–Korteweg equation):

\[ PWV = \sqrt{\frac{e.h}{2.r.p}} \]

“e” and “h” being the circumferential elastic modulus (~rigidity) and parietal thickness, “r” the arterial radius, and “p” the fluid density (~1.05 g/cm\(^3\)).

The Arteriograph® system (TensioMed Kft., Budapest, Hungary) estimates PWV from a single-site brachial-cuff oscillometric determination of the suprasystolic waveform at the brachial artery site [30]. Because the cuff is pressurized at least 35 mmHg over the actual systolic blood pressure, hemodynamic measurements are performed under “stop-flow / occluded artery”. Although PWV measured with the Arteriograph® has been validated against gold standards, there is still a controversy in the literature concerning the arterial pathway followed by the pressure wave. However, a recent study with MRI showed that the arterial pathway conditions covered by the Arteriograph® overlapped most of aortic root-bifurcation length, omitting only a few centimetres of proximal ascending aorta [31].

6. Heart rate variability

Numerous cardiovascular risk factors, including age, gender, obesity and fat distribution, lifestyle (including physical activity and nutrition), metabolic parameters, and components of MetS, may be capable of influencing autonomic nervous system (ANS) function. For this reason, psychophysiological research integrating heart rate variability (HRV) has increased during the last two decades, particularly given the fact that HRV is able to index cardiac vagal tone [32-34]. HRV is a measurement that represents the time variation between heartbeats. For example, a 60-beats per minute heart rate (HR) could mean 1 beat per second or it could mean an average of 1 beat every 0.5s, 1.5s, 0.5s, 1.5s, etc. Over the years, it has been used as a non-invasive method to analyze the regulation of the ANS of the heart [32]. The ANS
balance, that is the action of the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS), regulates our HR and BP, adapting to situations caused by internal and external agents [33].

Hence, a high capacity of variations has been described to adapt and respond satisfactorily to these stimuli that reflect a healthy neuro-cardiac interaction [34]. However, some chronic health conditions can cause or be caused by autonomic dysfunction that can reduce the body’s regulation capacity to respond optimally to different situations, like compensatory responses to BP variation, situations of stress, and physical exercise [35]. Low HRV, indicative of sympathetic predominance and parasympathetic reduction, has prognostic value for all-cause mortality and sudden cardiac death.

Thus, a reduced capacity to adapt to the internal and external stimuli could reflect a physiological malfunction of the ANS, leading to reduced HRV, a marker of health problems strongly associated with a high risk of mortality due to all causes [36]. Nevertheless, chronic health conditions are not solely responsible for reducing HRV; some physiological processes, like aging and the routine day-to-day sensations of modern man (mental fatigue and stress), can also acutely and chronically reduce HRV [37]. The mechanism by which reduced HRV is related to mortality is probably related to increased sympathetic activity and decreased parasympathetic activity, which reduces the threshold for ventricular fibrillation [32-35].

The simplified model of HR regulation shows that the sinus node is the principal physiological pacemaker and is innervated by efferences of both the sympathetic and parasympathetic branches and influenced, in turn, by different humoral factors. The sinus node acts after integrating all these elements and the relation between the sympathetic and parasympathetic stimuli is reflected in the variations of the intervals between heartbeats [38].

Different treatments exist to analyze the temporal variability of the intervals between heartbeats. Most of the studies are based on the treatment of the cardiac signal from linear methodologies, like the procedures based on the time domain (statistic and geometric methods) and the methodologies of the frequency domain (Fourier transforms and their variants). With the time-domain measurements, we learn the value of each heartbeat in a given moment of time as the duration of the R-R interval between consecutive heartbeats. In a continuous electrocardiogram (ECG), it is possible to detect each QRS complex and determine the normal-to-normal (N-N) intervals or between the R (R-R) curves and the instantaneous HR [39]. Total HRV is modulated by sympathetic, parasympathetic, and endocrine influences but mostly expresses circadian rhythm, which is not considered a vagal marker per se. On the other hand, short-term measures obtained under laboratory conditions, where subjects are usually in supine positions, do not capture circadian or sleep-related variations, but reflect mainly resting parasympathetic (vagal) variation in HR. Standardized conditions are crucial to short-term HRV measures.
However, the time-domain data do not provide information about the possible physiological origin of HRV. The parameters obtained in the time domain are influenced by changes in the activity of the SNS and of the PNS, a circumstance that makes these measurements not specific to concretely calculate the sympathetic-vagal balance [40]. The frequency analysis consists in breaking down the HR registry, which resembles a complex wave, so that the spectral components are obtained [41].

The ultra-low-frequency (ULF), very-low-frequency (VLF), low-frequency (LF), and high-frequency (HF) bands measurements tend to be expressed in absolute power values (ms$^2$), but LF and HF should also be expressed in normalized units (n.u.) that represent the relative value of the power of each of them in relation to the total power minus the VLF component. Although controversy exists, and further studies are needed, the activity of the parasympathetic efferences seems to be principally responsible for the HF, while the sympathetic and parasympathetic flows seem to determine the LF along with other regulatory mechanisms, like renin-angiotensin and baroreflex. The LF/HF ratio is considered the sympathetic-vagal balance or the modulation of the sympathetic reflex [42].

7. Autonomic dysfunction in sedentary individuals

Several lifestyle factors are capable of influencing ANS function, including diet, physical activity, and smoking, among others. Physical inactivity and unhealthy diet are considered major lifestyle risk factors for the development of several diseases, including cardiovascular disease. In sedentary individuals, apparently without other clinical complications, have reduced HRV indices at rest (indices that represent both PNS and SNS activity. According to the comparison made by Kiviniemi et al. [43], low cardiorespiratory fitness was a more important determinant of cardiac autonomic function than moderate-to-vigorous physical activity (MVPA; ≥3.5 METs, 2 wk) and body fat by bioimpedance. In addition, it has also been described that sedentary subjects with excess weight also show lower HRV [44].

Regarding the latter aspect, PNS activation can increase the basal metabolic rate, resulting in the production and secretion of leptin (diminishing food intake). Furthermore, blocking the PNS diminishes the basal metabolic rate by 11%, a rate responsible for 60% to 70% of our daily caloric expenditure [45]. As already described, reduced PNS activity is observed in individuals due to excess weight and sedentary conditions; however, it is not precisely known if this PNS reduction in activity is caused by mechanisms related to excess weight/physical inactivity, or if prior ANS dysfunction results in a change in body composition [46].

To complement the aforementioned, it has been described that accumulation of fat can induce dysfunction of ANS regulation in an overweight/sedentary individual; a hypothesis being postulated as one of the causes of the high prevalence of cardiovascular disease, especially in obese individuals [47]. Furthermore, reduced values of HRV indices are present in several chronic diseases related to obesity, like diabetes and hypertension. Analyzing HRV in apparently asymptomatic sedentary
subjects can serve as an early diagnosis of these diseases and serve to avoid complications with obesity [48].

8. Metabolic parameters

Metabolic parameters as total cholesterol, LDL-c, HDL-c, triglycerides, blood glucose, C-reactive protein, and others have been associated with ANS function. Accordingly, triglycerides, total cholesterol, and LDL-c appear to be inversely associated with ANS function, assessed by HRV. Conversely, it seems that increased HDL-c is positively associated with HRV. Nevertheless, some studies have failed to find associations between these metabolic parameters and HRV. Therefore, more studies are needed to analyse those associations [47,48].

Studies on ANS (assessed by HRV) and MetS have suggested that they are negatively associated [49-52]. However, these studies have been mainly conducted on middle-aged or elderly subjects [49-52], and most have not adjusted their analyses for physical activity, a potentially important confounder. Furthermore, there are no longitudinal studies addressing this topic in healthy young adults. Indeed, prospective studies are needed to answer some of the aforementioned questions. Therefore, the associations between MetS and HRV in young, healthy populations are under-studied.

8. Postprandial lipemia and cardiovascular health

MetS is therefore a matter of serious concern and we need to understand its etiology in order to improve strategies of treatment and prevention. In this regard, postprandial lipemia has increased in importance over the last few years as it has been demonstrated to influence the development of atherosclerosis. In addition, in modern times, fasting is not the typical physiological state of humans; in fact, they spend most of the time in the postprandial state [53].

Postprandial lipemia is defined as the time period (usually 6–8 hours) from last food intake. Many of the studies conducted have been performed in the post-absorptive stage, when the triglyceride metabolism and the lipid metabolism have reached equilibrium. Studies in said stage are a reflection of endogenous lipid metabolism, while studies carried out in the postprandial stage are a reflection of the metabolism of lipids incorporated exogenously. Zilversmit, in 1979 [54], postulated what has been called the hypothesis of the “atherogenic remnants”. This theory is based exclusively on the remnants of chylomicrons of exogenous origin, which can cause atherogenesis when enriched with cholesterol from the diet.

Recently, the “triglyceride intolerance” hypothesis has been formulated, according to which hypertriglyceridemia is potentially atherogenic through the cholesterol transferred from lipoproteins rich in cholesterol, such as low-density lipoprotein cholesterol (HDL-c) and high-density lipoprotein cholesterol (HDL-c) to lipoproteins rich in triglycerides, which due to this enrichment with cholesterol esters cannot be degraded and end up deposited in the vascular wall.
9. References


Approaches to Study Endothelial Responses to Shear Stress. Antioxid Redox Signal. 2016;25:389-400.


34. Shaffer F, McCraty R, Zerr CL. A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. Front Psychol. 2014;5:1040.


41. Laborde S, Mosley E, Thayer JF. Heart Rate Variability and Cardiac Vagal Tone in Psychophysiological Research - Recommendations for Experiment Planning, Data Analysis, and Data Reporting. Front Psychol. 2017;8:213.


Aims and layouts of the thesis

Study 1

Chapter 2

Title: Effectiveness of High-intensity interval exercise training (HIIT) Compared to Moderate Continuous Training (MCT) in Improving Vascular Parameters in Inactive Adults

Research aim: To determine the effects of moderate- versus high-intensity interval exercise training on vascular function parameters in physically inactive adults.

Hypothesis: It was hypothesized that the HIIT protocol (due to the higher intensity) would induce the greatest metabolic perturbations and, therefore, the largest acute responses of vascular function parameters compared to the MCT protocol performed alone.

Study 2

Chapter 3

Title: Similar Cardiometabolic Effects of High- and Moderate-Intensity Training Among Apparently Healthy Inactive Adults: A Randomized Clinical Trial.

Research aim: To compare the effects of high-intensity interval training and steady-state moderate-intensity training on clinical components of metabolic syndrome (MetS) in healthy physically inactive adults.

Hypothesis: It was hypothesized that the HIIT protocol (due to the higher intensity) would induce the greatest metabolic perturbations and, therefore, the largest acute responses of cardiometabolic parameters compared to the MCT protocol performed alone.
Study 3

Chapter 4

Title: Effect of Moderate Versus High-Intensity Interval Exercise Training on Heart Rate Variability Parameters in Inactive Latin-American Adults: A Randomised Clinical Trial.

Research aim: We investigated the effect of moderate versus high-intensity interval exercise training on the HRV indices in physically inactive adults.

Hypothesis: We hypothesized that HIIT would result in higher improvements in autonomic function as compared to the MCT protocol performed alone.

Study 4

Chapter 5

Title: Exercise and Postprandial Lipaemia: Effects on Vascular Health in Inactive Adults.

Research aim: We aimed to determine the effects of a 12-week HIT or a MCT program on postprandial metabolism and vascular function and arterial stiffness after high fat meal (HFM) ingestion in healthy, inactive Latin-American adults.

Hypothesis: It was hypothesized that HIT would be just as effective at improving metabolic parameters and vascular function after HFM ingestion as the MCT protocol in inactive Latin-American adults.

Study 5

Chapter 6

Title: Effects of Exercise Training Type and Intensity or Nutritional Guidance on Metabolic Syndrome Risk Factors, Ideal Cardiovascular Health Parameters,
Endothelial Function and Arterial Stiffness in Overweight Adults: Cardiometabolic HIIT-RT Study, A Randomized Controlled Trial.

Research aim: We aimed to investigate whether 12 weeks of high-intensity interval training (HIIT), resistance training (RT), concurrent training (CT=HIIT+RT) or nutritional guidance (NG) induced improvements in metabolic syndrome (MetS) risk factors, vascular function parameters and ideal cardiovascular health (CVH) in sedentary and overweight adults, and to compare the training adaptations between intervention groups.

Hypothesis: It was hypothesized that the HIIT protocol (due to the higher intensity) would induce the greatest metabolic perturbations and, therefore, the largest acute responses of cardiometabolic parameters compared to the RT, CT or NG protocol performed alone.
Chapter 2

Effectiveness of HIIT Compared to Moderate Continuous Training in Improving Vascular Parameters in Inactive Adults
1. Introduction

Strong evidence shows that physical inactivity (<150 min/wk of moderate-intensity activity or <75 min/wk of high-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]. Physical inactivity has a deleterious effect that is comparable to smoking and obesity and is now recognized as the fourth leading risk factor for global mortality, accounting for 6% of all deaths [2].

Growing evidence suggests that exercise training improves vascular structure and nitric oxide bioavailability and reduces CVD risk factors; improvements in endothelial function may explain a large proportion of the risk reduction [3]. A number of factors appear to influence the acute effects of exercise on endothelial function, including sex, exercise intensity and duration, and the timing of post-exercise vascular function measurements [3]. Training protocols involving traditional moderate continuous training (MCT) and high-intensity training (HIT) can improve endothelial function [4,5] a response largely mediated by acute elevations in blood flow and laminar shear stress during individual exercise bouts [6,7]. In line with this, a growing body of evidence has demonstrated comparable or superior improvements in cardiovascular function using low-volume HIT compared to MCT [5].

Additionally, three sessions of 4 min of high-intensity exercise per week (12 min/week) was sufficient to improve aortic reservoir pressure (an independent predictor of CVD), and thus may be a time-efficient exercise modality for reducing cardiovascular risk in individuals with metabolic syndrome [5,8]. Furthermore, it was suggested that the ability of HIT to restore vascular homeostasis through the enhancement of shear stress-induced nitric oxide bioavailability may be another important mechanism that explains the protective role of exercise against non-communicable disease development [9]. Interestingly, despite this evidence, few randomized trials have directly evaluated the effects of sustained MCT or HIT on the cardiometabolic health of inactive adults [4,9,10].

There exists an inter-individual variability in vascular function, such that under the same stimulus, some subjects may achieve benefits, and are considered responders (Rs), whereas others may exhibit a worsened response or remain unchanged, and are considered non-responders (NRs) [11,12]. Both genetic and environmental factors have been described to explain this previously reported phenomenon [13,14]. However, all of these studies are primarily endurance or resistance training-based [9,15] and most have not explored other exercise modalities such as HIT [16].

In Latin-American populations, information about optimal exercise timing for improving vascular function parameters is scarce. There is no consensus regarding optimal exercise timing for improving vascular function parameters. Additionally, determining the prevalence of NRs after an exercise program is relevant to optimize and predict responses in different populations (e.g., athletes or individuals with risk factors).
The purpose of this secondary randomized clinical trial analysis was to compare the effects of MCT versus HIT on vascular function in physically inactive adult Latin-Americans. Identifying the training regimen that has the most beneficial effects on each parameter could potentially lead to enhanced precision in prescribing exercise training intensity to achieve optimal outcomes in this population [16].

2. Material and methods

2.1 Sample and Procedures

Details of the study design and methods of the primary HIT-Heart Study trial have been described elsewhere (ClinicalTrials.gov ID: NCT02738385) [17,18]. Informed consent was obtained from each participant. The protocol was based on the Helsinki Declaration Accord (World Medical Association for Human Subjects). Moreover, ethical approval was obtained from the University of Santo Tomás (ID 27-0500-2015). Endothelial function and fitness parameters were assessed at baseline and over 12 weeks of training. Briefly, the HIT-Heart Study conducted in 2013–2015 tested the efficacy of MCT versus HIT in changing biomarkers of endothelial and cardiovascular health.

Participants (n=20) were recruited at the University of Rosario (Bogota, Colombia) from February 2015 to May 2016. Inclusion criteria were individuals aged 18–45 years who were inactive (<150 min·wk⁻¹ of moderate-intensity activity or 75 min·wk⁻¹ of vigorous-intensity activity by applied a short version of the self-reported Global Physical Activity Questionnaire) and had a body mass index (BMI) ≥18 and ≤30 kg/m². We excluded participants if they had a history of cardiovascular disease and related morbidities, diabetes mellitus 1 or 2, thyroid dysfunction, or cancer or if they were pregnant or smoked. All participants provided written informed consent before participating in the study. Participants were randomly assigned via a computer-generated, concealed, fixed block randomisation procedure to MCT (n = 10) or HIT (n = 11) groups. Data were obtained prior to randomisation by treating physiotherapists and physiologist, and then 12 weeks later by blinded assessors. Assessments were taken at baseline (Week 0) and 12 weeks after randomisation for all outcomes by experienced and blinded physiotherapists or exercise physiologist.

2.2 Interventions

Moderate-continuous training (MCT) group

The MCT protocol involved walking on a treadmill with the deck inclined to reach the desired intensity. Each preparatory period started with an exercise dose of 6 kcal·kg⁻¹·week⁻¹, which was increased progressively by 2 kcal·kg⁻¹·week⁻¹ until week 4 and was then maintained at 12 kcal·kg⁻¹·week⁻¹ for weeks 5 to 12. Exercise training sessions were designed to elicit a response in the acceptable moderate range, i.e., 60–75% of HRR and were adjusted according to ratings on the Borg scale [17,18]. The rating of perceived exertion used was 12 to 15-point single-item scale ranging from 6 to 20 (6 “No exertion” and 20 “Maximum exertion”). 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). Exercise was performed in 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 (Figure 1).

![Figure 1. Schematic representation of a 4 × 4 HIT session or 30–40 min MCT session.](image)

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

The HIT protocol involved fast walking and running on a treadmill with the deck inclined to reach the desired intensity. We calculated training energy expenditures according to participants’ age ranges associated with meeting the consensus public health recommendations from the Cardiometabolic HIT-RT Study [17,18]. Each preparatory period started with an exercise dose of 6 kcal·kg⁻¹·week⁻¹, which was increased progressively by 2 kcal·kg⁻¹·week⁻¹ until week 4 and was then maintained at 12 kcal·kg⁻¹·week⁻¹ for weeks 5 to 12. The overall goal for the HIT group was to perform exercise sessions in 4 × 4 min intervals at 85–95% of HRR (with the target zone maintained for at least two min), interspersed with a 4-min recovery period at 75-85% of HRR. The speed and inclination of the treadmill were continuously adjusted to ensure that participants trained at the correct intensity. During each exercise session, participants adhered to the 12 kcal·kg⁻¹·week⁻¹ energy expenditure format, which was equivalent to 300 kcal of energy expended by the end of the training and cool-down (5 min) periods, with a total exercise time ranging from 35 to 55 min. Exercise was performed in three sessions per week. During the supervised intervention, HR and Borg ratings were measured as described for the MCT group.
We selected 6 to 12 kcal·kg\(^{-1}\)·week\(^{-1}\) per week because this dose of kcal·kg\(^{-1}\)·week\(^{-1}\) has produced changes in VO\(_2\)peak that placed approximately 70% of the initial sedentary population above the cut point for a low level of fitness [17,18], as defined by both the American College of Sports Medicine (ACSM) [19] and the American Heart Association [20] guidelines for cardiovascular disease reduction.

The intensity to run/walk was related to a range of 85–95 % (HIT) or 60–75% (MCT) of the maximum predicted heart rate according to the widely known equation (Karvonen), and the rest period was considered under a heart rate of 75-85% to HIT group of this marker. Thus, using the heart rate and oxygen consumption data obtained from the baseline fitness (cardiorespiratory uptake) test, the heart rate associated with an oxygen consumption of approximately 60% (MCT) and approximately 75-85% (HIT) were prescribed for each participant [19].

2.3 Endothelial function arterial wall parameters measures

The primary outcome measure was endothelial function, as measured by flow mediated-dilation (FMD), aortic pulse wave velocity (PWV) and the augmentation index (AIx). FMD was measured as described in previous studies from our group [21] in the Colombian population using the protocol reported by Atkinson et al. [22]. The same operator performed all Doppler ultrasound (Mindray M-9® DS USA; Mahwah, NJ) examinations using a 7.5-MHz linear array probe to locate and interrogate determine flow velocity profiles in the right brachial artery. Ultrasound images were obtained after 20 min of rest in a supine position in a dark, climate-controlled, quiet room (22–24°C), with the participants arm immobilized and slightly supinated and elevated. A duplex ultrasound image of the brachial artery was obtained approximately midway between the antecubital fossa and shoulder. Blood velocity was assessed with the Doppler angle of insonation ≤ 60°. The baseline longitudinal image of the artery was acquired for 30 seconds and then the blood pressure cuff was inflated to 50 mm Hg above systolic pressure for 5 minutes. The longitudinal image of the artery was recorded continuously until 3 minutes after cuff deflation. Pulsed Doppler velocity signals were obtained for 20 seconds at baseline and for 10 seconds immediately after cuff deflation. The intra-session coefficient of variations was ≤1% for the baseline diameter. The technical error of measurement was 1.23% for baseline diameter, 1.77% for maximum diameter and 20% for %FMD. Images were recorded on a DVD player for subsequent measurements by one observer blinded to the study design. FMD was expressed as % change =[(maximum - baseline diameter) / baseline diameter] × 100. Normalized brachial artery FMD (FMDn) was calculated according allometric relationship between Dbase and peak diameter (Dpeak) [22].

PWV was measured by analyzing the oscillometric pressure curves registered from the upper arm with arteriographic computer program (Arteriograph Software v.1.9.9.2; TensioMed, Budapest, Hungary). The algorithm measuring blood pressure in the arteriography device has been validated [23]. PWV was calculated as the jugulum-to-symphysis distance (m) divided by the return time (return time/2) (s). For PWV measurements, the two recordings with the lowest standard deviation were chosen. The standard deviation was calculated on the basis of every heartbeat.
during a period of 8 s. The Alx was also calculated as the ratio of the difference between the first pulse 1 and second pulse 2 systolic peaks relative to the central pulse pressure, expressed as a percentage [(pulse 2 - pulse 1/central pulse pressure) x100]. The arteriograph calculates the Alx on the basis of the formula = (Alx% pulse 2 - pulse 1/central pulse pressure) x100 and thus provides the brachial/aortic Alx without applying a transfer function. The R value as an estimate of the measurement errors for the repeat measurements between two sessions (n=6) was low for the arteriograph (1.18 m·s⁻¹).

2.4 Secondary outcomes

**Anthropometric measurements:** After completing another general information questionnaire, participants were instructed to wear shorts and a T-shirt to the physical exam. They were also required to remove all worn jewelry and metal objects. Once the subjects were barefoot and in their underwear, their body weight (kg) was measured using an electric scale (Model Tanita® BC-420®, Tokyo, Japan) with a range of 0–200 kg and with an accuracy of within 100 g. Height was measured with a portable stadiometer with a precision of 0.1 cm and a range of 0–2.5 m (Seca® 274, Hamburg, Germany). Body mass index (BMI) was calculated as the body weight in kilograms divided by the square of height in meters (kg/m²).

**Cardiopulmonary exercise testing and training intensity:** A maximal incremental test was performed by each participant on a treadmill (Precor TRM® 885, Italy) using a ramp protocol that simulates field running described by Ramírez-Vélez [18]. The criteria for exercise termination followed the American College of Sports Medicine recommendations [19], identified by an exercise physiologist who was present during each test. Maximum pulmonary oxygen uptake was defined as the mean cardiorespiratory uptake of the last 30s of exercise; the maximum HR was registered at the exercise peak.

Although diet was not controlled, participants met with the study’s dietician for nutritional assessment and counselling at baseline, and an individualized iso-energetic nutrition intervention plan was developed from the baseline food intake assessment according to participant preferences. This plan was standardized at 1300 to 1500 kcal·day⁻¹ (50–55% carbohydrates, 30–35% total fat, <7% saturated fat and 15–22% protein), distributed across 3-4 meals per day [19].

Physical activity performed outside of the supervised exercise sessions (daily physical activity) was measured using Global Physical Activity Questionnaire for a 10 and 12 weeks [24]. MET-minutes/week were used to estimate the duration and intensity of physical activity during intervention.

2.5 Sample size

The measurement of FMD, validated in several population studies, was selected as the critical variable to calculate the sample size [25,26]. A randomized clinical trial of the effect of aerobic training on FMD resulted in a standardized effect size (ES) of 0.3 to 0.6 for improvement in endothelial function [27]. An a priori power analysis estimated that a total sample size of 10 participants in each
group would detect a 0.4 standardized ES for a between-group difference in improvements in FMD ($\beta = 0.80$, $\alpha = 0.05$ for a two-tailed test).

2.6 Statistical Analysis

To retain the data of all randomly allocated participants, an intention-to-treat analysis (all randomly assigned patients) was performed. Prior to the planned statistical analyses, a preliminary analysis was conducted (Kolmogorov-Smirnov test) to confirm the normality of the data. We used a generalized linear model (GLM) with repeated measures to analyze the influence of the different doses of exercise training on components of endothelial function and physical fitness outcomes [2 (group) x 2 (test time)]. Cohen’s $d$ for ESs were also calculated to determine the magnitude of the group differences. ESs were classified as small, small-to-medium, and medium-to-large effects (<0.20, 0.2–0.6 and 0.6–1.2, respectively) [28], and $\eta^2$ group x time interaction ESs were calculated as the between-group sum of squares divided by the total sum of squares and interpreted as follows: small (0.01); small-to-medium (0.01–0.10); and medium-to-large effect (0.10–0.25).

To classify the participants as Rs or NRs for improvements in FMDn/PWV, the typical error (TE) was calculated, similar to the approach in our recent study [11,13]. TE was calculated using the following equation: $TE = SDdiff/\sqrt{2}$, where $SDdiff$ is the variance (standard deviation) of the difference in scores observed between the 2 repeats of each test. A NR was defined as an individual who failed to demonstrate a decrease or increase (whichever represented a beneficial change) that was greater than 2 times the TE away from zero. Chi-squared ($\chi^2$) tests were used to assess the differences between the prevalence of NRs pre- and post-intervention for each group. All reported P values are two-sided (P<0.05). Statistical analyses were conducted using PASW Statistics 17 for Windows (SPSS, Inc., Chicago, Illinois).

3. Results

Figure 1 shows the CONSORT flowchart of the randomized clinical trial. A total of 28 potential physically inactive subjects were assessed for eligibility. Seven of them were excluded because they did not meet the inclusion criteria. Ten participants were randomly allocated to the MCT group, and 11 were allocated to the HIT group. After allocation, one participant in the MCT group withdrew for reasons unrelated to this study (lack of time due to work schedule).
Table 1 presents the within- and between-group differences in vascular parameters following the training program. Peak brachial artery diameter significantly increased in the HIT group (+0.3 [0.1] mm) and MCT group (+0.2 [0.2] mm), with a lower effect (Cohen’s $d = 0.10$). There was a medium-to-large group x time interaction effect on PWV [between-group mean difference = -0.5 (CI 95% = -0.3 to 1.3) m·s$^{-1}$; $\eta^2 = 0.60$; $p<0.01$], indicating positive adaptations following HIT compared with those following MCT. There were no significant treatment effects on other vascular parameters.
Table 1. Intent-to-Treat Analysis of anthropometric and vascular function parameters at baseline and changes after 12 weeks

<table>
<thead>
<tr>
<th></th>
<th>Groups</th>
<th>From Baseline to 12-week, Mean (95% CI)</th>
<th>MCT effect p value, (Effect size)</th>
<th>HIT effect p value, (Effect size)</th>
<th>( \eta^2 ) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline, Mean (SD)</td>
<td>After 12 weeks, Mean (SD)</td>
<td>Within-Group Change, Mean (SD)</td>
<td>Between-Group Difference in Change, Mean (Effect size)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MCT (n = 10)</td>
<td>HIT (n = 11)</td>
<td>MCT (n = 9)</td>
<td>HIT (n = 11)</td>
<td></td>
</tr>
<tr>
<td>Anthropometric</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>69.3 (15.3)</td>
<td>66.8 (10.9)</td>
<td>68.6 (13.5)</td>
<td>66.7 (10.5)</td>
<td>-0.6 (-1.9)</td>
</tr>
<tr>
<td></td>
<td>(25.2)</td>
<td>(24.8)</td>
<td>(25.6)</td>
<td>(24.1)</td>
<td>(0.179) (0.05)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.6 (3.6)</td>
<td>25.5 (4.2)</td>
<td>23.4 (3.0)</td>
<td>24.4 (4.2)</td>
<td>0.2 (1.1)</td>
</tr>
<tr>
<td></td>
<td>(6.4)</td>
<td>(6.3)</td>
<td>(6.1)</td>
<td>(6.2)</td>
<td>(-0.1) (-1.6)</td>
</tr>
<tr>
<td>Vascular function</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( D_{base} ), mm</td>
<td>3.0 (0.6)</td>
<td>2.7 (0.4)</td>
<td>3.2 (0.6)</td>
<td>2.9 (0.5)</td>
<td>0.2 (0.2)</td>
</tr>
<tr>
<td></td>
<td>(0.4)</td>
<td>(0.3)</td>
<td>(0.4)</td>
<td>(0.3)</td>
<td>(-0.1) (-1.9)</td>
</tr>
<tr>
<td>FMD, %</td>
<td>7.2 (3.3)</td>
<td>7.6 (5.5)</td>
<td>6.1 (2.7)</td>
<td>9.1 (5.3)</td>
<td>-1.0 (1.9)</td>
</tr>
<tr>
<td></td>
<td>(2.5)</td>
<td>(3.0)</td>
<td>(1.8)</td>
<td>(3.2)</td>
<td>(-2.9) (1.7)</td>
</tr>
<tr>
<td>( D_{peak} ), mm</td>
<td>3.2 (0.5)</td>
<td>3.0 (0.5)</td>
<td>3.4 (0.6)</td>
<td>3.3 (0.6)</td>
<td>0.2 (0.2)</td>
</tr>
<tr>
<td></td>
<td>(0.3)</td>
<td>(0.3)</td>
<td>(0.4)</td>
<td>(0.4)</td>
<td>(-0.1) (-1.9)</td>
</tr>
<tr>
<td>( D_{diff} )</td>
<td>0.2 (0.5)</td>
<td>0.3 (0.4)</td>
<td>0.1 (0.4)</td>
<td>0.3 (0.5)</td>
<td>-0.01 (0.08)</td>
</tr>
<tr>
<td></td>
<td>(0.3)</td>
<td>(0.2)</td>
<td>(0.3)</td>
<td>(0.2)</td>
<td>(-0.1) (-1.9)</td>
</tr>
<tr>
<td>FMDn, %</td>
<td>6.5 (2.9)</td>
<td>7.4 (5.7)</td>
<td>5.0 (5.4)</td>
<td>8.1 (4.8)</td>
<td>1.6 (0.7)</td>
</tr>
<tr>
<td></td>
<td>(1.7)</td>
<td>(1.6)</td>
<td>(1.3)</td>
<td>(1.7)</td>
<td>(-2.2) (1.3)</td>
</tr>
<tr>
<td>PWV, m·s(^{-1})</td>
<td>6.7 (0.8)</td>
<td>7.1 (1.2)</td>
<td>6.8 (0.9)</td>
<td>6.7 (1.6)</td>
<td>1.0 (0.8)</td>
</tr>
<tr>
<td></td>
<td>(1.6)</td>
<td>(1.3)</td>
<td>(1.3)</td>
<td>(1.6)</td>
<td>(-0.3) (-1.4)</td>
</tr>
<tr>
<td>AIx (aortic), %</td>
<td>41.7 (10.4)</td>
<td>24.5 (32.7)</td>
<td>41.7 (16.5)</td>
<td>22.3 (28.9)</td>
<td>0.01 (-2.1)</td>
</tr>
<tr>
<td></td>
<td>(2.1)</td>
<td>(6.5)</td>
<td>(1.6)</td>
<td>(1.9)</td>
<td>(-2.1) (1.2)</td>
</tr>
<tr>
<td>AIx (brachial), %</td>
<td>16.5 (5.2)</td>
<td>25.1 (16.5)</td>
<td>38.7 (69.0)</td>
<td>26.3 (14.6)</td>
<td>22.2 (-1.1)</td>
</tr>
<tr>
<td></td>
<td>(6.5)</td>
<td>(6.5)</td>
<td>(14.6)</td>
<td>(14.6)</td>
<td>(-1.98 to 0.66)</td>
</tr>
</tbody>
</table>

HIT, 4 x 4-min High-intensity interval training; MCT, Moderate intensity continuous training; Within-group effect size Cohen’s \( d \); between-group effect size \( \eta^2 \); D, Diameter; FMD, Flow-mediated vasodilation; FMDn, Normalized flow-mediated vasodilation; PWV, Pulse wave velocity; AIx, Augmentation index; * \( d \) Cohen significant difference at \( p < 0.01 \)
Figure 3A and 3B show the mean values for individual changes in FMDn (%) and PWV in both groups. Regarding FMDn (%), the analysis showed a NR prevalence of 66% (6 cases) in the MCT group and 18% (2 cases) in the HIT group (p=0.013). There was no significant difference in the prevalence of NRs for PWV between the MCT and the HIT group (66% versus 33%, p=0.051). These results are clinically relevant change and could be used as threshold value in individuals with characteristics like those of this study.

Figure 3. Differences in the prevalence of non-responders in vascular parameters after 12 weeks training.
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 2. Compliance with the study intervention was adequate, with 32/36 (89%) of participants receiving supervised exercise training. 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. Between 10 to 12-week, walking differences over time in both groups was MCT group 945 min vs HIT group 514 min, (p < 0.001), but this difference was evident from high-intensity physical activity levels (MCT group 885 min vs HIT group 1168 min, p < 0.001)."

**Table 2.** Attendance to prescribed exercise sessions and self-reported physical activity

<table>
<thead>
<tr>
<th>Variable</th>
<th>MCT (n = 9)</th>
<th>HIT (n = 11)</th>
<th>Group effect (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adherence (% of prescribed sessions completed), mean (SD)</td>
<td>98.7 (3.7)</td>
<td>98.4 (2.8)</td>
<td>0.969</td>
</tr>
<tr>
<td>Total number of sessions completed, mean (SD)</td>
<td>32.5 (1.3)</td>
<td>32.5 (0.9)</td>
<td>0.993</td>
</tr>
<tr>
<td>Total time spent training (min) per week, mean (SD)</td>
<td>1100 (258)</td>
<td>1031 (147)</td>
<td>0.043</td>
</tr>
<tr>
<td>International Physical Activity Questionnaire</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking MET-minutes/week, mean (SD)</td>
<td>945 (1890)</td>
<td>514 (1014)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Moderate MET-minutes/week, mean (SD)</td>
<td>200 (276)</td>
<td>128 (260)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vigorous MET-minutes/week, mean (SD)</td>
<td>885 (712)</td>
<td>1168 (588)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

SD, standard deviation

**4. Discussion**

To the best of our knowledge, this is the first randomized clinical trial studying the effects of exercise training intensity on vascular parameters and individual responses in physically inactive adults from a Latin-American population. These findings suggest that exercise training induces potent stimuli leading to improvements in vascular parameters (i.e., decrease in arterial wall thickness and increase in endothelial function). However, not all of the measured vascular functions responded in the same way to the type of exercise investigated, suggesting the presence of different regulatory mechanisms and time courses for induction.

HIT and MCT on a treadmill have been previously shown to be highly effective in patients with metabolic syndromes [29]. Additionally, exercise training has been shown to be an effective therapeutic strategy for vascular function improvement in different clinical populations [30]. A previous meta-analysis showed that HIT is more potent than MIT in enhancing FMDn, with a mean difference of 2.26% [31]. Specifically, this review suggested that a 4 x 4 HIT protocol three times per week for at least 12 weeks is an effective form of exercise for enhancing vascular functions. Our study showed a mean difference of 2.9% in vascular functions between groups; however, the difference was not significant. Along the same line, our data indicate that while brachial artery diameter increased as a result of exercise, arterial function assessed by FMD decreased at high levels of exercise (Table 1).
It is conceivable that substantial and/or sustained increases in shear forces that occur during exercise bouts may be associated with attenuated FMD, because stimulation of vasodilation post-exercise may result in the inhibition of related biochemical pathways [10]. In line with this, a meta-analysis of prospective studies reported a 13% reduction in the risk of cardiovascular events with a 1% increase in FMD; therefore, the magnitude of change in FMD following HIT (pre- vs. post-HIT +1.9%) was deemed to be clinically significant in our study.

Differences in exercise and experimental protocols in our study may have also contributed to discrepancies in our findings; however, this hypothesis remains to be tested. Our study showed that exercise intensity influences FMDn response; however, FMDn following exercise was attenuated in the MCT group but enhanced in the HIT group. Siasos et al. [7] suggested that both acute HIT and MCT can favorably affect endothelial function in healthy young adults, indicating another cardioprotective effect of exercise preventing the progression of atherosclerosis. The effects of these intense exercise regimens on FMDn reflect a combination of hemodynamic changes and endothelial nitric oxide-dependent mechanisms [4,10]. Exercise induces increases in blood flow, and augmented blood flow causes vasodilation, which directly impacts the magnitude of FMDn [22,32].

Regarding arterial wall parameters, aerobic exercise seems to significantly improve arterial stiffness, and this effect is enhanced at higher intensities of aerobic exercise and in participants with greater baseline arterial stiffness [31-33]. PWV is widely recognized as a direct marker of arterial stiffness [33]. Augmentation and the AIx are being more frequently used in studies as parameters of wave reflection [34]. In addition, an increase in PWV is linked with increased rates of cardiovascular incidences related to increased left ventricular afterload and wasted left ventricular energy [31,33]. A previous systematic review and meta-analysis of RCTs reported that every 1 m·s⁻¹ increase in PWV is associated with a 12–14% increase in the risk of cardiovascular events and a 13–15% increase in the risk of CVD mortality. On the other hand, it was reported that aerobic exercise reduced PWV by 0.63 m·s⁻¹, which may be translated into an 8% reduction in cardiovascular events and a 9% reduction in cardiovascular mortality. Furthermore, subgroup analyses suggested that there may be bigger effects on PWV and, consequently, on cardiovascular events and mortality of aerobic exercise in higher risk participants (with PWV ≥ 8 m/s at baseline) and with longer durations of aerobic exercise (> 10 weeks) [31,33]. To the best of our knowledge, this was the first study to investigate alterations in PWV and the AIx after HIT in physically inactive adults from a Latin-American population.

Evidence from systematic reviews and experimental studies has demonstrated a positive effects of various exercise modalities (aerobic, resistance and combined) on endothelial functions [7,29,31], but there are controversies regarding the effects of HIT on indices pertaining to arterial stiffness and wave reflection [7,31,35]. The mechanism by which HIT significantly reduces PWV more than MCT does could be associated with reduced exposure of the vasculature to reactive oxygen species that are often observed during high-volume exercise [36]. It is also possible that the higher volume of exercise in the HIT group may have resulted in the requirement of longer time for PWV recovery from repeated high-intensity exercise bouts, thereby providing a more accurate
representation of the cumulative effect of exercise intervention. However, the rate of NR was different between MCT and HIT in terms of reduced PWV and AIx in favor of high-intensity training, and this difference was also observed within each group with regard to other cardiometabolic and performance-related co-variables. These results may help identify the vascular wall that is more responsive and, conversely, the wall that is more resistant to the arterial stiffness-lowering effects of HIT [35].

According to the study by Siasos et al. [7], different intensities of aerobic exercise have different effects on central and peripheral arterial stiffness. In our study, despite differences between groups in terms of PWV, there was a trend for a greater reduction in these parameters following HIT. It could be hypothesized that the lack of effect on PWV may be due to normal PWV at baseline in most of the subjects (85%) (PWV ≥ 8 m/s at baseline). Additionally, aerobic exercise seems to have a greater effect on peripheral than on central indices of arterial stiffness [7], which could justify our findings. These discrepancies between findings could also be due to differences in exercise modes or durations of HIT intervals; Ramos et al. [8] and Sawyer et al. [15] suggest that metabolic responses to HIT vary depending on the duration of the work-rest intervals.

On the other hand, the phenomenon of NR has been explored on performance variables [1] using endurance [12], resistance (RT) [37], or HIT [11] in different age groups such as children [13], adults [29], and older populations [38]. Regarding FMDn (individual responses), our analysis showed an NR rate of 66% (6 cases) in the MCT group and 18% (2 cases) in the HIT group (P = 0.013). This information can be useful when there are more than one risk factor to improve in physically inactive populations, and this knowledge can be useful for choosing exercise interventions with low rates of NR and high rates of improvements in particular outcomes. The data from some studies support our conclusion that exercise intensity plays an important role in modulating adaptations in vascular functions in response to exercise [4,31,32]. In line with this, several previous studies have reported increases [39,40] decreases [27] or lack of change [10] in FMD following different exercise protocols. Unfortunately, none of these studies on exercise interventions reported on the rate of NR. Although some misleading studies have claimed the lack of non-responders in 4-week training intervals [1], more recently, this phenomenon has been confirmed after 6 weeks and 6-8-months of exercise by relevant authors in the field [1].

In any case, the term “NR” may be related more to semantics, as the authors demonstrate a lack of response in some of the chosen outcomes (e.g., VOpeak, lean body mass, leg strength) across participants. Even the authors of reports that refute the so-called ‘myth’ of exercise non-response might agree that the term “NR” depends solely on the chosen clinical outcomes and that a non-responder for one outcome may not be a non-responder in another outcome [1,11]. As technology advances and our understanding of the mechanisms driving exercise responses improves, scientists can continue to narrow the focus on clinical outcomes that are critical for improving the health of an individual, and healthcare practitioners can thus recommend exercise regimens on an individual basis rather than broadly suggesting the same exercise regimens for everyone.

More specifically, it has been suggested that HIT may impair endothelium-dependent vasodilation due to an increase in reactive oxygen species, resulting in a reduction in NO bioavailability. Additionally, responses in FMD are inversely proportional
to baseline arterial diameter. Further studies are necessary to establish optimal exercise training interventions for improving vascular health assessed by measuring FMD. Additionally, differences between the effects of different exercise regimens could be due to variability in their ability to generate greater blood flow through vessels supplying oxygen to the working muscles, which could in turn promote greater shear stress-induced nitric oxide bioavailability [39] and induce favorable endothelial adaptations [40].

In this context, several biologically plausible mechanisms may be used to explain the effects of exercise on the modulation of endothelial functions and arterial stiffness. It is widely known that exercise has the potential to reduce oxidative stress by increasing the efficiency of the antioxidant system, eventually improving endothelial dysfunction [40]. The main physiological mechanisms involve the up-regulation of endothelial nitric oxide synthase activity, as demonstrated in cell culture, animal and human studies, with a subsequent reduction in the expression of nicotinamide adenine dinucleotide (phosphate) (NAD(P)H)-dependent oxidase and the stimulation of free radical-scavenging systems that affect the levels of copper/zinc-containing superoxide dismutase, extracellular superoxide dismutase, glutathione peroxidase and glutathione [37]. Therefore, the improvements in arterial stiffness and endothelial functions in response to ischemia may be related to the direct effects of repeated bouts of HIT on the vasculature body weight or BMI.

The strengths of this study include the use of state of the art measures of vascular functions, physical fitness, and metabolic biomarkers with supervised exercise training in a non-clinical setting. In addition, adherence to the intervention was ≈89%. All subjects completed 32 of 36 exercise sessions, and research technicians supervised each session while HR was being monitored.

A primary limitation of this study was the lack of a true control group without exercise. 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 [29]. Due to this and other limitations (e.g., relatively small sample size and single site design), it is important to not over-interpret the results of this RCT [17]. Other limitations of this study include the lack of control over tobacco usage. Moreover, there is no gold standard measurement for arterial parameters. Additionally, indices other than post-occlusion reactive hyperemia flux were not assessed in the present study. Lastly, we cannot determine the directions of the associations nor any causality observed in this study with absolute certainty.

5. Conclusion

In summary, based on the results of the present study, physically inactive adults in both HIT and MCT groups experienced changes in FMD. The rate of response was significantly different between the groups regrading FMD but not PWV. The sustained change in PWV in the HIT group may represent vascular adaptation or endothelial fatigue. This study demonstrates the efficacy of high-intensity exercise in enhancing the cardioprotective effects of exercise on the progression of atherosclerosis in a physically inactive population.
6. References


19. AMERICAN COLLEGE OF SPORTS MEDICINE. Position stand: the recommended quantity and quality of exercise for developing and maintaining cardiorespiratory and


22. Atkinson G. Shear rate normalization is not essential for removing the dependency of flow-mediated dilation on baseline artery diameter: past research revisited. Physiol Meas 2014;35:1825-1835.


Chapter 3

Similar Cardiometabolic Effects of High- and Moderate-Intensity Training Among Apparently Healthy Inactive Adults: A Randomized Clinical Trial
1. Introduction

Disorders of the metabolic system have a key pathophysiological role in the early stages of excess adiposity, elevated blood pressure, insulin resistance, abnormal glucose metabolism and dyslipidemia (elevated triglyceride levels and low-density lipoprotein cholesterol [LDL-c], and reduced high-density lipoprotein cholesterol [HDL-c]), producing coronary vasoconstriction, increasing cardiac oxygen consumption and leading to fatal events [1,2]. This cluster of findings is recognized as metabolic syndrome (MetS) [3] and strongly predicts the risk of developing type 2 diabetes, hypertension and cardiovascular disease (CVD), which remains the leading cause of death worldwide [4-8].

Recently, Barceló [9] estimated that the number of CVD deaths in Latin America will increase by more than 60% between 2000 and 2020, while CVD deaths will increase by only 5% in high-income countries during the same period. The findings of the INTERHEART case-control study in Latin America showed that abdominal obesity, dyslipidemia, and hypertension were associated with high population-attributable risks of 48.5%, 40.8%, and 32.9%, respectively [10]. In the same retrospective study, daily consumption of fruits or vegetables and regular PA reduced the risk of acute myocardial infarction. Therefore, interventions aimed at the reduction of modifiable risk factors are thought to be the most effective way to prevent the onset of MetS and potentially CVD in Latin America.

On the other hand, MetS is determined by genetic predisposition as well as environmental factors that may promote its development, such as low levels of physical activity (PA), large volumes quantities of sedentary time (sitting), and poor eating habits [5,8]. The adoption and maintenance of PA are critical foci in the metabolic health management and overall health of individuals with potential medical risks, including acute complications such as cardiac events, hypoglycemia, and hyperglycemia. Strong evidence shows that physical inactivity (<150 min·wk⁻¹ of moderate-intensity PA or 75 min·wk⁻¹ of vigorous-intensity PA) are jointly associated with increased cardiometabolic morbidity and mortality in a dose-dependent manner [11,12]. Currently, physical inactivity is the fourth leading risk factor for global mortality and is comparable in that respect to smoking and obesity, accounting for 6% of all deaths [13]. Experimental studies indicate that physical inactivity and sedentary time result in alterations in cardiovascular [14] and metabolic biomarkers [15,16].

Systematic reviews [17-19] have found that physically inactive adults who participate in supervised interval training in clinical settings improve their exercise capacity, quality of life, maximal oxygen consumption (VO₂max) and metabolic control. A growing body of evidence has demonstrated comparable or greater improvements in cardiovascular function using low-volume high-intensity training (HIT) compared to traditional moderate-intensity continuous training (MCT) [17,18,20,21]. Furthermore, participation in HIT reduces risk factors that are associated with MetS, bringing improvement in features such as the oxidative metabolism–dependent energy system, metabolic capacity, qualitative profile of skeletal muscle fiber type, muscle mass, and fiber diameter [2,22-24]. In primary
prevention, Pattyn et al. [2] shown that endurance training has a favourable effect on most of the cardiovascular risk factors associated with the MetS such as: a mean reduction in abdominal obesity, blood pressure decrease and a mean increase in HDL-c. In this same line, in previously clinical trials [22,25-28] has been investigated the effect of exercise in different populations and for single cardiovascular risk factors, but none have specifically focused on the insufficient PA and the concomitant effect of HIT on all associated cardiovascular risk factors. However, few randomized trials have directly evaluated the effects of MCT or HIT on cardiometabolic health among inactive adults [2,25-28].

Although the epidemiologic transition and epidemic of CVD have been well documented in Latin Americans [29-31], relatively little research on their PA [32-34] and physical fitness exists. Moreover, Latin American countries [6,7] have a similar or even greater prevalence of MetS among adults than developed countries [8]. In this context, ethnicity and age has been associated with the development of MetS specially in Hispanic population [1,3,6,7]. According to the definition of the National Cholesterol Education Program—Adult Treatment Panel III of the United States, the prevalence of MetS in adults was: 32% in Hispanic Americans; 22% in African Americans; and 24% in European Americans [3]. In Colombia, Martínez-Torres et al. [32] reported the predisposing factors for having a MetS included: being male, over 25 years old and overweight or obese, all of them related to metabolic disorders as previously described in apparently healthy women [31]. In addition, the public policy recommendations also highlight the need for healthy adults to have an activity plan that integrates preventative and therapy recommendations [39-41]. For this reason, a randomized clinical trial (RCT) comparing different intensities of exercise training in adults with insufficient PA with a large age range and different ethnic groups are clinically relevant because it can provide evidence for a precise [21,35-37], prescribed intensity of exercise training to achieve optimal outcomes in this population [39-41].

Therefore, the purpose of this RCT was to compare the effects of MCT and HIT on the risk factors for MetS among apparently healthy physically inactive adults. We hypothesized that HIT and MCT would induce similar reductions in the risk factors for MetS and similar increases in exercise capacity when training frequency and session duration were equal in both types of training.

2. Methods

2.1 Study design and setting

The High Interval Intensity Training and ideal cardiovascular Heart Study (HIIT-Heart Study) was an RCT (ClinicalTrials.gov ID: NCT02738385) that included physically inactive Colombian adults who were randomly allocated to either an MCT group or an HIT group. The study was performed in accordance with the Declaration of Helsinki (2000) and was approved by the local office of the Medical Research Ethics Committee at the University of Santo Tomás (ID 27-0500-2015). Cardiometabolic health parameters and physical fitness outcomes were assessed at baseline and 12 weeks later. We provide an overview of the methods per the Consolidated Standards of Reporting Trials (CONSORT) checklist [38].
2.2 Participants and recruitment

This RCT was conducted at the University of Rosario and the University of Santo Tomás (Bogota, Colombia) from February 2015 to May 2016. Participants aged 18–45 years who were inactive and had a body mass index (BMI) ≥18 and ≤30 kg/m$^2$ and who were willing and almost immediately available to participate in the study were recruited from the Centre of Studies in Physical Activity Measurements (in Spanish, CEMA) via posted study recruitment flyers at community centers, study recruitment announcements at the CEMA, and word of mouth. 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 [39]. We have recently published a complete description of the HIIT-Heart Study design, methods, and primary outcomes for our current cohort [21]. Participants were required to sign a written informed consent form.

2.3 Blinding and randomization

Random allocation into the two study groups was performed by the CEMA at the University of Rosario in Bogotá, Colombia using block randomization with a block size of four. As each consecutive participant entered this RCT, he/she was randomly allocated to either the MCT group or the HIT group according to a computer-generated group allocation sequence. The randomization sequence was not concealed from the investigator who was responsible for assigning participants to groups. The principal investigators and statisticians were blinded to treatment allocation throughout the trial protocol.

2.4 Interventions

Both groups participated in the cardiometabolic program as recommended by both the American College of Sports Medicine (ACSM) [40] and the AHA [39,41] guidelines for ideal cardiovascular health and disease reduction. At the beginning of the training protocol, we measured the participants’ weight to determine the weekly energy expenditure that was necessary to achieve their target of 12 kcal·kg$^{-1}$·week$^{-1}$ (iso-energetic).

The MCT and HIT interventions lasted 12 weeks, with three sessions per week consisting of fast walking or running on a treadmill with the deck inclined to reach the desired intensity. HR was recorded during each session using an HR monitor (Polar Pacer, USA). In addition, Borg ratings were measured during each exercise session. An initial 2-week preparatory phase of training was performed to bring participants up to a 6 kcal·kg$^{-1}$·week$^{-1}$ goal (~150 kcal per session or equivalent to 6 Mets), which was progressively increased by 2 kcal·kg$^{-1}$·week$^{-1}$ until week 4 and was then maintained at 12 kcal·kg$^{-1}$·week$^{-1}$ for weeks 5 through 12 (~300 kcal per session or equivalent to 10 Mets). The duration of each individual session depends on the number of visits required to reach the target kcal·kg$^{-1}$·week$^{-1}$.

**Moderate-intensity continuous training (MCT) group:** Exercise training sessions were designed to elicit a response in the acceptable moderate-to-vigorous range, i.e., 55–75% heart rate reserve, and were adjusted according to ratings on the Borg scale. Each session consisted of a warm-up (5 min), followed by 15-55 min of
treadmill walking/running (15-35 min during the 2-week preparatory phase) and a final relaxation/cool-down period (10 min).

**High-intensity training (HIT) group:** We calculated the training energy expenditure for participants’ age ranges to meet the consensus public health recommendations included in the HIIT-RT Study [21]. A complete description of the design and methods has been published elsewhere [21]. During the 2-week preparatory phase, subjects warmed up at 65% heart rate reserve (5 min), then performed 4 × 4 min intervals at 60–80% heart rate reserve interspersed with 4 min of active recovery at 55% heart rate reserve. During weeks 3-12, subjects performed 4 × 4 min intervals at 85–95% heart rate reserve (remaining in the target zone for at least 2 min) interspersed with 4 min of active recovery at 65% heart rate reserve and a cool-down (5 min), with a range of total exercise time ranging from 35 to 55 min (including warm-up and cool-down). We selected 6 to 12 kcal·kg⁻¹·week⁻¹ per week because this dose of kcal/kg/week has produced changes in VO₂max that placed about 70% of the initially sedentary population above the cut point for low fitness, as defined in by both the ACSM) [40] and the AHA [39,41] guidelines for cardiovascular disease reduction.

Participants in both groups were supervised during each exercise training session by an investigator or research assistant. Exercise training was conducted at the “CEMA” fitness center on the campus of the University of Rosario, which contained the treadmills needed to complete the prescribed exercise programs. Each participant was instructed to inform the supervisor immediately if he or she experienced any unusual symptoms during 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 the study. All participants reported that they adhered 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 in energy expenditure was calculated for each participant according to both the HR and the number of minutes spent exercising during the training sessions. The trainers were physical therapists and physical educators with experience developing and monitoring exercise programs with clinical populations. Adherence to the exercise program was encouraged by the exercise professional who supervised each of the group sessions. To maximize adherence to the training program, the trainer supervised no more than 3–5 participants simultaneously.

Although diet was not controlled, participants met with the study dietician for nutrition assessment and counselling at baseline, and an individualized iso-energetic nutrition intervention plan was developed from the baseline food intake assessment according to participant preferences. This plan was standardised at 1300 to 1500 kcal·day⁻¹ (50–55% carbohydrates, 30–35% total fat, <7% saturated fat and 15–22% protein), distributed across 3-4 meals per day.
2.5 Data collection and outcome measures

The outcome measures were assessed at baseline and 12-week follow-up by personnel who were blinded to the treatment allocation. The data were recorded on standardized forms and entered into a secured Microsoft Excel Access database that included quality control checks (e.g., range checks, notifications of missing data).

Anthropometric and body composition variables were collected at the same time in the morning, between 7:00 a.m. and 10:00 a.m. Body weight and height were measured following standard procedures with an electronic scale (Tanita® BC544, Tokyo, Japan) and a mechanical stadiometer platform (Seca® 274, Hamburg, Germany), respectively. BMI was calculated as body weight in kilograms divided by the square of height in meters (kg/m²). Waist circumference (WC) was measured at the narrowest point between the lower costal border and the iliac crest using a tape measure (Ohaus® 8004-MA, New Jersey, USA). In cases in which this point was not evident, WC was measured at the midpoint between the last rib and the iliac crest [43]. We measured each variable twice and used the average unless the first and second measures varied ≥ 1%. In such cases, we used the median of three measurements. In all measures, we found very good test-retest reliability [body weight (intra-class correlation, ICC = 0.983), height (ICC = 0.973), BMI (ICC 0.897), and WC (ICC = 0.967)]. The percentages of body fat mass and lean mass were obtained using the Tetrapolar Bioelectrical Impedance Analysis (BIA) system (SECA mBCA 515®, HANS E. RÜTH S.A, Hamburgo Alemania), with subjects standing barefoot on the metal contacts. This method was previously validated by experts in the field [42]. Our lab’s analysis showed strong agreement between the two methods as reflected in the range of BF%. This result shows that BIA and dual-energy X-ray absorptiometry are comparable methods for measuring body composition with higher or lower body fat percentages (unpublished data). Before testing, the participants were required to adhere to the following instructions from the BIA manufacturer [42]: i) not to eat or drink within 4 h of the test, ii) not to consume caffeine or alcohol within 12 h of the test, iii) not to take diuretics within 7 days of the test, iv) not to perform physical exercise within 12 h of the test, and v) to urinate within 30 min of the test. BIA measurements were performed at 50 kHz with a 0.8 mA sine wave constant current under standard conditions [42]. The measurement was made twice, and the average value was used. Inter-observer variability was R=0.89. BIA has been extensively used as the gold standard against other body composition methods in subjects from the same region of origin as the current participants [42].

Blood pressure was measured using an electronic oscillometric device (Riester Ri-Champion model, Jungingen, Germany) according to the recommendations of the Association for the Advancement of Medical Instrumentation [44]. Prior to blood pressure monitoring, the accuracy of the device was tested using a standard mercury sphygmomanometer in a random subsample (n=25) to ensure that there was no consistent difference (>10 mmHg) in blood pressure. To calculate the mean arterial pressure, the diastolic blood pressure was added, and the sum was added to the systolic blood pressure. Inter-observer variability was R=0.96.
Blood samples were collected between 5:30 and 7:00 am by two experienced phlebotomists after ≥ 12 h of fasting. Blood samples were obtained from an antecubital vein, and analyses were subsequently completed within one day of collection. The biochemical profile included plasma lipid triglycerides, total cholesterol, HDL-c, LDL-c, and glucose (measured by enzymatic colorimetric methods). Inter-assay reproducibility (coefficient of variation) was determined via 10 replicate analyses of 5 plasma pools over 15 days and was 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. Additional outcomes in this study were participant adherence and adverse events. Total exercise time was defined as the total time spent on exercise training during the study. Data on participant adherence to the prescribed exercise training variables are expressed in the intervention section.

We used the revised International Diabetes Federation (IDF) [45] criteria for MetS: (i) increased waist circumference (males ≥ 94 cm and females ≥ 80 cm); (ii) increased triglycerides (≥ 150 mg/dl); (iii) reduced HDL-c (males < 40 mg/dl and females < 50 mg/dl); (iv) increased blood pressure (≥ 130 mm Hg systolic or ≥ 85 mm Hg diastolic); and (v) increased fasting glucose (≥ 100 mg/dl). To test the effects of exercise training on MetS, we used a continuous Z-score, rather than a series of dichotomous scores. This concept has been proposed by other researchers to represent and detect overall metabolic changes more accurately for several reasons [22,45]. Firstly, the continuous score would be more sensitive to small and large changes that do not change the IDF criteria [22]. Secondly, the continuous score would be less sensitive to small changes that occur in the vicinity of the diagnostic criteria for any one variable [22]. Thus, composite continuum score of MetS risk has been observed in several adult studies and has been demonstrated to be a good method to assess overall cardiometabolic risk [34]. The MetS Z-score was calculated from individual subject data, IDF criteria [45], and standard deviations using data from the entire subject cohort at baseline. The equation used was MetS Z-score = ([♂40 or ♀50 – HDL-c]/SD multiplied by (-1)) + ([triglycerides – 150]/SD) + ([fasting plasma glucose – 100]/SD) + ([WC – ♂94 or ♀80]/SD) + ([mean blood pressure – 100]/SD).

2.6 Statistical Analysis

To retain the data of all randomly allocated participants, an intention-to-treat analysis (all randomly assigned patients) was performed. Prior to the planned statistical analyses, a preliminary analysis was conducted (Kolmogorov-Smirnov test) to confirm the normality of the data. Once it was confirmed that the sample data satisfied the normality assumption, statistical analyses relevant to our main research interest were conducted. t-tests for continuous variables and Chi-square for categorical variables were used to investigate any possible differences in baseline characteristics and adherence between the groups. We used a generalized linear model (GLM) to analyze the influence of the different doses of exercise training on MetS components and body composition outcomes with repeated measures [2 (group) x 2 (test time)]. Inter-group differences in changes with time were tested using the impaired t-test. Cohen’s d effect sizes (ES) were also calculated to determine the magnitude of the group differences. ES was
classified as small, medium, and large as <0.20, 0.2–0.6 and 0.6–1.2, respectively [46]. The significance of the interactions effects between variables was tested using Spearman correlation analyses and denoted as $r_s$. All reported P values were two-sided (P<0.05). Statistical analyses were conducted using PASW Statistics 17 for Windows (SPSS, Inc., Chicago, Illinois).

3. Results

The baseline characteristics of the MCT group, HIT group and total sample are outlined in Table 1. The t-test or Chi-square indicated that no statistically significant differences in the baseline characteristics (P>0.05) existed between the groups.

Table 1. Baseline participant characteristics

<table>
<thead>
<tr>
<th></th>
<th>Total sample (n = 20)</th>
<th>MCT (n = 9)</th>
<th>HIT (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8 (40.0)</td>
<td>5 (55.6)</td>
<td>3 (27.3)</td>
</tr>
<tr>
<td>Female</td>
<td>12 (60.0)</td>
<td>4 (44.4)</td>
<td>8 (72.7)</td>
</tr>
<tr>
<td>Age, mean (sd), y</td>
<td>31.8 (7.8)</td>
<td>31.4 (6.4)</td>
<td>32.1 (9.0)</td>
</tr>
<tr>
<td>Race/ethnicity, N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black or Afro-Colombian</td>
<td>18 (90.0)</td>
<td>7 (77.7)</td>
<td>11 (100)</td>
</tr>
<tr>
<td>Others (Indigenous)</td>
<td>2 (10.0)</td>
<td>2 (22.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Socioeconomic level, N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-mid</td>
<td>11 (55.0)</td>
<td>5 (55.5)</td>
<td>6 (54.5)</td>
</tr>
<tr>
<td>Mid-high</td>
<td>9 (45.0)</td>
<td>4 (45.5)</td>
<td>5 (45.4)</td>
</tr>
<tr>
<td>Education, N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>1 (5.0)</td>
<td>0 (0.0)</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>Technical</td>
<td>1 (5.0)</td>
<td>1 (11.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>University</td>
<td>18 (90.0)</td>
<td>8 (88.9)</td>
<td>10 (90.9)</td>
</tr>
<tr>
<td>Occupation, N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Student/work</td>
<td>15 (80.0)</td>
<td>7 (77.7)</td>
<td>9 (81.8)</td>
</tr>
<tr>
<td>Housewife</td>
<td>5 (20.0)</td>
<td>2 (22.3)</td>
<td>3 (18.2)</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>4 (20.0)</td>
<td>3 (33.3)</td>
<td>1 (10.0)</td>
</tr>
<tr>
<td>Married/de facto</td>
<td>16 (80.0)</td>
<td>6 (66.3)</td>
<td>10 (90.0)</td>
</tr>
<tr>
<td>Height, mean (sd), m</td>
<td>1.67 (0.06)</td>
<td>1.69 (0.05)</td>
<td>1.68 (0.09)</td>
</tr>
</tbody>
</table>

BMI, body mass index

Table 2 list the effects of the exercise interventions on MetS components. For MetS Z-score a significant main effect of time was observed in MCT (P=0.009, ES=0.82) and HIT (P=0.015, ES=0.55) groups. The difference between groups was −2.795 [95% CI: 1.276-4.311, P=0.001]; time x group (P=0.001). In addition, we calculated the frequency of the MetS risk factors at each time point and the average number of MetS risk factors for each training group. The average number of cardiometabolic risk factors changed by −0.133 in the MCT group (P=0.040); ES=0.67 and 0.018 in the HIT group (P=0.294); ES =0.13 (no significant difference between groups= −0.152; P=0.227). There was a significant increase in fasting glucose from week 0 to week 12 in the MCT group (P=0.039); ES=0.19 and the HIT group (P=0.001); ES=0.29. Although the t-test did not reveal significant differences between the groups (1.6 mg [95% CI: −8.5 -11.8; P=0.078),
a meaningful ES increase was observed in favor of the MCT group, ES=1.19. Mean blood pressure significantly decreased from week 0 to week 12 in the HIT group (P=0.019 ES=0.24), as did WC (P=0.006 ES=0.27) and TG (P=0.012 ES=0.39) in the MCT group.
### Table 2. Intent-to-treat analysis of IDF criteria for MetS characteristics and body composition at baseline and changes after 12 weeks

<table>
<thead>
<tr>
<th></th>
<th>Groups</th>
<th>From Baseline to 12-week, Mean (95% CI)</th>
<th>MCT effect p value (ES)</th>
<th>HIT effect p value (ES)</th>
<th>Time x group p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
<td>Whiting-Group Changed</td>
<td>Between-Group Difference in Change</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MCT (n = 9)</td>
<td>HIT (n = 11)</td>
<td>MCT (n = 9)</td>
<td>HIT (n = 11)</td>
<td></td>
</tr>
<tr>
<td>MetS Z-score</td>
<td>0.668 (1.856)</td>
<td>-2.351 (2.384)*</td>
<td>1.546 (1.575)</td>
<td>-1.249 (-1.629)</td>
<td>0.009 (0.82)</td>
</tr>
<tr>
<td></td>
<td>0.878 (-0.112)</td>
<td>(1.998) (2.344)</td>
<td>(-1.133) (-0.152)</td>
<td>(-0.294 to -0.004)</td>
<td>0.015 (0.294)</td>
</tr>
<tr>
<td>Average risk factors per subject</td>
<td>0.244 (0.260)</td>
<td>0.164 (0.121)</td>
<td>(0.200)</td>
<td>0.018</td>
<td>0.227 (0.14)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>81.9 (12.2)</td>
<td>75.4 (7.6)</td>
<td>(3.0)</td>
<td>0.3</td>
<td>0.006 (0.085)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>170.1 (41.8)</td>
<td>159.4 (47.4)</td>
<td>(37.4)</td>
<td>33.9</td>
<td>0.102 (0.211)</td>
</tr>
<tr>
<td>High-density lipoprotein (mg/dL)</td>
<td>43.0 (14.1)</td>
<td>46.9 (9.6)</td>
<td>(6.8)</td>
<td>10.4</td>
<td>0.074 (0.422)</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>134.1 (82.2)</td>
<td>100.4 (36.8)</td>
<td>-23.8</td>
<td>-8.0</td>
<td>0.012 (0.387)</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>82.3 (13.7)</td>
<td>78.3 (5.6)</td>
<td>(65.1)</td>
<td>30.9</td>
<td>0.455 (0.20)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>116.8 (5.1)</td>
<td>116.2 (6.5)</td>
<td>(14.7)</td>
<td>5.8</td>
<td>0.039 (0.001)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>72.3 (7.0)</td>
<td>71.0 (8.7)</td>
<td>(-3.8)</td>
<td>-3.7</td>
<td>0.014 (0.029)</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>87.3 (6.0)</td>
<td>86.0 (7.6)</td>
<td>(-3.8)</td>
<td>-3.9</td>
<td>0.222 (0.283)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.3 (15.3)</td>
<td>66.8 (10.9)</td>
<td>(-0.6)</td>
<td>-0.1</td>
<td>0.060 (0.019)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.6 (3.6)</td>
<td>25.5 (4.2)</td>
<td>0.2</td>
<td>1.1</td>
<td>0.179 (0.353)</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>49.7 (9.3)</td>
<td>44.9 (5.8)</td>
<td>-0.3</td>
<td>1.1</td>
<td>0.237 (0.007)</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>27.4 (7.3)</td>
<td>31.3 (12.2)</td>
<td>(1.2)</td>
<td>(1.2)</td>
<td>0.001 (0.010)</td>
</tr>
</tbody>
</table>

Data in Mean (SD); BMI, body mass index.
* Difference between groups at baseline.
For various anthropometric and body composition variables and the MetS Z-score after 12 weeks of training are presented in Table 3. Negative correlations were observed between the MetS Z-score, weight ($r_s=-0.627$, $P=0.011$), BMI ($r_s=-0.756$, $P<0.001$) and body fat ($r_s=-0.858$, $P<0.001$) in the HIT group. There were no significant correlations in the MCT group.

**Table 3.** Partial correlation between MetS Z-score and anthropometric/body composition characteristics after 12 weeks of program training

<table>
<thead>
<tr>
<th></th>
<th>MCT</th>
<th>HIT</th>
<th>MCT effect (p value)</th>
<th>HIT effect (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>-0.042</td>
<td>-0.627</td>
<td>0.915</td>
<td>0.011</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>0.001</td>
<td>-0.756</td>
<td>1.000</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>0.150</td>
<td>-0.858</td>
<td>0.700</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>-0.150</td>
<td>0.382</td>
<td>0.700</td>
<td>0.247</td>
</tr>
</tbody>
</table>

Data represent Spearman correlation coefficients. BMI, body mass index

4. Discussion

To our knowledge, this is the first RCT to compare the effects of different modes of exercise training on the clinical risk factor profile for MetS among apparently healthy physically inactive Latin American adults. The present study demonstrates that HIT was a more potent stimulus than MCT at improving a sensitive cluster of MetS risk factors, although it failed to significantly improve individual factors compared with MCT. Additionally, HIT produced stronger and moderately significant changes in MetS Z-score in terms of weight, BMI, and body fat.

There are divergent findings regarding MetS risk factors and HIT compared with MCT programs [11,18,20,47-49]. Our study showed a higher MetS Z-score reduction after HIT than after MCT. The lowering of the MetS Z-score by supervised training is similar to what others have found in at risk patients [22,50-52]. In addition, we found that HIT or MCT significantly reduced individual risk factors as others have found previously [22,52]. These include reducing triglycerides levels, fat mass, abdominal obesity and mean blood pressure [2]. However, the MCT group had a higher baseline MetS Z-score than the HIT group, resulting in a greater improvement (ES=0.82). In contrast to the current results, the RUSH-Study, which was performed with 81 middle-aged healthy men, showed similar positive effects on the MetS Z-score when HIT and MCT were compared [50]. However, in the aforementioned research, the HIT intervention included work intervals 3-fold longer than in the current study and thus a more prevalent aerobic component in the former, closer to MCT-induced adaptive loads [18].

Regarding unhealthy populations, studies have shown divergent findings. Confirming our results, Tjønna et al. [51] observed fewer subjects with MetS and fewer MetS risk factors in adults diagnosed with MetS after 16 weeks of HIT compared with MCT. In contrast, Johnson et al. [52] did not confirm the superiority of HIT compared with MCT in overweight and obese populations. Similarly,
Earnest et al. [53] observed similar improvements in the MetS Z-score and the number of MetS risk factors between overweight males who participated in HIT and MCT. Due to methodological differences across studies (i.e., sex; age; initial health, weight and fitness status; prescribed medication; type and intensity of exercise, or interval duration; length of the exercise program) and the impact of such differences on outcomes [54-56], it is difficult to draw general conclusions. These and other possible factors need to be studied. The mechanism through which HIT had a greater effect than MCT on metabolic biomarkers compared to MCT is not clear. In the current study, participants in the HIT completed 4×4 min of exercise up to 95% of HRmax three days per week for 12 weeks, while the MCT group trained at only 55-75% of HRmax. In this context, we speculate that both training intensities might induce additive improvement in the oxidative metabolism–dependent energy system, metabolic capacity, qualitative profile of skeletal muscle fiber type, muscle mass and fiber diameter [54-56], although with potentially greater impact after HIT than after MCT. Further research is needed to reach a consensus.

No differences (time x group) were found in single MetS risk factors changes between HIT and MCT, although a significant increase in fasting glucose from baseline to post-exercise training was observed in both groups. However, levels of fasting glucose were within the healthy range. Although there are limitations to comparing Cohen’s scores in our study, the Cohen’s $d$ value suggests important clinical applicability. Overall, we were unable to detect consistent superiority of HIT versus MCT programs (or vice versa) on MetS in healthy adults [18].

Furthermore, the beneficial effects of exercise on MetS Z-score were achieved without concomitant lean mass gainer, however, a decrease in fat mass was associated with reductions in the MetS Z-score ($r_s=-0.858, P<0.001$) in the HIT group, which emphasizes meaningfulness of this change in body composition. Interestingly, the WC decreased significantly in both groups, however, $t$-test did not reveal significant differences between the groups. Changes in body composition, or more precisely, changes in abdominal obesity and fat mass seem to be an important factor when an exercise intervention for reducing CVD markers is planned. In the present study we showed that a significant reduction in MetS Z-score is possible also in the absence of change in lean mass.

The strengths of this study included the use of a novel Z-score to evaluate the effects of different exercise programs on the risk of MetS; this scoring method provides an increased level of sensitivity. Each subject completed at least 32 of 36 exercise sessions, and researchers supervised each session while the subjects’ HR was being monitored.

A primary limitation of this study was the lack of a true non-exercise control group. Thus, we were unable to determine causality in our interpretation of the observed exercise-induced improvements in cardiometabolic health parameters within the groups. Second, as a common tool to assess body weight and relevant body composition parameters, BIA was used in the present study. However, it is not
the “gold standard” body composition measure. Due to this and other limitations (e.g. relatively small sample size; single site design), it will be important not to over-interpret the results of this RCT. Lastly, we cannot determine the directions of the associations nor causality observed in this study with absolute certainty. Future studies may consider tighter regulation of these factors to control their effects during a relatively longer intervention.

5. Conclusion

HIT and MCT offer similar metabolic and cardiovascular protection against single MetS risk factors but not the average risk factors per subject. These effects could be enhanced with a reduction in fat mass that was observed only when HIT was performed. Thus, the improvement in the cardiovascular profile achieved in the present study may be an effective strategy for reduction in MetS Z-score and improving the health trajectory of physically inactive adults.

6. References


Chapter 4

Effect of Moderate Versus High-Intensity Interval Exercise Training on Heart Rate Variability Parameters in Inactive Latin-American Adults: A Randomised Clinical Trial
1. Introduction

Disorders of the autonomic nervous system have a key pathophysiological role in the early stages of essential hypertension, myocardial infarction and chronic heart failure by producing coronary vasoconstriction, increasing cardiac oxygen consumption and leading to fatal events [15,56]. Heart rate variability (HRV) is of increasing interest because it is a marker of cardiovascular autonomic function and because reduced HRV is a direct predictor of cardiovascular risk and all-cause mortality [13,58]. Additionally, the parasympathetic withdrawal quantitated by HRV is associated with reduced coronary flow reserve and antedates episodes of dynamic myocardial ischemia [37]. HRV refers to the periodic changes in heart rate and serves as an index of the activity level of the autonomic nervous system [26].

HRV can be evaluated by time and frequency domain indices. Accordingly, it can be represented in a time domain in which R-R intervals (in milliseconds) are plotted against time (in seconds) [53]. Among the most used indices, the standard deviation of normal beat-to-beat (R–R) intervals (SDNN) has been suggested to reflect global variability, and the root-mean-square of successive R–R intervals (rMSSD) and high-frequency (HF) power have been linked to vagal activity [8].

Strong evidence shows that physical inactivity, i.e., <150 min/wk. of moderate activity or 75 min/wk. of vigorous activity, can increase the risk of many adverse health conditions, including major non-communicable diseases such as coronary artery disease (CAD), metabolic syndrome and breast and colon cancers [9,34]. Currently, physical inactivity has a deleterious effect that is comparable to smoking and obesity. It is now recognised as the fourth-leading risk factor for global mortality and accounts for 6% of all deaths [36]. Furthermore, physical inactivity is associated with decreased HRV, particularly HF power, thus reflecting reduced cardiovascular autonomic control [24]. Experimental studies have indicated that sedentary time results in alterations of cardiovascular health and HRV and during prolonged bed-rest [25,28,54].

On the other hand, cross-sectional reports in different population suggest that regular aerobic exercise training is associated with improved HRV [1], however, studies examining the effect of moderate or high intensity on HRV are mixed and incomplete with respect to clinically recommended training paradigms. In the clinical setting, previous systematic reviews have found that inactivity physical adults who participate in supervised interval training can experience improvements in HRV, exercise capacity, quality of life, maximal oxygen consumption (VO₂max) and cardiac remodelling [3,18,50]. Additionally, high-intensity exercise has been shown can positively modify the sympathovagal control of HRV toward facilitating a persistent increase in parasympathetic tone, known to be associated with a better prognosis in non-communicable diseases patients [50].

Interestingly, few studies showed comparable or superior improvements in cardiovascular function using low-volume, high-intensity training (HIT) compared to traditional moderate continuous training (MCT) [18,23,47,50,59]. However, the effects of MCT, HIT or a combination of the two (MCT/HIT) program on HRV
indices, which is clinically the current standard in inactive adults, has yet to be established [5,12,21,42].

In the Latin-American population, a region that has undergone a well-documented epidemiologic transition and epidemic of obesity [30,40,52], relatively little research on physical activity [49] and physical fitness exists [17,41,48]. A randomised clinical trial comparing different intensities of exercise training in inactive adults is clinically relevant because it could provide evidence for a precise, prescribed intensity of exercise training for optimal outcomes in this population [20,33,34,59]. Given this knowledge gap, the aim of the current randomised clinical trial was to compare the effect of MCT versus HIT on HRV indices in physically inactive adults.

2. Materials and Methods

2.1 Experimental Approach to the Problem

The HIIT-Heart Study is a substudy of the ‘High Intensity Interval- vs Moderate Training on Biomarkers of Endothelial and Cardiovascular Health in Adults’ study (registered at ClinicalTrials.gov, registration number: NCT02738385) in which the aim was to compare the efficacy of different volumes of HIT and traditional training in reducing risk factors constituting the CAD. In this substudy, we report changes in HVR variables that were only recorded at our local site (Bogotá, Colombia). A participant flow diagram is shown in Figure 1. The overall objective of the substudy “HIIT-Heart” is to quantify the dose-effect of different exercise intensities (i.e. moderate-intensity and low volume vigorous-intensity), on HRV indices (primary outcome), and on physiologic response (heart rate, blood pressure), body composition (BMI, waist circumference, body fat, lean mass) and VO₂peak in adults (secondary outcomes).

2.2 Participants and recruitment

This randomised clinical trial was conducted at the University of Rosario in Bogota and Santo Tomás University, Colombia, between February 2015 and May 2016. Primary and secondary 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 [11].

Participants were recruited from the Centre of Studies in Physical Activity Measurements (in Spanish, CEMA) by posting study recruitment flyers at community centres, by study recruitment announcements at CEMA and by word-of-mouth. Subjects are eligible to participate if they are located in the metropolitan region with available time (1 hour per day) to support the trial. Additional eligibility criteria include participants were aged 18–45, were inactive (<150 min/wk. of moderate-intensity activity or 75 min/wk. of vigorous-intensity activity), had a body mass index ≥18 and ≤30 kg/m² and identified as being willing and having almost immediate availability.

Risks were minimised by ruling out contraindications to the testing and training protocols via a health history and a thorough physical examination before
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 [35]. 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), 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 ≥118 mg/dL) or use of any prescribed drugs, any active use of illegal or illicit drugs or inability to participate due to a physical impairment. In addition, we confirmed by two exercise physiologists, subjects if they had alteration in ventricular function and/or cardiomyopathy, through a standard 12-lead ECG at rest and every 3-min of the maximum treadmill exercise test. All subjects remained under usual medical care and clinical follow-up (i.e., regular appointments with a physician) throughout the protocol. Written informed consent was obtained for all subjects, and ethical approval was granted by the local office for Research Ethics Committee at University of Santo Tomás (ID 27-0500-2015). Additionally, each participant completed an informed consent document outlining the experiment that was approved by the Institutional Review Board. The study conforms to the principles outlined in the Declaration of Helsinki.

2.3 Blinding and randomisation

Randomisation into the two study arms was performed by the CEMA at University of Rosario, Bogotá, Colombia, using block randomisation with block sizes of four. As each participant consecutively entered this randomised clinical trial, he/she was randomly allocated to either the MCT or the HIT group according to the computer-generated allocation sequence. The randomisation 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. Furthermore, 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.

2.4 Interventions

The participants assigned to the intervention group participated in the cardiometabolic programme as recommended by the Colombian guidelines COLDEPORTES (in Spanish, Departamento Administrativo del Deporte, la Recreacion, la Actividad Fisica y el Aprovechamiento del Tiempo Libre) [29], and AHA [35,39] for cardiovascular health promotion and disease reduction. At the beginning of the training protocol, we obtained the participants' weight to determine the weekly energy expenditure necessary to achieve their target of 12-kcal·kg·
1·week⁻¹ (iso-energetic). It was expected that the gradual increase in total energy expenditure would minimise fatigue, soreness, injuries and attrition.

After inclusion, patients performed a maximal cardiopulmonary exercise test on a maximum treadmill exercise test (Precor TRM 885, Italy) following the modified Balke protocol [1] and physiological parameters (\(\dot{V}O_2\), HR and Borg ratings) from the test were used to set exercise intensity. Based on averaged HRmax and \(\dot{V}O_2\)peak, the participants were classified according to normative values, referenced to age and sex [1,39]. MCT and HIT interventions lasted 12 weeks, with three sessions per week, consisting in fast walking or running on a treadmill with the deck inclined to reach the desired intensity. HR was recorded each session using a HR monitor (Polar Electro, Kempele, Finland). In addition, rating of perceived exertion (RPE) were also measured in each exercise session. An initial 2-week preparatory phase of training was performed to bring participants up to a 6-kcal·kg⁻¹·week⁻¹ goal (~150 kcal per session), which was increased progressively 2-kcal·kg⁻¹·week⁻¹ until week 4, and was then maintained at 12-kcal·kg⁻¹·week⁻¹ for weeks 5 to 12 (~300 kcal per session).

**Moderate-continuous training (MCT) group**

Exercise training sessions were designed to elicit a response in the acceptable moderate-to-vigorous range, i.e., 55–75% HRmax/RPE of 11–15 on Borg scale. Sessions consisted of a warm-up (5 min), followed by 15-55 min of treadmill walking/running (15-35 min during the 2-week preparatory phase), and a final relaxation/cool-down period (10 min).

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

We calculated the training energy expenditure for participants’ age ranges associated with meeting the consensus public health recommendations from the Cardiometabolic HIIT-RT Study [47]. A complete description of the design and methods has been published elsewhere [47]. During the 2-week preparatory phase subjects warmed up at 65% HRmax (5 min), then perform 4 × 4 min intervals at 60–80% HRmax/RPE of 13–15 on Borg scale, interspersed with 4 min of recovery at 55% HRmax/RPE of 11–13 on the Borg scale. During weeks 3-12 subjects perform 4 × 4 min intervals at 85–95% HRmax/RPE of 15–17 on Borg scale (with the target zone maintained for at least two minutes), interspersed with 4 min recovery at 65% HRmax, and a cool-down (5 min), with a range total exercise time of 35 to 55 min (with warm-up and cool-down).

Both groups were required to attend two supervised sessions with an exercise physiologist at the University of Rosario at a fitness centre “CEMA”, which contained the treadmills needed to complete the prescribed exercise programmes. 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 this study. 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 both the HR and the minutes spent exercising during the training sessions. Trainers were physical therapists and physical educators with experience developing and monitoring exercise programmes among clinical populations. 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, a maximum of 3–5 participants were trained simultaneously. Each participant met with the study dietician for nutrition assessment and counselling. An individualised nutrition intervention plan was developed from the baseline food intake assessment according to participant preferences [47]. This plan consisted of a standardised meal consisting of 1300 to 1500 kcal (50–55% carbohydrates, 30–35% total fat, <7% saturated fat and 15–22% protein).

2.5 Experimental procedure

Prior to the procedure, participants were instructed to refrain from strenuous activities for at least 48 h, and caffeine and alcohol for at least 24 h before all tests. Subsequently, participants reported for testing following an overnight fast, consuming only water, and refraining supplement intake that morning. All measurements were tested on two different days in climate controlled room between the hours of 07:00 and 10:00 h.

Primary outcome measures

The primary outcome measure was HRV measured between 07:00 and 08:00 h for 25 minutes in a semi-dark room (22–23°C) following a 12-hour fast. HRV measurements were conducted at the same time (±1 hour) of day for each assessment period. We used a two-channel ECG signal detected by a Heart Rate Monitor (Polar Electro, Kempele, Finland) and transmitted online to a PC through Polar Advantage Interface receiver. We quantified HRV from the last 5 minutes of R–R interval recording. First, we examined the parasympathetic nervous system by calculating the square root of the mean of the sum of the squares of differences between adjacent R–R intervals (rMSSD). rMSSD is considered to be a stable measure of parasympathetic modulations of heart rate [14]. Mean R–R intervals were recorded at a rate of 250 Hz. Second, SDNN was measured, reflecting the cyclic components responsible for variability in the period of recording and reflective of both sympathetic and parasympathetic tone. Third, frequency domain was analysed in three absolute and log normalized frequency bands define as: HF and LF. In the frequency domain, oscillations of RR intervals were examined within the low-frequency (LF: 0.04–0.15 Hz) and high-frequency bands (HF: .0.15–0.40 Hz). The sympatho-vagal balance was obtained by the ratio of the power LF to HF (LF/HF) bands. All index are described and used in previous reports [14,45,53]. The resulting R–R intervals were analysed in the time domain, in the frequency domain using spectral analysis (Fast Fourier Transform), and nonlinearly through the Poincare’ plot (Kubios HRV Analysis v 2.0, Biosignal Analysis and Medical Imaging Group at
the Department of Applied Physics, University of Kuopio, Kuopio, Finland). In-house testing revealed near perfect ($r = 0.99$) correlations between these methods and electrocardiographs. These differences were also similar to what was reported by other applications that assess HRV via heart rate monitors. The reproducibility of 24-hour derived HRV indices was ICC = 0.86 to frequency domain parameters and ICC = 0.95 to time domain parameters.

**Secondary outcomes**

**Anthropometric and body composition measurements:** Body weight was measured using electronic scales (Tanita® BC544, Tokyo, Japan) with a low technical error of measurement (TEM = 0.510%). Height was measured using a mechanical stadiometer platform (Seca® 274, Hamburg, Germany; TEM = 0.01%). Body mass index (BMI) was calculated as the body weight in kilogrammes divided by the square of height in meters ($kg/m^2$). The waist circumference (WC) was measured at the narrowest point between the lower costal border and the iliac crest using a tape measure (Ohaus® 8004-MA, New Jersey, USA; TEM = 0.05%). In cases where this point was not evident, it was measured at the midpoint between the last rib and the iliac crest. 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. The percentages of body fat mass and mass muscle were obtained using the Tetrapolar Bioelectrical Impedance Analysis (BIA) system (BF-350, Tanita Corp, Tokyo, Japan). Before testing, participants were required to adhere to these BIA manufacturer’s instructions: 1) to not eat or drink within 4 h of the test; 2) to not consume caffeine or alcohol within 12 h of the test, 3) to not take diuretics within 7 days of the test; 4) to not do physical exercise within 12 h of the test, and; 5) to urinate within 30 min of the test. An electrical current of 50 kHz was passed through the participant and resistance and reactance were measured. To ensure data quality, the equipment was calibrated daily using a known calibration standard, in accordance with the manufacturer’s instructions [6]. 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. The reproducibility of our data was R=0.98.

**Cardiorespiratory fitness:** $\dot{V}O_2$peak was determined using a maximum treadmill exercise test (Precor TRM 885, Italy) following the modified Balke protocol, which has been extensively used [47] 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 [47]. 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 AHA guidelines for ‘Indications for Terminating Exercise Testing’ [35]. Maximal oxygen uptake was defined as the highest recorded $\dot{V}O_2$peak after two of three criteria were met: 1) a plateau in VO$_2$ 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 [4,49]. The reproducibility of our data was R=0.92.

**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 [55]. Two blood pressure readings were taken separated by a 10-min interval. Inter-observer variability was R=0.96.

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. These data 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 presented in the interventions section. However, self-reported physical activity was measured using the recent physical activity questionnaire. 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 [4]. The outcome was assessed in METs (units of metabolic equivalence) per week. This questionnaire was administered immediately before and after the training period and at 12 weeks following the completion of the exercise intervention.

### 2.6 Statistical Analysis

To retain data of all randomly allocated participants, an intention-to-treat analysis population (all randomly assigned patients) was performed. Before the planned statistical analyses, a preliminary analysis was conducted (Shapiro–Wilk test) to confirm data distribution normality. Primary and secondary outcomes values are reported as mean (±) standard deviation (SD) or 95% confidence interval (CI 95%) unless otherwise specified. Due to their skewed distribution, the following variables were log-transformed before analyses: SDNN, rMSSD, HF, LF and LF/HF ratio. To aid interpretation, data were back-transformed from the log scale for presentation in the results.

Adherence to the exercise program for both groups was expressed as the total number of training days that each participant completed of the prescribed number of training days and total exercise time during the 12-week supervised exercise program. Once it was confirmed that the sample data satisfied the
normality assumption, statistical analyses relevant to our main research interest were conducted. A t-test was used to investigate any possible differences in baseline characteristics and adherence between the groups. We used a generalised linear model (GLM) to analyse the influence of the differing doses of exercise training on HRV characteristics. The Dunnett-Hsu test allows for specific multiple pair-wise comparisons while still protecting against type I statistical errors.

Change between the pre- and post-measures was calculated for each outcome variable of interest. For normal data, effect sizes (ES) were calculated using Cohen’s d (between group: \( d = \frac{M_1 - M_2}{spooled} \), where \( M_1 \) and \( M_2 \) are the mean changes (\( M_{post} - M_{pre} \)) for each group, and spooled is the pooled standard deviation of changes from each group; within group: \( d = \frac{M_d}{S_d} \), where \( M_d \) is the mean difference from pre-to-post and \( S_d \) is the standard deviation of differences between subjects), which was defined as small, medium, and large for 0.20, 0.50, and 0.80, respectively. Finally, we used a subgroup analysis to compare dose-response effects across predefined baseline groups. Significance of interaction relationships between variables was tested using a Spearman correlation analysis and denoted as \( r_s \). All reported P values are two-sided (\( P < 0.05 \)).

In order to determine TEM for HRV indices, blood pressure and cardiorespiratory fitness, a experiment trial involving six recreationally active participants (Four males; two females, age, 29±1 yrs; BMI, 26±9 kg/m\(^2\); \( \text{VO}_2\text{peak} \), 38±7 ml·kg·min\(^{-1}\)) reported to the lab on two separate occasions separated by at least a week and the resulting values were utilized to calculate TEM (data no shown). The body composition variables were used as the indicator of precision by TEM. It is based on at least two measurements taken of the same subject by the same observer (intraobserver variability). The TEM was estimated from the results of an ANOVA as the square root of the within-subject mean square.

The coefficient of reliability (R) and ICC estimates the proportion of between-subject variance in a measured population that is free from measurement error to HRV indices, blood pressure and cardiorespiratory fitness. Measures of ‘R’ can be used to match the relative reliability of different anthropometric measurements, as well as of the same measurements in different age groups and to estimate sample size requirements in anthropometric studies.

‘R’ as a percentage (R%) was calculated using the following equation: \( R \% = 1 - (\text{total TEM}^2 / \text{SD}^2) \). Intraclass correlation coefficients (ICC) were also determined from the mean square values of the ANOVA. In order to establish criteria to categorize participants as ‘responders’ or ‘non-responders’, the biological variability (i.e. ICC and R) were established to determine the TEM. In this study, non-responses were determined using two times the TEM of measurement for SDNN (0.007 ms), rMSSD (0.003 ms), and rr interval length (0.005 ms). Statistical analyses were conducted using SPSS version 22 (IBM, Armonk, New York, USA).
3. Results

Table 1 and Figure 1, lists the effects of the exercise interventions on HRV and physiological parameters. Difference between groups were observed on SDNN change, with 3.4 (8.9) ms in the MCT group and 29.1 (7.6) ms in the HIT group (difference between groups 32.6 [95% CI, 24.9 to 40.4; p = 0.01]; d = 1.14 [95% CI, 0.19 to 2.00]) and in the LF/HF ratio, with a change of 0.5 (0.9) ms in the MCT group and 0.5 (0.4) ms in HIT group (P between groups = 0.016; d =0.01 [95% CI, −0.88 to 0.88]). Additionally, the percentage of body fat did not change in the MCT group (0.8); d = 0.01 [95% CI, −0.92 to 0.92], whereas it decreased by 1.1 percent in the HIT group −1.1 (1.5); d = 0.10 [95% CI, −0.73 to 0.93], (difference between groups 1.2 [95% CI, 0.1 to 2.4 P = 0.04]; d = −0.88 [95% CI, −1.81 to 0.03]). There were no significant treatment effects on other parameters.

Figure 1. A and B, within-subject effect sizes (Cohen’s d ± 95% CI) following 12 weeks of program training by groups. B and D, magnitude-based effect sizes (ES ± 95% CI) between-group difference in change. ■ HIT group; ● MCT group.
Table 1. Intent-to-Treat Analysis of indices of HRV and physiologic characteristics at baseline and changes after 12 weeks

<table>
<thead>
<tr>
<th>Time domain</th>
<th>Baseline</th>
<th>Follow-up</th>
<th>From Baseline to 12-week, Mean (95% CI)</th>
<th>MCT effect (p value)</th>
<th>HIT effect (p value)</th>
<th>Time x group (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Groups</td>
<td></td>
<td>Whiting-Group Change</td>
<td>Between-Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MCT (n = 9)</td>
<td>HIT (n = 11)</td>
<td>MCT (n = 9)</td>
<td>Difference in Change</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time domain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td>65.2 (21.6)</td>
<td>74.9 (35.5)</td>
<td>61.7 (12.6)</td>
<td>3.4 (8.9)</td>
<td>29.1 (7.6)</td>
<td>32.6 (24.9 to 40.4)</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(11)</td>
<td>(9)</td>
<td>(9)</td>
<td>(9)</td>
<td>(9)</td>
</tr>
<tr>
<td>rMSSD (ms)</td>
<td>58.3 (30.9)</td>
<td>68.8 (39.9)</td>
<td>59.5 (22.1)</td>
<td>1.2 (8.8)</td>
<td>11.4 (11.5)</td>
<td>10.2 (0.3 to 20.1)</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(11)</td>
<td>(9)</td>
<td>(9)</td>
<td>(9)</td>
<td>(9)</td>
</tr>
<tr>
<td>Frequency domain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF (ms)</td>
<td>1673.2 (2074.7)</td>
<td>1547.8 (1399.7)</td>
<td>652.7 (1349.2)</td>
<td>107.0 (457.3)</td>
<td>759.8 (-148.6 to 1668.1)</td>
<td>0.832 (0.842 0.897)</td>
</tr>
<tr>
<td></td>
<td>(903.5)</td>
<td>(964.5)</td>
<td>(946.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LF (ms)</td>
<td>1422.0 (1141.2)</td>
<td>954.0 (903.5)</td>
<td>184.3 (43.3)</td>
<td>202.3 (176.6)</td>
<td>18.0 (-109.3 to 145.3)</td>
<td>0.697 (0.543 0.245)</td>
</tr>
<tr>
<td></td>
<td>(725.5)</td>
<td>(964.5)</td>
<td>(946.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LF/HF (ms)</td>
<td>1.1 (0.9)</td>
<td>1.5 (1.5)</td>
<td>0.5 (0.9)</td>
<td>0.5 (0.4)</td>
<td>0.05 (-0.61 to 0.71)</td>
<td>0.612 (0.407 0.016)</td>
</tr>
<tr>
<td></td>
<td>(1.8)</td>
<td>(1.9)</td>
<td>(1.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physiologic characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>62.0 (7.6)</td>
<td>58.6 (9.6)</td>
<td>59.3 (9.1)</td>
<td>-2.6 (-1.5)</td>
<td>-5.9 (0.8)</td>
<td>-3.2 (-4.4 to -2.1)</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(9)</td>
<td>(8)</td>
<td></td>
<td>(8)</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>116.8 (5.1)</td>
<td>116.2 (6.5)</td>
<td>113.0 (7.6)</td>
<td>-3.8 (-7.6)</td>
<td>-3.7 (-6.8 to 6.5)</td>
<td>-0.2 (-0.6 to 0.0)</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(9)</td>
<td>(9)</td>
<td></td>
<td>(9)</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>72.3 (7.0)</td>
<td>71.0 (8.7)</td>
<td>67.8 (8.7)</td>
<td>-4.4 (8.5)</td>
<td>-4.0 (6.8)</td>
<td>-0.4 (-7.7 to 6.8)</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(9)</td>
<td>(9)</td>
<td></td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>Anthropometric and body composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>81.9 (12.2)</td>
<td>75.4 (7.6)</td>
<td>79.5 (10.6)</td>
<td>-1.7 (3.0)</td>
<td>0.3 (2.6)</td>
<td>-2.1 (-4.7 to 0.5)</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(9)</td>
<td>(8)</td>
<td></td>
<td>(8)</td>
<td></td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>49.7 (9.3)</td>
<td>44.9 (5.8)</td>
<td>49.4 (8.3)</td>
<td>-0.3 (1.2)</td>
<td>1.1 (1.2)</td>
<td>-1.4 (-2.5 to -0.2)</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(6)</td>
<td>(5)</td>
<td></td>
<td>(5)</td>
<td></td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>27.4 (7.3)</td>
<td>31.3 (12.2)</td>
<td>27.4 (6.6)</td>
<td>0.0 (0.9)</td>
<td>-1.2 (1.5)</td>
<td>1.2 (0.0 to 2.4)</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(9)</td>
<td>(11.5)</td>
<td></td>
<td>(9)</td>
<td></td>
</tr>
<tr>
<td>Cardiorespiratory fitness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO2peak (ml·kg·min⁻¹)</td>
<td>37.1 (7.6)</td>
<td>36.1 (7.6)</td>
<td>43.6 (9.0)</td>
<td>6.5 (9.3)</td>
<td>7.5 (9.3)</td>
<td>1.0 (27.2 to 6.2)</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(9)</td>
<td>(9)</td>
<td></td>
<td>(9)</td>
<td></td>
</tr>
</tbody>
</table>
Spearman correlation ($r_s$) characteristics for various physiologic variables and HRV indices after 12 weeks of program training are presented in Table 2. We observed a moderate negative correlation between BMI and rMSSD in the MCT group ($r_s = -0.667; p < 0.05$).

Table 2. Partial correlation between physiologic characteristics and indices of heart rate variability after 12 weeks of exercise training.

<table>
<thead>
<tr>
<th>Physiologic characteristics</th>
<th>MCT Time Domain</th>
<th>MCT Frequency Domain</th>
<th>HIT Time Domain</th>
<th>HIT Frequency Domain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rMSSD</td>
<td>SDNN</td>
<td>HF</td>
<td>LF</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.619</td>
<td>-0.268</td>
<td>-0.611</td>
<td>-0.159</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>-0.667*</td>
<td>-0.267</td>
<td>-0.633</td>
<td>-0.167</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>-0.385</td>
<td>-0.452</td>
<td>-0.418</td>
<td>-0.435</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>-0.567</td>
<td>-0.517</td>
<td>-0.583</td>
<td>-0.650</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>-0.433</td>
<td>0.050</td>
<td>-0.467</td>
<td>0.183</td>
</tr>
<tr>
<td>Cardiorespiratory fitness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO$_2$peak (ml·kg·min$^{-1}$)</td>
<td>0.323</td>
<td>0.349</td>
<td>0.264</td>
<td>0.162</td>
</tr>
</tbody>
</table>

Data represent Spearman correlation coefficients. * P<0.05 ** P<0.001

BMI, body mass index; SDNN, standard deviation of RR intervals; rMSSD, root mean square successive difference of RR intervals; HF, high frequency spectral power; LF, low frequency spectral power

When analysing the HRV change from baseline to 12-week follow-up, we observed negative correlations between rMSSD and waist circumference ($r_s = -0.747; p < 0.001$), changes in SDNN ($r_s = -0.720; p < 0.05$) and HF$_{Ln}$ ($r_s = -0.700; p < 0.05$), in the HIT group. Finally, we observed a stronger correlation between Ln rMSSD and R-R interval in the HIT group ($r_s = 0.834; p < 0.05$) Figure 2A. We also observed a non-significant correlation between Ln rMSSD and R-R interval in the MCT group ($r_s = 0.396; p = 0.290$), Figure 2B.

Figure 3 shows differences on prevalence of ‘responders’ and ‘non-responders’ based on relevant HRV indices after the 12-week supervised exercise. However, no significant effect size difference was found between ‘responders’ and ‘non-responders’ prevalence for the HRV variables: ∆SDNN (ms) 33.3% versus 63.6% ($d = 0.35$ (CI 95%, $-0.53$ to $1.24$) $p = 0.206$), ∆rMSSD (ms) 44.4% versus 63.6% ($d = 0.19$ (CI 95%, $-0.63$ to $1.03$) $p = 0.180$), and ∆rr interval length (ms) 66.6% versus 63.6% ($d = 0.10$ (CI 95%, $-0.79$ to $0.74$) $p=0.155$) in the MCT and HIT groups, respectively.
Figure 2. The relationship between the R-R interval length and the natural logarithm of the square of the mean sum of the squared differences between R-R intervals (rMSSD) after 12 weeks of program training by groups.
Figure 3. Individual patterns of response following 12 weeks of program training by groups.
4. Discussion

To our knowledge, this is the first randomised clinical trial on the effect of exercise training intensity on HRV in physically inactive adults from the Latin-American population. Our findings suggest that HIT was a more effective medium-term strategy to increase HRV, specifically SDNN and LF/HF ratio, than MCT was. Collectively, the magnitude of the change for both training groups was not significantly different in the remaining parameters, suggesting that either training protocol may provide similar medium-term benefits in cardiovascular health. Additionally, we did not find differences in the ‘responder’ prevalence in relation to improvements HRV in any of these or secondary outcomes.

Increasing attention is being focused on the role of the autonomic nervous system in health and disease [51]. Exercise has been reported to be effective in improving HRV because exercise serves to reduce the activity of sympathetic nervous system while increasing the activity of the parasympathetic nervous system [10]. Specifically, aerobic exercise training increases cardiac vagal modulation via functional and structural adaptations in cardiovascular system (e.g., stroke volume) [38,45]. Our study showed an increase in SDNN in the HIT group compared to the MCT group. SDNN reflects the cyclic components responsible for variability in the period of recording and is reflective of both sympathetic and parasympathetic tone [27]. In this sense, studies in adults with Type 2 diabetes also did not report improvements in HRV after 12 [32] or 16 weeks [16] of aerobic exercise programs, although improvements did occur in programs of 24 weeks [43].

Thus, HRV improvement may be affected by the length of the exercise period. Therefore, HIT seems to favour a greater impact on neurocardiac activity than MCT in the medium-term [44]. The mechanism by which HIT has greater effects on the markers of cardiac autonomic outflow compared to MCT is not clear. It may be that supramaximal exercise generates higher catecholamine concentrations compared to lower-intensity exercise [31]. This finding may contribute to the observed autonomic modulation [1]. Furthermore, the higher catecholamine levels could explain major reductions in percentage of body fat in the HIT group, as catecholamines would stimulate lipolysis, which is primarily responsible for fat release from adipose tissue fat stores [57]. However, the observed increase in HRV is consistent with a study in middle-aged men following 2 weeks of HIT (4–6 x 30 s of all-out cycling efforts with 4-min recovery) compared to aerobic training (40–60 min at 60% of peak workload) [33].

Our findings also indicate differences between groups in LF/HF ratio changes. Although the mechanisms are not clear, this result could be explained by a larger increase in vagal- or baroreflex-mediated modulation of the sinoatrial node with HIT compared with MCT [33]. Additionally, differences in the hemodynamic oscillations experienced during the exercise sessions could be involved as could alterations, according to several authors, of intrinsic HR, S-A node sensitivity [7] and/or alterations of myocardial phenotype [2]. HIT might be an efficient short-term strategy to improve cardiac autonomic function and may
have an important antiarrhythmic effect [22]. Therefore, the results of the present study indicate that the decrease in sympathetic activity after HIT is smaller than the increase in parasympathetic activity.

Another finding of the current study is our demonstration of variability in the individual responses following different training protocols (MCT and HIT). Several reports have recommended that before individuals are classified as responders or non-responders, it is important to determine if variability in the individual responses within the experimental condition are greater than within-subject variation [7,59,60]. Specifically, our results demonstrated that intervention protocols which differ in intensity, time, and metabolic demand, like MCT and HIT, can induce different adaptive responses in HRV indices, blood pressure and cardiorespiratory fitness within a given individual [7]. This indicates that following the same stimulus, some subjects may achieve positive benefits (i.e., responders – ‘R’), whereas other subjects may experience a worsened or unchanged response after training (i.e., non-responders – ‘NR’). Environmental and genetic factors have been described as the main reasons for this phenomenon [7]. Thus, it is relevant to understand the unexplored environmental factors that may be related to eliciting an increased or decreased NR incidence to plan future well-designed genetic studies.

Additionally, we observed a stronger correlation between Ln rMSSD and R-R interval in the HIT group ($r_s = 0.834; p < 0.05$, i.e., positive training adaptation). However, for the first time in this study, we demonstrate how these variables can also change during positive adaptation to HIT. In this context, parasympathetic tone is likely maintained and/or increased. As such, the expected increases in Ln rMSSD (a measure of vagal modulation) were blunted by the high levels of vagal tone and parasympathetic saturation in the case of the HIT group. In this case, vagal saturation and decreases in cardiac parasympathetic indices of HRV after regular training can be related to positive, healthy outcomes [15,22,33,34]. The main changes observed in vagal-related indices with additional HIT training may well be due to the greater training intensity needed for HRV change in healthy participants [22].

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 [19], or generate similar improvements [20]. Contrasting our results, studies using treadmill protocols have not shown any difference in body weight and composition between these isocaloric programmes [7]. Our results support that HIT interventions are superior in terms of enhancing fat oxidation than MCT [19,20]. 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 [37,50] and type 2 diabetic subjects [32]. Considering the sedentary lifestyle of the population, obesity and the risk for non-communicable diseases are increasing, the knowledge of a more effective mode of training (i.e., training modes as endurance, HIT, or other protocols that achieve a reduced amount of responders – ‘R’ prevalence after training interventions), in accordance with the profile of individuals (i.e., physically inactive,
unhealthy individuals, or athletes) and achievement of improvements in their risk factors may be useful information for practitioners, public health exercise programs, and populations with/at risk of CAD. This may positively affect disease morbidity, mortality and health care expenditures [35].

The strengths of this study included state of the art measures of HRV, physical fitness and supervised exercise training in a non-clinical setting. Additionally, adherence to the intervention was approximately 98%. All subjects completed 32 of the 36 exercise sessions, and research technicians supervised each session while HR was being monitored. A primary limitation of this study was the lack of a true non-exercising control group. Thus, we are unable to determine causality in our interpretation of the observed exercise-induced improvements in cardiovascular health parameters. However, among studies comparing HIT and MCT that included a control group, no changes in autonomic function were observed in the control group [44]. Second, BIA was used in the present study as a common tool to assess body weight and the relevant parameters of body composition. However, BIA is not the “gold standard” in body composition measurement. Future studies may consider tighter control of these factors such that the effects of these different factors may be isolated and identified in a relatively longer intervention.

5. Conclusion

These data underline the importance of a multidisciplinary approach aiming at promoting HIT exercise programme in physically inactive adults. For the practitioners/clinicians or trainer working with inactive populations should promote HIT exercise longer than 12 weeks in order to improve outcomes in cardiovascular health, due to HRV is a direct predictor of cardiovascular risk and all-cause mortality. Additional randomised controlled trials are required to elucidate the mechanisms responsible for these results in physically inactive adults and other populations, such as metabolic syndrome, obese, or insulin resistance adults.

6. Reference


Chapter 5

Exercise and Postprandial Lipaemia: Effects on Vascular Health in Inactive Adults
1. Introduction

Postprandial lipemia (PPL) is defined as the elevation of circulating triglyceride-rich lipoproteins after high-fat meal (HFM) consumption. Evidence has suggested that these exaggerated elevations of triglycerides levels are linked to impairment of endothelial function, characterized by imbalance between the actions of vasodilators and vasoconstrictors (1). Although the pathophysiology of endothelial dysfunction has not been fully elucidated, reduced nitric oxide and increased oxidative stress appears to be the mechanisms involving in the reduction of the vasodilatory response (2).

Endothelial dysfunction induced by PPL has been established as an early and reversible predictor of atherosclerotic disease and cardiac events (3,4). Thus, since humans spend a considerable part of the day in a postprandial state, interventions that can reduce the magnitude and duration of this metabolic state might be beneficial in the prevention of cardiovascular diseases (CVD).

Evidence has suggested that exercise training prior to high-fat meal ingestion has an attenuating effect on postprandial metabolism (5,6). Along the same lines, previous studies have reported that energy expenditure through prior exercise is related to the magnitude of this effect (7,8). Mestek et al. (2008) reported that isocaloric sessions before a meal mitigate PPL independently of the intensity of the exercise session (9). By contrast, it was previously reported that the magnitude of PPL was influenced by prior exercise intensity (5,10). Thus, the effect of the intensity of the exercise on postprandial response remains inconsistent.

With regard to postprandial endothelial function, evidence has shown that a single bout of exercise prior to consuming an HFM improves fasting and postprandial endothelial function compared with a resting control condition (11–13). Thus, it has been reported that acute moderate- and high-intensity exercise has transient benefits for macrovascular endothelial function in both fasting and postprandial states and that these effect may be due to the improvement of antioxidant status (14,15). However, the limited prior studies carried out to investigate the effect of exercise intensity have produced inconsistent findings (15–17).

The abovementioned studies have investigated the protective effect of exercise performed a few hours before consumption of an HFM on postprandial metabolism and endothelial function, focusing on the acute effect of exercise. Thus, although 12 weeks training program has been established as a protocol to assess the chronic effect (18), the potential impact on postprandial metabolism and vascular function after HFM have not been previously investigated. A narrative review summarizes the current literature on the possible contribution of medium- to long-term physical training in the reduction of postprandial response, which has yielded inconclusive data (18). Interestingly, a recent systematic review and meta-analysis evidenced that performing even a short period (∼4 min) of high-intensity exercise has greater benefits than moderate-intensity exercise in terms of cardiometabolic risk factors (19).
Considering that most adults do not meet the public health recommendation of at least 150 minutes per week of moderate intensity exercise and taking into account that habitual physical activity declines during middle age (20), it is of special interest to identify how much high intensity exercise is required to optimize vascular function in adulthood.

Thus, we hypothesized that medium-term exercise could attenuate the postprandial decrement in metabolism and endothelial function and that this effect would differ according to exercise intensity. On this basis, we aimed to determine the effect of 12 weeks of high-intensity training (HIT) and moderate continuous training (MCT) on postprandial metabolism and vascular function and arterial stiffness after HFM ingestion in healthy, inactive Latin-American adults.

2. Methods

2.1 Study design and setting

Details of the study design and methods of the primary HIT-Heart Study trial have been described elsewhere (ClinicalTrials.gov ID: NCT02738385) (21). 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, Colombia (ID 27-0500-2015). Postprandial biochemical and vascular function responses were assessed at baseline and over 12 weeks of training. We have provided an overview of the methods as per the Consolidated Standards of Reporting Trials (CONSORT) checklist (22).

2.2 Participants

Participants (n=20) were recruited at the University of Rosario (Bogota, Colombia) from February 2015 to May 2016. Subjects are eligible to participate if they are located in the metropolitan region with available time (1 hour per day) to support the trial. Inclusion criteria were individuals aged 18–45 years who were inactive (<150 min·wk⁻¹ of moderate-intensity activity or 75 min·wk⁻¹ of vigorous-intensity activity), had a body mass index (BMI) ≥18 and ≤30 kg/m² and identified as being willing and having almost immediate availability. Individuals with a history of any medical condition identified by the American Heart Association as an absolute contraindication to exercise testing were excluded from this study (23).

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 mmHg or diastolic blood pressure 95 mmHg), gastrointestinal disease (including self-reported chronic hepatitis or cirrhosis, any episode of alcoholic hepatitis or alcoholic pancreatitis within the 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 ≥118 mg·dl⁻¹) or use of any prescribed drugs, any active use of illegal or illicit drugs, or inability to participate because of a physical impairment. In addition, we confirmed by 2 exercise physiologists, subjects if they had alteration in ventricular function and/or
cardiomyopathy, through a standard 12-lead electrocardiography (ECG) at rest and every 3 minutes of the maximum treadmill exercise test. All subjects remained under usual medical care and clinical follow-up (i.e., regular appointments with a physician) throughout the protocol. All participants provided written informed consent before participating in the study.

2.3 Blinding and randomization

The coordinating Research Center for the Physical Activity Measurement (CEMA) in Bogotá randomized the procedures with randomization software using randomly permuted blocks. Group allocation was conducted via an online system in which the details of eligible participants were entered to obtain group assignments (i.e., 3:2 or 2:3). Assessors were blinded to study group assignments.

2.4 Interventions

After inclusion, patients performed a maximal cardiopulmonary exercise test on a maximum treadmill exercise test (Precor TRM 885, Precor Corp., Rome, Italy) following the modified Balke protocol and physiological parameters (maximal O\textsubscript{2} consumption (VO\textsubscript{2}), heart rate (HR), and Borg ratings) from the test were used to set exercise intensity. Based on averaged maximum HR (HR\textsubscript{max}) and VO\textsubscript{2}\textsubscript{peak}, the participants were classified according to normative values, referenced to age and sex (24). MCT and HIT interventions lasted 12 weeks, with 3 sessions per week, consisting in fast walking or running on a treadmill with the deck inclined to reach the desired intensity. Heart rate was recorded each session using an HR monitor (Polar Electro, Kempele, Finland). In addition, rating of perceived exertion (RPE) was also measured in each exercise session.

**Moderate continuous training (MCT) group:** Each preparatory period started with an exercise dose of 6 kcal·kg\textsuperscript{-1}·week\textsuperscript{-1}, which was increased progressively by 2 kcal·kg\textsuperscript{-1}·week\textsuperscript{-1} until week 4 and was then maintained at 12 kcal·kg\textsuperscript{-1}·week\textsuperscript{-1} for weeks 5 to 12, which was equivalent to 300 kcal of energy expended by the end of the training and cool-down (3 min) periods, with total exercise time ranging from 45 to 55 min. Exercise training sessions were designed to elicit a response in the acceptable moderate-to-vigorous range, i.e., 60–75% of HRR, and were adjusted according to ratings on the Borg scale (25). Exercise was performed in three sessions per week. During the supervised intervention, HR was recorded using an HR monitor (Polar Pacer, USA) to ensure compliance with the exercise stimulus at the predetermined target HR zone.

**High-intensity training (HIT) group:** We calculated training energy expenditures according to participants’ age ranges and set the target energy expenditures to meet the consensus public health recommendations from the Cardiometabolic HIT-RT Study (25). Each preparatory period started with an exercise dose of 6 kcal·kg\textsuperscript{-1}·week\textsuperscript{-1}, which was increased progressively by 2 kcal·kg\textsuperscript{-1}·week\textsuperscript{-1} until week 4 and was then maintained at 12 kcal·kg\textsuperscript{-1}·week\textsuperscript{-1} for weeks 5 to 12. The overall goal for the HIT group was to perform exercise sessions in 4 × 4-min intervals at 85–95% of HRR (with the target zone maintained for at least 2 min),
interspersed with a 4-min recovery period at 75–85% of HRR. During each exercise session, participants adhered to the 12 kcal·kg⁻¹·week⁻¹ energy expenditure format, which was equivalent to 300 kcal of energy expended by the end of the training and cool-down (3 min) periods, with total exercise time ranging from 32 to 45 min. Exercise was performed in three sessions per week. During the supervised intervention, HR and Borg ratings were measured as described for the MCT group.

Both groups were instructed to refrain from exercise training and to avoid changing their physical activity levels outside this study. All participants reported adhering to these instructions. Although diet was not controlled, participants met with the study dietician for nutrition assessment and counseling at baseline, and an individualized iso-energetic nutrition intervention plan was developed from the baseline food intake assessment according to participant preferences. This plan was standardized at 1300–1500 kcal·day⁻¹ (50–55% carbohydrates, 30–35% total fat, <7% saturated fat and 15–22% protein), distributed across 3–4 meals per day.

2.5 Data collection and outcome measures

Experimental Procedure

All measurements were tested at baseline and 12-week follow-up by personnel who were blinded to the treatment allocation. To control for confounding variables, we instructed the subjects to i) fast for 10-12 h, ii) abstain from exercise for 24 h, iii) abstain from caffeine, tobacco, and vitamin supplements for 12 h, and iv) awake between 0600 and 0700 h, all prior to each treatment condition. The HFM, previously used in other studies (26), consisted of a breakfast containing 1049 calories, 79 g of fat, 31 g of saturated fat, 4.5 g of trans fat, 666 mg of cholesterol, 69 g of carbohydrates, 31 g of protein, and 2.220 mg of sodium, adjusted by individual body weight. The effects of the HFM were measured in a fasted state and 60, 120, 180, and 240 min postprandially. Figure 1 represents the schedule of experimental events for each subject.

Figure 1. Schedule of experimental events for each subject. HIT, High-intensity interval training; MCT, Moderate-intensity continuous training; HFM, High-fat meal.

Discontinuous arrows represent capillary blood samples and assessment of endothelial function.

Biochemical parameters

Blood samples were obtained from an antecubital vein. The biochemical profile included plasma total cholesterol, high-density lipoprotein cholesterol
(HDL-c), triglycerides and glucose (measured by enzymatic colorimetric methods). Inter-assay reproducibility (coefficient of variation) was determined via ten replicate analyses of five plasma pools over 15 days and was shown to be 2.0, 3.2, 2.6 and 1.5% for total cholesterol, HDL-c, triglycerides and serum glucose, respectively.

Vascular function and arterial stiffness measures

All subjects were tested at the same time of day and after 10-12 hours of fasting, with no caffeine intake for at least 12 hours and consumption of a low nitrate diet for 48 hours. Vascular function and arterial stiffness, as measured by flow-mediated vasodilation (FMD), aortic pulse wave velocity (PWV) and the augmentation index (AIx) were measured. FMD was measured as described in previous studies from our group in the Colombian population (26) using the guidelines reported by Atkinson et al. (27). The same operator performed all Doppler ultrasound (Mindray M-9® DS USA; Mahwah, NJ) examinations using a 7.5-MHz linear array probe. Ultrasound imaging of the brachial artery was performed with the athletes in the supine position after 15 min of rest, with the arm abducted approximately 80° from the body and the forearm supinated. The ultrasound probe (7.5-MHz) was positioned with a 60° insonation angle in a longitudinal plane at a site 1-3 cm proximal to the antecubital fossa to visualize the anterior and posterior lumen-intima interfaces to measure diameter and central flow velocity (pulsed Doppler). After the baseline images were recorded, a blood pressure cuff, positioned on the arm, was inflated to 200mmHg for 5 min. To assess FMD, images were acquired continuously for 3 minutes after cuff deflation, during reactive hyperemia period. Brachial artery diameter recording was restarted at least 30 seconds before cuff deflation and continued for 3 min thereafter. The peak artery diameter and the time to reach this peak after cuff deflation were recorded. Images were recorded on a DVD for subsequent measurements by one observer blinded to the study design. FMD was calculated as the percent rise of peak diameter from the preceding baseline diameter and was measured every 1 min after deflation for 3 min. Normalized brachial artery FMD (FMDn) was calculated according allometric relationship between Dbase and peak diameter (Dpeak) (27). The intra-session coefficient of variations was ≤1% for the baseline diameter. Reliability was estimated by intra-class correlation coefficients (ICCs) based on four baseline measurements (n=8 subjects), yielding ICC values of 0.91 for baseline diameter and 0.83 for FMD (own data). The technical error of measurement was 1.23% for baseline diameter, 1.77% for maximum diameter and 20% for %FMD.

PWV was measured by analyzing the oscillometric pressure curves registered from the upper arm. Patient data and the measured distance between the jugulum and the symphysis were registered in an arteriographic computer program (Arteriograph Software v.1.9.9.2; TensioMed, Budapest, Hungary). A tape measure was used to measure the distance between the jugulum and the symphysis, i.e., 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 (28). PWV was calculated as the jugulum-to-symphysis distance (m) divided by half the return time (return time/2) (s). For PWV measurements, the two
recordings with the lowest standard deviations were chosen. The standard deviation was calculated on the basis of all heartbeats during a period of 8 s.

The AIx was also calculated as the ratio of the difference between the systolic peaks of the first pulse (1) and second pulse (2) relative to the central pulse pressure, expressed as a percentage \[\frac{(pulse\ 2\ -\ pulse\ 1)}{central\ pulse\ pressure}\times 100\]. The arteriograph calculates the AIx on the basis of the formula: \(AIx = \frac{(pulse\ 2 - pulse\ 1)}{central\ pulse\ pressure}\times 100\). Thus, it provides the brachial/aortic AIx without applying a transfer function. The R value, used as an estimate of the measurement errors for the repeated measurements between two sessions, was low for the arteriograph (1.18 m·s⁻¹).

### 2.6 Statistical Analysis

To retain the data of all randomly allocated participants, we performed an intention-to-treat analysis (all randomly assigned patients). The Shapiro–Wilk test was used to verify data distribution normality. Once it was confirmed that the sample data satisfied the normality assumption, statistical analyses relevant to our main research interest were conducted. Outcome values are reported as the mean with standard deviation (SD) unless otherwise specified. The area under the curve (AUC), expressed in arbitrary units (au) via the trapezoidal method, was calculated and used to analyze the response to the training protocols. The effect of training on AUC measures was analyzed by two-way analysis of variance. In addition, the percent change (Δ%) between HIT and MCT and the effect of HFM consumption at fasted state and 60, 120, 180, and 240 minutes postprandially were calculated for each variable and were analyzed using ANCOVA test including their baseline measurement as co-variable. A criterion alpha level of \(P \leq 0.05\) was used to determine statistical significance. All data are reported as the mean ± standard deviation. Statistical analyses were conducted using PASW Statistics 17 for Windows (SPSS, Inc., Chicago, Illinois).

### 3. Results

**Biochemistry and endothelial response**

Postprandial biochemical and endothelial function responses with summary measures of these responses after 12 weeks of HIT or MCT are shown in Table 1. A significant difference in glucose between 0 min (fasted state) and 120 min postprandially in HIT group was identified (\(P=0.035\)). Likewise, FMD (%) was significantly different between the fasted state and 60 min after an HFM in the HIT group (\(P=0.042\)).
Table 1. Intent-to-treat analysis of the effect of 12 weeks of HIT or MCR on postprandial lipemia biochemical and vascular function response after HFM ingestion.

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>0 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
<th>0 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>151.3 (21.0)</td>
<td>159.0 (28.1)</td>
<td>159.0 (30.2)</td>
<td>163.2 (30.0)</td>
<td>164.8 (20.2)</td>
<td>153.1 (29.09)</td>
<td>158.6 (25.1)</td>
<td>165.1(25.1)</td>
<td>166.8 (25.0)</td>
<td>167.1 (29.4)</td>
</tr>
<tr>
<td>High-density lipoprotein (mg/dL)</td>
<td>46.0 (14.1)</td>
<td>47.3 (14.6)</td>
<td>46.1 (12.7)</td>
<td>48.1 (16.1)</td>
<td>48.7 (15.9)</td>
<td>42.1 (9.5)</td>
<td>44.2 (11.6)</td>
<td>42.5 (12.2)</td>
<td>41.6 (12.6)</td>
<td>40.5 (10.9)</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>108.1 (35.8)</td>
<td>118.7 (41.7)</td>
<td>142.1 (53.7)</td>
<td>177.2 (91.5)</td>
<td>171.7 (84.5)</td>
<td>117.7 (33.1)</td>
<td>132.4 (31.1)</td>
<td>196.8 (56.1)</td>
<td>222.7 (68.2)</td>
<td>222.5 (91.2)</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>76.4 (11.0)</td>
<td>78.8 (12.9)</td>
<td>88.5 (8.8)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.3 (7.8)</td>
<td>86.3 (5.0)</td>
<td>85.9 (6.3)</td>
<td>93.6 (12.4)</td>
<td>94.2 (7.8)</td>
<td>93.3 (10.3)</td>
<td>89.5 (10.7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vascular function parameters</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>D&lt;sub&gt;base&lt;/sub&gt;, mm</td>
<td>2.7 (0.4)</td>
<td>2.8 (0.3)</td>
<td>2.7 (0.3)</td>
<td>2.7 (0.4)</td>
<td>2.8 (0.3)</td>
<td>3.1 (0.5)</td>
<td>3.2 (0.4)</td>
<td>3.2 (0.5)</td>
<td>3.3 (0.5)</td>
<td>3.4 (0.4)</td>
</tr>
<tr>
<td>FMD, %</td>
<td>13.4 (4.6)</td>
<td>6.3 (7.3)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.3 (5.3)</td>
<td>12.0 (5.8)</td>
<td>10.4 (4.6)</td>
<td>9.4 (4.0)</td>
<td>6.1 (3.9)</td>
<td>7.5 (3.1)</td>
<td>7.3 (5.7)</td>
<td>6.7 (4.5)</td>
</tr>
<tr>
<td>D&lt;sub&gt;peak&lt;/sub&gt; mm</td>
<td>3.0 (0.3)</td>
<td>2.9 (0.3)</td>
<td>3.1 (0.3)</td>
<td>3.0 (0.3)</td>
<td>3.1 (0.4)</td>
<td>3.4 (0.5)</td>
<td>3.4 (0.5)</td>
<td>3.4 (0.5)</td>
<td>3.6 (0.5)</td>
<td>3.6 (0.4)</td>
</tr>
<tr>
<td>D&lt;sub&gt;pulse&lt;/sub&gt;</td>
<td>0.3 (0.2)</td>
<td>0.1 (0.1)</td>
<td>0.4 (0.3)</td>
<td>0.3 (0.2)</td>
<td>0.3 (0.2)</td>
<td>0.3 (0.2)</td>
<td>0.2 (0.1)</td>
<td>0.2 (0.1)</td>
<td>0.2 (0.2)</td>
<td>0.2 (0.1)</td>
</tr>
<tr>
<td>nFMD, %</td>
<td>13.5 (6.3)</td>
<td>13.4 (4.6)</td>
<td>11.7 (6.1)</td>
<td>11.8 (7.3)</td>
<td>10.1 (5.1)</td>
<td>8.1 (4.1)</td>
<td>9.4 (4.0)</td>
<td>6.5 (3.2)</td>
<td>6.3 (5.2)</td>
<td>5.5 (4.2)</td>
</tr>
<tr>
<td>PWV, m·s&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>6.6 (1.5)</td>
<td>7.0 (1.6)</td>
<td>6.6 (2.4)</td>
<td>6.7 (1.2)</td>
<td>6.8 (1.0)</td>
<td>6.7 (0.9)</td>
<td>6.5 (1.0)</td>
<td>6.7 (1.0)</td>
<td>6.5 (1.0)</td>
<td>6.7 (1.0)</td>
</tr>
<tr>
<td>AIx (aortic), %</td>
<td>26.3 (14.6)</td>
<td>14.7 (9.4)</td>
<td>15.9 (12.1)</td>
<td>18.3 (12.5)</td>
<td>19.1 (9.2)</td>
<td>38.7 (69.0)</td>
<td>7.6 (4.2)</td>
<td>3.6 (16.0)</td>
<td>9.5 (4.1)</td>
<td>11.2 (7.0)</td>
</tr>
<tr>
<td>AIx (brachial), %</td>
<td>-22.3 (28.9)</td>
<td>-45.2 (18.7)</td>
<td>-42.7 (24.0)</td>
<td>-38.1 (24.8)</td>
<td>-36.5 (18.2)</td>
<td>-41.7 (16.5)</td>
<td>-59.3 (8.5)</td>
<td>-34.6 (45.0)</td>
<td>-55.5 (8.2)</td>
<td>-52.0 (13.8)</td>
</tr>
<tr>
<td>PP(mHg)</td>
<td>45.5 (7.7)</td>
<td>49.8 (7.3)</td>
<td>45.9 (8.5)</td>
<td>48.5 (10.4)</td>
<td>47.4 (7.4)</td>
<td>45.1 (7.6)</td>
<td>46.7 (6.4)</td>
<td>51.0 (8.4)</td>
<td>46.0 (6.6)</td>
<td>48.8 (7.2)</td>
</tr>
<tr>
<td>SBPao (mmHg)</td>
<td>107.6 (14.2)</td>
<td>102.4 (7.7)</td>
<td>102.6 (12.9)</td>
<td>105.8 (10.2)</td>
<td>106.3 (9.6)</td>
<td>104.3 (7.3)</td>
<td>103.6 (8.4)</td>
<td>104.5 (6.1)</td>
<td>105.0 (8.4)</td>
<td>109.3 (9.6)</td>
</tr>
<tr>
<td>PPao (mmHg)</td>
<td>40.6 (6.6)</td>
<td>39.0 (7.1)</td>
<td>39.6 (7.1)</td>
<td>39.7 (6.3)</td>
<td>39.3 (5.1)</td>
<td>30.7 (12.9)</td>
<td>33.8 (5.3)</td>
<td>38.0 (6.9)</td>
<td>35.0 (6.2)</td>
<td>36.8 (6.1)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>112.5 (9.1)</td>
<td>113.2 (6.4)</td>
<td>111.5 (8.4)</td>
<td>114.6 (7.2)</td>
<td>114.3 (6.6)</td>
<td>113.0 (7.6)</td>
<td>116.4 (9.1)</td>
<td>118.6 (6.1)</td>
<td>117.0 (8.1)</td>
<td>121.2 (10.8)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>67.0 (10.3)</td>
<td>63.4 (8.8)</td>
<td>65.6 (11.4)</td>
<td>66.1 (9.1)</td>
<td>67.0 (8.0)</td>
<td>67.8 (9.4)</td>
<td>69.7 (6.2)</td>
<td>66.5 (6.6)</td>
<td>71.0 (11.7)</td>
<td>72.4 (6.8)</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>82.0 (9.2)</td>
<td>80.7 (7.4)</td>
<td>80.8 (9.7)</td>
<td>82.2 (7.0)</td>
<td>82.9 (6.7)</td>
<td>82.8 (7.8)</td>
<td>85.3 (6.8)</td>
<td>83.7 (5.2)</td>
<td>86.2 (10.2)</td>
<td>88.7 (7.7)</td>
</tr>
</tbody>
</table>

Values are participant characteristics at baseline, mean (SD). HIT, 4 x 4-min High-intensity interval training; MCT, Moderate-intensity continuous training; D<sub>base</sub>, Diameter; FMD, Flow-mediated vasodilation; nFMD, Normalized flow-mediated vasodilation; PWV, Pulse wave velocity; AIx, Augmentation index; PP, Pulse Pressure; SBPao, Central systolic blood pressure; PPao, Pulmonary artery occlusion pressure. Whole group repeated measures ANOVA, a<sub>=</sub> 0 min vs 60 min; b<sub>=</sub> 0 min vs 120 min.
Figure 2 lists the effects of HIT and MCT conditions on total cholesterol, triglycerides and glucose postprandial responses with summary measures of these responses. The total cholesterol response expressed as AUC \((0-240)\) was lower following HIT than following MCT, but no significant differences were observed (8%, \(P>0.05\)). Similarly, triglycerides AUC \((0-240)\) was also lower after HIT compared with MCT; a trend toward significance was found (24%, \(P=0.076\)). Note that AUC \((0-240)\) for the glucose response was significantly lower following HIT than MCT (10%, \(P=0.008\)).

Figure 2. Total cholesterol, TG and glucose responses to PPL (left) and incremental AUC (right) after 12 weeks of HIT and MCT.
The effects on FMD, nFMD and PWV postprandial responses after HIT and MCT with summary measures of these responses are represented in Figure 3. FMD and nFMD AUC (0-240) were statistically higher following HIT than following MCT (46.9%, P=0.021 and 67.3%, P=0.009, respectively). PWV AUC (0-240) did not differ following HIT and MCT (2.3%, P>0.05).

Figure 3. FMD (%), FMDn (%) and PWV responses to PPL (left) and incremental AUC (right) after 12 weeks of HIT and MCT.

4. Discussion

The aim of this study was to investigate the effects of chronic MCT and HIT on postprandial lipemia and vascular function and arterial stiffness after HFM consumption in inactive adults. The main finding of this study is that a 12-week regimen of HIT reduces glucose concentrations and exerts greater post-HFM
endothelial function than MCT, supporting the idea that the effect of chronic exercise on postprandial response is dependent on exercise intensity (29).

Existing evidence has shown that acute exercise increases FMD following an HFM (30,12). Nevertheless, the present study is the first to demonstrate that medium-term exercise training prevented the decline in FMD induced by PPL, supporting the protective effect of regular exercise on vascular function. This is clinically relevant since endothelial function is an independent risk factor of cardiovascular diseases (3). Interestingly, the higher AUC FMD in the HIT condition compared with MCT after an HFM indicates that HIT may provide major vascular benefits in inactive adults (P=0.009).

In agreement with our results, previous studies that focused on the effect of acute exercise demonstrated that FMD remained greater after an HFM following a single bout of HIT compared with MCT (15,31). Note that, although it is of special interest to calculate incremental AUCs from the curve drawn from hourly measurements up to 4 hours using the trapezoid rule, most previous studies have not included these data (32). In contrast to our study, a recent research conducted in 11 physically active young men reported that FMD response did not differ between the two conditions (16). The differences in training status between this study population (physically active) and our study cohort (inactive) might explain the inconsistent findings, since it has been shown that FMD responses after an HFM may differ between active and inactive subjects (33). Thus, based on our results and previous research, it can be hypothesized that exercise attenuates the negative effects of an HFM on endothelial cell function in an inactive population. Further studies investigating the mechano-sensory mechanisms contributing to the effect of exercise on vascular function as well as possible interactions among molecular pathways are required (34).

The mechanism by which chronic exercise training can modulate postprandial endothelial function is unclear. Regular exercise has been proposed to decrease PPL and therefore reduce postprandial oxidative stress by maintaining low lipoprotein levels (35). An alternative mechanistic explanation is that regular exercise might increase antioxidant capacity, leading to maintenance of endothelial function (15). Indeed, a substantial increase in exercise intensity has been linked to greater protection of vascular function against oxidative stress, supporting the possibility that HIT might trigger larger vascular effects at the cellular and molecular levels (15).

Likewise, exercise might exert a positive effect on endothelial function by stimulating the production and bioavailability of nitric oxide (NO), since physical activity induces the activity of endothelial nitric oxide synthase (eNOS), increases the capacity of the cellular antioxidant system and diminishes the formation of reactive oxygen species (ROS) (36). In addition, it has been demonstrated that a single session of exercise increased circulating and intramuscular free radical levels (37), which may lead to inactivation of NO and consequently affect endothelium-mediated vasodilation (38). It seems that acute exercise mediates the oxidant-antioxidant balance in favor of antioxidants, resulted in the maintenance of vascular function; a similar effect was observed from the co-ingestion of antioxidants (39).
Thus, it is tempting to speculate that the effects of different intensities of exercise on postprandial FMD are related to changes in antioxidant status.

On the other hand, medium-term HIT decreased the glucose response over the postprandial observation period by 10% compared with MCT, indicating that the magnitude of postprandial glucose response was dependent on exercise intensity (P=0.008). Conversely, recent research did not find differences between the two training protocols regarding postprandial glucose levels (10,15,16). However, it is especially relevant to consider that these studies have only examined the effect of postprandial glucose level after acute exercise. Thus, it is possible that only medium or long-term training have a substantial effect on postprandial glucose response.

Additionally, we found similar total cholesterol, HDL and triglyceride responses after HFM in both training conditions, suggesting that medium-term exercise training intensity might not play an important role in the postprandial decrement in these marker responses. In prior studies focusing on acute exercise, significant differences between HIT and MCT have been shown for triglycerides but not for total cholesterol or HDL levels (10,15,16). In this context, results from preliminary studies have suggested that the positive effect of exercise training on PPL might be short lived, demonstrating variations in the effect sizes for exercise training performed within 24 hours prior to HFM ingestion and for exercise training performed more than 24 hours pre-prandial (8,40). Thus, we might hypothesize that postprandial triglyceride response might be short lived, showing a relevant effect only after acute exercise.

This study had some limitations. Due to the high sensitivity of endothelium to nutritional changes, it would be ideal to administrate isocaloric meals to participants at least three days before the measurement of endothelial function. In this study, although diet was not controlled, a dietician provided an individualized iso-energetic nutrition intervention plan. Second, since endothelial function is well known to be affected by age and training status and our study cohort was composed of healthy, inactive mature adults, this could imply that our findings may not be generalizable to other populations with different characteristics. A final possible limitation is that we did not examine other factors such as antioxidant status that might ameliorate postprandial response, and this area remains open to future research.

The main strength of our study is that, to our knowledge, this is the first randomized clinical trial on the effect of exercise-training intensity on biochemical parameters and endothelial functional responses to HFM in inactive adults from the Latin-American population. In addition, we provided measurements of these PPL responses at multiple time points in order to describe their time course after chronic exercise.

5. Conclusion

In summary, the novel finding of this study was that medium-term supervised physical training may mitigate endothelial dysfunction and glucose response induced by PPL. Exercise intensity seems to play an important role in these protective effects,
suggesting that high-intensity training might be the most effective in reducing postprandial glucose levels and attenuating vascular impairments. Therefore, medium-term HIT is an effective strategy to reduce CVD.

6. References


27. Atkinson G. Shear rate normalization is not essential for removing the dependency of flow-mediated dilation on baseline artery diameter: past research revisited. Physiol Meas. 2014;35(9):1825-35.


2002;959:82–92.


Chapter 6

Effects of Exercise Training Type and Intensity or Nutritional Guidance on Metabolic Syndrome Risk Factors, Ideal Cardiovascular Health Parameters, Endothelial Function and Arterial Stiffness in Overweight Adults: Cardiometabolic HIIT-RT Study, A Randomized Controlled Trial
1. Introduction

Obesity is a major public health problem worldwide [1]. The prevalence of overweight/obesity is rapidly increasing and, in the Latin-American population, it is estimated that more than 19% of adults are obese [2]. Excess body weight is an independent risk predictor for cardiometabolic disease, including heart disease, diabetes, hypertension, chronic kidney disease or stroke [1]. In addition, excessive accumulation of body fat has been related to the metabolic syndrome (MetS), a clustering of central obesity, hyperglycemia or hyperinsulinemia, dyslipidemia, and elevated blood pressure [3], thus increasing cardiovascular risk [4]. A recent estimation of the prevalence of MetS among U.S. adults is 34.3% [5].

Regular physical activity and nutritional guidance are among the therapeutic actions used to reduce MetS in adults [6]. According to the World Health Organization (WHO) and the American College of Sports Medicine, the recommended exercise prescription for improving and maintaining health is at least 150 min of moderate-intensity physical activity (40–60% maximum oxygen uptake, VO2max) or 75 min of vigorous-intensity physical activity (60–85% VO2max) per week for healthy adults [7]. Despite the importance of engaging in regular exercise, most adults fail to meet these recommendations, reporting lack of time as a major barrier for being physically active [8]. Accordingly, identifying more time-efficient modes of exercise training is a major area of interest.

High-intensity interval training (HIIT), characterized by brief, intermittent bursts of vigorous activity interspersed by periods of low-intensity exercise, has become a popular and more time-efficient alternative to traditional exercise strategies [9]. The impact of HIIT on cardiometabolic risk factors such as body composition, metabolic parameters, vascular function and physical fitness has been investigated extensively in overweight-obese populations [10–16]. Compared with moderate-intensity continuous exercise, HIIT might result in a superior or equal improvement in body composition, cardiovascular health (CVH) and cardiorespiratory fitness [17–21]. Resistance training (RT), a type of strength training, has also been shown to be effective in improving several cardiometabolic risk factors including insulin resistance/hyperglycemia, dyslipidemia, hypertension and obesity [22–24].

Previous studies investigating the effects of concurrent aerobic and RT on cardiometabolic health have produced inconclusive findings [14, 25–28]. For example, Ho et al. showed that 12 weeks of combination exercise training yielded greater benefits for body composition and cardiorespiratory fitness than moderate-intensity aerobic and RT modalities in overweight/obese adults [14]. By contrast, Willis et al. reported that a program of combined aerobic training and RT in middle-aged overweight/obese adults failed to significantly reduce fat or body mass over aerobic training alone, suggesting that aerobic training is the optimal mode of exercise for reducing these parameters. They also found that a program including RT was needed to increase lean mass in this group [25]. Interestingly, Kemmler et al. reported that a high-intensity aerobic and resistance exercise program affected MetS risk factors and significantly lowered the severity of MetS in thirty-two elderly...
females (69.5 ± 4.3 years), supporting that high intensity is more strongly inversely related to the MetS than low-intensity exercise [29]. However, the potential greater benefits of specific concurrent HIIT and RT across cardiometabolic markers over these modalities alone has not been widely investigated and thus the optimization of HIIT adaptations with RT remains an important goal for clinical research [30].

Nutritional guidance has been recently postulated as an effective intervention for promoting cardiometabolic health [31]. Indeed, nutritional guidance with adequate nutrition has been shown to improve insulin resistance, reduce oxidative stress and lipid profile and prevent excess weight, hypertension, type 2 diabetes, and low grade chronic inflammation [32]. Nevertheless, the potential effects of nutritional guidance on cardiometabolic health when directly compared with different training modalities remain to be determined.

To the best of our knowledge, the effect of different exercise intervention programs on sedentary and overweight males has been scarcely investigated. This is understandable, because such investigations require a multidisciplinary team and long-term study to detect the changes occurring in this population. Against this background, the aim of the present study was to investigate whether 12 weeks of HIIT, RT, CT or a nutritional guidance plan induced improvements in MetS risk factors, vascular function and ideal CVH in sedentary and overweight males, and to compare the training adaptations between intervention groups.

2. Methods

2.1 Study design

The Cardiometabolic HIIT-RT study is a single blind, randomized controlled 2 × 2 factorial trial (ClinicalTrials.gov ID: NCT02715063) conducted from March 2016 to June 2017 in Bogotá, Colombia. The study was approved by the Research Ethics Committee of The University of Manuela Beltran (ID 06-1006-2014) and complied with the revised ethical guidelines of the Declaration of Helsinki (revision of 2013). Randomization was performed by a third party using variable permuted block sizes with computer-generated random numbers. Details of sample calculation, randomization, characteristics of participants, design, methods and measurements of the Cardiometabolic HIIT-RT study have been published elsewhere [33]; however, the most relevant information is briefly described below.

2.2 Participants

The study included a total of 51 sedentary subjects (no participation in exercise more than once a week for the previous six months), aged 30–50 years, with abdominal obesity (waist circumference [WC] ≥90 cm [men] ≥80 cm [women]) or excess weight, body mass index ≥ 25 and ≤ 30 kg/m², identified as being willing and with almost immediate availability was enrolled. Subjects were recruited from a private health care institution (Clinica Rangel Pereira, IPS) and the Rosario University in Bogotá. Before being enrolled in the study, all participants were informed of the purpose and risk of the study and signed an informed consent form.
2.3 Exercise training intervention

1. Nutritional guidance (NG): Without exercise training. Participants received counseling about goals for CVH, as well as monitoring CVH over time in the Colombia population, key signs and symptoms, diet and screening for cardiometabolic risk factors. All participants received NG four times during the program: twice in individual sessions (baseline and after 12 weeks) and twice in groups (fourth and eighth weeks). Diets were monitored by means of three-day dietary recall, in accordance with the standards of the American Dietetic Association. The prescribed NG was based on an exchange list, by reducing 250 kilocalories (kcal) from the calorie total in the diet, in order to promote a 250 g reduction per week in body mass (0.5 to 1.0 kg per month). Total energy intake in kilocalories and the amounts of each nutrient (carbohydrates, fat, and protein in grams) were assessed at baseline (0, 4 and 12 week) using a 24-h weighed dietary record method. The assessment was carried out by trained registered clinical dietitians (A.H), and the scoring was controlled by one researcher (R.R.V).

2. High-intensity interval training (HIIT) group: All HIIT sessions were preceded with a 5-min warm-up and ended with a 4-min cool-down at 65% heart rate maximum (HRmax) until the subject expended between 400 and 500 kcal. The HIIT protocol consisted of four bouts of 4-min intervals at 85–95% HRmax interspersed with 4 min of active recovery at 75–85% HRmax. Participants were instructed to reach their target HR for each interval within the first 2 min of the 4-min interval. We calculated the training energy expenditure with the consensus public health recommendations from WHO [34] and the US Department of Health and Human Services [35]. HR monitors (A3, Polar Electro OY, Finland) were used to adjust workload to achieve the target HR. In addition, a rating of perceived exertion was also measured during each exercise session (15–17 during high intensity and 11–13 during recovery).

3. Resistance training (RT) group: The RT session was initiated with ~12–15 repetitions per set of six exercises that targeted all the major muscle groups at high intensity. A 60-s recovery was permitted as many times as needed according to the subject’s weight until the subject expended between 400 and 500 kcal at 50 to 70% of one-repetition maximum (1RM).

4. Concurrent training (CT = HIIT+RT) group: The CT group did the 50% aerobic training program plus the 50% RT program during each session. The energy expenditure associated with the physical training prescribed for the CT group was therefore ~400 to 500 kcal/session [33].

2.4 Training intensity and energy expenditure during the exercise session

In terms of exercise intensity, the actual intensity values were reported as the mean of HR measured in the HIIT and CT groups and as the average value of workload and repetitions determined in the acute session in the RT group. The intensity of the HIIT or CT group was based on the percentage of each individual’s HRmax derived from a maximal treadmill test. Research staff monitored and recorded compliance with target HR and energy expenditure during the sessions.
2.5 Blood draws and analysis

Participants arrived at the Rosario University CEMA-Laboratory between 6:00 and 9:00 following a 10- to 12-h overnight fast. Participants were reminded to maintain standardized conditions (i.e., a hydrated state and abstaining from caffeine and alcohol consumption for 36 h). The following blood parameters were measured: (i) high-density lipoprotein cholesterol (HDL-C), (ii) triglycerides, (iii) low-density lipoprotein cholesterol (LDL-C), (iv) total cholesterol, (v) fasting glucose, and (vi) the metabolic regulators glucose and hemoglobin A1C (HbA1c) (by enzymatic colorimetric methods). All determinations were measured by using Cardiocheck® (Polymer Technology Systems, PTS, Indianapolis, IN, USA) and A1CNow+® (Bayer Diabetes care, Sunnyvale, CA, USA).

2.6 Blood pressure and heart rate

Systolic and diastolic blood pressure were recorded using an automatic monitor (Omron HEM® 7114TM, Omron Healthcare Co. Ltd.) in duplicate after 15 minutes of rest, with the subjects in a seated position and with both feet resting on the floor [36]. The mean arterial pressure (MAP) was calculated using the following formula: MAP = (systolic blood pressure + (2 x diastolic blood pressure)) / 3. Resting HR was measured using a chest monitor (V-800®, Polar Electro Inc., Kempele, Finland) after subjects had rested in the supine position for at least 15 min.

2.7 Anthropometry and body composition

Body mass (Tanita® BC-418, Tokyo, Japan) and height (Seca® 274, Hamburg, Germany) were measured in duplicate using standard protocols. BMI was calculated with the following formula: BMI = body weight (kg)/height squared (m²). WC was measured to the nearest 1 mm with a flexible steel tape measure (Lufkin W606PM®, Parsippany, NJ, USA) placed midway between the lowest rib and the iliac crest while participants were in a standing position at the end of an exhalation, in accordance with the International Society for the Advancement of Kinanthropometry guidelines [37]. The technical error of measurement values was less than 2% for all anthropometric variables. Whole body fat and lean mass, trunk fat mass index, muscle index and appendicular muscle mass were measured by dual-energy X-ray absorptiometry (QDR-1500, Hologic Corp., Software version 7.10, Waltham, MA).

2.8 Cardiorespiratory fitness and muscular strength

At 48 h after the start of the training period, the VO₂max of inactive subjects was determined 24 h before the acute intervention using a maximum treadmill exercise test (Precor TRM 885, Italy). Exercise capacity was evaluated according to treadmill exercise test duration, which was used to estimate aerobic consumption expressed in metabolic equivalents (METs), based on well-characterized regression equations recommended by the American College of Sports Medicine. In addition, previous studies demonstrated that treadmill test time correlates well (r=0.92) with VO₂max [38, 39]. Blood pressure was recorded at rest, at each stage change, at peak exercise, and during recovery using a standardized cuff sphygmomanometer.
Regarding muscular strength, 1RM was measured for six different exercises: bicep screw curl, triceps extension, dumbbell side lateral raise, military press, dumbbell squat and dumbbell front lunge, which were implemented based on similar procedures [33]. The 1RM was performed in six resistance exercises and was conducted between 09:00 and 11:00 a.m.; the highest load of three attempts per exercise was reported. The 50–70% value of the 1RM was used to determine the workload during the sessions for the RT and CT groups.

2.9 Endothelial function measures

Endothelial function was measured by flow mediated-dilation (FMD), aortic pulse wave velocity (PWV) and the augmentation index (AIx). FMD was measured as described previously by our group [40] using the guidelines reported by Corretti et al. [41]. The intra-session coefficient of variations was ≤1% for the baseline diameter. The technical error of measurement was 1.23% for baseline diameter, 1.77% for maximum diameter and 20% for %FMD. Images were recorded on a DVD player for subsequent measurements by an observer blinded to the study design. FMD was expressed as % change=[(maximum – baseline diameter) / baseline diameter] × 100. Normalized brachial artery FMD (FMDn) was calculated according to the allometric relationship between base diameter (Dbase) and peak diameter (Dpeak) [42]. PWV was measured by analyzing the oscillometric pressure curves registered from the upper arm with an arteriographic computer program (Arteriograph Software v.1.9.9.2; TensioMed, Budapest, Hungary). The algorithm measuring blood pressure in the arteriography device has been previously validated [43]. PWV was calculated as the jugulum-to-symphysis distance (m) divided by the return time (return time/2) (s). The arteriograph calculates the Alx on the basis of the formula (Alx% pulse 2 - pulse 1/central pulse pressure) ×100 and thus provides the brachial/aortic Alx without applying a transfer function. The R value as an estimate of the measurement errors for the repeat measurements between two sessions (n=6) was low for the arteriograph (1.18 m·s⁻¹).

2.10 Ideal CVH behaviors and CVH risk factors

The metrics for ideal CVH in children and adolescents defined by the American Heart Association (AHA) were followed as precisely as possible (Supplemental Table S1).

Supplement Table 1. Definition of the Ideal Cardiovascular Health Metrics (>20 Years of Age) as Defined by the American Heart Association and the Criteria Used in this Study.

<table>
<thead>
<tr>
<th>Health behaviors</th>
<th>Ideal Metric, AHA Definition</th>
<th>Ideal Metric, Definition in this Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>Never tried; never smoked whole cigarette</td>
<td>Never smoked a cigarette</td>
</tr>
<tr>
<td>Body mass index</td>
<td>&lt; 25kg/m²</td>
<td>&lt;25kg/m²</td>
</tr>
<tr>
<td>Physical activity</td>
<td>≥60 min of moderate- or vigorous-intensity activity every day</td>
<td>Exercise capacity was dichotomized to high vs. low based on estimated aerobic consumption (cut-off point</td>
</tr>
<tr>
<td>Diet</td>
<td>4–5 components:</td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>--------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fruit and vegetables: ≥4.5 cups/d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fish: 2 or more 3.5-oz servings/wk</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fiber-rich whole grains: 3 or more 1-oz-equivalent servings/d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium: &lt;1500 mg/d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sugar-sweetened beverages: ≤450 kcal (36 oz)/wk</td>
<td></td>
</tr>
</tbody>
</table>

| Health factors | Total cholesterol: <170 mg/dL (<4.40 mmol/L) | <170 mg/dL |
|                | Blood pressure: <120/<80 mmHg                  | <120/<80 mmHg |
|                | Plasma glucose: <100 mg/dL (<5.6 mmol/L)       | <100 mg/dL or HbA1c < 5.7%  |

Data on smoking were collected via self-reported questionnaires (number of cigarettes smoked per day). Ideal smoking status was determined as non-smoker or quit smoking ≥12 months. Although the AHA relies on physical activity to determine active habits, we used estimated cardiorespiratory fitness (CRF), due to its robust association with cardiovascular risk factors and ideal CVH in this population [44]. Exercise capacity was dichotomized to high (Ideal CVH) versus low (non-ideal CVH) based on estimated CRF (cut-off point of VO$_2$ max > 35 mL/kg/min in women or > 40 mL/kg/min in men).

BMI was classified using WHO criteria (normal: 18.5 to 24.9 kg/m$^2$; overweight: 25.0 to 29.9 kg/m$^2$; and obese: ≥30 kg/m$^2$) [45]. A seven-day recall was the dietary assessment tool used to assessed the Mediterranean diet (MetDiet) quality. As suggested by Thanapoulou et al., [46] the total score was divided into two categories of Mediterranean diet quality: (1) ≤8 points = poor diet quality; and ≥9 points = good diet quality (optimal Mediterranean diet style). Participants who had at least ≥9 points were categorized as having an ideal healthy diet, whereas adults with 8 points were classified as having a non-ideal healthy diet. Glucose fasting, total cholesterol and blood pressure were included as CVH risk factors.

**2.11 AHA criteria**

The AHA guidelines [47] were used to construct an ideal CVH index based on 7 metrics and using the cut-off points for adults, with participants receiving one point for the presence of each ideal metric. The ideal behaviors defined by the AHA were as follows: BMI <25 kg/m$^2$, CRF (VO$_2$max >35 mL/kg/min in women or >40 mL/kg/min in men), non-smoking status (either never having smoked), and consumption of a dietary pattern that promotes ideal CVH. The factors were classified as an untreated systolic
blood pressure <120 mmHg and diastolic blood pressure <80 mmHg, untreated total cholesterol ≤200 mg/dL, and untreated fasting blood glucose <100 mg/dL or HbA1c <5.7%.

Finally, the participants were categorized into 1 of 3 health levels based on the number of CVH metrics in the ideal range that they exhibited; the healthiest level (favorable ideal CVH score) was defined as having between 5 and 7 metrics in the ideal range; the intermediate level, 3 to 4 metrics; and the unfavorable level, 0 to 2 metrics. These cut-off points have been used in previous international studies [48, 49].

2. 12 Cardiometabolic parameters

We calculated a composite cardiometabolic z-score that reflects a continuous score of the five metabolic abnormalities. The cardiometabolic z-score was calculated from subjects’ data, based on the International Diabetes Federation [50], and standard deviations using data from the entire subject cohort at baseline. The equation used was: MetScore = ([HDL-C: ♂≤40 or ♀≤50 mg/dL]/SD*-1) + ([TG: 150 mg/dL]/SD) + ([fasting glucose: 100 mg/dL]/SD) + ([WC: ♂≥94 or ♀≥80 cm]/SD) + ([MAP: 100 mmHg]/SD). The mean of this continuously distributed cardiometabolic z-score was therefore zero by definition.

2.13 Statistical analyses

Baseline demographics were summarized as means and standard deviations, and between group differences were examined using ANOVA for continuous data. Categorical data were summarized as frequencies and percentages, and group differences at baseline were examined using lineal $\chi^2$ tests. The mean change in each group was reported as the estimated margin of the mean, as assessed by 95% confidence intervals (CI) with adjustment for kcal for diet, sex and baseline values as covariates using an unstructured covariance matrix for the repeated measures. Within-group differences were considered significant when the 95% CI did not include zero. In the per-protocol mixed model analyses, we used 95% CI and $p$ values (<0.05) for the intergroup comparisons, for each outcome measure across group × time interaction factors. Cohen’s $d$ for effect size was also calculated to determine the magnitude of the group differences. The effect size was classified as small, medium, and medium-to-large effects (<0.20, 0.2–0.6 and 0.6–1.2, respectively), and partial $\eta^2$ was considered small if $\eta^2<0.04$, and large if $\eta^2 >0.36$ in interaction effect analysis [51].

To examine the cumulative effects of the 7 CVH metrics, we created a dichotomized variable for each component of the health metrics: “ideal” was coded as 1, and “poor” was coded as 0. The total ideal CVH metrics score of each individual ranged from 0 to 7. Changes in CVH metrics were calculated by subtracting the total score for the metrics obtained in pre- from the total score obtained in post-intervention. Participants were divided into three categories based on the changes in the 7 CVH metrics. Categorical CVH metrics were described as percentages and were compared using $\chi^2$ tests. The significance level adopted to reject the null hypothesis was $P < 0.05$. All analyses were performed using the SPSS software package (Version 24, IBM, New York, USA).
3. Results

3.1 Characteristics of the subjects

Figure 1 shows the CONSORT flow diagram of study progression. A total of 80 participants were eligible after assessment. Reasons for eligible subjects declining to participate included 'lack of time' (n=5), and 'personal reasons' (n=3). Of the remaining 72 participants, 18 were randomized into each of the following intervention groups: i) NG; ii) HIIT; iii) RT; and iv) CT (HIIT and RT protocol).

Baseline parameters are shown in Table 2. In total, 9 patients had abnormalities in total cholesterol levels, 86.2% had low HDL-C levels, 43.1% had high triglyceride levels, and 39.7% had HbA1c >5.6%. In total, 72.4% of participants were not currently smoking, 5.2% had normal weight, 46.6% had a healthy VO2max, and 32.8% had a healthy diet by MetDiet score. Most study participants reached ideal health status for the following cardiovascular factor metrics: total cholesterol (84.5%), fasting glucose (87.9%), and blood pressure (89.7%). Other details of vascular function, exercise and diet parameters are shown in Table 2.
Table 2. Characteristics of the subjects (n=58)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex, men n (%)</strong></td>
<td>23 (39.7)</td>
</tr>
<tr>
<td><strong>Morphological parameters, mean (SD)</strong></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>40.78 (7.06)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>79.55 (12.30)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>162.51 (7.94)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>30.04 (3.49)</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>92.68 (9.49)</td>
</tr>
<tr>
<td>Fat mass index, kg/m²</td>
<td>11.61 (2.90)</td>
</tr>
<tr>
<td>Trunk fat mass, g</td>
<td>16.42 (4.36)</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>46.01 (8.28)</td>
</tr>
<tr>
<td>Fat-free mass, kg/m²</td>
<td>18.02 (1.84)</td>
</tr>
<tr>
<td>Appendicular skeletal muscle mass, kg/m²</td>
<td>7.82 (1.07)</td>
</tr>
<tr>
<td><strong>Cardiometabolic risk factors parameters, mean (SD)</strong></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>167.29 (34.51)</td>
</tr>
<tr>
<td>High total cholesterol, n (%)</td>
<td>9 (14.5)</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>37.15 (9.53)</td>
</tr>
<tr>
<td>Low HDL-C, n (%)</td>
<td>50 (86.2)</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>99.42 (31.30)</td>
</tr>
<tr>
<td>High LDL-C, n (%)</td>
<td>25 (43.9)</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>172.37 (108.65)</td>
</tr>
<tr>
<td>High triglycerides, n (%)</td>
<td>25 (43.1)</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>89.68 (7.92)</td>
</tr>
<tr>
<td>High glucose, n (%)</td>
<td>7 (12.1)</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.45 (0.47)</td>
</tr>
<tr>
<td>High HbA1c, n (%)</td>
<td>23 (39.7)</td>
</tr>
<tr>
<td>Cardiometabolic z-score</td>
<td>0.767 (2.98)</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>116.12 (10.30)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>73.01 (9.87)</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>87.37 (9.66)</td>
</tr>
<tr>
<td><strong>Vascular function parameters, mean (SD)</strong></td>
<td></td>
</tr>
<tr>
<td>(D_{base}), mm</td>
<td>32.0 (4.98)</td>
</tr>
<tr>
<td>FMD, %</td>
<td>9.81 (6.62)</td>
</tr>
<tr>
<td>(D_{peak}) mm</td>
<td>35.08 (5.36)</td>
</tr>
<tr>
<td>(D_{diff})</td>
<td>5.58 (3.54)</td>
</tr>
<tr>
<td>FMDn, %</td>
<td>2.68 (1.77)</td>
</tr>
<tr>
<td>PWV, m·s(^{-1})</td>
<td>7.33 (1.06)</td>
</tr>
<tr>
<td>AIx (aortic), %</td>
<td>24.52 (18.57)</td>
</tr>
<tr>
<td>AIx (brachial), %</td>
<td>-19.89 (27.03)</td>
</tr>
<tr>
<td><strong>AHA Ideal CVH criteria, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td><em>Behavior metrics</em></td>
<td></td>
</tr>
<tr>
<td>Not currently smoking</td>
<td>42 (72.4)</td>
</tr>
<tr>
<td>BMI &lt;25 kg/m²</td>
<td>3 (5.2)</td>
</tr>
<tr>
<td>(VO_2)max, (men &gt;40, women &gt;35 mL/kg/min)</td>
<td>27 (46.6)</td>
</tr>
<tr>
<td>Healthy diet by MetDiet &gt;8 points</td>
<td>19 (32.8)</td>
</tr>
<tr>
<td><em>Factors metrics</em></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol &lt;200 mg/dL</td>
<td>49 (84.5)</td>
</tr>
</tbody>
</table>
3.2 Training compliance

Training compliance (% of total sessions completed; mean) for each training group was the following: HIIT, 95%; RT, 96%; CT, 88%. There were no differences in training compliance between intervention groups (P = 0.671).

3.3 Changes in body composition parameters

The results of the intention to treatment (ITT) analysis in body composition are shown in Supplemental Table S2 and Figure 2. We found a decrease in total body fat (%) from PRE to POST intervention both in HIIT (-3.082, CI95% = -4.20 to -1.95; ES= 0.663; P < 0.001) and RT (-3.273, CI95% = -5.35 to -1.18; ES = 0.392; P < 0.001), and for trunk fat mass (g) both in HIIT (-2.004, CI95% = -3.27 to -0.72; ES = 0.392; P < 0.001) and RT (-2.007, CI95% = -3.74 to -0.26; ES = 0.258; P < 0.001). In the per-protocol analyses, none of the interventions significantly changed body fat (%) (F(interaction)=1.628; P = 0.198) or trunk fat mass (F(interaction)=1.217; P = 0.313).
Figure 2. Training response in body composition parameters between intervention groups. NG, nutritional guidance; HIIT, high-intensity interval training; RT, resistance training; Concurrent training (HIIT+RT) group; Data shown are mean changes and individual participant responses. ES, effect size.

3.4 Changes in metabolic parameters

With regards to metabolic parameters (Figure 3), we found a decrease in cholesterol (-16.833, CI95% = -29.51 to -4.15; ES = 0.289; P < 0.001) and LDL-c (-25.727, CI95% = -44.52 to -6.92; ES=0.329; P < 0.001) levels from PRE to POST in HIIT and in triglyceride levels in the NG group (-45.250, CI95% = -72.91 to -17.58; ES = 0.294; P < 0.001). There were statistically significant decreases for
cardiometabolic z-score in the four groups after the intervention (time effect $F_{(49.12)}$; ES = range 0.365–0.468; all $P$ values < 0.001); however, the training response (mean changes) difference between the four groups was not statistically significant (interaction effect $F_{(0.261)}$; $P = 0.853$).

**Figure 3.** Training response in metabolic parameters between intervention groups. NG, nutritional guidance; HIIT, high-intensity interval training; RT, resistance training; Concurrent training (HIIT+RT) group; Data shown are mean changes and individual participant responses. ES, effect size.
3.5 Changes in vascular function parameters

The exercise effort test results for the four intervention groups are shown in Figure 4. After 12 weeks of supervised training, all three exercise programs significantly increased FMD (%), in the following increasing order: HIIT group (5.442, CI95% = 3.234 to 7.649; ES = 0.584; P < 0.001); RT group (6.427, CI95% = 2.340 to 10.510; ES = 0.393; P < 0.001); and CT group (7.450, CI95% = 4.032 to 10.860; ES = 0.590; P < 0.001), (time effect F (47.57); P < 0.001). In the RT group, there was a medium effect on PWV (m·s⁻¹) (-0.382, CI95% = -0.620 to -0.138; ES = 0.391; P < 0.001) and brachial Alx (%) (15.209, CI95% = 3.260 to 27.150; ES = 0.298; P < 0.001, interaction effect F (3.505); P = 0.021), indicating positive adaptations in the RT group compared with the CT group. There were no significant treatment effects on other vascular parameters.

![Figure 4. Training response in vascular function parameters between intervention groups. NG, nutritional guidance; HIIT, high-intensity interval training; RT, resistance training;](attachment:image_url)
Concurrent training (HIIT+RT) group; Data shown are mean changes and individual participant responses. ES, effect size.

3.6 Changes in exercise parameters

Figure 5 shows the results for HRrest (bpm), VO$_2$max, (mL/kg/min) and handgrip strength (kg) for the four groups. With regards to the HRrest after the intervention, the only significant result was observed in the RT group (PRE 62.3 (11.2) vs. POST 57.0 (8.3); mean difference -5.364, CI95% = -10.21 to -0.51; ES = 0.243; P < 0.0001). When adjusted for kcal diet, sex and baseline values, three exercise modalities improved VO$_2$max: HIIT +8.375; ES =0.579, RT +4.145; ES =0.579, and CT +6.370; ES =0.579; all P < 0.001. The improvement difference between the groups was statistically significant between the HIIT and NG groups (P = 0.028). There were also significant differences in handgrip strength betweenPRE and POST training measures: HIIT +3.158; ES =0.209, RT +6.336; ES =0.625, and CT +3.160; ES =0.254; all P < 0.001. The improvement difference between the groups was not statistically significant, interaction effect F (0.344) P = 0.794.

![Figure 5](image.png)

**Figure 5.** Training response in exercise parameters between intervention groups. NG, nutritional guidance; HIIT, high-intensity interval training; RT, resistance training; Concurrent training (HIIT+RT) group; Data shown are mean changes and individual participant responses. ES, effect size.
3.7 Change in CVH score (behaviors and factors) metrics according to AHA criteria

We compared the baseline characteristics of the 7 categories cardiovascular behaviours and factors metrics in Figure 6. Only the NG group had an increase in the percentage of the pre-value in the healthy diet metric, whereas HIIT and CT groups had an improvement in VO$_2$max (P < 0.05).

**Figure 6.** Changes after the 12-wk follow-up in ideal cardiovascular health metrics criteria according to AHA by intervention groups. NG, nutritional guidance; HIIT, high-intensity
interval training; RT, resistance training; Concurrent training (HIIT+RT) group; Data shown are unadjusted prevalence (%).

The estimated changes of 3 health categories based on the number of CVH metrics are shown as a percentage of the pre-value in Figure 7. The HIIT and RT groups had a 33.4% (P = 0.032) and 41.6% (P = 0.020) increase in >5 metrics, respectively.

Figure 7. Changes after the 12-wk follow-up in categorical ideal cardiovascular health metrics criteria according to AHA by intervention groups. NG, nutritional guidance; HIIT, high-intensity interval training; RT, resistance training; Concurrent training (HIIT+RT) group; Data shown are unadjusted prevalence (%).

4. Discussion

Our aim was to compare the effects of 12 weeks of HIIT, RT, CT (HIIT+RT) or NG on MetS risk factors, ideal CVH parameters and vascular function in a cohort of sedentary and overweight adults. The main findings of this study are that 12 weeks of HIIT leads to significant improvements in VO2max as compared with NG, and also that RT stimulates a greater increase in arterial stiffness than CT. Moreover, both HIIT and RT modalities increase ideal CVH metrics, supporting the positive effect of both training programs for CVH in sedentary and overweight males.

Exercise training is a well established means of enhancing vascular health [52]. Consistent with previous studies [15, 53, 54], our data reveal that all three exercise regimens improve FMD. Furthermore, RT was found to improve PWV and aortic AIx, supporting the concept that different types of exercise training might produce
diverse adaptations of arterial stiffness in obese and sedentary adults [55]. Indeed, we observed that aortic AIx was higher in the RT than in the CT group. This is of particular interest since high AIx, a measure of pulse wave reflections influencing the central blood pressure, predicts mortality and cardiovascular events [56]. Although RT programs have shown beneficial effect on arterial stiffness [57, 53, 58], a recent study found that a 8-week period of RT did not change arterial stiffness in individuals with MetS or healthy controls [59]. Considering that the aforementioned study used a shorter training period, it could be hypothesized that only longer RT interventions have a substantial effect on arterial stiffness. It has been suggested that the role of different exercise training interventions on vascular function might be mediated by the synthesis of molecular mediators, changes in neurohormonal release and/or oxidant/antioxidant balance [52]. Nevertheless, the specific role of RT in arterial stiffness in overweight and sedentary adults is unclear and warrants further investigation.

With regards to body composition parameters, although no significant difference in training response was found between HIIT, RT, CT and NG, we found decrease in total body fat and trunk fat mass after the HIIT and RT interventions. The HIIT program yielded the largest improvements in total body fat (ES=0.663) and trunk fat mass (ES=0.392), indicating that HIIT is an optimal mode of exercise for fat loss in sedentary and overweight adults. Also, HIIT has been reported to be the more time-efficient approach to achieve the beneficial effects of exercise on body composition [13].

All four regimens led to reductions in WC (NG=-1.69 cm, HIIT=-4.39 cm, RT=-3.95 cm, and CT=-2.86 cm). It has been suggested that WC reduction is an important component that influences adult MetS, as it reflects abdominal fat excess and is closely related to cardiovascular diseases. The potential mechanisms underlying the HIIT-induced fat loss effect are unknown but might include increased exercise and post-exercise fatty acid oxidation and suppressed appetite [60]. Therefore, the data of the present study strongly suggest that the response of overweight adults to a program of exercise training consists of a change in visceral obesity and a decrease in the risk factors for MetS.

No changes in metabolic parameters were observed between the four intervention groups; however, we found significant decreases in total cholesterol and LDL-c from baseline to post-exercise in the HIIT group. Previous studies examining HIIT protocols for blood lipids have also demonstrated positive changes [10–13, 60]. In agreement with previous research [10, 61], we failed to find any favorable change in HDL-c levels in overweight and sedentary adults. By contrast, Tjonna et al. reported that HDL-c increased in middle-aged adults in response to 16 weeks of aerobic interval training [62]. It is relevant to consider, however, that this study was conducted in a cohort of adults with MetS and very low baseline HDL-c values. The lack of consistent results might also be explained by differences in HIIT programs, and it is possible that only long-term training has a substantial effect on HDL-c response.
Regarding cardiometabolic health, significant decreases in the cardiometabolic z-score were identified in the four groups after the intervention, although the training response difference between the groups was not statistically significant. Considering that HIIT yielded the largest improvement in cardiometabolic z-score (ES=0.468), it seems that HIIT could be a more effective intervention for improving cardiometabolic risk in overweight and sedentary adults. Existing research has indeed shown that high intensity exercise is more strongly inversely related with MetS when compared with low-intensity exercise [29, 63].

Despite the similarity in volume and duration of the HIIT and RT interventions with the CT program, we saw a greater VO2max, (ES=0.579) improvement with HIIT than with RT (ES=0.263), CT (ES=0.326) or NG (ES=0.001). Nevertheless, the improvement difference between the groups was statistically significant only between HIIT and NG groups (P = 0.028). This is consistent with previous studies [11, 18, 21, 22]. Moreover, our results overall are in line with other studies [11,12,19,25,28] in that all groups responded positively to exercise and presented an increase in VO2max or METs and a decrease in HRrest. Improvements in CRF parameters were also demonstrated through a decrease in HRrest and the use of more intense workloads. Better heart and muscle function likely played a role in this improved performance [63].

We found an inverse relationship between aerobic fitness and fat content, and both were significantly related to the lipid profile [62,70]. This reinforces the importance of supervised exercise training as a non-pharmacologic strategy for reversing the adverse effects of lack of exercise among overweight adults, thereby preserving fat-free mass in sedentary and overweight males.

Ideal CVH metrics are inversely associated with cardiovascular events, supporting the use of these metrics as a useful tool to predict cardiovascular disease risk [64]. To the best of our knowledge, this study is the first to investigate the effect of different exercise training modalities and NG on CVH metrics in a population of middle-age overweight adults. We found an increase in healthy diet metrics after 12-weeks of NG and an improvement in VO2max after HIIT and CT. Indeed, there was an increase of 33.4% and 41.6% in CVH metrics in HIIT and RT groups, respectively. In line with our findings, recent systematic reviews and meta-analyses concluded that HIIT can improve some cardiometabolic risk factors in overweight/obese populations [13, 19]. Similarly, available data indicate that an RT program is also an effective exercise modality for reducing the risk of cardiovascular disease [65]. Both exercise protocols and NG were effective in promoting important changes to a number of health-related parameters. Accordingly, a 12-week training program comprising HIIT or RT should be recommended for overweight and sedentary adults in order to improve their CVH.

**Limitations**

This study had some limitations. First, since endothelial function is well known to be affected by age and training status, and our study cohort comprised overweight and sedentary middle-aged adults, this could imply that our findings may not be generalizable to other populations with different characteristics. Another limitation is
the lack of dietary control during the course of the intervention. However, we continually reminded subjects of their commitment to maintain their current dietary habits in order to minimize the influence of diet.

The main strength of our study is that it is the first RCT, to our knowledge, on the effect of 12 weeks of HIIT, RT, CT or NG on MetS risk factors, ideal CVH and vascular function in adults from the Latin-American population. Secondly, there was high exercise compliance and we used state-of-the-art measures of cardiovascular fitness and metabolic/endothelial function. Moreover, body composition parameters were assessed by dual-energy X-ray absorptiometry, considered the current “gold standard” for body composition measurement.

5. Conclusion

A 12-week training program of HIIT resulted in greater cardiorespiratory fitness than NG, whereas an RT program improved arterial stiffness over CT, supporting the notion that different exercise training regimens might produce different adaptations of arterial stiffness in obese and sedentary adults. Both HIIT and RT programs increase ideal CVH metrics, supporting the positive effect of both training programs on CVH in sedentary and overweight males.

6. References


42. Atkinson G (2014) Shear rate normalization is not essential for removing the dependency of flow-mediated dilation on baseline artery diameter: past research revisited. Physiol Meas 35:1825–1835.


1. Exercise and vascular function in humans (Study I)

Physical inactivity is associated with increased oxidative stress, endothelial dysfunction and atherosclerosis, whereas physical training and aerobic capacity are associated with a low risk of cardiovascular disease and mortality [1,2]. Aerobic exercise is therefore strongly recommended for both healthy people and for patients with cardiovascular disease to improve cardiovascular health and reduce the risk of premature death [3]. It has been observed, both in sedentary youth and those who are physically trained, that a single session of physical exercise (five series of 5 minutes of running at 90% VO$_{2\text{max}}$) is enough to provoke a significant increase of FMD and NO bioavailability during the following 48 hours after ending the session [4]. A similar effect on the vasodilating capacity of the endothelium was also described in patients with chronic heart failure, who showed increased shear stress after an acute session of moderate exercise (25 min in cycling) [5].

This increase in the endothelium’s dilating response seems determined by two different effects of the physical exercise, but closely related to each other: on one side is the powerful mechanical stimulus provoked by exercise on the vascular wall; on the other is the increase of the metabolic needs in the active muscles that require the capture of energy substrates to maintain fiber contraction [6]. In both phenomena, NO seems to play a principal role in determining endothelial response upon exercise, as demonstrated from the acute increase in NO synthesis and use during and immediately after physical exercise [7].

Increased levels of physical activity and cardiorespiratory capacity induced by exercise is directly related to reduced morbidity and mortality in coronary heart disease. Among the possible mechanisms that determine these benefits of exercise in the general population there is improved shear stress [8]. Clarkson et al. [9] observed a significant increase of the endothelium-dependent vasodilatation in the brachial artery of young and healthy individuals after engaging in a 10-week training program of moderate intensity that included aerobic exercise (4.8 km running per day) and anaerobic exercise (strength exercises of the upper body).

This observation in a healthy population was then reinforced by the finding of significantly higher endothelium-mediated vasodilatation in elderly adults (between 61 and 83 years of age) with a high level of physical training (> 40 ml·kg$^{-1}$ min$^{-1}$ of VO$_{2\text{max}}$) with relation to their sedentary peers (≤ 27 ml·kg$^{-1}$ min$^{-1}$ of VO$_{2\text{max}}$) [10,11].

It is relevant to highlight that in these studies the type of exercise analyzed always conditioned significant effort of the lower limbs, with a vasodilating effect that
evidently was not only limited to the vessels next to the active muscle mass. Hence, this evidence suggests that the local activity of the muscle groups of the lower half of the body constitutes a potent NO stimulus at the whole-body level; and, consequently, demonstrates the importance of physical forces (like increased cardiac frequency, blood pressure, blood viscosity, etc.), which these types of sports induce on the vascular bed in tissue that is not necessarily active.

2. Physiological mechanisms of vascular response induced by exercise in humans (Study I-II-VI)

During exercise, increased blood flow rates through the vessels are produced, which causes an increase of the friction forces on the vascular wall. The mechanical stress resulting from this phenomenon is directly related to the blood’s velocity and viscosity [12] and it constitutes the principal stimulus for acute endothelial production of NO with the aim of proportionally increasing the vessel’s diameter. At the molecular level, the mechanical stress causes an increase in the messenger RNA (mRNA) and protein transcription of the eNOS modulated by aperture of K+ channels in the endothelial cell. This phenomenon, along with the accumulation of nitrite (a stable compound that results from NO degradation), increases the capacity of the cell exposed to the stimulus of producing NO and, subsequently, the vessel’s dilatation [13].

The intensity of the mechanical stress generated is, hence, proportional to the duration and intensity of the physical exercise performed, as well as to the muscle mass involved in the effort [14]. Additionally, the type of exercise also directly modulates the intensity of the friction forces and, consequently, NO production [15]. This peculiarity (dependent on the type of exercise) is due to the differences provoked in blood flow behavior and, particularly, to the oscillation between an antegrade systolic and retrograde diastolic flow provoked in each type of physical exercise. Higher oscillation between these flows determines an increase in friction forces, which explains the findings of greater vasodilatation mediated by NO in the inactive upper limbs after performing lower-limb exercises, such as cycling, running, and walking [16,17], where the total flow is even lower than in upper-limb exercises (but with a higher retrograde component in the flow) [18].

During exercise, NO is also a potential modulator of the skeletal muscle metabolism that favors the cellular capture of fuels, like glucose. In this sense, different studies in animal models have noted that increased NO synthesis during exercise responded to the muscle’s need to preserve the energy reserves favoring the entry of glucose into the muscle [19,20]. However, this evidence was confirmed recently in humans through a study conducted by Bradley et al. [21] in which an inhibitor of NOS (L-N3-monomethyl Arginine citrate, L-NMMA) infused into the femoral artery during aerobic exercise on a cycle ergometer reduced glucose capture by 48% compared to the infusion of a control saline solution. In this sense, the exact mechanisms that explain the NO-mediated glucose capture are still partially understood, although it is known that they act independently and parallel to
the mechanisms induced by insulin (like glucose transporter-4, GLUT-4 activation); which are not significantly affected when eNOS is experimentally inhibited [22].

Different studies on the behavior of the coronary and peripheral vasculature in animals suggest that a short period of physical exercise increases NOS activity, as well as NO production and bioactivity, causing an effect that compensates the increase of the mechanical stress induced by the effort [23]. Notwithstanding, after the first weeks, the vascular wall structure could be remodeled by the effect of NO and possibly other mediators, whose result is a chronic increase of the vessel’s light [24]. Consequently, mechanical stress would result structurally normalization, and the activity of the vasodilating system of NO would return to levels prior to starting the exercise program [25] (Figure 1).

Figure 1. Shear stress signals derived from endothelial cells by exercise training. Note: High shear stress induced by physical exercise promotes several beneficial cardiovascular effects. Via mechano-receptor stimulation, high shear stress up-regulates and stimulates proteins in the Akt/eNOS pathway and NO production, and down-regulates AT1R and NADPH oxidase subunits. High shear stress may inhibit p47 translocation, leading to a decrease in NADPH oxidase activity and diminished ROS generation. Modified from Ref [23].

This hypothesis has also been founded on studies that found no modifications in baseline NO production and in vasodilatation in highly trained athletes [26]. However, although it seems logical to expect vascular adaptation to mechanical stress induced by systemic exercise, this hypothesis does not contemplate other variables that can constantly increase the need for NO synthesis. This would be the case of the time of day in which the physical exercise takes place, which could substantially increase the degree of mechanical stress on the vascular wall [27];
also, constant variations in the metabolic needs of the musculature derived from the progression of loads and training intensities, training of new muscle groups, and of the rest-training periods that are part of the natural history of the sporting life of athletes. Even other external factors, like dietary supplementation before, during, or after the effort, could modify the vasodilating response generated by the exercise.

3. Physical exercise as a strategy to improve cardiovascular risk factors (Study III-VI)

Considering that the health benefits of physical exercise can only be seen with regular practice, a positive relationship between intensity training and the reduction of the risk and improvement in metabolic parameters-related risk factors is expected [28]. On the other hand, it is well-known that sedentarism is a risk factor for the development of several degenerative diseases. In fact, a physical inactivity is negatively related to most of the metabolic syndrome components; therefore, increasing physical exercise could prevent and/or treat the metabolic syndrome [29].

It has been suggested that high-and moderate aerobic training at least twice a week for at least six weeks may improve the metabolic profile and cardiovascular fitness [30]. HIIT may not reduce total cholesterol and LDL-C fraction levels, but it changes the quality of LDL-C subfractions, thereby increasing the concentrations of large LDL-C and reducing the concentration of small LDL-C. However, the recommendation of moderate physical activity does not seem to prevent the syndrome of reaching pandemic levels and the optimal training regimen remains to be defined.

In same line, the most significant effects of physical activities are observed in HDL-C and triglyceride levels [31]. Previous meta-analyses demonstrated that dynamic moderate intensity exercise decreases blood pressure in hypertensive individuals [32], causes a significant reduction in blood lipids and lipoproteins in patients with hyperlipidaemia, reduces glycated haemoglobin (HbA1c; percentage) in patients with type 2 diabetes mellitus and is associated with improved body composition in obese individuals [33,34].

The benefits of physical exercise on insulin sensitivity have been demonstrated with both predominantly aerobic exercises and predominantly strength exercises [33]. However, the mechanism by which these modalities of exercise promote an improvement on the insulin sensitivity seems to be different, indicating that the combination of two exercise modalities may potentiate this effect. The positive changes found in our study can be explained by better body fat distribution (reductions in total body mass and waist circumference, and unchanged fat-free mass) and increased physical fitness due to training. Physical training and nutritional guidance probably produced a healthy lifestyle.
4. Physiological mechanisms of HRV induced by exercise in humans (Study IV)

There are various potential adaptations which explain HIIT-induced positive changes in the autonomic functioning of heart. One of the potential mechanism underlying HIIT induced cardiac vagal tone may be Angiotensin II. Angiotensin II inhibits cardiac vagal activity [35]. Sedentary or physically inactive individuals have higher plasma rennin activity than athletes [36]. Therefore, sedentary individuals have higher Angiotensin II.

Exercise causes suppression of Angiotensin II which may, to some extent, mediate enhancement of cardiac vagal tone [37]. Research have also suggested that exercise-induced nitric oxide bioavailability mediate changes in cardiac vagal tone and inhibit sympathetic influences [38]. Furthermore, HIT induced increased baroreflex sensitivity and reduction in arterial stiffness may also be considered adaptation enhances cardiac vagal tone [39].

5. Physiological mechanisms prior intensity of exercise on postprandial metabolism and vascular function (Study V)

Regular exercise has long been regarded as an important component of a healthy lifestyle. Every adult should accumulate 30 min or more of moderate-intensity exercise on most, preferably all, days of the week. The scientific evidence clearly demonstrates that regular, moderate-intensity exercise provides substantial health benefits and the novel aspect of these reports was that the activity can be accumulated through a minimum of 10min per bout to attain 30 min or more for general health benefit [40]. Intermittent exercise also confers substantial benefits, among those that stand the reduce postprandial lipaemia (considered an emerging risk factor for cardiovascular disease). Acute exercise can significantly reduce the postprandial TG response in adults, which may contributing to the improve on endothelial dysfunction, insulin resistance and oxidative stress [41,42], but the underlying mechanisms responsible for this beneficial effect, which are independent of traditional cardiovascular risk factors, remain poorly understood [43]. Besides, physical exercise may reduce postprandial hyperlipemia levels for up to eight hours after a HMF [44]. Several exercise variations have been tested, such as CMT and HIT [2,45-47].

In a study carried out by Bond et al. [48] 2 weeks of HIIT in adolescents improved HRV and FMD but not traditional cardiovascular risk factors (triglycerides, cholesterol, glucose, insulin, and blood pressure) in fasting and postprandial states. In this study, each training session consisted of eight to ten 1-min repetitions of cycling at 90% peak power interspersed with 75 s of unloaded cycling. These favorable changes were lost after 3 days of training suggesting that regularly performing high-intensity exercise is needed to maintain these benefits.
These findings are similar to those of Gill et al. [49], who used prior exercise, but more specifically those of Padilla et al. [50], study that used MCT following a HFM to attenuate the impaired endothelial dysfunction. Even though Padilla et al. and Tyldum et al. demonstrated an increase in FMD following exercise it is difficult to interpret these findings since in the combination of meal and exercise only two measurements were taken, preprandial and two hours following both the meal and the exercise. While results of several studies suggest that influence of physical exercise on the endothelium [51,52], its functions, and its interactions [53] with blood components [54]. Physical exercise has positive effects on endothelial function by stimulating the production and bioavailability of NO [55], given that exercise induces the activity of eNOS, increases the capacity of the cellular antioxidant system and diminishes the formation of ROS.

However, the mechanisms responsible for the reduction in triglycerides in the present study are unclear. One possibility is that TG are cleared faster from the circulation following acute exercise, a process mediated by an acute increase in the activity of skeletal muscle lipoprotein lipase (LPL) in a time course consistent with the postprandial reduction of TG. Gill et al. [56] found that individuals who had the greatest increase in LPL activity following exercise tended to have the largest reductions in PPL. This suggests that increases in LPL activity are not essential to attenuating postprandial lipemia after prior exercise but, where increases occur, they can have a strong influence on the magnitude of the reduction.

The exercise attenuation of fasting and postprandial triglycerides may also be mediated by changes in insulin sensitivity. A recent investigation in healthy adults found that the addition of protein corrected the endothelial dysfunction associated with a HFM [57]. The authors concluded that the effect was mediated by insulin. For consistency with the current guidelines regarding physical activity and health [58,59] the present study employed moderate levels of activity both exercise intensity and exercise volume in this study are consistent with the guidelines [40].

7. References


dysfunction in patients with chronic heart failure: systemic effects of lower-limb

lower limb exercise on forearm vascular function: contribution of nitric oxide. Am

19. Balon TW, Nadler JL. Evidence that nitric oxide increases glucose transport in

20. Roberts CK, Barnard RJ, Scheck SH, Balon TW. Exercise-stimulated glucose
transport in skeletal muscle is nitric oxide dependent. Am J Physiol.

leg glucose uptake but not blood flow during dynamic exercise in humans.

22. Hayashi T, Wojtaszewski JF, Goodyear LJ. Exercise regulation of glucose

23. Di Francescomarino S, Sciartilli A, Di Valerio V, Di Baldassarre A, Gallina S. The

training in patients with cardiovascular disease: focus on skeletal muscle,
endothelium, and myocardium. Am J Physiol Heart Circ Physiol. 2017;313:H72-
H88.

25. Szostak J, Laurant P. The forgotten face of regular physical exercise: a 'natural'

Mathers JC. Exercise modalities and endothelial function: a systematic review
2015;45:279-96.

27. Gliemann L, Nyberg M, Hellsten Y. Nitric oxide and reactive oxygen species in
limb vascular function: what is the effect of physical activity? Free Radic Res.
2014;48:71-83.

28. Torres-Leal FL, Capitani MD, Tirapegui J. The effect of physical exercise and


Chapter 8

Conclusions, practical applications and future perspectives

Study 1 (Chapter 2)

**Conclusion 1:** Under the conditions of the present study, physically inactive adults in both groups experienced changes in FMD. The rate of response was significantly different between exercise groups for FMDn measure but not PWV outcome. The sustained change in PWV in the HIT group may represent a signal of vascular adaptation or endothelial fatigue.

**Practical application 1:** This study demonstrates the efficacy of HIT in enhancing the cardioprotective effects of exercise on the progression of atherosclerosis in a physically inactive population.

**Future perspective 1:** Identifying the training regimen that has the most beneficial effects on each parameter could potentially lead to enhanced precision in prescribing exercise training intensity to achieve optimal outcomes in this population. Under the conditions of the present study, physically inactive adults in both groups experienced changes in FMD. Not all vascular function measured responded the same to this type of exercise, suggesting different regulatory mechanisms and time courses for induction.

Study 2 (Chapter 3)

**Conclusion 2:** Among apparently healthy physically inactive adults, HIT and MCT offer similar cardiometabolic protection against single MetS risk factors but differ in their effect on average risk factors per subject.

**Practical application 2:** The improvement in the cardiovascular profile achieved in the present study may be an effective strategy for reduction in MetS Z-score and improving the health trajectory of physically inactive adults.

**Future perspective 2:** Changes in body composition, or more precisely, changes in abdominal obesity and fat mass seem to be an important factor when an exercise intervention for reducing CVD markers is planned. In the present study we showed that a significant reduction in MetS Z-score is possible also in the absence of change in lean mass.

Study 3 (Chapter 4)

**Conclusion 3:** In inactive adults, this study showed that a 12-week HIT training program could increase short-term HRV, mostly in vagally mediated indices such as SDNN and HF/LFLn ratio power.
Practical application 3: These data underline the importance of a multidisciplinary approach aiming at promoting HIT exercise programme in physically inactive adults. For the practitioners/clinicians or trainer working with inactive populations should promote HIT exercise longer than 12 weeks in order to improve outcomes in cardiovascular health, due to HRV is a direct predictor of cardiovascular risk and all-cause mortality.

Future perspective 3: Additional randomised controlled trials are required to elucidate the mechanisms responsible for these results in physically inactive adults and other populations, such as metabolic syndrome, obese, or insulin resistance adults.

Study 4 (Chapter 5)

Conclusion 4: Exercise intensity plays an important role in these protective effects, suggesting that HIT might be more effective than MCT in reducing postprandial glucose levels and attenuating vascular impairments.

Practical application 4: The novel finding of this study was that medium-term supervised physical training may mitigate endothelial dysfunction and glucose response induced by PPL. Exercise intensity seems to play an important role in these protective effects, suggesting that high-intensity training might be the most effective in reducing postprandial glucose levels and attenuating vascular impairments. Therefore, medium-term HIT is an effective strategy to reduce CVD.

Future perspective 4: Exercise was found to be associated with beneficial effects on biomarkers endothelial function in the postprandial state. Randomized clinical trials assessing the effect of MCT or HIT on hard outcomes of cardiovascular disease and MetS are warranted.

Study 5 (Chapter 6)

Conclusion 5: A 12-week training program of HIT resulted in greater cardiorespiratory fitness than nutritional guidance, whereas an resistance training program improved arterial stiffness over concurrent training, supporting the notion that different exercise training regimens might produce different adaptations of arterial stiffness in obese and sedentary adults.

Practical application 5: Designing HIT or resistance training intervention programs might be a beneficial strategy for improving cardiac autonomic dysfunction induced by an HFM, thus reducing cardiovascular disease.

Future perspective 5: Both HIT and resistance training programs increase ideal cardiovascular health metrics, supporting the positive effect of both training programs on cardiovascular health in sedentary and overweight males.
8. Conclusiones, aplicaciones prácticas y perspectivas futuras

Estudio 1 (Capítulo 2)

Conclusión 1. Bajo las condiciones de este estudio, los adultos físicamente inactivos en ambos grupos vieron cambios en la dilatación dependiente del endotelio (DDE). Sin embargo, la tasa de respuesta fue significativamente diferente entre los grupos de ejercicio para la medición de DDE-normalizada pero no para el resultado de la velocidad deinda de pulso (VOP). El cambio sostenido en VOP en el grupo HIT puede representar una señal de adaptación vascular o fatiga endotelial.

Aplicación práctica 1. Este estudio demuestra la eficacia del HIT en parámetros asociados a factores de riesgo cardiometabólicos en adultos físicamente inactivos.

Perspectiva futura 1. Identificar el régimen de entrenamiento que presenta los mejores efectos sobre cada parámetro cardiovascular podría, potencialmente, conducir a una mayor precisión en la prescripción de intensidad de entrenamiento de ejercicio para lograr resultados óptimos en esta población. Bajo las condiciones de este estudio, los adultos físicamente inactivos en ambos grupos vieron cambios en la DDE. Sin embargo, no todos los programas de ejercicio inducen mejoría o superioridad biológica, lo que sugiere que diferentes mecanismos regular los efectos.

Estudio 2 (Capítulo 3)

Conclusión 2. En adultos aparentemente saludables y físicamente inactivos, tanto el HIT, como el entrenamiento continuo ofrecen similar protección cardiometabólica contra factores de riesgo asociados al síndrome metabólico (SM), pero sus efectos, difieren a nivel individual.

Aplicación práctica 2. El mejoramiento en el perfil cardiovascular y en la composición corporal logrado en este estudio puede ser una estrategia efectiva para la reducción del puntaje Z del SM en adultos físicamente inactivos.

Perspectiva futura 2. Los cambios en composición corporal, o de manera más precisa, los cambios en la obesidad abdominal y la masa grasa parecen ser un factor importante cuando se planifica una intervención de ejercicios para reducir los marcadores de enfermedad cardiovascular. En este estudio mostramos que una reducción en la puntuación Z en MetS también es posible sin modificar la masa muscular.

Estudio 3 (Capítulo 4)

Conclusión 3. En adultos inactivos y con exceso de peso, se demostró que un programa de entrenamiento de HIT de 12 semanas podría incrementar la variabilidad de la frecuencia cardiaca (VFC) en el corto plazo, principalmente en índices mediados vagalmente, como la desviación estándar de los valores de NN (SDNN) y relación HF/LF (indicador fiable del balance autonómico en reposo).
Aplicación práctica 3. Estos datos resaltan la importancia de un enfoque multidisciplinario con el objetivo de promover un programa de ejercicio de HIT en adultos físicamente inactivos. Los practicantes/clínicos o entrenadores que trabajan con poblaciones inactivas podrían promover programas de HIT por más de 12 semanas, con el propósito de mejorar el balance autonómico debido a que una menor VFC se considera un predictor directo de riesgo cardiovascular y mortalidad por todas las causas.

Perspectiva futura 3. Se requieren ensayos controlados aleatorios adicionales para dilucidar los mecanismos responsables de estos resultados en adultos físicamente inactivos y otras poblaciones, tales como adultos con síndrome metabólico, obesos o con resistencia a la insulina.

Estudio 4 (Capítulo 5)

Conclusión 4. La intensidad del ejercicio juega un papel importante en estos efectos protectores, sugiriendo que el HIT podría ser más efectivo que el entrenamiento continuo de intensidad moderada en la reducción de los niveles de glucosa postprandial y atenuación de deterioro vascular.

Aplicación práctica 4. El hallazgo novedoso de este estudio fue que el entrenamiento físico supervisado a mediano plazo puede mitigar la disfunción endotelial y la respuesta de glucosa inducida por un estado de lipemia post-prandial. Así pues, se demostró que la intensidad del ejercicio parece jugar un papel importante en estos efectos, lo cual sugiere que el HIT podría ser el más efectivo para reducir los niveles de glucosa postprandial y atenuar deterioro vascular inducido por la lipemia post-prandial. Por lo tanto, el HIT a mediano plazo es una estrategia efectiva para reducir los mecanismos asociados al estado de estrés oxidativo que se asocia la lipemia post-prandial.

Perspectiva futura 4. Se encontró que un protocolo HIT induce mayores efectos beneficiosos sobre la función endotelial y en los biomarcadores relacionados a un estado de lipemia postprandial. Se hacen necesarios ensayos clínicos aleatorios que evalúen mecanismo que explique los efectos del entrenamiento HIT en sujetos con exceso de peso y sedentarios.

Estudio 5 (Capítulo 6)

Conclusión 5. Un programa de entrenamiento de 12 semanas de HIT incremento en mayor medida la capacidad cardiorrespiratoria que un protocolo de orientación nutricional o combinado (HIT+fuerza), mientras que un programa de entrenamiento de fuerza muscular mejoró en mayor medida la rigidez arterial, apoyando la noción de que diferentes regímenes de entrenamiento podrían producir diferentes adaptaciones en diversos marcadores metabólicos en sujetos con exceso de peso y sedentarios.

Aplicación práctica 5. Nuestros resultados muestran evidencias de que el entrenamiento HIT o fuerza muscular y sin cambios en la alimentación, disminuye la adiposidad corporal/abdominal y mejoran la rigidez vascular, al mismo tiempo que incrementa el fitness cardiorrespiratorio (VO2max) en adultos colombianos.
sedentarios con exceso de peso corporal. Se ha reportado que el VO$_{2\text{max}}$ es un predictor independiente de todas las causas de mortalidad y el riesgo de muerte se reduce un 13% por cada 3.5 ml/kg*min de VO$_2$ o METs que se incrementa. De acuerdo a esto, consideramos que estos participantes redujeron su riesgo de muerte por ECV pues encontramos una mejoría de 2 METs.

**Perspectiva futura 5.** Este trabajo demuestra que un programa supervisado de HIT o de fuerza sin modificar la ingesta dietética son una estrategia útil para reducir algunos de los factores de riesgo más relevantes asociados a mortalidad por todas las causas.
Chapter 8

Relevant papers
calcium and parathyroid hormone levels were normal in our patient.

This case, in addition to previous reports, highlights the importance of regular medication review for prolonged PPI usage. PPIs should be prescribed at the lowest effective dose and only for as long as clinically indicated. Besides PPIH, PPIs have also been associated with other adverse effects such as the risk of *Clostridium difficile* infection, pneumonia, and bone loss or fractures. This was reflected in the American Geriatric Society 2015 updated Beers criteria where PPIs were listed as potentially inappropriate medications to be avoided in patients 65 years and older.

References


Zi Ying Koh, MBBS, MRCP, MMed
Department of Geriatric Medicine
Tan Tock Seng Hospital
Singapore, Singapore

Stephanie Wan Min Lim, BSc(Pharm)
Department of Pharmacy
Tan Tock Seng Hospital
Singapore, Singapore

Wee Shiong Lim, MBBS, MRCP, MMed, MHPE
Department of Geriatric Medicine
Tan Tock Seng Hospital
Singapore, Singapore

http://dx.doi.org/10.1016/j.jamda.2017.04.009

---

**Exercise for Disease Prevention and Management: A Precision Medicine Approach**

To the Editor:

Lack of engagement in sufficient weekly physical activity (PA) is a major risk factor for morbidity and premature mortality. Estimates from 2012 indicated that not meeting PA public health recommendations is responsible for more than 5 million deaths globally each year. Furthermore, insufficient PA was estimated to account for more than 11% of the US aggregated health care expenditures in 2014, which translates to approximately $120 billion per year or an excess $1400 in per capita costs. Although low- and middle-income countries bear 75% of the disease burden associated with physical inactivity, more than 80% of health care costs and 60% of indirect costs occur in high-income countries.

Despite numerous trials and programs, PA levels in the United States have remained flat for decades, with most adults not meeting aerobic (48%) and muscle-strengthening (70%) guidelines. In fact, when assessed objectively, noncompliance with PA guidelines constitutes the most prevalent of the “hard” risk factors for major chronic diseases. Evidence shows that PA has a significant role, in many cases comparable or superior to drug interventions, in the prevention and treatment of more than 40 noncommunicable chronic diseases, such as obesity, heart disease, diabetes, hypertension, cancer, depression, Alzheimer disease, arthritis, and osteoporosis. However, despite the substantial health and economic burden associated with physical inactivity and the fact that ample evidence supports the effectiveness of PA promotion by the health care sector, PA promotion is not yet a standard of care for disease prevention and management in health care settings.

Precision/personalized medicine is an emerging approach for prevention as well as more effective diagnosis and disease treatment that takes into account variability in genes, environment, and lifestyle for each individual. Precision medicine will enable health care providers to more accurately define the optimal treatment and prevention strategies for a particular disease phenotype and if widely implemented may lead to large clinical and public health improvements both in the United States and globally. Despite an initial focus on “genes, drugs, and disease” aspects related to social, environmental, and behavioral disease determinants are increasingly being emphasized. This viewpoint emphasizes the importance of integrating personalized PA and exercise prescriptions as part of precision lifestyle and behavioral medicine.

**Toward a Precise PA Prescription Era**

Precision PA and exercise prescriptions can help address the substantial variability in individual patient response to health-related fitness outcomes and tailoring of exercise programs to the individual phenotype of each patient. In this context, there are 2 main components: a short-term focus on improving chronic disease–related declines in functional capacity and a longer-term aim to generate knowledge applicable to the whole range of maintaining optimal health and preventing diseases.

Physical inactivity is a key factor contributing to the onset of muscle mass and function decline (ie, sarcopenia), which in turn appears to be a vital aspect related to frailty. Poor health, disability, and dependency do not need to be the inevitable consequences of aging. PA, as an intervention, is one of the most important components in improving the functional capacity of frail seniors. Accordingly, an important conceptual idea for frailty is that the focus should be on functionality and not on the diagnosis of disease for older patients. Furthermore, provision of PA interventions is relatively free of potential unwanted side effects caused by common medications that are prescribed in patients with multiple comorbidities. In this line, PA is a very promising intervention for the modulation of both health span and life span in a number of species. Substantial evidence already exists in support of multipronged PA and exercise counseling, prescription, and referral strategies. Despite the overwhelming scientific evidence that PA improves the health of the population, society in general and health professionals in particular are not yet clear on how to...

---

Descargado para Anonymous User (n/a) en ClinicalKey Espanol Colombia, Ecuador & Peru Flood Relief de ClinicalKey.es por Elsevier en junio 28, 2017.

Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2017. Elsevier Inc. Todos los derechos reservados.
approach this matter. Although an initial good step, PA and exercise prescriptions as a form of medicine are much more than just walking. Unfortunately, exercise programs are typically developed for the average person controlling relative intensity efforts (ie, “one-size-fits-all” approach), with less consideration for the differences between individuals. This would necessarily involve an individualized prescription according to the functional capacity of the person, with specific recommendations about the dose (intensity, volume, and frequency), similar to those of other medications. Indeed, researchers have recognized the substantial variability in patient response to physical exercise interventions and have sought to understand these differences.

For example, in cancer disease there is increasing interest in health-related fitness outcomes by exercise researchers that makes the application of precision medicine (ie, the focus on genetic and molecular subgroups) much more relevant. Nevertheless, there are some differences between exercise and medical interventions that may have implications for the application of precision medicine to exercise oncology. On the other hand, several reports pertain to “average data,” and there is wide interindividual variability in response to exercise training (IVRET), which has mainly been explored in endurance-based studies.

The IVRET implies that under the same stimulus, some individuals may achieve benefits, who are considered responders, whereas others may exhibit a worsened or unchanged response after training, termed nonresponders. In the era of precision medicine, IVRET in the magnitude of response to supervised exercise training (subject-by-training interaction; “individual response”) has received increasing scientific interest. This is an exciting time to help patients combat the increasingly recognized impact of aging as well as noncommunicable diseases on the broad-based benefits of PA while targeting exercise prescriptions and programs using precision behavioral and lifestyle medicine approaches.

References


Robinson Ramírez-Vélez, PhD
Centro de Estudios para la Medición de la Actividad Física «CEMA»
Escuela de Medicina y Ciencias de la Salud
Universidad del Rosario
Bogotá, Colombia
Felipe Lobelo, MD, PhD
Hubert Department of Global Health
Rollins School of Public Health
Emory University, and, Exercise is Medicine Global Research and Collaboration Center
Atlanta, GA
Mikel Izquierdo, PhD
Department of Health Sciences, Public University of Navarre
CIBER de Fragilidad y Envejecimiento Saludable (CB16/10/00315)
Pamplona, Navarre, Spain

http://dx.doi.org/10.1016/j.jamda.2017.04.012
High Intensity Interval- vs Resistance or Combined- Training for Improving Cardiometabolic Health in Overweight Adults (Cardiometabolic HIIT-RT Study): study protocol for a randomised controlled trial

Robinson Ramírez-Vélez 1*, Alejandra Hernandez 1, Karem Castro 1, Alejandra Tordecilla-Sanders 1, Katherine González-Ruíz 1,2, Jorge Enrique Correa-Bautista 1, Mikel Izquierdo 3 and Antonio García-Hermoso 4

Abstract

Background: Although evidence shows the positive health effects of physical activity, most of the adult population in Colombia are sedentary. It is, therefore, important to implement strategies that generate changes in lifestyle behaviours. This protocol describes a study in which we will compare the effects of 12 weeks of high-intensity interval training (HIIT), resistance training (RT) or combined training (HIIT + RT) on the improvement of body composition, endothelial function, blood pressure, blood lipids, and cardiorespiratory fitness in a cohort of sedentary, overweight adults (aged 30–50 years).

Methods/design: Sixty sedentary, overweight adults attending primary care in Bogotá, Colombia will be included in a factorial randomised controlled trial. Participants will be randomly assigned to the following intervention groups: (1) non-exercise group: usual care with dietary support, (2) HIIT group: 4 x 4-min intervals at 85–95% maximum heart rate (HRmax) (with the target zone maintained for at least 2 minutes), interspersed with a 4-min recovery period, at 65% HRmax, (3) RT group: completing a resistance circuit (including upper and lower muscle groups) as many times as needed according to subject’s weight until an expenditure of 500 kcal at 40–80% of one-rep max (1RM) has been achieved, and (4) combined group: HIIT + RT. The primary end point for effectiveness is vascular function as measured by flow-mediated vasodilatation 1 week after the end of exercise training.

Discussion: The results of this study will provide new information about the possible effect of the programme in improving the cardiometabolic health of overweight adults, making a more efficient use of an adult’s resources over time.

Trial registration: ClinicalTrials.gov ID: NCT02715063. Registered on 8 March 2016.

Keywords: Exercise, Risk factor, Cardiovascular disease, Overweight

* Correspondence: robin640@hotmail.com; robinson.ramirez@urosario.edu.co
1Centro de Estudios en Medición de la Actividad Física (CEMA), Escuela de Medicina y Ciencias de la Salud, Universidad del Rosario, Cra. 24 No. 63C-69, Bogotá, D.C, Colombia

© 2016 Ramírez-Vélez et al. Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Background
The prevalence of obesity has increased worldwide [1] among both children and adults, and obesity is associated with an increased risk for cardiovascular diseases (CVD) [2, 3]. Substantial evidence indicates that increased body weight and body fat distribution are associated with a higher frequency of adverse health consequences, including hypertension, CVD, metabolic disorders, osteoarthritis, gallbladder stone disease, asthma and multiple malignancies [4, 5]. International organisations [6, 7] and previous epidemiological cross-sectional studies have suggested that individuals with a large accumulation of body fat in the abdominal region are at greater risk for the development of metabolic syndrome [8, 9]. In addition to obesity, hypertension, dyslipidaemia and low cardiorespiratory fitness (CRF) are also modifiable risk factors associated with the risk for CVD [10, 11]. Furthermore, it has become increasingly clear that low CRF may exacerbate the risk of CVD mortality, and that increasing peak CRF to higher than 5 peak metabolic equivalents (MET) can reduce and perhaps eliminate the mortality rate associated with dyslipidaemia, obesity, type 2 diabetes mellitus and hypertension [12].

The health benefits of exercise training are well documented; it is necessary for healthy growth and development because it optimises cardiometabolic function and prevents chronic disease [13, 14]. Additionally, its benefits are not only biological, but also include psychosocial advantages [15]. The most recent guidelines promoted by the World Health Organisation (WHO) recommend a minimum of 150 min of moderate-intensity physical activity (3 to <6 MET) or 75 min of vigorous-intensity physical activity (≥6 MET per week or any equivalent combination for health benefits, and 300 min of moderate-intensity physical activity or 150 min of vigorous-intensity physical activity per week for additional health benefits [16, 17].

Previous studies have reported improvement in endothelial function in different disorders, such as obesity, diabetes mellitus and metabolic syndrome, by increasing the production and bioactivity of nitric oxide [18–21]. In addition, a growing body of evidence has demonstrated comparable or superior improvements in cardiometabolic health outcomes using low-volume, high-intensity interval training (HIIT) compared to traditional moderate-intensity continuous training (MICT) [18, 19]. Other studies have revealed a strong relationship between vascular function and CRF [20, 21]. Thus, because HIIT is a potent method of improving CRF, several systematic and narrative reviews that have investigated the impact of HIIT relative to MICT on vascular function in clinical patients have emerged over recent years [18, 19, 22]. HIIT provides rapid physiological adaptations, as indicated by improvements in maximal oxygen uptake (VO₂max), anaerobic threshold and stroke volume [23, 24]. Instead, it was suggested that the ability of HIIT 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 [25].

Interestingly, despite the prevalence of obesity and the existing multiple position stands promoting exercise for the treatment of obesity, there are few randomised trials that have directly compared the effects of sustained resistance training (RT), HIIT, or a combination of the two (RT + HIIT) to be as effective or more effective for improving cardiometabolic health in adults [26, 27]. Most of the published studies addressing RT and fat mass changes have compared RT to an inactive control group and not to HIIT. Furthermore, existing studies have not directly studied comparable amounts of HIIT and RT. A recent randomised controlled trial (RCT) suggests that adding plyometric exercises to a HIIT programme may be more beneficial than HIIT alone in young obese women [28]. Given the increasing burden of chronic disease, more research is needed to better understand the effect of different exercise modalities on these risk factors [29].

Thus, this paper describes the rationale, design, and methodologies used in a factorial randomised controlled trial (Cardiometabolic HIIT-RT Study), wherein we hypothesised that HIIT and combined training would result in greater improvements in vascular function compared to RT and usual clinical care.

Methods/design
Study design and setting
The present study is a RCT (ClinicalTrials.gov ID: NCT02715063). The Cardiometabolic HIIT-RT Study is a single-blind, randomised controlled, 2 × 2 factorial trial. The study received ethical approval from the Medical Research Ethics Committee of The University of Manuela Beltran (ID 06-1006-2014). Random allocation to treatment will be performed at the individual level.

Procedures
Participants
Participants aged 30–50 years, who are sedentary (no participation in exercise more than once a week for the previous 6 months) with abdominal obesity: waist circumference (WC) at least 90 cm for men, and at least 80 cm for women, or with excess weight: body mass index (BMI) at least 25 kg/m² for men and 35 kg/m² for women, and who are identified as being willing and with almost immediate availability, will be enrolled. Eligible subjects for the present study and those interested in participating will be invited to a pre-test that includes an interview in a private health care institution (Clinica
Rangel Pereira IPS) and further assessments performed at the Centre of Studies in Physical Activity Measurements (in Spanish, CEMA), School of Medicine and Health Sciences, University of Rosario, Bogotá, Colombia. Risks will be minimised by ruling out contraindications to the testing and training protocols via a health history and thorough physical examination prior to the testing sessions. Inclusion and exclusion criteria are provided in Table 1.

**Recruitment**

Consecutive men or women with abdominal obesity or excess weight will be recruited from a private health care institution (Clinica Rangel Pereira, IPS) and one primary care institution (Universidad del Rosario, IPS) that receives referrals from both medical consultants in secondary care and primary care general practitioners in the capital district of Bogotá, Cundinamarca Department in the Andean region. This region is located at approximately 4°35’56” N 74°04’51” W and at an elevation of approximately 2625 m (min: 2500 m; max: 3250 m) above sea level [30]. Subjects who are interested in participating will be approached with further information and screened for pre-participation exercise habits using a cardiovascular and musculoskeletal checklist (i.e. the patient’s medical history, disease history, physical fitness, and more). A member of the research team will follow up with a phone call to screen for eligibility and explain the main requirements of the study. All participants will provide written informed consent before entering the study.

**Blinding and randomisation methods**

The randomisation into the four study arms will be performed by the Centre of Studies in Physical Activity Measurements at University of Rosario, Bogotá, Colombia, using block randomisation with a block size of four. Eligible participants will be randomly assigned after completing the baseline measurements to either the control or exercise training groups. The principal investigator will coordinate the allocation sequence, and randomisation will be computer-generated. All participants and study personnel (including investigators, trainers, and statisticians) will be blinded to treatment allocation throughout the trial protocol. Access to the allocation code will be restricted to one study statistician who will not perform the final study analyses. Randomisation will be conducted independently using sealed opaque envelopes. These procedures are also detailed in the study operations manual. Moreover, the importance of maintaining the blinding and allocation concealment will be reinforced by regularly scheduled conference calls at the sites and daily meetings with the field investigators (Fig. 1).

**Table 1 Inclusion/exclusion criteria**

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central obesity: waist circumference ≥90 cm (men); ≥80 cm (women), or with excess weight: body mass index ≥25 kg/m² (men) and ≤35 kg/m² (women)</td>
<td>Systemic infections</td>
</tr>
<tr>
<td>Meets at least one criteria for metabolic syndrome (IDF 2006): triglycerides ≥150 mg/dl; HDLc &lt;40 mg/dL (men); &lt;50 mg/dL (women); high blood pressure ≥130/85 mmHg and/or fasting plasma glucose ≥100 mg/dL</td>
<td>Weight loss or gain of &gt;10 % of body weight in the past 6 months for any reason</td>
</tr>
<tr>
<td>Written informed consent</td>
<td>Currently taking medication that suppresses or stimulates appetite</td>
</tr>
<tr>
<td>Interested in improving cardiovascular health and physical fitness</td>
<td>Uncontrolled hypertension: systolic blood pressure 160 mmHg or diastolic blood pressure 95 mmHg on treatment</td>
</tr>
<tr>
<td></td>
<td>Gastrointestinal disease, including self-reported chronic hepatitis or cirrhosis, any episode of alcoholic hepatitis or alcoholic pancreatitis within the past year, inflammatory bowel disease requiring treatment within the past year, recent or abdominal surgery (e.g. gastrectomy)</td>
</tr>
<tr>
<td></td>
<td>Asthma</td>
</tr>
<tr>
<td></td>
<td>Diagnosed diabetes (type 1 or 2), fasting impaired glucose tolerance (blood glucose 118 mg/dL), or use of any antidiabetic medications</td>
</tr>
<tr>
<td></td>
<td>Currently taking antidepressant, steroid, or thyroid medication, unless dosage unstable (no change for 6 months)</td>
</tr>
<tr>
<td></td>
<td>Current exerciser (&gt;30 min organised exercise per week).</td>
</tr>
<tr>
<td></td>
<td>Indication of unsuitability of current health for exercise protocol (Physical Activity Readiness Questionnaire, PARQ)</td>
</tr>
<tr>
<td></td>
<td>Any other conditions which, in the opinion of the investigators, would adversely affect the conduct of the trial</td>
</tr>
</tbody>
</table>
**Intervention**

The participants who are randomly assigned to the intervention group will participate in the cardiometabolic programme.

1. **Usual clinical care group**

   This group will receive usual clinical care according to the consensus recommendations of the national goals for cardiovascular health promotion and disease reduction of the American Heart Association [31] and Colombian guidelines COLDEPORTES (in Spanish, Departamento Administrativo del Deporte, la Recreación, la Actividad Física y el Aprovechamiento del Tiempo Libre) [32]. Participants will receive counselling about goals for cardiovascular health, as well as monitoring cardiovascular health over time in the Colombian population, key signs and symptoms, diet and screening for cardiometabolic risk factors. This group will be asked to maintain their level of activity during the 12-week study period.

2. **High-intensity interval training (HIIT) group**

   The HIIT protocol will be completed with fast walking or running on a treadmill with the deck inclined to reach the desired intensity. We will calculate the training energy expenditure for participants' age ranges associated with meeting the consensus public health recommendations from the WHO [16] and the US Department of Health and Human Services [17]. Each preparatory period starts with an exercise dose of 6 kcal kg\(^{-1}\) week\(^{-1}\), which will increase progressively by 2 kcal kg\(^{-1}\) week\(^{-1}\) until week 4, where it will remain at 12 kcal kg\(^{-1}\) week\(^{-1}\) for weeks 5 to 12.

   **Preparatory training phase: weeks 1–4**

   To initiate our study we will use a 4-week preparatory phase of training to bring all participants up their 300-kcal goal for the session. To accomplish this they will warm up at 65% of maximum heart rate (HRmax) (5 min); exercise for 4 × 4-min intervals at 60–80% HRmax, interspersed with a 4-min recovery period at 55% HRmax, at a frequency of three times per week.

   **Protocol of interval training: weeks 5–12**

   The overall goal for the HIIT group is to perform exercise sessions in 4 × 4-min intervals at 85–95% HRmax (with the target zone maintained for at least 2 minutes), interspersed with a 4-min recovery period at 65% HRmax. During each exercise session, participants will adhere to the 12-kcal kg\(^{-1}\) week\(^{-1}\) energy expenditure format, equivalent to 500 kcal of expended energy at the end of training and cool-down (5 min), with a range total exercise time of 35 to 45 min (Fig. 2). Exercise will be performed for three sessions per week. During the supervised intervention, we will record HR using an HR monitor (Polar Pacer, Lake Success, NY, USA) to ensure compliance with the exercise stimulus at the predetermined target HR zone. In addition, HR and Borg ratings will also be measured in each exercise session.

3. **Resistance training group**

   **Preparatory training phase: weeks 1–4**
All participants will complete a base resistance training protocol during the 2 weeks prior to the training intervention (Table 2). This phase will encompass a total of three workouts (Monday, Wednesday, and Friday) during the 4 weeks. During the adaptation phase, the subjects will expend energy up to 300 kcal at 20–50% of one repetition maximum (1RM), for 2 × 20–30 repetitions with 1-min rest intervals. During each exercise session, participants will adhere to the 6–8 kcal kg⁻¹ week⁻¹ energy expenditure format. The purpose of the preparatory phase will be to instruct proper lifting technique, familiarise participants with all exercises, and ensure that the participants began the study with a comparable training base.

**RT protocol: weeks 5–12**

After the preparatory phase, participants will expend 500 kcal of energy during the protocol training phase at 40–80% of 1RM, for 4 × 20–30 repetitions and 1-min rest intervals. The RT protocol will be used to complete a resistance circuit (including upper and lower muscle groups; eight exercises); all participants will expend energy up to the 500-kcal goal. The entire workout will last approximately 30–40 minutes, depending on the number of exercises (Table 2), at a frequency of three times per week. Each session is preceded and followed by a gradual warm-up and cool-down period (both of 10-min duration and consisting of walking and light, static stretching (avoiding muscle pain) in most muscle groups). The cool-down period also includes relaxation and stretching exercises. The RT protocol will be performed through the full range of motion normally associated with correct technique for each exercise, engaging the major muscle groups (abdominal, dorsal, shoulder, upper and lower limb muscles). As a general rule and to avoid potential risks, we will avoid (1) activities that include Valsalva’s mechanism, (2) ballistic and plyometric movements, and (3) positions of extreme muscular tension. This resistance training programme has been used before, successfully promoting strength and muscle gains in middle-aged and elderly populations with a variety of conditions (e.g. diabetes type II and obesity) [33, 34].

### 4. Combined training group

This group will receive both the HIIT and RT protocols as described above. Therefore, the energy expenditure associated with the physical training prescribed for the vigorous-intensity group will be approximately 1500 kcal/week.

Overall, we will monitor each subject’s HR (FS1, Polar Electro Oy, Kempele, Finland) during the exercise sessions. We will estimate the energy expenditure during the exercise sessions by calibrating the energy expenditure to the HR during the VO₂max tests performed at the baseline and post-intervention time points. The regression of the energy expenditure will be calculated for each participant according to HR and minutes spent exercising during the training sessions. Trainers will be physical therapists and physical educators with experience developing and monitoring exercise programmes among clinical populations. All protocol training will be performed under observation and supervision in an exercise laboratory with complete and strict monitoring of the amount of exercise completed in each session. Adherence to the exercise programme will be encouraged by the exercise professional who supervises each of the group sessions. To maximise adherence to the training programme each session will have a maximum of three to five participants training simultaneously. Attendance at supervised sessions includes compliance with target HR and expenditure energy and will be monitored and recorded by research staff. Each participant will meet with the study dietician for nutrition assessment and counselling, and an individualised nutrition intervention plan will be developed from the baseline food intake assessment according to participant preferences [35]. This plan is a standardised meal consisting of 1300 to 1500 kcal (50–55% carbohydrates, 30–35% total fat, less than 7% saturated fat and 15–22% protein). At the beginning of the training protocol, we will obtain the participants’ weight to determine weekly energy expenditure necessary to achieve their target of 12 kcal kg⁻¹ week⁻¹ (iso-energetic). It is expected that the
A gradual increase in total energy expenditure will minimise fatigue, soreness, injuries, and attrition.

Data collection and outcome measures
Outcome measures will be assessed at baseline and at 12-week follow-up by personnel blinded to the treatment allocation. Data will be recorded on standardised forms and entered into a secured-access database that contains quality control checks (e.g. range checks, notification of missing data).

The primary outcome measure is endothelial function as measured by flow-mediated dilatation (FMD). The secondary outcome variables include HR variability, pulse wave velocity (PWV), weight, BMI, WC, body composition, body temperature, biochemical profile, health-related physical fitness, self-perceived health, health-related quality of life (HRQL), and side effects. Other variables of interest include 24-hour dietary recall, lifestyle and demographic characteristics (Table 3 and Figure 3).

Primary outcome measures

Endothelial function FMD will be measured with the technique introduced by Ramírez-Vélez et al. [36] in the
Colombian population using the guidelines reported by Corretti et al. [37]. The diameter of the brachial artery will be assessed using a high-resolution ultrasound device (Acuson Sequoia C512, Acuson Siemens, Mountain View, CA, USA), equipped with a 7.5–10-MHz linear array transducer and an integrated electrocardiography package. The ultrasound procedures will be performed with the subject resting quietly in a supine position for at least 10 min. Measurements will be performed in the morning (06.00–08.00 hours) due to diurnal variation in a climate-controlled room (18–20 °C) with the lights dimmed. All measurements will be taken at the end of diastole as observed by electrocardiogram. First, the diameter of the right brachial artery will be searched in a cross-sectional view and then scanned over a longitudinal section 5 to 10 cm proximal to the right elbow. The diameter of the brachial artery will be measured from the anterior to the posterior intima-lumen interface at a fixed distance, calculating the mean diameter from four cardiac cycles. After this, a pneumatic tourniquet placed around the right forearm will be rapidly inflated to at least 50 mmHg above the systolic blood pressure for 5 min. A sudden release of the cuff will induce an increase in blood flow in the brachial artery located proximal to the tourniquet. During reactive hyperaemia, shear stress will increase, causing endothelium-dependent vasodilatation, mainly due to endothelial release of nitric oxide [38, 39]. This secondary dilatation enhances and prolongs the reactive hyperaemic phase. FMD of the brachial artery will be measured 45–60 s after cuff release. The change in diameter caused by the increased flow will be calculated as the percentage change relative to the baseline measurement (FMD%). The intra-session coefficients of variation will be up to 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 date). Images will be recorded on a DVD player for subsequent measurements by one observer blinded to the study.

**Secondary outcomes**

**Heart rate variability** Measurement of HR variability will be performed according to current recommendations by the European Society of Cardiology using an evaluated share-ware (Kubios, vers. 2.0; http://kubios.uef.fi/) [40]. The variations in interbeat intervals in the time domain will be quantified by the mean values and standard deviations of normal interbeat intervals in the supine position.

**Aortic pulse wave velocity (PWV) and augmentation index (Alx)** Both PWV and Alx will be measured with the oscillometric method using the occlusion technique. Patient data and the measured distance between the jugulum and the symphysis will be registered in the arteriography-programmed computer (TensioMed Software v.1.9.9.2; TensioMed, Budapest, Hungary). A tape measure will be used for measuring the distance

<table>
<thead>
<tr>
<th>Programme variable</th>
<th>Preparatory phase</th>
<th>Volume</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise prescription</td>
<td>20–40 % 1RM</td>
<td>40 % 1RM</td>
<td>30–50 % 1RM</td>
</tr>
<tr>
<td>Training intensity</td>
<td>2 × 20–30 repetitions</td>
<td>2 × 20–30 repetitions</td>
<td>2 × 20–30 repetitions</td>
</tr>
<tr>
<td>Training volume</td>
<td>1 min</td>
<td>30 s</td>
<td>1 min</td>
</tr>
<tr>
<td>Energy expenditure</td>
<td>300 kcal</td>
<td>500 kcal</td>
<td></td>
</tr>
<tr>
<td>Rest time</td>
<td>Weeks 1 to 4</td>
<td>Weeks 1 to 4</td>
<td></td>
</tr>
<tr>
<td>Specific exercises</td>
<td>Barbell squats</td>
<td>Dumbbell squats</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Split squats</td>
<td>Adductor</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biceps curls</td>
<td>Dumbbell lateral</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>raises shoulder</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dumbbell military</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>shoulder press</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dumbbell triceps</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>curls</td>
<td></td>
</tr>
</tbody>
</table>

Volume = sets × repetitions, 1RM one repetition maximum
between the jugulum and the symphysis, namely the aortic distance. The cuff will be placed on the patient’s upper arm and connected to the device. Pressure variations in the cuff will influence a pressure receptor and the signal, and will then be transferred via an infrared port to the computer. The algorithm measuring blood pressure in the arteriography device has been validated [41]. PWV will be 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 will be chosen. The standard deviation will be calculated from every heartbeat during a period of 8 s. Both aortic Alx (Alxao) and brachial Alx (Alxbr) will be calculated as:

\[
100 \times \left( \frac{\text{early systolic pressure peak} - \text{late systolic pressure peak}}{\text{pulse pressure}} \right)
\]

The return time is the difference (in ms) between the first (early systolic pressure peak) and reflected systolic wave (late systolic peak) and is related to the stiffness of the aorta. The PWV and Alx will be presented as the mean values from two recordings. The algorithm for estimation of central systolic blood pressure (cSBP) has been derived from the relationship between invasively measured cSBP and the SBP in the brachial artery, and cSBP estimated by arteriography correlates well with invasively measured cSBP [41]. Blood pressure will be measured at the same time of the day using a validated digital automatic blood pressure monitor (OMRON M6, Omron Health Care Co., Ltd., Kyoto, Japan) according to the International Protocol of the European Society of Hypertension [42].

**Morphological component** Anthropometric variables will be assessed by a nutritionist in accordance to the International Society for the Advancement of Kinanthropometry (ISAK) guidelines [43]. Variables will be collected at the same time in the morning, between 00.70 and 00.80 hours, following an overnight fast of at least 10–12 hours. The body weight of the subjects will be measured when the subjects are in underwear and without shoes, using electronic scales (Tanita® BC544, Tokyo, Japan). The height of the subjects will be measured using a mechanical stadiometer platform (Seca® 274, Hamburg, Germany). The BMI of the subjects will be calculated as the body weight in kilograms divided by the square of the height in metres. The WC will be measured as the narrowest point between the lower costal border and the iliac crest; in case this is not evident, it will be measured at the midpoint between the last rib and the iliac crest using a tape measure (Ohaus® 8004-MA, Parsippany, NJ, USA). The waist-to-height ratio will be computed by dividing WC by height, and this provides a surrogate measure of central body fat. We will take each measure twice and use the average measure obtained, unless the first and second measures vary by more than 1 %, in which case we will use the median of three measurements.

**Body composition** We will also measure fat mass, lean body mass, abdominal adipose tissue and bone mineral density by conducting a dual-energy X-ray absorptiometry

<table>
<thead>
<tr>
<th>Type of outcomes</th>
<th>Specific outcomes</th>
<th>Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary outcomes</td>
<td>Endothelial function as measured by flow-mediated vasodilatation</td>
<td>X</td>
</tr>
<tr>
<td>Secondary outcomes</td>
<td>Vascular function: heart rate variability, aortic pulse wave velocity, augmentation index and blood pressure</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Morphological component: weight, height, BMI, waist circumference</td>
<td>X, X</td>
</tr>
<tr>
<td></td>
<td>Body composition: fat mass and non-bone lean body mass</td>
<td>X, X</td>
</tr>
<tr>
<td></td>
<td>Body temperature</td>
<td>X, X</td>
</tr>
<tr>
<td></td>
<td>Plasma samples and biochemical analysis: LDL cholesterol (LDL-c), HDL cholesterol (HDL-c), total cholesterol, triglycerides, glucose, glycated haemoglobin (HbA1c)</td>
<td>X, X</td>
</tr>
<tr>
<td></td>
<td>Fitness component: self-reported fitness, 1RM, hand-grip and peak uptake of volume of oxygen</td>
<td>X, X</td>
</tr>
<tr>
<td></td>
<td>Self-perceived health: health-related quality of life</td>
<td>X, X</td>
</tr>
<tr>
<td></td>
<td>Recent Physical Activity Questionnaire</td>
<td>X, X, X</td>
</tr>
<tr>
<td></td>
<td>Demographic information</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Side effects</td>
<td>X, X, X</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of outcomes</th>
<th>Specific outcomes</th>
<th>Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary outcomes</td>
<td>Endothelial function as measured by flow-mediated vasodilatation</td>
<td>X</td>
</tr>
<tr>
<td>Secondary outcomes</td>
<td>Vascular function: heart rate variability, aortic pulse wave velocity, augmentation index and blood pressure</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Morphological component: weight, height, BMI, waist circumference</td>
<td>X, X</td>
</tr>
<tr>
<td></td>
<td>Body composition: fat mass and non-bone lean body mass</td>
<td>X, X</td>
</tr>
<tr>
<td></td>
<td>Body temperature</td>
<td>X, X</td>
</tr>
<tr>
<td></td>
<td>Plasma samples and biochemical analysis: LDL cholesterol (LDL-c), HDL cholesterol (HDL-c), total cholesterol, triglycerides, glucose, glycated haemoglobin (HbA1c)</td>
<td>X, X</td>
</tr>
<tr>
<td></td>
<td>Fitness component: self-reported fitness, 1RM, hand-grip and peak uptake of volume of oxygen</td>
<td>X, X</td>
</tr>
<tr>
<td></td>
<td>Self-perceived health: health-related quality of life</td>
<td>X, X</td>
</tr>
<tr>
<td></td>
<td>Recent Physical Activity Questionnaire</td>
<td>X, X, X</td>
</tr>
<tr>
<td></td>
<td>Demographic information</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Side effects</td>
<td>X, X, X</td>
</tr>
</tbody>
</table>

1RM one repetition maximum, BMI body mass index
scan (DEXA) (Hologic, QDR 4500 W). An experienced DEXA technologist who is blinded to the study randomisation will perform the DEXA imaging studies.

**Body temperature** We will use a thermo-infrared camera (FLIR Thermacam E60, FLIR systems, Boston, MA, USA). Images in the frontal plane will be taken from the anterior and posterior sides, according to the thermal image acquisition criteria described by Ring and Ammer [44]. During the measurement process, participants will remain in their underwear and will maintain a steady orthostatic position during the image acquisitions. The distance from the camera to the subjects will be 2.5 m. The study will be conducted according to the guidelines of the American Academy of Thermography [45]. A single investigator, trained in the use of thermographic devices, will obtain the images.

**Plasma samples and biochemical analysis** A fasting blood sample will be obtained from the cubital vein in the early morning at the clinical care session attended by the participants in the subset. The biochemical profile will include: (1) plasma lipid triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) (by enzymatic colourimetric methods); and (2) the metabolic regulators glucose and haemoglobin A1C (HbA1c) (by enzymatic colourimetric methods). All determinations will be analysed in serum using a Cardiocheck® and A1CNow® system.

**Self-reported fitness** Self-reported fitness will be determined by the International Fitness Scale (IFIS), which is a questionnaire validated in European [46] and Colombian adults [47]. IFIS consists of a Likert-type scale (range 1–5) with five response options (very poor, poor, average, good, and very good) about perceived overall fitness; its main components include CRF, muscular strength, speed and agility, and flexibility (http://www.helenastudy.com/IFIS). IFIS has shown ‘high’ validity and ‘moderate’ to ‘good’ reliability in young adults [48]. In Colombian adults, the internal consistency and reproducibility of IFIS items was high (Cronbach’s alpha = 0.80) and the averaged ICC range was 0.90–0.96 [47].

**Health-related physical fitness** Physical fitness will be measured using tests that have previously shown high validity and reliability levels. It will be determined using a maximum treadmill exercise test (Pprec TRM 885, Treveso, Italy) following the modified Balke protocol [49], which has been extensively used [50, 51] and validated [52]. The treadmill test will use a ramp protocol where the inclination is constant (5.5 %) and the speed increased by 0.5 km/h every minute, starting at 4 km/h. Each session will begin with a 5- to 10-min warm-up at 50 W. We will ask participants to refrain from smoking for 2 hours before the test, and from drinking alcohol or doing any vigorous- or moderate-intensity activities for 48 h before the test. HRmax will be used to determine the training intensity for each participant. We will measure blood pressure prior to and during the test. Exercise will be terminated if participants are fatigued, or earlier if they fulfill the ACSM’s guidelines for ‘Indications for Terminating Exercise Testing’ [53]. CRF is defined as the highest recorded VO2 value (VO2max) after two of three criteria are met: (1) a plateau in VO2 after increase in workload, (2) a respiratory exchange ratio >1.10, and (3) a maximal HR within 10 bpm of their age-predicted maximum. Muscular fitness will be assessed using the hand-grip test (maximum hand-grip strength assessment) using a standard adjustable handle analogue hand-grip dynamometer T-18 TKK SMEDLY III® (Takei Scientific Instruments Co., Ltd, Niigata, Japan). Participants will be given a brief demonstration and verbal instructions for the test and, if necessary, the dynamometer will be adjusted according to the subject’s hand size according to predetermined protocols. Hand-grip strength will be assessed with the subject in a standing position with their shoulders adducted and neutrally rotated, and their arms parallel but not in contact with their body. The participants will be asked to squeeze the handle for a maximum of 3–5 s, but no verbal encouragement will be given during the test. Two trials will be allowed in each limb, and the average score will be recorded as peak hand-grip strength (kg). Thus, the values of hand-grip strength presented will combine the results of left- and right-handed subjects, without consideration of hand dominance. Hand-grip will be adjusted by allographic parameters defined by Jarić [54] (dynamometry/weight0.67). Strength in the eight exercises will be assessed at baseline and immediately after the intervention ends. A general warm-up consisting of riding a cycle ergometer for 5 min at a self-selected resistance will precede strength testing. Standardised procedures will be used to predict a one-rep max (1RM) from reps-to-fatigue barbell squats, dumbbell adductor squats, split squats, lateral adductor squat, biceps curls, dumbbell lateral shoulder raises, dumbbell military shoulder press, and dumbbell triceps curls from each participant’s performance during the preparatory training phase [55]. Progressive overload will be achieved by increasing the load when all prescribed repetitions (for a particular exercise) are achieved on two consecutive workouts [56].

**Recent Physical Activity Questionnaire (RPAQ)** Self-reported physical activity is measured using the RPAQ. This assesses physical activity across four domains (domestic, recreational, work, commuting) over the...
previous month. 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 [57].

**Health-related quality of life (HRQL)** HRQL will be measured by the SF Community - short-form survey (SF-12®) Colombian version for physical and mental domains' summary scores and eight subscales (including vitality) [58]. The internal consistency of the HRQL items is moderate (Cronbach’s alpha = 0.70).

**Sociodemographic information** Baseline sociodemographic values, which could act as covariates or confounds for the tested treatment modality, will also be collected. The surveys will include questions on age, education, occupation, income, health history, and alcohol consumption, among others.

**Dietary assessment** To determine the average habitual energy and macronutrient intake, a detailed 24-h diet record will be obtained from all subjects 1 weekday and 1 weekend day during the 1-week baseline period. The Food Intake Analysis Software (FAO/INFOODS, Report of the Technical Workshop on Standards for Food Composition Data Interchange, Rome 2004) and national food composition tables (for specific foods) will be used to analyse total energy and macronutrient intake of each subject’s 24-h diet.

**Side effects and monitoring** The study will be conducted according to good clinical practice and standard operating procedures. It will be monitored by the Human Rights Committee at the Universidad Manuela Beltrán Coordinating Centre composed of experts in physical exercise, sports medicine, physical therapists, physical educators and clinical epidemiologists. Interim monitoring reports will be submitted to the experts, focusing on patient intake, adherence to the protocol, baseline comparability of treatment groups, completeness of data retrieval, and adverse events. All adverse events will also be reported to the Universidad Manuela Beltrán Ethics Committee. To standardise the study procedures, an operations manual has been written, and comprehensive training sessions will be held prior to the initiation of the trial. An independent researcher will be in charge of auditing all assessment staff to record all of these events for the participants over the study period.

**Power calculation and sample size** The aim of this study is to obtain data on the effects of HIIT, RT or combined training that will result in similar improvements in cardiometabolic health compared to the usual clinical care group. The measurement of FMD, validated in several population studies, was selected as the critical variable to calculate the sample size [59, 60]. To calculate the required sample size, we will use the formula for the comparison of two means: \( n = \frac{(A + B)^2}{2 \times DIF^2} \), where \( n \) is the sample size required in each group, \( DIF = \) size of desired difference between groups, \( A \) and \( B \) depend on the desired significance level and desired power, respectively. Using estimates obtained from the literature [59, 60] and our previously performed study [61, 62], a sample size of 12 subjects in each group will be needed to reach a power of 80% to detect a difference in means in the FMD of 2% in the FMD after 12 weeks of training, assuming a \( DIF = 2.7 \) using a two-sample \( t \) test with a 0.05 two-sided significance level. Assuming a drop-out rate of 15%, the total minimal sample size has been increased to 15 subjects for each group. We believe that this sample size is feasible and realistic based on our previous experiences in RCTs [33, 34, 59, 60, 63, 64].

**Statistical analyses** The final data will be analysed using IBM SPSS 22.0 (SPSS, Inc., Chicago, IL, USA) and SAS software (SAS Institute Inc., Cary, NC, USA). An exploratory analysis will be performed to determine the frequency, range, variability, and distribution type for each variable to use the most appropriate statistical test when comparisons will be necessary. These analyses permit the assessment of the primary analysis of the data and will be undertaken using the principle of intention-to-treat (ITT). The ITT analysis for this study will include all participants, including those who are not fully compliant and those with missing outcome data. Because this is an experimental design with two measures of the primary and secondary outcomes, the first at baseline \( x_0 \) (\( t_0 = \) weeks) and the second after interventions \( x_1 \) (\( t_1 = 12 \) weeks) in four study groups, a comparative analysis between these measures to establish differences will be executed. To perform these comparisons, one-way ANOVA or Kruskal-Wallis tests will be applied when appropriate. Subsequently, a multivariate analysis will be carried out, and the autocorrelation between repeated measures will be taken into account. We will use longitudinal analysis methods, such as a generalised estimating equation approach, to control the differences among measures at baseline and to incorporate incomplete observations into this analysis. Finally, we will investigate if there is an interaction between the two interventions for the primary outcome at 12 weeks of supervised training HIIT, RT or combined training (HIIT + RT). For these
analyses, we will include appropriate interaction terms in the models [38, 65]. The trial is not powered to detect these interactions and is likely to have poor precision for the interaction terms. We plan to report regression coefficients for the interaction terms and their 95 % confidence intervals. However, we aim to recruit 15 participants per group (a total of 60) to accommodate for a 20 % attrition rate and elimination due to non-compliance.

Dissemination
We will disseminate the results of our study via presentations at international conferences and publications in peer-reviewed journals. The study will be implemented and reported in line with Standard Protocol Items for Randomised Trials [66] Additional file 1.

Discussion
This protocol describes a study in which we will compare the effects of 12 weeks of HIIT, RT or combined training on improvements in body composition, endothelial function, blood pressure, blood lipids, and cardiovascular fitness in a cohort of sedentary, overweight adults (aged 30–50 years).

Latin American countries are experiencing different stages of nutrition transition. Although the prevalence of undernutrition is declining at different rates, the prevalence of overweight is dramatically increasing [67]. In addition, physical inactivity and sedentary lifestyles are also a preventable behaviour associated with obesity, but the evidence on this issue remains mixed [68], and the results might not be generalisable to all world regions [69]. Thus, determining whether HIIT + RT can be a viable public health approach to improve cardiometabolic health is warranted, particularly given a recent finding that HIIT added to plyometric exercise is more consistently associated with improvements in metabolic abnormalities than HIIT alone.

We believe that our trial could help to determine which type of programme better improves the cardiometabolic health of overweight adults and makes more efficient use of an adult’s resources over time. In summary, the primary objective of the trial is to contribute to the growing body of literature about physical exercise interventions and cardiometabolic health among sedentary, overweight adults living in Bogotá, which will be useful for designing innovative and time-efficient preventive measures among the Colombian population.

Trial status
The Cardiometabolic HIIT-RT Study began recruiting patients in March 2016 and will close recruitment in October 2016. Data collection will be completed in June 2017.

Additional file

Additional file 1: SPIRIT 2013 Checklist: recommended items to address in a clinical trial protocol and related documents. (DOC 122 kb)

Abbreviations
HR, heart rate; HRmax, maximum heart rate; HRQL, health-related quality of life; IFIS, International Fitness Scale; ISAK, International Society for the Advancement of Kinanthropometry; LDL-c, low-density lipoprotein cholesterol; MET, metabolic equivalents; MICT, moderate-intensity continuous training; PWV, pulse wave velocity; PWV, pulse wave velocity; RPAQ, Recent Physical Activity Questionnaire; RT, resistance training; SF-12™ SF Community - short-form survey; VO₂max, maximal oxygen uptake; WC, waist circumference

Funding
This study is supported by FLUR (Code QDN-BG-001) from the Universidad del Rosario. The funding source has no role in study design; in data collection, analysis and interpretation; in the writing of the report; or in the decision to submit the paper for publication.

Availability of supporting data
The study data have legal and ethical restrictions imposed by the authors’ IRB (Manuela Beltran University).

Competing interests
The authors declare that they have no competing interests.

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

Ethics approval and consent to participate
Prior to the study, researchers and field practitioners performed ten theoretical and practical sessions to standardise the evaluation process and to minimise inter-observer variability. A comprehensive verbal description of the nature and purpose of the study will be given to the participants. The study’s health professional is responsible for ensuring that the subject understands the potential risks and benefits of participating in the study. All participants will provide written informed consent before entering the study. The study will be conducted according to the ethical standards established in the 1961 Declaration of Helsinki (as revised in Hong Kong in 1989 and in Edinburgh, Scotland, in 2000) and Colombian Health Authorities under the Law of Data Protection (Resolution 8430/93). The appropriate signatures and dates on the informed consent form must be obtained before administration of the intervention. The Research Ethics Review Board at the University of Manuela Beltrán approved the survey protocol (Code N° 06-1006-2014, Resolución 8430 de 1993; Ministerio de Salud de Colombia).


Background: Strong evidence shows that physical inactivity 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. We aimed to determine the effects of moderate- versus high-intensity interval exercise training on vascular function parameters in physically inactive adults.

Methods: Twenty inactive adults were randomly allocated to receive either moderate continuous training (MCT group; 60-75% of their heart rate reserve (HRR)) or high-intensity interval training (HIT group; 4 min at 85-95% of peak HRR). Vascular function (Normalization of brachial artery flow-mediated dilation, FMDn [%], aortic pulse wave velocity, PWV [m·s−1], AIx, augmentation index) was measured at baseline and over 12 weeks of training.

Results: FMD changed by -1.0 (6.3) % in the MCT group and 1.9 (6.2) % in the HIT group (no significant difference between groups: 2.9 [95% CI, −3.0 to 8.8; η2 = 0.15, p=0.131]. PWV changed by 0.1 in the MCT group but decreased by −0.4 in the HIT group (η2 = 0.39, p <0.01 interaction), and there was not a significant difference in the prevalence of NR for PWV between the MCT and HIT groups (66% versus 33%, P = 0.051). Regarding FMDn (%), an analysis showed that the prevalence of NR was 66% (6 cases) in the MCT group and 18% (2 cases) in the HIT group (P = 0.013).

Conclusions: Under the conditions of the present study, physically inactive adults in both groups experienced changed in FMD. The rate of response was significant different between exercise groups for FMDn measure but not PWV outcome. The sustained change in PWV in the HIT group may represent a signal of vascular adaptation or endothelial fatigue.
Effectiveness of HIIT Compared to Moderate Continuous Training in Improving Vascular Parameters in Inactive Adults

Robinson Ramírez-Vélez¹
E-mail: robin640@hotmail.com // robinson.ramirez@urosario.edu.co
Paula Andrea Hernández-Quiñones²
E-mail: paulaculturafisica@yahoo.com
Alejandra Tordecilla-Sanders¹
E-mail: alesanders_0615@hotmail.com
Cristian Álvarez³,⁴
E-mail: cristian.alvarez@ulagos.cl
Rodrigo Ramírez-Campillo⁴
E-mail: r.ramirez@ulagos.cl
Mikel Izquierdo⁵
E-mail: mikel.izquierdo@gmail.com
Jorge Enrique Correa-Bautista¹
E-mail: jorge.correa@urosario.edu.co
Antonio García-Hermoso⁶
E-mail: antonio.garcia.h@usach.cl
Ronald G. García⁷,⁸,⁹
E-mail: rgarcia@nmr.mgh.harvard.edu

¹ Centro de Estudios para la Medición de la Actividad Física «CEMA», Escuela de Medicina y Ciencias de la Salud, Universidad del Rosario, Bogotá D.C, Colombia.
² Grupo GICAEDS. Programa de Cultura Física, Deporte y Recreación, Universidad Santo Tomás, Bogotá, D.C, Colombia.
³ Department of Physical Activity Sciences, Universidad de Los Lagos, Osorno
⁴ Research Nucleus in Health, Physical Activity and Sports, Universidad de Los Lagos, Osorno
⁵ Department of Health Sciences, Public University of Navarre, CIBER de Fragilidad y Envejecimiento Saludable (CB16/10/00315), Tudela, Navarre, Spain.
⁶ Laboratorio de Ciencias de la Actividad Física, el Deporte y la Salud, Universidad de Santiago de Chile, USACH, Santiago, Chile.
⁷ Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
⁸ Neurovascular Science Group, Fundación Cardiovascular de Colombia, Floridablanca, Santander, Colombia
⁹ Connors Center for Women’s Health and Gender Biology, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, USA

ClinicalTrials.gov ID: NCT02738385 (https://clinicaltrials.gov/ct2/show/NCT02738385)
Date of registration: April 14, 2016
Abbreviated title: Exercise Training on Vascular Function in Inactive Adults

Corresponding:
Robinson Ramírez-Vélez, PhD. Universidad del Rosario, Bogotá, D.C, Colombia. Phone: +57 (1) 2970200 ext. 3428. E-mail: robin640@hotmail.com // robinson.ramirez@urosario.edu.co
Abstract

Background: Strong evidence shows that physical inactivity 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. We aimed to determine the effects of moderate- versus high-intensity interval exercise training on vascular function parameters in physically inactive adults.

Methods: Twenty inactive adults were randomly allocated to receive either moderate continuous training (MCT group; 60-75% of their heart rate reserve (HRR) or high-intensity interval training (HIT group; 4 min at 85-95% of peak HRR). Vascular function (Normalization of brachial artery flow-mediated dilation, FMDn [%], aortic pulse wave velocity, PWV [m·s⁻¹], Alx, augmentation index) was measured at baseline and over 12 weeks of training.

Results: FMD changed by -1.0 (6.3) % in the MCT group and 1.9 (6.2) % in the HIT group (no significant difference between groups: 2.9 [95% CI, −3.0 to 8.8; η² = 0.15, p=0.131]. PWV changed by 0.1 in the MCT group but decreased by −0.4 in the HIT group (η² = 0.39, p <0.01 interaction), and there was not a significant difference in the prevalence of NR for PWV between the MCT and HIT groups (66% versus 33%, P = 0.051). Regarding FMDn (%), an analysis showed that the prevalence of NR was 66% (6 cases) in the MCT group and 18% (2 cases) in the HIT group (P = 0.013).

Conclusions: Under the conditions of the present study, physically inactive adults in both groups experienced changed in FMD. The rate of response was significant different between exercise groups for FMDn measure but not PWV outcome. The sustained change in PWV in the HIT group may represent a signal of vascular adaptation or endothelial fatigue.

Key words: Aerobic exercise; arterial stiffness; cardiovascular disease prevention; endothelial dysfunction; intermittent training; sedentarism

Background

Strong evidence shows that physical inactivity (<150 min/wk of moderate-intensity activity or <75 min/wk of high-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\textsuperscript{1,2}. Physical inactivity has a deleterious effect that is comparable to smoking and obesity and is now recognized as the fourth leading risk factor for global mortality, accounting for 6\% of all deaths\textsuperscript{2}.

Growing evidence suggests that exercise training improves vascular structure and nitric oxide bioavailability and reduces CVD risk factors; improvements in endothelial function may explain a large proportion of the risk reduction\textsuperscript{3}. A number of factors appear to influence the acute effects of exercise on endothelial function, including sex, exercise intensity and duration, and the timing of post-exercise vascular function measurements\textsuperscript{3}. Training protocols involving traditional moderate continuous training (MCT) and high-intensity training (HIT) can improve endothelial function\textsuperscript{4,5}, a response largely mediated by acute elevations in blood flow and laminar shear stress during individual exercise bouts\textsuperscript{6,7}. In line with this, a growing body of evidence has demonstrated comparable or superior improvements in cardiovascular function using low-volume HIT compared to MCT\textsuperscript{5}.

Additionally, three sessions of 4 min of high-intensity exercise per week (12 min/week) was sufficient to improve aortic reservoir pressure (an independent predictor of CVD), and thus may be a time-efficient exercise modality for reducing cardiovascular risk in individuals with metabolic syndrome\textsuperscript{5,8}. Furthermore, it was suggested that the
ability of HIT to restore vascular homeostasis through the enhancement of shear stress-
induced nitric oxide bioavailability may be another important mechanism that explains the
protective role of exercise against non-communicable disease development\(^9\). Interestingly,
despite this evidence, few randomized trials have directly evaluated the effects of
sustained MCT or HIT on the cardiometabolic health of inactive adults\(^4,9,10\).

There exists an inter-individual variability in vascular function, such that under the
same stimulus, some subjects may achieve benefits, and are considered responders (Rs),
whereas others may exhibit a worsened response or remain unchanged, and are considered
non-responders (NRs)\(^11,12\). Both genetic and environmental factors have been described to
explain this previously reported phenomenon\(^13,14\). However, all of these studies are
primarily endurance or resistance training-based\(^9,15\) and most have not explored other
exercise modalities such as HIT\(^16\).

In Latin-American populations, information about optimal exercise timing for
improving vascular function parameters is scarce. There is no consensus regarding optimal
exercise timing for improving vascular function parameters. Additionally, determining the
prevalence of NRs after an exercise program is relevant to optimize and predict responses in
different populations (e.g., athletes or individuals with risk factors).

The purpose of this secondary randomized clinical trial analysis was to compare the
effects of MCT versus HIT on vascular function in physically inactive adult Latin-
Americans. Identifying the training regimen that has the most beneficial effects on each
parameter could potentially lead to enhanced precision in prescribing exercise training
intensity to achieve optimal outcomes in this population\(^16\).
Methods

Sample and Procedures

Details of the study design and methods of the primary HIT-Heart Study trial have been described elsewhere (ClinicalTrials.gov ID: NCT02738385)\textsuperscript{17,18}. Informed consent was obtained from each participant. The protocol was based on the Helsinki Declaration Accord (World Medical Association for Human Subjects). Moreover, ethical approval was obtained from the University of Santo Tomás (ID 27-0500-2015). Endothelial function and fitness parameters were assessed at baseline and over 12 weeks of training. Briefly, the HIT-Heart Study conducted in 2013–2015 tested the efficacy of MCT versus HIT in changings biomarkers of endothelial and cardiovascular health (see Figure Supplement 1 for CONSORT diagram).

** Insert Figure Supplement 1 **

Participants (n=20) were recruited at the University of Rosario (Bogota, Colombia) from February 2015 to May 2016. Inclusion criteria were individuals aged 18–45 years who were inactive (<150 min·wk\textsuperscript{-1} of moderate-intensity activity or 75 min·wk\textsuperscript{-1} of vigorous-intensity activity by applied a short version of the self-reported Global Physical Activity Questionnaire) and had a body mass index (BMI) \(\geq 18\) and \(\leq 30\) kg/m\textsuperscript{2}. We excluded participants if they had a history of cardiovascular disease and related morbidities, diabetes mellitus 1 or 2, thyroid dysfunction, or cancer or if they were pregnant or smoked. All participants provided written informed consent before participating in the study. Participants were randomly assigned via a computer-generated, concealed, fixed block randomisation procedure to MCT (n = 10) or HIT (n = 11) groups. Data were obtained prior to
randomisation by treating physiotherapists and physiologist, and then 12 weeks later by
blinded assessors. Assessments were taken at baseline (Week 0) and 12 weeks after
randomisation for all outcomes by experienced and blinded physiotherapists or exercise
physiologist.

**Interventions**

*Moderate-continuous training (MCT) group*

The MCT protocol involved walking on a treadmill with the deck inclined to reach
the desired intensity. Each preparatory period started with an exercise dose of 6 kcal·kg\(^{-1}\)·week\(^{-1}\), which was increased progressively by 2 kcal·kg\(^{-1}\)·week\(^{-1}\) until week 4 and was then
maintained at 12 kcal·kg\(^{-1}\)·week\(^{-1}\) for weeks 5 to 12. Exercise training sessions were designed
to elicit a response in the acceptable moderate range, i.e., 60–75% of HRR and were adjusted
according to ratings on the Borg scale\(^{17,18}\). The rating of perceived exertion used was 12 to
15-point single-item scale ranging from 6 to 20 (6 “No exertion” and 20 “Maximum
exertion”). 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). Exercise was
performed in 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 (Figure 1).

** Insert Figure 1 **

*High-intensity training (HIT) group*

The HIT protocol involved fast walking and running on a treadmill with the deck
inclined to reach the desired intensity. We calculated training energy expenditures according
to participants’ age ranges associated with meeting the consensus public health recommendations from the Cardiometabolic HIT-RT Study\textsuperscript{17,18}. Each preparatory period started with an exercise dose of 6 kcal·kg\(^{-1}\)·week\(^{-1}\), which was increased progressively by 2 kcal·kg\(^{-1}\)·week\(^{-1}\) until week 4 and was then maintained at 12 kcal·kg\(^{-1}\)·week\(^{-1}\) for weeks 5 to 12. The overall goal for the HIT group was to perform exercise sessions in 4 × 4 min intervals at 85–95\% of HRR (with the target zone maintained for at least two min), interspersed with a 4-min recovery period at 75-85\% of HRR. The speed and inclination of the treadmill were continuously adjusted to ensure that participants trained at the correct intensity. During each exercise session, participants adhered to the 12 -kcal·kg\(^{-1}\)·week\(^{-1}\) energy expenditure format, which was equivalent to 300 kcal of energy expended by the end of the training and cool-down (5 min) periods, with a total exercise time ranging from 35 to 55 min. Exercise was performed in three sessions per week. During the supervised intervention, HR and Borg ratings were measured as described for the MCT group.

We selected 6 to 12 kcal·kg\(^{-1}\)·week\(^{-1}\) per week because this dose of kcal·kg\(^{-1}\)·week\(^{-1}\) has produced changes in VO\(_2\)peak that placed approximately 70\% of the initial sedentary population above the cut point for a low level of fitness\textsuperscript{17,18}, as defined by both the American College of Sports Medicine (ACSM)\textsuperscript{19} and the American Heart Association\textsuperscript{20} guidelines for cardiovascular disease reduction.

The intensity to run/walk was related to a range of 85–95 \% (HIT) or 60–75\% (MCT) of the maximum predicted heart rate according to the widely known equation (Karvonen), and the rest period was considered under a heart rate of 75-85\% to HIT group of this marker. Thus, using the heart rate and oxygen consumption data obtained from the baseline fitness (cardiorespiratory uptake) test, the heart rate associated with an oxygen consumption of
approximately 60% (MCT) and approximately 75-85% (HIT) were prescribed for each participant\(^\text{19}\).

**Endothelial function arterial wall parameters measures**

The primary outcome measure was endothelial function, as measured by flow-mediated-dilation (FMD), aortic pulse wave velocity (PWV) and the augmentation index (AIx). FMD was measured as described in previous studies from our group\(^\text{21}\) in the Colombian population using the protocol reported by Atkinson et al\(^\text{22}\). A detailed description of the FMD technique can be found in a previous study\(^\text{21}\). FMD was expressed as % change =\[(maximum - baseline diameter) / baseline diameter\] × 100. Normalized brachial artery FMD (FMDn) was calculated according allometric relationship between Dbase and peak diameter (Dpeak)\(^\text{22}\).

PWV was measured by analyzing the oscillometric pressure curves registered from the upper arm with arteriographic computer program (Arteriograph Software v.1.9.9.2; TensioMed, Budapest, Hungary). The algorithm measuring blood pressure in the arteriography device has been validated\(^\text{23}\). A detailed description of the PWV and AIx technique can be found in a previous study\(^\text{21}\). The R value as an estimate of the measurement errors for the repeat measurements between two sessions (n=6) was low for the arteriograph (1.18 m·s\(^{-1}\)).

**Secondary outcomes**

**Anthropometric measurements:** After completing another general information questionnaire, participants were instructed to wear shorts and a T-shirt to the physical exam. They were also required to remove all worn jewelry and metal objects. Once the subjects were barefoot and in their underwear, their body weight (kg) was measured using an electric
scale (Model Tanita® BC-420®, Tokyo, Japan) with a range of 0–200 kg and with an accuracy of within 100 g. Height was measured with a portable stadiometer with a precision of 0.1 cm and a range of 0–2.5 m (Seca® 274, Hamburg, Germany). Body mass index (BMI) was calculated as the body weight in kilograms divided by the square of height in meters (kg/m²).

Cardiopulmonary exercise testing and training intensity: A maximal incremental test was performed by each participant on a treadmill (Precor TRM® 885, Italy) using a ramp protocol that simulates field running described by Ramírez-Vélez. The criteria for exercise termination followed the American College of Sports Medicine recommendations, identified by an exercise physiologist who was present during each test. Maximum pulmonary oxygen uptake was defined as the mean cardiorespiratory uptake of the last 30s of exercise; the maximum HR was registered at the exercise peak.

Although diet was not controlled, participants met with the study’s dietician for nutritional assessment and counselling at baseline, and an individualized iso-energetic nutrition intervention plan was developed from the baseline food intake assessment according to participant preferences. This plan was standardized at 1300 to 1500 kcal·day⁻¹ (50–55% carbohydrates, 30–35% total fat, <7% saturated fat and 15–22% protein), distributed across 3-4 meals per day.

Physical activity performed outside of the supervised exercise sessions (daily physical activity) was measured using Global Physical Activity Questionnaire for a 10 and 12 weeks. MET-minutes/week were used to estimate the duration and intensity of physical activity during intervention.

Sample size
The measurement of FMD, validated in several population studies, was selected as the critical variable to calculate the sample size\textsuperscript{25,26}. A randomized clinical trial of the effect of aerobic training on FMD resulted in a standardized effect size (ES) of 0.3 to 0.6 for improvement in endothelial function\textsuperscript{27}. An \textit{a priori} power analysis estimated that a total sample size of 10 participants in each group would detect a 0.4 standardized ES for a between-group difference in improvements in FMD ($\beta = 0.80$, $\alpha = 0.05$ for a two-tailed test).

\textit{Statistical Analysis}

To retain the data of all randomly allocated participants, an intention-to-treat analysis (all randomly assigned patients) was performed. Prior to the planned statistical analyses, a preliminary analysis was conducted (\textit{Kolmogorov-Smirnov} test) to confirm the normality of the data. We used a generalized linear model (GLM) with repeated measures to analyze the influence of the different doses of exercise training on components of endothelial function and physical fitness outcomes [2 (group) x 2 (test time)]. Cohen’s $d$ for ESs were also calculated to determine the magnitude of the group differences. ESs were classified as small, small-to-medium, and medium-to-large effects ($<0.20$, 0.2–0.6 and 0.6–1.2, respectively)\textsuperscript{28}, and $\eta^2$ group x time interaction ESs were calculated as the between-group sum of squares divided by the total sum of squares and interpreted as follows: small (0.01); small-to-medium (0.01–0.10); and medium-to-large effect (0.10–0.25).

To classify the participants as Rs or NRs for improvements in FMDn/PWV, the typical error (TE) was calculated, similar to the approach in our recent study\textsuperscript{11,13}. TE was calculated using the following equation: $TE = SD\text{diff}/\sqrt{2}$, where $SD\text{diff}$ is the variance...
(standard deviation) of the difference in scores observed between the 2 repeats of each test. A NR was defined as an individual who failed to demonstrate a decrease or increase (whichever represented a beneficial change) that was greater than 2 times the TE away from zero. Chi-squared ($\chi^2$) tests were used to assess the differences between the prevalence of NRs pre- and post-intervention for each group. All reported P values are two-sided (P<0.05). Statistical analyses were conducted using PASW Statistics 17 for Windows (SPSS, Inc., Chicago, Illinois).

**Results**

Supplement Figure S1 shows the flowchart of this randomized clinical trial. A total of 28 physical inactive subjects were assessed for eligibility, of which seven were excluded for not meeting the 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 for reasons unrelated to this study (i.e., lack of time due to work schedule).

Table 1 presents the within- and between-group differences in vascular parameters following the training program. Peak brachial artery diameter significantly increased in the HIT group (+0.3 [0.1] mm) and MCT group (+0.2 [0.2] mm), with a lower effect (Cohen’s $d = 0.10$). There was a medium-to-large group x time interaction effect on PWV [between-group mean difference = -0.5 (CI 95% = -0.3 to 1.3) m·s$^{-1}$; $\eta^2 = 0.60$; P<0.01], indicating positive adaptations following HIT compared with those following MCT. There were no significant treatment effects on other vascular parameters.
Figure 2A and 2B show the mean values for individual changes in FMDn (%) and PWV in both groups. Regarding FMDn (%), the analysis showed a NR prevalence of 66% (6 cases) in the MCT group and 18% (2 cases) in the HIT group (p=0.013). There was no significant difference in the prevalence of NRs for PWV between the MCT and the HIT group (66% versus 33%, p=0.051). These results are clinically relevant change and could be used as threshold value in individuals with characteristics like those of this study.

** Insert Figure 2 **

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 2. Compliance with the study intervention was adequate, with 32/36 (89%) of participants receiving supervised exercise training. 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. Between 10 to 12-week, walking differences over time in both groups was MCT group 945 min vs HIT group 514 min, (p < 0.001), but this difference was evident from high-intensity physical activity levels (MCT group 885 min vs HIT group 1168 min, p < 0.001).”

** Insert Table 2 **

Discussion

To the best of our knowledge, this is the first randomized clinical trial studying the effects of exercise training intensity on vascular parameters and individual responses in physically inactive adults from a Latin-American population. These findings suggest
that exercise training induces potent stimuli leading to improvements in vascular
parameters (i.e., decrease in arterial wall thickness and increase in endothelial function).
However, not all of the measured vascular functions responded in the same way to the type
of exercise investigated, suggesting the presence of different regulatory mechanisms and
time courses for induction.

HIT and MCT on a treadmill have been previously shown to be highly effective in
patients with metabolic syndromes\textsuperscript{29}. Additionally, exercise training has been shown to be
an effective therapeutic strategy for vascular function improvement in different clinical
populations\textsuperscript{30}. A previous meta-analysis showed that HIT is more potent than MIT in
enhancing FMDn, with a mean difference of 2.26\%\textsuperscript{31}. Specifically, this review suggested
that a 4 x 4 HIT protocol three times per week for at least 12 weeks is an effective form of
exercise for enhancing vascular functions. Our study showed a mean difference of 2.9\% in
vascular functions between groups; however, the difference was not significant. Along the
same line, our data indicate that while brachial artery diameter increased as a result of
exercise, arterial function assessed by FMD decreased at high levels of exercise (Table 1).

It is conceivable that substantial and/or sustained increases in shear forces that occur
during exercise bouts may be associated with attenuated FMD, because stimulation of
vasodilation post-exercise may result in the inhibition of related biochemical pathways\textsuperscript{30}. In
line with this, a meta-analysis of prospective studies reported a 13\% reduction in the risk of
cardiovascular events with a 1\% increase in FMDn; therefore, the magnitude of change in
FMD following HIT (pre- vs. post-HIT +1.9\%) was deemed to be clinically significant in
our study.
Differences in exercise and experimental protocols in our study may have also contributed to discrepancies in our findings; however, this hypothesis remains to be tested. Our study showed that exercise intensity influences FMDn response; however, FMDn following exercise was attenuated in the MCT group but enhanced in the HIT group. Siasos et al.\textsuperscript{7} suggested that both acute HIT and MCT can favorably affect endothelial function in healthy young adults, indicating another cardioprotective effect of exercise preventing the progression of atherosclerosis. The effects of these intense exercise regimens on FMDn reflect a combination of hemodynamic changes and endothelial nitric oxide-dependent mechanisms\textsuperscript{4,10}. Exercise induces increases in blood flow, and augmented blood flow causes vasodilation, which directly impacts the magnitude of FMDn\textsuperscript{22,32}.

Regarding arterial wall parameters, aerobic exercise seems to significantly improve arterial stiffness, and this effect is enhanced at higher intensities of aerobic exercise and in participants with greater baseline arterial stiffness\textsuperscript{31,33}. PWV is widely recognized as a direct marker of arterial stiffness\textsuperscript{33}. Augmentation and the Alx are being more frequently used in studies as parameters of wave reflection\textsuperscript{34}. In addition, an increase in PWV is linked with increased rates of cardiovascular incidences related to increased left ventricular afterload and wasted left ventricular energy\textsuperscript{31,33}. A previous systematic review and meta-analysis of RCTs reported that every 1 m·s\textsuperscript{-1} increase in PWV is associated with a 12–14% increase in the risk of cardiovascular events and a 13–15% increase in the risk of CVD mortality. On the other hand, it was reported that aerobic exercise reduced PWV by 0.63 m·s\textsuperscript{-1}, which may be translated into an 8% reduction in cardiovascular events and a 9% reduction in cardiovascular mortality. Furthermore, subgroup analyses suggested that there may be
bigger effects on PWV and, consequently, on cardiovascular events and mortality of aerobic exercise in higher risk participants (with PWV ≥ 8 m/s at baseline) and with longer durations of aerobic exercise (> 10 weeks)\textsuperscript{31,33}. To the best of our knowledge, this was the first study to investigate alterations in PWV and the AIx after HIT in physically inactive adults from a Latin-American population.

Evidence from systematic reviews and experimental studies has demonstrated a positive effects of various exercise modalities (aerobic, resistance and combined) on endothelial functions\textsuperscript{7,29,31}, but there are controversies regarding the effects of HIT on indices pertaining to arterial stiffness and wave reflection\textsuperscript{7,31,35}. The mechanism by which HIT significantly reduces PWV more than MCT does could be associated with reduced exposure of the vasculature to reactive oxygen species that are often observed during high-volume exercise\textsuperscript{36}. It is also possible that the higher volume of exercise in the HIT group may have resulted in the requirement of longer time for PWV recovery from repeated high-intensity exercise bouts, thereby providing a more accurate representation of the cumulative effect of exercise intervention. However, the rate of NR was different between MCT and HIT in terms of reduced PWV and AIx in the favor of high-intensity training, and this difference was also observed within each group with regard to other cardiometabolic and performance-related co-variables. These results may help identify the vascular wall that is more responsive and, conversely, the wall that is more resistant to the arterial stiffness-lowering effects of HIT\textsuperscript{35}.

According to the study by Siasos et al.\textsuperscript{7}, different intensities of aerobic exercise have different effects on central and peripheral arterial stiffness. In our study, despite differences
between groups in terms of PWV, there was a trend for a greater reduction in these parameters following HIT. It could be hypothesized that the lack of effect on PWV may be due to normal PWV at baseline in most of the subjects (85%) (PWV ≥ 8 m/s at baseline). Additionally, aerobic exercise seems to have a greater effect on peripheral than on central indices of arterial stiffness\(^7\), which could justify our findings. These discrepancies between findings could also be due to differences in exercise modes or durations of HIT intervals; Ramos et al.\(^8\) and Sawyer et al.\(^15\) suggest that metabolic responses to HIT vary depending on the duration of the work-rest intervals.

On the other hand, the phenomenon of NR has been explored on performance variables\(^1\) using endurance\(^{12}\), resistance (RT)\(^{37}\), or HIT\(^{11}\) in different age groups such as children\(^{13}\), adults\(^{29}\), and older populations\(^{38}\). Regarding FMDn (individual responses), our analysis showed an NR rate of 66% (6 cases) in the MCT group and 18% (2 cases) in the HIT group (\(P = 0.013\)). This information can be useful when there are more than one risk factor to improve in physically inactive populations, and this knowledge can be useful for choosing exercise interventions with low rates of NR and high rates of improvements in particular outcomes. The data from some studies support our conclusion that exercise intensity plays an important role in modulating adaptations in vascular functions in response to exercise\(^{4,31,32}\). In line with this, several previous studies have reported increases\(^{39,40}\) decreases\(^{27}\) or lack of change\(^{10}\) in FMD following different exercise protocols. Unfortunately, none of these studies on exercise interventions reported on the rate of NR. Although some misleading studies have claimed the lack of non-responders in 4-week training intervals\(^1\),
more recently, this phenomenon has been confirmed after 6 weeks and 6-8-months of exercise by relevant authors in the field.

In any case, the term “NR” may be related more to semantics, as the authors demonstrate a lack of response in some of the chosen outcomes (e.g., VOpeak, lean body mass, leg strength) across participants. Even the authors of reports that refute the so-called ‘myth’ of exercise non-response might agree that the term “NR” depends solely on the chosen clinical outcomes and that a non-responder for one outcome may not be a non-responder in another outcome. As technology advances and our understanding of the mechanisms driving exercise responses improves, scientists can continue to narrow the focus on clinical outcomes that are critical for improving the health of an individual, and healthcare practitioners can thus recommend exercise regimens on an individual basis rather than broadly suggesting the same exercise regimens for everyone.

More specifically, it has been suggested that HIT may impair endothelium-dependent vasodilation due to an increase in reactive oxygen species, resulting in a reduction in NO bioavailability. Additionally, responses in FMD are inversely proportional to baseline arterial diameter. Further studies are necessary to establish optimal exercise training interventions for improving vascular health assessed by measuring FMD. Additionally, differences between the effects of different exercise regimens could be due to variability in their ability to generate greater blood flow through vessels supplying oxygen to the working muscles, which could in turn promote greater shear stress-induced nitric oxide bioavailability and induce favorable endothelial adaptations. In this context, several biologically plausible mechanisms may be used to explain the effects of exercise on the modulation of endothelial functions and arterial stiffness. It is widely known that exercise has the potential to reduce
oxidative stress by increasing the efficiency of the antioxidant system, eventually improving endothelial dysfunction\textsuperscript{40}. The main physiological mechanisms involve the up-regulation of endothelial nitric oxide synthase activity, as demonstrated in cell culture, animal and human studies, with a subsequent reduction in the expression of nicotinamide adenine dinucleotide (phosphate) (NAD(P)H)-dependent oxidase and the stimulation of free radical-scavenging systems that affect the levels of copper/zinc-containing superoxide dismutase, extracellular superoxide dismutase, glutathione peroxidase and glutathione\textsuperscript{37}. Therefore, the improvements in arterial stiffness and endothelial functions in response to ischemia may be related to the direct effects of repeated bouts of HIT on the vasculature body weight or BMI.

The strengths of this study include the use of state of the art measures of vascular functions, physical fitness, and metabolic biomarkers with supervised exercise training in a non-clinical setting. In addition, adherence to the intervention was \( \approx 89\% \). All subjects completed 32 of 36 exercise sessions, and research technicians supervised each session while HR was being monitored. A primary limitation of this study was the lack of a true control group without exercise. 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\textsuperscript{29}. Due to this and other limitations (e.g., relatively small sample size and single site design), it is important to not over-interpret the results of this RCT\textsuperscript{17}. Other limitations of this study include the lack of control over tobacco usage. Moreover, there is no gold standard measurement for arterial parameters. Additionally, indices other than post-occlusion reactive hyperemia flux were not assessed in
the present study. Lastly, we cannot determine the directions of the associations nor any causality observed in this study with absolute certainty.

Identifying the training regimen that has the most beneficial effects on each parameter could potentially lead to enhanced precision in prescribing exercise training intensity to achieve optimal outcomes in this population. Under the conditions of the present study, physically inactive adults in both groups experienced changes in FMD. Not all vascular function measured responded the same to this type of exercise, suggesting different regulatory mechanisms and time courses for induction.

**Conclusion**

In summary, based on the results of the present study, physically inactive adults in both HIT and MCT groups experienced changes in FMD. The rate of response was significantly different between the groups regrading FMDn but not PWV. The sustained change in PWV in the HIT group may represent vascular adaptation or endothelial fatigue. This study demonstrates the efficacy of high-intensity exercise in enhancing the cardioprotective effects of exercise on the progression of atherosclerosis in a physically inactive population.

**Abbreviations**

MCT: Moderate intensity training; HIT: High Intensity interval training; HRR: Heart rate reserve; 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; ICC: Intra-class correlation coefficients; METs: Units of metabolic equivalence; NRs: Non-responders; Rs: Responders
Declarations

Ethics approval and consent to participate

Details of the study design and methods of the primary HIT-Heart Study trial have been described elsewhere (ClinicalTrials.gov ID: NCT02738385; April 14th, 2016). 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, Colombia (ID 27-0500-2015). All participants provided written informed consent before participating in the study.

Consent for publication

Not applicable.

Availability of data and materials

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

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.
Authors contributions

RR-V conceived and designed the project. RR-V, JEC-B, RG-G and PAH-Q reviewed the literature studies and conducted data extraction. RR-V and JEC-B conducted data analyses. RR-V, RG-G and MI were responsible for data interpretation. AT-S, MI and RR-V drafted the manuscript, and PAH-Q, and MI revised it critically for intellectual contributions. MI and RR-V coordinate the study development. All authors reviewed and edited the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We would like to thank and acknowledge the enthusiastic group of test participants who made this study possible.
References


22 Atkinson G. Shear rate normalization is not essential for removing the dependency of flow-mediated dilation on baseline artery diameter: past research revisited. Physiol Meas 2014;35:1825-1835.


Legend

**Figure Supplement 1.** CONSORT guidelines flow diagram for enrolment and randomisation HIT-Heart Study.

**Figure 1.** Schematic representation of a $4 \times 4$ HIT session or 30–40 min MCT session.

**Figure 2.** Differences in the prevalence of non-responders in vascular parameters after 12 weeks training.
### Table 1. Intent-to-Treat Analysis of anthropometric and vascular function parameters at baseline and changes after 12 weeks

<table>
<thead>
<tr>
<th>Groups</th>
<th>Anthropometric</th>
<th>Vascular function</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline, Mean (SD)</td>
<td>After 12 weeks, Mean (SD)</td>
</tr>
<tr>
<td></td>
<td>MCT (n = 10)</td>
<td>HIT (n = 11)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>69.3 (15.3)</td>
<td>66.8 (10.9)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.6 (3.6)</td>
<td>25.5 (4.2)</td>
</tr>
</tbody>
</table>

**Anthropometric**
- Weight, kg
- Body mass index, kg/m²

**Vascular function**
- $D_{base}$, mm
- $D_{peak}$ mm
- $D_{diff}$
- FMD, %
- FMDn, %
- PWV, m·s⁻¹
- AIX (aortic, %)
- AIX (brachial, %)

HIT: 4 x 4-min High-intensity interval training; MCT: Moderate intensity continuous training; Within-group effect size Cohen’s $d$; between-group effect size $\eta^2$; $D$: Diameter; FMD: Flow-mediated vasodilation; FMDn: Normalized flow-mediated vasodilation; PWV: Pulse wave velocity; AIX: Augmentation index; * $d$ cohen significant difference at $p < 0.01$
Table 2. Attendance to prescribed exercise sessions and self-reported physical activity

<table>
<thead>
<tr>
<th>Variable</th>
<th>MCT (n = 9)</th>
<th>HIT (n = 11)</th>
<th>Group effect (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adherence (% of prescribed sessions completed, mean (SD))</td>
<td>98.7 (3.7)</td>
<td>98.4 (2.8)</td>
<td>0.969</td>
</tr>
<tr>
<td>Total number of sessions completed, mean (SD)</td>
<td>32.5 (1.3)</td>
<td>32.5 (0.9)</td>
<td>0.993</td>
</tr>
<tr>
<td>Total time spent training (min) per week, mean (SD)</td>
<td>1100 (258)</td>
<td>1031 (147)</td>
<td>0.043</td>
</tr>
<tr>
<td>International Physical Activity Questionnaire (10 to 12 week)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking MET-minutes/week, mean (SD)</td>
<td>945 (1890)</td>
<td>514 (1014)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Moderate MET-minutes/week, mean (SD)</td>
<td>200 (276)</td>
<td>128 (260)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vigorous MET-minutes/week, mean (SD)</td>
<td>885 (712)</td>
<td>1168 (588)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

SD, standard deviation
85-95% HR

75-85% HR Recovery

60-75% HR

Time (min)

Warm-up 60-70% HR

Cool-down 60-70% HR

HIT group

MCT group
Figure 2

A

Training response (change) FMDn

non-responders
n= 6 (66%)

non-responders
n= 2 (18%)

B

Training response (change) m/sec

non-responders
n= 6 (66%)

non-responders
n= 3 (33%)

MCT group

HIT group
Click here to access/download
Supplementary Material
Suppl S1.tif
Erratum to: Similar cardiometabolic effects of high- and moderate-intensity training among apparently healthy inactive adults: a randomized clinical trial

Robinson Ramírez-Vélez1*, Alejandra Tordecilla-Sanders1, Luis Andrés Téllez-T1, Diana Camelo-Prieto2, Paula Andrea Hernández-Quiñonez1, Jorge Enrique Correa-Bautista1, Antonio García-Hermoso3, Rodrigo Ramirez-Campillo4,5,6 and Mikel Izquierdo7

Erratum to: J Transl Med (2017) 15:118
DOI 10.1186/s12967-017-1216-6

In the original version of this article [1], published on 30 May 2017, we noticed an error in Table 2. The lean mass (kg) and fat mass (%) corrected table is included in this erratum. These changes have no material impact on the conclusions of our paper. We apologize for any inconvenience caused to our readers.

*Correspondence: robin640@hotmail.com; robinson.ramirez@urosario.edu.co
1 Centro de Estudios para la Medición de la Actividad Física «CEMA», Escuela de Medicina y Ciencias de la Salud, Universidad del Rosario, Bogotá D.C, Colombia
Full list of author information is available at the end of the article
<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline MCT (n = 9)</th>
<th>Baseline HIT (n = 11)</th>
<th>Follow-up MCT (n = 9)</th>
<th>Follow-up HIT (n = 11)</th>
<th>From baseline to 12-week, mean (95% CI)</th>
<th>MCT effect p value (ES)</th>
<th>HIT effect p value (ES)</th>
<th>Time x group p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean mass (kg)</td>
<td>49.7 (9.3)</td>
<td>44.9 (5.8)</td>
<td>49.4 (8.3)</td>
<td>45.9 (5.9)</td>
<td>−0.3 (1.2)</td>
<td>1.1 (1.2)</td>
<td>−1.4 (−2.5 to −0.2)</td>
<td>0.237 (0.01)</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>274 (73)</td>
<td>31.3 (12.2)</td>
<td>274 (8.6)</td>
<td>30.1 (11.5)</td>
<td>0.0 (0.9)</td>
<td>−1.2 (1.5)</td>
<td>1.2 (0.0 to 2.4)</td>
<td>0.500 (0.03)</td>
</tr>
</tbody>
</table>
Author details
1 Centro de Estudios para la Medición de la Actividad Física «CEMA», Escuela de Medicina y Ciencias de la Salud, Universidad del Rosario, Bogotá D.C, Colombia. 2 Grupo GICAEDS, Facultad de Cultura Física, Deporte y Recreación, Universidad Santo Tomás, Bogotá D.C, Colombia. 3 Laboratorio de Ciencias de la Actividad Física, el Deporte y la Salud, Universidad de Santiago de Chile, USACH, Santiago, Chile. 4 Departamento de Ciencias de la Actividad Física, Universidad de Los Lagos, Osorno, Chile. 5 Núcleo de Investigación en Salud, Actividad Física y Deporte; Laboratorio de Medición y Evaluación Deportiva, Universidad de Los Lagos, Osorno, Chile. 6 Unidad de Fisiología Integrativa, Laboratorio del Ciencias del Ejercicio, Clínica MEDS, Santiago, Chile. 7 Departamento de Ciencias de la Actividad Física, Universidad de Los Lagos, Osorno, Chile. 8 Departamento de Ciencias de la Actividad Física, Universidad de Los Lagos, Osorno, Chile. 9 Departamento de Ciencias de la Actividad Física, Universidad de Los Lagos, Osorno, Chile.

The online version of the original article can be found under doi:10.1186/s12967-017-1216-6.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Published online: 13 June 2017

Reference
Similar cardiometabolic effects of high- and moderate-intensity training among apparently healthy inactive adults: a randomized clinical trial

Robinson Ramírez-Vélez1*, Alejandra Tordecilla-Sanders1, Luis Andrés Téllez-Téllez1, Diana Camelo-Prieto2, Paula Andrea Hernández Quiñónez1, Jorge Enrique Correa-Bautista1, Antonio García-Hermoso3, Rodrigo Ramirez-Campillo4,5,6 and Mikel Izquierdo7

Abstract

Background: Metabolic syndrome (MetS) increases the risk of morbidity and mortality from cardiovascular disease, and exercise training is an important factor in the treatment and prevention of the clinical components of MetS.

Objective: The aim was to compare the effects of high-intensity interval training and steady-state moderate-intensity training on clinical components of MetS in healthy physically inactive adults.

Methods: Twenty adults were randomly allocated to receive either moderate-intensity continuous training [MCT group; 60–80% heart rate reserve (HRR)] or high-intensity interval training (HIT group; 4 × 4 min at 85–95% peak HRR interspersed with 4 min of active rest at 65% peak HRR). We used the revised International Diabetes Federation criteria for MetS. A MetS Z-score was calculated for each individual and each component of the MetS.

Results: In intent-to-treat analyses, the changes in MetS Z-score were 1.546 (1.575) in the MCT group and −1.249 (1.629) in the HIT group (between-groups difference, P = 0.001). The average number of cardiometabolic risk factors changed in the MCT group (−0.133, P = 0.040) but not in the HIT group (0.018, P = 0.294), with no difference between groups (P = 0.277).

Conclusion: Among apparently healthy physically inactive adults, HIT and MCT offer similar cardiometabolic protection against single MetS risk factors but differ in their effect on average risk factors per subject.

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

Keywords: Randomised controlled trial, Exercise training, Metabolic syndrome, Intensity

Background

Disorders of the metabolic system have a key pathophysiological role in the early stages of excess adiposity, elevated blood pressure, insulin resistance, abnormal glucose metabolism and dyslipidemia [elevated triglyceride levels and low-density lipoprotein cholesterol (LDL-c), and reduced high-density lipoprotein cholesterol (HDL-c)], producing coronary vasoconstriction, increasing cardiac oxygen consumption and leading to fatal events [1, 2]. This cluster of findings is recognized as metabolic syndrome (MetS) [3] and strongly predicts the risk of developing type 2 diabetes, hypertension and cardiovascular disease (CVD), which remains the leading cause of death worldwide [4–8].

Recently, Barceló [9] estimated that the number of CVD deaths in Latin America will increase by more

*Correspondence: robin640@hotmail.com; robinson.ramirez@urosario.edu.co

1 Centro de Estudios para la Medición de la Actividad Física «CEMA», Escuela de Medicina y Ciencias de la Salud, Universidad del Rosario, Bogotá D.C, Colombia

Full list of author information is available at the end of the article

© The Author(s) 2017. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
than 60% between 2000 and 2020, while CVD deaths will increase by only 5% in high-income countries during the same period. The findings of the INTERHEART case–control study in Latin America showed that abdominal obesity, dyslipidemia, and hypertension were associated with high population-attributable risks of 48.5, 40.8, and 32.9%, respectively [10]. In the same retrospective study, daily consumption of fruits or vegetables and regular PA reduced the risk of acute myocardial infarction. Therefore, interventions aimed at the reduction of modifiable risk factors are thought to be the most effective way to prevent the onset of MetS and potentially CVD in Latin America.

On the other hand, MetS is determined by genetic predisposition as well as environmental factors that may promote its development, such as low levels of physical activity (PA), large volumes quantities of sedentary time (sitting), and poor eating habits [5, 8]. The adoption and maintenance of PA are critical foci in the metabolic health management and overall health of individuals with potential medical risks, including acute complications such as cardiac events, hypoglycemia, and hyperglycemia. Strong evidence shows that physical inactivity (<150 min week$^{-1}$ of moderate-intensity PA or 75 min week$^{-1}$ of vigorous-intensity PA) are jointly associated with increased cardiometabolic morbidity and mortality in a dose-dependent manner [11, 12]. Currently, physical inactivity is the fourth leading risk factor for global mortality and is comparable in that respect to smoking and obesity, accounting for 6% of all deaths [13]. Experimental studies indicate that physical inactivity and sedentary time result in alterations in cardiovascular [14] and metabolic biomarkers [15, 16].

Systematic reviews [17–19] have found that physically inactive adults who participate in supervised interval training in clinical settings improve their exercise capacity, quality of life, maximal oxygen consumption (VO$_2$max) and metabolic control. A growing body of evidence has demonstrated comparable or greater improvements in cardiovascular function using low-volume high-intensity training (HIT) compared to traditional moderate-intensity continuous training (MCT) [17, 18, 20, 21]. Furthermore, participation in HIT reduces risk factors that are associated with MetS, bringing improvement in features such as the oxidative metabolism–dependent energy system, metabolic capacity, qualitative profile of skeletal muscle fiber type, muscle mass, and fiber diameter [2, 22–24]. In primary prevention, Pattyn et al. [2] shown that endurance training has a favourable effect on most of the cardiovascular risk factors associated with the MetS such as: a mean reduction in abdominal obesity, blood pressure decrease and a mean increase in HDL-c. In this same line, in previously clinical trials [22, 25–28] has been investigated the effect of exercise in different populations and for single cardiovascular risk factors, but none have specifically focused on the insufficient PA and the concomitant effect of HIT on all associated cardiovascular risk factors. However, few randomized trials have directly evaluated the effects of MCT or HIT on cardiometabolic health among inactive adults [2, 25–28].

Although the epidemiologic transition and epidemic of CVD have been well documented in Latin Americans [29–31], relatively little research on their PA [32–34] and physical fitness exists. Moreover, Latin American countries [6, 7] have a similar or even greater prevalence of MetS among adults than developed countries [8]. In this context, ethnicity and age has been associated with the development of MetS specially in Hispanic population [1, 3, 6, 7]. According to the definition of the National Cholesterol Education Program–Adult Treatment Panel III of the United States, the prevalence of MetS in adults was: 32% in Hispanic Americans; 22% in African Americans; and 24% in European Americans [3]. In Colombia, Martínez-Torres et al. [32] reported the predisposing factors for having a MetS included: being male, over 25 years old and overweight or obese, all of them related to metabolic disorders as previously described in apparently healthy women [31]. In addition, the public policy recommendations also highlight the need for healthy adults to have an activity plan that integrates preventative and therapy recommendations [40–42]. For this reason, a randomized clinical trial (RCT) comparing different intensities of exercise training in adults with insufficient PA with a large age range and different ethnic groups are clinically relevant because it can provide evidence for a precise [21, 35–37], prescribed intensity of exercise training to achieve optimal outcomes in this population [40–42].

Therefore, the purpose of this RCT was to compare the effects of MCT and HIT on the risk factors for MetS among apparently healthy physically inactive adults. We hypothesized that HIT and MCT would induce similar reductions in the risk factors for MetS and similar increases in exercise capacity when training frequency and session duration were equal in both types of training.

Methods
Study design and setting
The High Interval Intensity Training and ideal cardiovascular Heart Study (HIIT-Heart Study) was an RCT (ClinicalTrials.gov ID: NCT02738385) that included physically inactive Colombian adults who were randomly allocated to either an MCT group or an HIT group. The study was performed in accordance with the Declaration of Helsinki [38] and was approved by the local office of the Medical Research Ethics Committee at the University of
Santo Tomás (ID 27-0500-2015). Cardiometabolic health parameters and physical fitness outcomes were assessed at baseline and 12 weeks later. We provide an overview of the methods per the Consolidated Standards of Reporting Trials (CONSORT) checklist [39].

Participants and recruitment
This RCT was conducted at the University of Rosario and the University of Santo Tomás (Bogota, Colombia) from February 2015 to May 2016. Participants aged 18–45 years who were inactive and had a body mass index (BMI) ≥18 and ≤30 kg/m² and who were willing and almost immediately available to participate in the study were recruited from the Centre of Studies in Physical Activity Measurements (in Spanish, CEMA) via posted study recruitment flyers at community centers, study recruitment announcements at the CEMA, and word of mouth. 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 [40]. We have recently published a complete description of the HIIT-Heart Study design, methods, and primary outcomes for our current cohort [21]. Participants were required to sign a written informed consent form.

Blinding and randomization
Random allocation into the two study groups was performed by the CEMA at the University of Rosario in Bogotá, Colombia using block randomization with a block size of four. As each consecutive participant entered this RCT, he/she was randomly allocated to either the MCT group or the HIT group according to a computer-generated group allocation sequence. The randomization sequence was not concealed from the investigator who was responsible for assigning participants to groups. The principal investigators and statisticians were blinded to treatment allocation throughout the trial protocol.

Interventions
Both groups participated in the cardiometabolic program as recommended by both the American College of Sports Medicine (ACSM) [41] and the AHA [40, 42] guidelines for ideal cardiovascular health and disease reduction. At the beginning of the training protocol, we measured the participants’ weight to determine the weekly energy expenditure that was necessary to achieve their target of 12 kcal kg⁻¹ week⁻¹ (iso-energetic).

The MCT and HIT interventions lasted 12 weeks, with three sessions per week consisting of fast walking or running on a treadmill with the deck inclined to reach the desired intensity. HR was recorded during each session using an HR monitor (Polar Pacer, USA). In addition, Borg ratings were measured during each exercise session. An initial 2-week preparatory phase of training was performed to bring participants up to a 6 kcal kg⁻¹ week⁻¹ goal (~150 kcal per session or equivalent to 6 Mets), which was progressively increased by 2 kcal kg⁻¹ week⁻¹ until week 4 and was then maintained at 12 kcal kg⁻¹ week⁻¹ for weeks 5 through 12 (~300 kcal per session or equivalent to 10 Mets). The duration of each individual session depends on the number of visits required to reach the target kcal kg⁻¹ week⁻¹.

Moderate-intensity continuous training (MCT) group
Exercise training sessions were designed to elicit a response in the acceptable moderate-to-vigorous range, i.e., 55–75% heart rate reserve, and were adjusted according to ratings on the Borg scale. Each session consisted of a warm-up (5 min), followed by 15–55 min of treadmill walking/running (15–35 min during the 2-week preparatory phase) and a final relaxation/cool-down period (10 min).

High-intensity training (HIT) group
We calculated the training energy expenditure for participants’ age ranges to meet the consensus public health recommendations included in the HIIT-RT Study [21]. A complete description of the design and methods has been published elsewhere [21]. During the 2-week preparatory phase, subjects warmed up at 65% heart rate reserve (5 min), then performed 4 × 4 min intervals at 60–80% heart rate reserve interspersed with 4 min of active recovery at 55% heart rate reserve. During weeks 3–12, subjects performed 4 × 4 min intervals at 85–95% heart rate reserve (remaining in the target zone for at least 2 min) interspersed with 4 min of active recovery at 65% heart rate reserve and a cool-down (5 min), with a range of total exercise time ranging from 35 to 55 min (including warm-up and cool-down). We selected 6–12 kcal kg⁻¹ week⁻¹ per week because this dose of kcal/kg/week has produced changes in VO₂max that placed about 70% of the initially sedentary population above the cut point for low fitness, as defined in by both the ACSM) [41] and the AHA [40, 42] guidelines for cardiovascular disease reduction.

Participants in both groups were supervised during each exercise training session by an investigator or research assistant. Exercise training was conducted at the “CEMA” fitness center on the campus of the University of Rosario, which contained the treadmills needed to complete the prescribed exercise programs. Each participant was instructed to inform the supervisor immediately if he or she experienced any unusual symptoms during 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 the study. All participants reported that they adhered 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 in energy expenditure was calculated for each participant according to both the HR and the number of minutes spent exercising during the training sessions. The trainers were physical therapists and physical educators with experience developing and monitoring exercise programs with clinical populations. Adherence to the exercise program was encouraged by the exercise professional who supervised each of the group sessions. To maximize adherence to the training program, the trainer supervised no more than 3–5 participants simultaneously. Although diet was not controlled, participants met with the study dietician for nutrition assessment and counselling at baseline, and an individualized iso-energetic nutrition intervention plan was developed from the baseline food intake assessment according to participant preferences. This plan was standardised at 1300–1500 kcal day⁻¹ (50–55% carbohydrates, 30–35% total fat, <7% saturated fat and 15–22% protein), distributed across 3–4 meals per day.

**Data collection and outcome measures**

The outcome measures were assessed at baseline and 12-week follow-up by personnel who were blinded to the treatment allocation. The data were recorded on standardized forms and entered into a secured Microsoft Excel Access database that included quality control checks (e.g., range checks, notifications of missing data).

Anthropometric and body composition variables were collected at the same time in the morning, between 7:00 a.m. and 10:00 a.m. Body weight and height were measured following standard procedures with an electronic scale (Tanita® BC544, Tokyo, Japan) and a mechanical stadiometer platform (Seca® 274, Hamburg, Germany), respectively. BMI was calculated as body weight in kilograms divided by the square of height in meters (kg/m²). Waist circumference (WC) was measured at the narrowest point between the lower costal border and the iliac crest using a tape measure (Ohaus® 8004-MA, New Jersey, USA). In cases in which this point was not evident, WC was measured at the midpoint between the last rib and the iliac crest [43]. We measured each variable twice and used the average unless the first and second measures varied ≥1%. In such cases, we used the median of three measurements. In all measures, we found very good test-retest reliability [body weight (intra-class correlation, ICC = 0.983), height (ICC = 0.973), BMI (ICC 0.897), and WC (ICC = 0.967)]. The percentages of body fat mass and lean mass were obtained using the Tetrapolar Bioelectrical Impedance Analysis (BIA) system (SECA mBCA 515®, HANS E. RÜTH S.A, Hamburgo Alemania), with subjects standing barefoot on the metal contacts. This method was previously validated by experts in the field [44]. Our lab’s analysis showed strong agreement between the two methods as reflected in the range of BF%. This result shows that BIA and dual-energy X-ray absorptiometry are comparable methods for measuring body composition with higher or lower body fat percentages (unpublished data). Before testing, the participants were required to adhere to the following instructions from the BIA manufacturer [44]: (1) not to eat or drink within 4 h of the test, (2) not to consume caffeine or alcohol within 12 h of the test, (3) not to take diuretics within 7 days of the test, (4) not to perform physical exercise within 12 h of the test, and (5) to urinate within 30 min of the test. BIA measurements were performed at 50 kHz with a 0.8 mA sine wave constant current under standard conditions [44]. The measurement was made twice, and the average value was used. Inter-observer variability was R = 0.89. BIA has been extensively used as the gold standard against other body composition methods in subjects from the same region of origin as the current participants [44].

Blood pressure was measured using an electronic oscillometric device (Riester Ri-Champion model, Jüngingen, Germany) according to the recommendations of the Association for the Advancement of Medical Instrumentation [45]. Prior to blood pressure monitoring, the accuracy of the device was tested using a standard mercury sphygmomanometer in a random subsample (n = 25) to ensure that there was no consistent difference (>10 mmHg) in blood pressure. To calculate the mean arterial pressure, the diastolic blood pressure was added and the sum was added to the systolic blood pressure. Inter-observer variability was R = 0.96.

Blood samples were collected between 5:30 and 7:00 a.m. by two experienced phlebotomists after ≥12 h of fasting. Blood samples were obtained from an antecubital vein, and analyses were subsequently completed within one day of collection. The biochemical profile included plasma lipid triglycerides, total cholesterol, HDL-c, LDL-c, and glucose (measured by enzymatic colorimetric methods). Inter-assay reproducibility (coefficient of variation) was determined via ten replicate analyses of five plasma pools over 15 days and was 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. Additional outcomes in this study were participant adherence and adverse events. Total exercise time was
defined as the total time spent on exercise training during the study. Data on participant adherence to the prescribed exercise training variables are expressed in the intervention section.

We used the revised International Diabetes Federation (IDF) [46] criteria for MetS: (i) increased waist circumference (males \( \geq 94 \) cm and females \( \geq 80 \) cm); (ii) increased triglycerides (\( \geq 150 \) mg/dl); (iii) reduced HDL-c (males \(<40\) mg/dl and females \(<50\) mg/dl); (iv) increased blood pressure (\( \geq 130 \) mm Hg systolic or \( \geq 85 \) mm Hg diastolic); and (v) increased fasting glucose (\( \geq 100 \) mg/dl). To test the effects of exercise training on MetS, we used a continuous Z-score, rather than a series of dichotomous scores. This concept has been proposed by other researchers to represent and detect overall metabolic changes more accurately and detect overall metabolic changes for several reasons [22, 46]. Firstly, the continuous score would be more sensitive to small and large changes that do not change the IDF criteria [22]. Secondly, the continuous score would be less sensitive to small changes that occur in the vicinity of the diagnostic criteria for any one variable [22]. Thus, composite continuum score of MetS risk has been observed in several adult studies and has been demonstrated to be a good method to assess overall cardiometabolic risk [34]. The MetS Z-score was calculated from individual subject data, IDF criteria [46], and standard deviations using data from the entire subject cohort at baseline. The equation used was MetS Z-score = \( \frac{(WC—94)}{SD} + \frac{(triglycerides—150)}{SD} + \frac{(fasting plasma glucose—100)}{SD} + \frac{150—HDL-c}{SD} + \frac{mean blood pressure—100}{SD} \).

**Statistical analysis**

To retain the data of all randomly allocated participants, an intention-to-treat analysis (all randomly assigned patients) was performed. Prior to the planned statistical analyses, a preliminary analysis was conducted (Kolmogorov–Smirnov test) to confirm the normality of the data. Once it was confirmed that the sample data satisfied the normality assumption, statistical analyses relevant to our main research interest were conducted. t-tests for continuous variables and Chi square for categorical variables were used to investigate any possible differences in baseline characteristics and adherence between the groups. We used a generalized linear model (GLM) to analyze the influence of the different doses of exercise training on MetS components and body composition outcomes with repeated measures [2 (group) \( \times \) 2 (test time)]. Intergroup differences in changes with time were tested using the impaired t test. Cohen's \( d \) effect sizes (ES) were also calculated to determine the magnitude of the group differences. ES was classified as small, medium, and large as \(<0.20, 0.2—0.6\) and \(0.6—1.2\), respectively [47]. The significance of the interactions effects between variables was tested using Spearman correlation analyses and denoted as \( r_s \). All reported P values were two-sided (\( P < 0.05 \)). Statistical analyses were conducted using PASW Statistics 17 for Windows (SPSS, Inc., Chicago, Illinois).

**Results**

Figure 1 shows the CONSORT flowchart of the randomized clinical trial. A total of 28 potential physically inactive subjects were assessed for eligibility. Seven of them were excluded because they did not meet the inclusion criteria. Ten participants were randomly allocated to the MCT group, and 11 were allocated to the HIT group. After allocation, one participant in the MCT group withdrew for reasons unrelated to this study (lack of time due to work schedule).

The baseline characteristics of the MCT group, HIT group and total sample are outlined in Table 1. The t-test or Chi square indicated that no statistically significant differences in the baseline characteristics (\( P > 0.05 \)) existed between the groups.

Table 2 list the effects of the exercise interventions on MetS components. For MetS Z-score a significant main effect of time was observed in MCT (\( P = 0.009, ES = 0.82 \)) and HIT (\( P = 0.015, ES = 0.55 \)) groups. The difference between groups was \(-2.795 (95\% CI 1.276—4.311, P = 0.001) \) time \( \times \) group (\( P = 0.001 \)). In addition, we calculated the frequency of the MetS risk factors at each time point and the average number of MetS risk factors for each training group. The average number of cardiometabolic risk factors changed by \(-0.133 \) in the MCT group (\( P = 0.040 \)); ES = 0.67 and 0.018 in the HIT group (\( P = 0.294 \); ES = 0.13 (no significant difference between groups = \(-0.152; P = 0.227 \)). There was a significant increase in fasting glucose from week 0 to week 12 in the MCT group (\( P = 0.039 \)); ES = 0.19 and the HIT group (\( P = 0.001 \); ES = 0.29. Although the t-test did not reveal significant differences between the groups (1.6 mg (95% CI \(-8.5—11.8; P = 0.078 \)), a meaningful ES increase was observed in favor of the MCT group, ES = 1.19. Mean blood pressure significantly decreased from week 0 to week 12 in the HIT group (\( P = 0.019 ES = 0.24 \)), as did WC (\( P = 0.006 ES = 0.27 \)) and TG (\( P = 0.012 ES = 0.39 \) in the MCT group.

\( r_s \) for various anthropometric and body composition variables and the MetS Z-score after 12 weeks of training are presented in Table 3. Negative correlations were observed between the MetS Z-score, weight (\( r_s = -0.627, P = 0.011 \)), BMI (\( r_s = -0.756, P < 0.001 \)) and body fat (\( r_s = -0.858, P < 0.001 \)) in the HIT group. There were no significant correlations in the MCT group.
No adverse events were reported over the course of this investigation. There were differences in the total exercise time between groups (MCT, 1100 ± 258 min; HIT, 1031 ± 147 min, training days (MCT, 35.5 ± 1.3 days; HIT, 35.4 ± 0.9 days).

Discussion
To our knowledge, this is the first RCT to compare the effects of different modes of exercise training on the clinical risk factor profile for MetS among apparently healthy physically inactive Latin American adults. The present study demonstrates that HIT was a more potent stimulus than MCT at improving a sensitive cluster of MetS risk factors, although it failed to significantly improve individual factors compared with MCT. Additionally, HIT produced stronger and moderately significant changes in MetS Z-score in terms of weight, BMI, and body fat.

There are divergent findings regarding MetS risk factors and HIT compared with MCT programs [11, 18, 20, 48–50]. Our study showed a higher MetS Z-score reduction after HIT than after MCT. The lowering of the MetS Z-score by supervised training is similar to what others have found in at risk patients [22, 51–53]. In addition, we found that HIT or MCT significantly reduced individual risk factors as others have found previously [22, 53]. These include reducing triglycerides levels, fat mass, abdominal obesity and mean blood pressure [2]. However, the MCT group had a higher baseline MetS Z-score than the HIT group, resulting in a greater improvement (ES = 0.82). In contrast to the current results, the RUSH-Study, which was performed with 81 middle-aged healthy men, showed similar positive effects on the MetS Z-score when HIT and MCT were compared [51]. However, in the aforementioned research, the HIT intervention included work intervals threefold longer than in the current study and thus a more prevalent aerobic component in the former, closer to MCT-induced adaptive loads [18].
Regarding unhealthy populations, studies have shown divergent findings. Confirming our results, Tjønna et al. [52] observed fewer subjects with MetS and fewer MetS risk factors in adults diagnosed with MetS after 16 weeks of HIT compared with MCT. In contrast, Johnson et al. [53] did not confirm the superiority of HIT compared with MCT in overweight and obese populations. Similarly, Earnest et al. [54] observed similar improvements in the MetS Z-score and the number of MetS risk factors between overweight males who participated in HIT and MCT. Due to methodological differences across studies (i.e., sex; age; initial health, weight and fitness status; prescribed medication; type and intensity of exercise, or interval duration; length of the exercise program) and the impact of such differences on outcomes [55–57], it is difficult to draw general conclusions. These and other possible factors need to be studied. The mechanism through which HIT had a greater effect than MCT on metabolic biomarkers compared to MCT is not clear. In the current study, participants in the HIT completed 4 × 4 min of exercise up to 95% of HRmax three days per week for 12 weeks, while the MCT group trained at only 55–75% of HRmax. In this context, we speculate that both training intensities might induce additive improvement in the oxidative metabolism–dependent energy system, metabolic capacity, qualitative profile of skeletal muscle fiber type, muscle mass and fiber diameter [55, 56, 58], although with potentially greater impact after HIT than after MCT. Further research is needed to reach a consensus.

No differences (time × group) were found in single MetS risk factors changes between HIT and MCT, although a significant increase in fasting glucose from baseline to post-exercise training was observed in both groups. However, levels of fasting glucose were within the healthy range. Although there are limitations to comparing Cohen's scores in our study, the Cohen's d value suggests important clinical applicability. Overall, we were unable to detect consistent superiority of HIT versus MCT programs (or vice versa) on MetS in healthy adults [18].

Furthermore, the beneficial effects of exercise on MetS Z-score were achieved without concomitant lean mass gains, however, a decrease in fat mass was associated with reductions in the MetS Z-score \( (r_s = -0.858, P < 0.001) \) in the HIT group, which emphasizes meaningfulness of this change in body composition. Interestingly, the WC decreased significantly in both groups, however, \( t \)-test did not reveal significant differences between the groups. Changes in body composition, or more precisely, changes in abdominal obesity and fat mass seem to be an important factor when an exercise intervention for reducing CVD markers is planned. In the present study we showed that a significant reduction in MetS Z-score is possible also in the absence of change in lean mass.

The strengths of this study included the use of a novel Z-score to evaluate the effects of different exercise programs on the risk of MetS; this scoring method provides an increased level of sensitivity. Each subject completed at least 32 of 36 exercise sessions, and researchers supervised each session while the subjects’ HR was being monitored.

A primary limitation of this study was the lack of a true non-exercise control group. Thus, we were unable to determine causality in our interpretation of the observed exercise-induced improvements in cardiometabolic health parameters within the groups. Second, as a common tool to assess body weight and relevant body composition parameters, BIA was used in the present study. However, it is not the “gold standard” body composition measure. Due to this and other limitations (e.g., relatively small sample size; single site design), it will be important not to over-interpret the results of this RCT. Lastly, we cannot determine the directions of the associations nor causality observed in this study with absolute certainty. Future studies may consider tighter regulation of these factors to control their effects during a relatively longer intervention.
Table 2  Intent-to-treat analysis of IDF criteria for MetS characteristics and body composition at baseline and changes after 12 weeks

<table>
<thead>
<tr>
<th></th>
<th>Groups</th>
<th>From baseline to 12-week, mean (95% CI)</th>
<th>MCT effect P value (ES)</th>
<th>HIT effect P value (ES)</th>
<th>Time x group P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>MCT (n = 9)</td>
<td>HIT (n = 11)</td>
<td>Between-group difference in change</td>
</tr>
<tr>
<td><strong>Mets Z-score</strong></td>
<td></td>
<td></td>
<td>MCT (n = 9)</td>
<td>HIT (n = 11)</td>
<td></td>
</tr>
<tr>
<td>Average risk factors</td>
<td></td>
<td></td>
<td>MCT (n = 9)</td>
<td>HIT (n = 11)</td>
<td></td>
</tr>
<tr>
<td>per subject</td>
<td></td>
<td></td>
<td>MCT (n = 9)</td>
<td>HIT (n = 11)</td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td></td>
<td></td>
<td>MCT (n = 9)</td>
<td>HIT (n = 11)</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td></td>
<td></td>
<td>MCT (n = 9)</td>
<td>HIT (n = 11)</td>
<td></td>
</tr>
<tr>
<td>High-density lipoprotein (mg/dL)</td>
<td></td>
<td></td>
<td>MCT (n = 9)</td>
<td>HIT (n = 11)</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td></td>
<td></td>
<td>MCT (n = 9)</td>
<td>HIT (n = 11)</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td></td>
<td></td>
<td>MCT (n = 9)</td>
<td>HIT (n = 11)</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td></td>
<td></td>
<td>MCT (n = 9)</td>
<td>HIT (n = 11)</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td></td>
<td></td>
<td>MCT (n = 9)</td>
<td>HIT (n = 11)</td>
<td></td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td></td>
<td></td>
<td>MCT (n = 9)</td>
<td>HIT (n = 11)</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
<td>MCT (n = 9)</td>
<td>HIT (n = 11)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td>MCT (n = 9)</td>
<td>HIT (n = 11)</td>
<td></td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td></td>
<td></td>
<td>MCT (n = 9)</td>
<td>HIT (n = 11)</td>
<td></td>
</tr>
<tr>
<td>Body fat (%)</td>
<td></td>
<td></td>
<td>MCT (n = 9)</td>
<td>HIT (n = 11)</td>
<td></td>
</tr>
</tbody>
</table>

**SD data in mean, BMI body mass index**

* Difference between groups at baseline
Univ consolidated standards of randomized clinical trials; CVD: cardiovascular
Centro de Estudios para la Medición de la Actividad Física; CONSORT:
in
ACSM: American College of Sports Medicine; AHA: American Heart Associa‑
Abbreviations

study may be an effective strategy for reduction in MetS
in the cardiovascular profile achieved in the present
enhanced with a reduction in fat mass that was observed
only when HIT was performed. Thus, the improvement
in the cardiovascular profile achieved in the present
study may be an effective strategy for reduction in MetS
Z-score and improving the health trajectory of physically
inactive adults.

Conclusion
HIT and MCT offer similar metabolic and cardiovascu‑
lar protection against single MetS risk factors but not the
average risk factors per subject. These effects could be
enhanced with a reduction in fat mass that was observed
only when HIT was performed. Thus, the improvement
in the cardiovascular profile achieved in the present
study may be an effective strategy for reduction in MetS
Z-score and improving the health trajectory of physically
inactive adults.

Abbreviations
ACSM: American College of Sports Medicine; AHA: American Heart Associa‑
Al: bioelectrical impedance analysis; BMI: body mass index; CEMA: in
Spanish, Centro de Estudios para la Medición de la Actividad Física; CONSORT:
consolidated standards of randomized clinical trials; CVD: cardiovascular
disease; ES: effect sizes; GLM: generalized linear model; HDL-c: high-density
lipoprotein cholesterol; HIT-Heart Study: high interval intensity training and
ideal cardiovascular heart study; HIT: high-intensity training; HR: heart rate;
HRR: heart rate reserve; ICC: intra-class correlation; IDF: International Diabetes
Federation; LDL-c: low-density lipoprotein cholesterol; MCT: moderate-inten‑
sity continuous training; MetS: metabolic syndrome; PA: physical activity; RCT:
randomized clinical trial; rS: spearman correlation; VO2max: maximal oxygen
consumption; WC: waist circumference.

Authors’ contributions
RR-V analyzed the clinical data and wrote the manuscript. AG-H designed the
study and reviewed the manuscript. JEC-B, AT-S, LAT-T, DC-P, PAH-Q, RR-C and
MI performed clinical studies and analyzed their clinical data. All authors read
and approved the final manuscript.

Author details
1 Centro de Estudios para la Medición de la Actividad Física « CEMA», Escuela
de Medicina y Ciencias de la Salud, Universidad del Rosario, Bogotá D.C,
Colombia. 2 Grupo GICAEDS, Facultad de Cultura Física, Deporte y Recreación,
Universidad Santo Tomás, Bogotá D.C, Colombia. 3 Laboratorio de Ciencias
de la Actividad Física, el Deporte y la Salud, Universidad de Santiago de Chile,
USACH, Santiago, Chile. 4 Departamento de Ciencias de la Actividad Física,
Universidad de Los Lagos, Osorno, Chile. 5 Núcleo de Investigación en Salud,
Actividad Física y Deporte; Laboratorio de Medición y Evaluación Deportiva,
Universidad de Los Lagos, Osorno, Chile. 6 Unidad de Fisiología Integrativa,
Laboratorio del Ciencias del Ejercicio, Clínica MEDS, Santiago, Chile. 7 Depart‑
ment of Health Sciences, Public University of Navarre, CIBER de Fragilidad y
Envejecimiento Saludable (CB16/10/00315), Pamplona, Navarre, Spain.

Acknowledgements
Not applicable.

Table 3 Partial correlation between MetS Z‑score and anthropometric/body composition characteristics after 12 weeks of program training

<table>
<thead>
<tr>
<th></th>
<th>MCT</th>
<th>HIT</th>
<th>MCT effect (P value)</th>
<th>HIT effect (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>-0.042</td>
<td>-0.627</td>
<td>0.915</td>
<td>0.011</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.001</td>
<td>-0.756</td>
<td>1.000</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>0.150</td>
<td>-0.858</td>
<td>0.700</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>-0.150</td>
<td>0.382</td>
<td>0.700</td>
<td>0.247</td>
</tr>
</tbody>
</table>

Data represent Spearman correlation coefficients
BMI: body mass index

References
1. Townsend N, Nichols M, Scarborough P, Rayner M. Cardiovascular disease in
2. Pattyn N, Cornelissen VA, Esghjri SRT, Vanhees L. The effect of exercise on
2013;43:121–33.
2012;380(9838):294–305.

Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
Please contact author for data requests.

Ethics approval and consent to participate
This study was approved by the Institutional Review Board of University of Santo Tomás (ID 27-0500-2015).

Funding
This study was part of the project entitled “Body Adiposity Index and Biomark‑
ers of Endothelial and Cardiovascular Health in Adults” and “High Interval
Intensity Training and Ideal cardiovascular Heart Study (HIT-Heart Study)”,
which was funded by Centre for Studies on Measurement of Physical Activity,
School of Medicine and Health Sciences, Universidad del Rosario (Code No.
FIUR DN-BG001). The funder had no role in the study design, data collection,
data analysis and interpretation, preparation of the manuscript, or deci‑
sion to publish. We are also grateful for the substantial contributions of our
research team (Dr. Katherine Gonzalez‑Ruiz, Mr. David Chaparro and Dr. Diana
Diaz‑Vidal).

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in pub‑
lished maps and institutional affiliations.

Received: 27 February 2017   Accepted: 17 May 2017
Published online: 30 May 2017


Effect of Moderate- Versus High-Intensity Interval Exercise Training on Heart Rate Variability Parameters in Inactive Latin-American Adults: A Randomized Clinical Trial

Robinson Ramírez-Velez,1 Alejandra Tordecilla-Sanders,1 Luis A. Téllez-T,2 Diana Camelo-Prieto,2 Paula A. Hernández-Quinónez,1 Jorge E. Correa-Bautista,1 Antonio García-Hermoso,3 Rodrigo Ramírez-Campillo,4 and Mikel Izquierdo5

1Center of Studies in Physical Activity Measurements, School of Medicine and Health Sciences, University of Rosario, Bogotá, District Capital, Colombia; 2GICAEDS Group, Faculty of Physical Culture, Sport and Recreation, Saint Thomas University, Bogotá, District Capital, Colombia; 3School of Physical Activity, Sport and Health Sciences, University of Santiago, Chile, USACH, Santiago, Chile; 4Department of Physical Activity Sciences, University of Los Lagos, Osorno, Chile; and 5Department of Health Sciences, Public University of Navarra, Center for Biomedical Research in Network (CIBER) of Fragility and Healthy Aging (CB16/10/00315), Pamplona, Navarre, Spain

ABSTRACT

Ramírez-Velez, R, Tordecilla-Sanders, A, Téllez-T, LA, Camelo-Prieto, D, Hernández-Quinónez, PA, Correa-Bautista, JE, García-Hermoso, A, Ramírez-Campillo, R, and Izquierdo, M. Effect of moderate- versus high-intensity interval exercise training on heart rate variability parameters in inactive Latin-American adults: a randomized clinical trial. J Strength Cond Res XX(X): 000–000, 2017—We investigated the effect of moderate versus high-intensity interval exercise training on the heart rate variability (HRV) indices in physically inactive adults. 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%, which consisted of walking on a treadmill at 60–80% of the maximum heart rate (HRmax) until the expenditure of 300 kcal. The HIT group ran on a treadmill for 4 minutes at 85–95% peak HRmax and had a recovery of 4 minutes at 65% peak HRmax until the expenditure of 300 kcal. Supine resting HRV indices (time domain: SDNN = SD of normal-to-normal intervals; rMSSD = root mean square successive difference of R-R intervals and frequency domain: HFLn = high-frequency spectral power; LF = low-frequency spectral power and HF/LF ratio) were measured at baseline and 12 weeks thereafter. The SDNN changes were 3.4 (8.9) milliseconds in the MCT group and 29.1 (7.6) milliseconds in the HIT group (difference between groups 32.6 (95% confidence interval, 24.9 to 40.4 [p = 0.01]). The LF/HF% ratio changes were 0.19 (0.03) milliseconds in the MCT group and 0.13 (0.01) milliseconds in the HIT group (p between groups = 0.016). No significant group differences were observed for the rMSSD, HF, and LF parameters. In inactive adults, this study showed that a 12-week HIT training program could increase short-term HRV, mostly in vagally mediated indices such as SDNN and HF/LF ratio power. Trial registration. ClinicalTrials.gov NCT02739385 https://clinicaltrials.gov/ct2/show/NCT01796275, registered on March 23, 2016.

KEY WORDS randomized controlled trial, autonomic nervous system, cardiac autonomic control

INTRODUCTION

Disorders of the autonomic nervous system have a key pathophysiological role in the early stages of essential hypertension, myocardial infarction, and chronic heart failure by producing coronary vasoconstriction, increasing cardiac oxygen consumption, and leading to fatal events (15,56). Heart rate variability (HRV) is of increasing interest because it is a marker of cardiovascular autonomic function and because reduced HRV is a direct predictor of cardiovascular risk and all-cause mortality (13,58). In addition, the parasympathetic withdrawal quantitated by HRV is associated with reduced coronary flow reserve and antedates episodes of dynamic myocardial ischemia (37). Heart rate variability refers to the periodic changes in heart rate (HR) and serves as an index of the activity level of the autonomic nervous system (26).
Heart rate variability can be evaluated by time and frequency domain indices. Accordingly, it can be represented in a time domain in which R-R intervals (in milliseconds) are plotted against time (in seconds) (53). Among the most used indices, the SD of normal beat-to-beat (R-R) intervals (SDNN) has been suggested to reflect global variability, and the root-mean-square of successive R-R intervals (rMSSD) and high-frequency (HF) power have been linked to vagal activity (8).

Strong evidence shows that physical inactivity, i.e., <150 min·wk⁻¹ of moderate activity or 75 min·wk⁻¹ of vigorous activity, can increase the risk of many adverse health conditions, including major noncommunicable diseases such as coronary artery disease (CAD), metabolic syndrome, and breast and colon cancers (9,34). Currently, physical inactivity has a deleterious effect that is comparable with smoking and obesity. It is now recognized as the fourth leading risk factor for global mortality and accounts for 6% of all deaths (36). Furthermore, physical inactivity is associated with decreased HRV, particularly HF power, thus reflecting reduced cardiovascular autonomic control (24). Experimental studies have indicated that sedentary time results in alterations of cardiovascular health and HRV and during prolonged bed-rest (25,28,54).

On the other hand, cross-sectional reports in different population suggest that regular aerobic exercise training is associated with improved HRV (1); however, studies examining the effect of moderate or high intensity on HRV are mixed and incomplete with respect to clinically recommended training paradigm. In the clinical setting, previous systematic reviews have found that inactive physical adults who participate in supervised interval training can experience improvements in HRV, exercise capacity, quality of life, maximal oxygen consumption (VO₂max), and cardiac remodeling (3,18,50). In addition, high-intensity exercise has been shown to positively modify the sympathovagal control of HRV toward facilitating a persistent increase in parasympathetic tone, known to be associated with a better prognosis in noncommunicable diseases patients (50).

Of interest, few studies showed comparable or superior improvements in cardiovascular function using low-volume, high-intensity training (HIT) compared with traditional moderate continuous training (MCT) (18,23,47,50,59). However, the effects of MCT, HIT, or a combination of the 2 (MCT·HIT) program on HRV indices, which is clinically the current standard in inactive adults, have yet to be established (5,12,21,42).

In the Latin-American population, a region that has undergone a well-documented epidemiologic transition and epidemic of obesity (30,40,52), relatively little research on physical activity (49) and physical fitness exists (17,41,48). A randomized clinical trial comparing different intensities of exercise training in inactive adults is clinically relevant because it could provide evidence for a precise, prescribed intensity of exercise training for optimal outcomes in this population (20,33,34,59). Given this knowledge gap, the aim of the current randomized clinical trial was to compare the effect of MCT versus HIT on HRV indices in physically inactive adults.

**Materials and Methods**

**Experimental Approach to the Problem**

The HIIT-Heart Study is a substudy of the “High Intensity Interval-vs. Moderate Training on Biomarkers of Endothelial and Cardiovascular Health in Adults” study (registered at ClinicalTrials.gov, registration number: NCT02738385) in which the aim was to compare the efficacy of different volumes of HIT and traditional training in reducing risk factors constituting the CAD. In this substudy, we report changes in HRV variables that were only recorded at our local site (Bogotá, Colombia). A participant flow diagram is shown in Figure 1. The overall objective of the substudy “HIIT-Heart” is to quantify the dose-effect of different exercise intensities (i.e., moderate-intensity and low-volume vigorous-intensity), on HRV indices (primary outcome), and on physiologic response (HR, blood pressure), body composition (body mass index [BMI], waist circumference [WC], body fat, and lean mass) and peak oxygen consumption (VO₂peak) in adults (secondary outcomes).

**Participants and Recruitment**

This randomized clinical trial was conducted at the University of Rosario in Bogota and Santo Tomás University, Colombia, between February 2015 and May 2016. Primary and secondary 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 (11).

Participants were recruited from the Centre of Studies in Physical Activity Measurements (in Spanish, CEMA) by posting study recruitment flyers at community centers, by study recruitment announcements at CEMA, and by word-of-mouth. Subjects are eligible to participate if they are located in the metropolitan region with available time (1 hour per day) to support the trial. Additional eligibility criteria include participants who were aged 18–45, were inactive (<150 min·wk⁻¹ of moderate-intensity activity or 75 min·wk⁻¹ of vigorous-intensity activity), had a BMI ≥18 and ≤30 kg·m⁻², and identified as being willing and having almost immediate availability.

Risks were minimized by ruling out contraindications to the testing and training protocols via a health history and a thorough physical examination before 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 (35). 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 mmHg or diastolic blood pressure 95 mmHg), gastrointestinal disease (including self-reported chronic hepatitis or cirrhosis, any episode of alcoholic hepatitis or alcoholic pancreatitis within the 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 ≥118 mg·dl⁻¹) or use of any prescribed drugs, any active use of illegal or illicit drugs, or inability to participate because of a physical impairment. In addition, we confirmed by 2 exercise physiologists, subjects if they had alteration in ventricular function and/or cardiomyopathy, through a standard 12-lead electrocardiography (ECG) at rest and every 3 minutes of the maximum treadmill exercise test. All subjects remained under usual medical care and clinical follow-up (i.e., regular appointments with a physician) throughout the protocol. Written informed consent was obtained for all subjects, and ethical approval was granted by the local office for Research Ethics Committee at University of Santo Tomás (ID 27-0500-2015). In addition, each participant completed an informed consent document outlining the experiment that was approved by the institutional review board. The study conforms to the principles outlined in the Declaration of Helsinki.

**Blinding and Randomization**

Randomization into the 2 study arms was performed by the CEMA at University of Rosario, Bogotá, Colombia, using block randomization with block sizes of 4. As each participant consecutively entered this randomized clinical trial, the subject was randomly allocated to either the MCT or the HIT group according to the computer-generated allocation sequence. 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. Furthermore, 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 assigned to the intervention group participated in the cardiometabolic program as recommended by the Colombian guidelines COLDEPORTES (in Spanish, Departamento Administrativo del Deporte, la Recreacion, la Actividad Fisica y el Aprovechamiento del Tiempo Libre) (29) and AHA (35,39) for cardiovascular health promotion and disease reduction. At the beginning of the training protocol, we obtained the participants’ weight to determine the weekly energy expenditure necessary to achieve their target of 12-kcal·kg⁻¹·wk⁻¹ (iso-energetic). It was expected that the gradual increase in total energy expenditure would minimize fatigue, soreness, injuries, and attrition.

After inclusion, patients performed a maximal cardiopulmonary exercise test on a maximum treadmill exercise test (Pecor TRM 885, Pecor Corp, Rome, Italy) following the modified Balke protocol (1) and physiological parameters (VO₂, HR, and Borg ratings) from the test were used to set exercise intensity. Based on averaged maximum HR (HRmax) and VO₂peak, the participants were classified according to normative values, referenced to age and sex (1,39). Moderate continuous training and HIT interventions lasted 12 weeks, with 3 sessions per week, consisting in fast walking or running on a treadmill with the deck inclined to
reach the desired intensity. Heart rate was recorded each session using an HR monitor (Polar Electro, Kempele, Finland). In addition, rating of perceived exertion (RPE) was also measured in each exercise session. An initial 2-week preparatory phase of training was performed to bring participants up to a 6-kcal·kg⁻¹·wk⁻¹ goal (~150 kcal per session), which was increased progressively 2-kcal·kg⁻¹·wk⁻¹ until week 4, and was then maintained at 12-kcal·kg⁻¹·wk⁻¹ for weeks 5–12 (~300 kcal per session).

**Moderate Continuous Training (MCT) Group.** Exercise-training sessions were designed to elicit a response in the acceptable moderate-to-vigorous range, i.e., 55–75% HRmax/RPE of 11–15 on Borg scale. Sessions consisted of a warm-up (5 minutes), followed by 15–55 minutes of treadmill walking/running (15–35 minutes during the 2-week preparatory phase), and a final relaxation/cool-down period (10 minutes).

**High-Intensity Training (HIT) Group.** We calculated the training energy expenditure for participants’ age ranges associated with meeting the consensus public health recommendations from the Cardiometabolic HIIT-RT Study (47). A complete description of the design and methods has been published elsewhere (47). During the 2-week preparatory phase, subjects warmed up at 65% HRmax (5 minutes), then performed 4 × 4 minutes intervals at 60–80% HRmax/RPE of 13–15 on Borg scale, interspersed with 4 minutes of recovery at 55% HRmax/RPE of 11–13 on the Borg scale. During weeks 3–12, subjects perform 4 × 4 minutes intervals at 85–95% HRmax/RPE of 15–17 on Borg scale (with the target zone maintained for at least 2 minutes), interspersed with 4 minutes recovery at 65% HRmax, and a cool-down (5 minutes), with a total range exercise time of 35–55 minutes (with warm-up and cool-down).

Both groups were required to attend 2 supervised sessions with an exercise physiologist at the University of Rosario at a fitness centre “CEMA,” which contained the treadmills needed to complete the prescribed exercise programs. Each participant was instructed to immediately inform the supervisor if he or she experienced any unusual symptoms while exercising and to consult a physician if needed. Participants were instructed to refrain from exercise training and to avoid changing their physical activity levels outside this study. 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 postintervention time points. The regression of the energy expenditure was calculated for each participant according to both the HR and the minutes spent exercising during the training sessions. Trainers were physical therapists and physical educators with experience developing and monitoring exercise programs among clinical populations. Adherence to the exercise program was encouraged by the exercise professional who supervised each of the group sessions. To maximize adherence to the training program, a maximum of 3–5 participants were trained simultaneously. Each participant met with the study dietician for nutrition assessment and counseling. An individualized nutrition intervention plan was developed from the baseline food intake assessment according to participant preferences (47). This plan consisted of a standardized meal consisting of 1,300–1,500 kcal (50–55% carbohydrates, 30–35% total fat, <7% saturated fat, and 15–22% protein).

**Experimental Procedure.** Before the procedure, participants were instructed to refrain from strenuous activities for at least 48 hours, and caffeine and alcohol for at least 24 hours before all tests. Subsequently, participants reported for testing after an overnight fast, consuming only water, and refraining supplement intake that morning. All measurements were tested on 2 different days in climate controlled room between 07:00 and 10:00 hours.

**Primary Outcome Measures.** The primary outcome measure was HRV measured between 07:00 and 08:00 hours for 25 minutes in a semi-dark room (22–23°C) after a 12-hour fast. Heart rate variability measurements were conducted at the same time (±1 hour) of the day for each assessment period. We used a 2-channel ECG signal detected by an HR monitor (Polar Electro, Kempele, Finland) and transmitted online to a PC through Polar Advantage Interface receiver. We quantified HRV from the last 5 minutes of R-R interval recording. First, we examined the parasympathetic nervous system by calculating the square root of the mean of the sum of the squares of differences between adjacent R-R intervals (rMSSD). rMSSD is considered to be a stable measure of parasympathetic modulations of HR (14). Mean R-R intervals were recorded at a rate of 250 Hz. Second, SDNN was measured, reflecting the cyclic components responsible for variability in the period of recording and reflective of both sympathetic and parasympathetic tone. Third, frequency domain was analyzed in 3 absolute and log normalized frequency bands defined as HF and LF. In the frequency domain, oscillations of R-R intervals were examined within the low-frequency (LF: 0.04–0.15 Hz) and high-frequency bands (HF: 0.15–0.40 Hz). The sympathovagal balance was obtained by the ratio of the power LF to HF (LF/HF) bands. All indices are described and used in previous reports (14,45,53). The resulting R-R intervals were analyzed in the time domain, in the frequency domain using spectral analysis (fast Fourier transform), and nonlinearly through the Poincare’ plot (Kubios HRV Analysis v 2.0, Biosignal Analysis and Medical Imaging Group at the Department of Applied Physics, University of Kuopio, Kuopio, Finland). In-house testing revealed near-perfect (r = 0.99) correlations between these methods and electrocardiographs. These differences were also similar to what was reported by other applications that assess HRV through HR monitors. The reproducibility of 24-hour derived HRV...
eat or drink within 4 hours of the test; (b) to not consume
adhere to these BIA manufacturer’s instructions: (a) to not
Tokyo, Japan). Before testing, participants were required to
Impedance Analysis (BIA) system (BF-350; Tanita Corp,
muscle were obtained using the Tetrapolar Bioelectrical
measurements. The percentages of body fat mass and mass
divided by the square of height in meters (kg m$^{-2}$) 2). The WC
index was intraclass correlation coefficient (ICC) = 0.86 to
height domain parameters and ICC = 0.95 to time
domain parameters.

Secondary Outcomes. Anthropometric and Body Compo-
mension Measurements. Body weight was measured using
electronic scales (Tanita BC544; Tanita Corp., Tokyo, Japan)
with a low technical error of measurement (TEM = 0.510%).
Height was measured using a mechanical stadiometer plat-
form (Seca 274; Hamburg, Germany; TEM = 0.01%). Body
mass index was calculated as the body weight in kilograms
divided by the square of height in meters (kg m$^{-2}$). The WC
was measured at the narrowest point between the lower
costal border and the iliac crest using a tape measure (Ohaus
8004-MA; Ohaus Corp., Parsippany, NJ, USA; TEM = 0.05%). In cases where this point was not evident, it was
measured at the midpoint between the last rib and the iliac
crest. We measured each variable twice and used the average
measure obtained unless the first and second measures var-
ied by more than 1%, in which case we used the median of 3
measurements. The percentages of body fat mass and mass
muscle were obtained using the Tetrapolar Bioelectrical
Impedance Analysis (BIA) system (BF-350; Tanita Corp,
Tokyo, Japan). Before testing, participants were required to
adhere to these BIA manufacturer’s instructions: (a) to not
eat or drink within 4 hours of the test; (b) to not consume
(47) in people inactive. The treadmill test used a ramp pro-
tocol where the inclination is constant (5.5%) and the speed
increases by 0.5 km h$^{-1}$ every minute, starting at 4 km h$^{-1}$
(47). Each session began with a 5–10 minutes warm-up at 50
W. We asked participants to refrain from smoking 2 hours
before the test, and from drinking alcohol or doing any vig-
orous or moderate-intensity activities 48 hours before the
test. HRmax was used to determine the training intensity
for each participant. We measured blood pressure before
and during the test. Exercise was terminated if participants
were fatigued, or earlier if they fulfilled the AHA guidelines
for “Indications for Terminating Exercise Testing” (35). Max-
imal oxygen uptake was defined as the highest recorded
VO$_2$peak after 2 of the 3 criteria were met: (a) a plateau in
VO$_2$ after increase in workload; (b) a respiratory exchange
ratio $>$1.10, and (c) a maximal HR within 10 b min$^{-1}$ 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 exer-
cise testing as described in this study is reliable and is a stan-
dard for measuring exercise capacity (4,49). The
reproducibility of our data was $R = 0.98$.

Cardiorespiratory Fitness. VO$_2$peak was determined using
a maximum treadmill exercise test (Precor TRM 885; Precor
Corp., Rome, Italy) following the modified Balke protocol,
which has been extensively used

Resting Blood Pressure. Blood pressure was measured
using an electronic oscillometric device (Ri-Champion
model; Riester Corp., Jungingen, Germany) after being

<table>
<thead>
<tr>
<th>TABLE 1. Baseline participant characteristics.*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Total sample (n = 20)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Age, mean (SD), (y)</td>
</tr>
<tr>
<td>Race/ethnicity, n (%)</td>
</tr>
<tr>
<td>Black or Afro-Colombian</td>
</tr>
<tr>
<td>Others (indigenous)</td>
</tr>
<tr>
<td>Socioeconomic level, n (%)</td>
</tr>
<tr>
<td>Low-mid</td>
</tr>
<tr>
<td>Mid-high</td>
</tr>
<tr>
<td>Education, n (%)</td>
</tr>
<tr>
<td>Secondary</td>
</tr>
<tr>
<td>Technical</td>
</tr>
<tr>
<td>University</td>
</tr>
<tr>
<td>Occupation, n (%)</td>
</tr>
<tr>
<td>Student/work</td>
</tr>
<tr>
<td>Housewife</td>
</tr>
<tr>
<td>Marital status</td>
</tr>
<tr>
<td>Single</td>
</tr>
<tr>
<td>Married/de facto</td>
</tr>
<tr>
<td>Weight, mean (SD) (kg)</td>
</tr>
<tr>
<td>Height, mean (SD) (m)</td>
</tr>
<tr>
<td>BMI, mean (SD) (kg m$^{-2}$)</td>
</tr>
</tbody>
</table>

*MCT = moderate-intensity continuous training; HIT = high-intensity interval training; BMI = body mass index.
TABLE 2. Intent-to-treat analysis of indices of heart rate variability and physiologic characteristics at baseline and changes after 12 wk.*†

<table>
<thead>
<tr>
<th>Time domain</th>
<th>Baseline</th>
<th>Follow-up</th>
<th>MCT (n = 9)</th>
<th>HIT (n = 11)</th>
<th>MCT (n = 9)</th>
<th>HIT (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDNN (ms)</td>
<td>65.2 (21.6)</td>
<td>74.9 (35.5)</td>
<td>61.7 (12.6)</td>
<td>104.1 (43.1)</td>
<td>59.5 (22.1)</td>
<td>80.2 (51.5)</td>
</tr>
<tr>
<td>rMSSD (ms)</td>
<td>58.3 (30.9)</td>
<td>68.8 (39.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency domain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF (ms)</td>
<td>1,673.2 (2074.7)</td>
<td>1,547.8 (1,399.7)</td>
<td>1020.4 (725.5)</td>
<td>1,654.8 (1857.1)</td>
<td>1,624.4 (964.5)</td>
<td>1,138.3 (948.8)</td>
</tr>
<tr>
<td>LF (ms)</td>
<td>1,422.0 (1,141.2)</td>
<td>954.0 (903.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LF/HF (ms)</td>
<td>1.1 (0.9)</td>
<td>1.5 (1.5)</td>
<td>1.7 (1.8)</td>
<td>2.1 (1.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physiologic characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (b·m⁻¹)</td>
<td>62.0 (7.6)</td>
<td>58.6 (9.6)</td>
<td>59.3 (9.1)</td>
<td>52.7 (8.7)</td>
<td>59.3 (9.1)</td>
<td>52.7 (8.7)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>116.8 (5.1)</td>
<td>116.2 (6.5)</td>
<td>113.0 (7.6)</td>
<td>112.5 (9.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>72.3 (7.0)</td>
<td>71.0 (8.7)</td>
<td>67.8 (9.4)</td>
<td>67.0 (10.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthropometric and body composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.3 (15.3)</td>
<td>66.8 (10.9)</td>
<td>68.6 (13.5)</td>
<td>66.7 (10.5)</td>
<td>68.6 (13.5)</td>
<td>66.7 (10.5)</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>23.6 (3.6)</td>
<td>25.5 (4.2)</td>
<td>23.4 (3.0)</td>
<td>24.4 (4.2)</td>
<td>23.4 (3.0)</td>
<td>24.4 (4.2)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>81.9 (12.2)</td>
<td>75.4 (7.6)</td>
<td>79.5 (10.6)</td>
<td>75.7 (8.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>27.4 (7.2)</td>
<td>31.2 (12.1)</td>
<td>27.4 (6.5)</td>
<td>30.0 (11.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>24.0 (5.9)</td>
<td>21.1 (3.5)</td>
<td>24.2 (5.1)</td>
<td>22.0 (3.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiorespiratory fitness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO₂peak (ml·kg⁻¹·min⁻¹)</td>
<td>37.1 (7.6)</td>
<td>36.1 (7.6)</td>
<td>43.6 (9.0)</td>
<td>43.6 (9.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From baseline to 12-wk, mean (95% CI)

<table>
<thead>
<tr>
<th>MCT (n = 9)</th>
<th>HIT (n = 11)</th>
<th>Between-group difference in change</th>
<th>MCT effect, p</th>
<th>HIT effect, p</th>
<th>Time × group, p</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4 (8.9)</td>
<td>29.1 (7.6)</td>
<td>32.6 (24.9 to 40.4)</td>
<td>0.838</td>
<td>0.097</td>
<td>0.013</td>
</tr>
<tr>
<td>1.2 (8.8)</td>
<td>11.4 (11.5)</td>
<td>10.2 (0.3 to 20.1)</td>
<td>0.724</td>
<td>0.721</td>
<td>0.576</td>
</tr>
<tr>
<td>652.7 (1,349.2)</td>
<td>107.0 (457.3)</td>
<td>759.8 (–148.6 to 1,686.1)</td>
<td>0.832</td>
<td>0.842</td>
<td>0.897</td>
</tr>
<tr>
<td>184.3 (43.3)</td>
<td>202.3 (176.6)</td>
<td>18.0 (–109.3 to 145.3)</td>
<td>0.697</td>
<td>0.543</td>
<td>0.245</td>
</tr>
<tr>
<td>0.5 (0.9)</td>
<td>0.5 (0.4)</td>
<td>0.05 (–0.61 to 0.71)</td>
<td>0.612</td>
<td>0.407</td>
<td>0.016</td>
</tr>
<tr>
<td>–2.6 (–1.5)</td>
<td>–5.9 (0.8)</td>
<td>–3.2 (–4.4 to –2.1)</td>
<td>0.512</td>
<td>0.150</td>
<td>0.118</td>
</tr>
<tr>
<td>–3.8 (7.6)</td>
<td>–3.7 (6.5)</td>
<td>–0.2 (–6.8 to 6.5)</td>
<td>0.222</td>
<td>0.283</td>
<td>0.906</td>
</tr>
<tr>
<td>–4.4 (8.5)</td>
<td>–4.0 (6.8)</td>
<td>–0.4 (–7.7 to 6.8)</td>
<td>0.274</td>
<td>0.339</td>
<td>0.960</td>
</tr>
<tr>
<td>–0.6 (1.9)</td>
<td>–0.1 (1.6)</td>
<td>–0.5 (–2.2 to 1.2)</td>
<td>0.179</td>
<td>0.353</td>
<td>0.451</td>
</tr>
<tr>
<td>0.2 (0.7)</td>
<td>1.1 (3.2)</td>
<td>–0.9 (–1.4 to 3.3)</td>
<td>0.190</td>
<td>0.130</td>
<td>0.879</td>
</tr>
<tr>
<td>–1.7 (3.0)</td>
<td>0.3 (2.6)</td>
<td>–2.1 (–4.7 to 0.5)</td>
<td>0.070</td>
<td>0.357</td>
<td>0.672</td>
</tr>
<tr>
<td>0.0 (0.8)</td>
<td>–1.1 (1.5)</td>
<td>1.2 (0.1 to 2.4)</td>
<td>0.500</td>
<td>0.010</td>
<td>0.048</td>
</tr>
<tr>
<td>0.1 (0.0)</td>
<td>0.9 (0.0)</td>
<td>0.8 (–0.3 to 1.3)</td>
<td>0.363</td>
<td>0.032</td>
<td>0.292</td>
</tr>
<tr>
<td>6.5 (9.3)</td>
<td>6.5 (9.3)</td>
<td>0.5 (–7.2 to 6.2)</td>
<td>0.035</td>
<td>0.012</td>
<td>0.745</td>
</tr>
</tbody>
</table>

*MCT = moderate-intensity continuous training; HIT = high-intensity interval training; SDNN = SD of RR intervals; rMSSD = root mean square successive difference of R-R intervals; HF = high-frequency spectral power; LF = low-frequency spectral power; BMI = body mass index.
†Data in mean (SD).
seated in a quiet room for 10 minutes with their back supported and feet on the ground according to the International Protocol of the European Society of Hypertension (55). Two blood pressure readings were taken separated by a 10-minute interval. Interobserver variability was $R = 0.96$.

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. These data were used to assess each group’s adherence to the exercise program. Total exercise time was defined as the 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 presented in the interventions section. However, self-reported physical activity was measured using the recent physical activity questionnaire. This questionnaire assesses physical activity across 4 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 (4). The outcome was assessed in METs (units of metabolic equivalence) per week. This questionnaire was administered immediately before and after the training period and at 12 weeks after the completion of the exercise intervention.

### Statistical Analyses

To retain data of all randomly allocated participants, an intention-to-treat analysis population (all randomly assigned patients) was performed. Before the planned statistical analyses, a preliminary analysis was conducted (Shapiro-Wilk test) to confirm data distribution normality. Primary and secondary outcomes values are reported as mean ($\pm$) $SD$ or 95% confidence interval (CI 95%) unless otherwise specified. Because of their skewed distribution, the following variables were log-transformed before analyses: SDNN, rMSSD, HF, LF, and LF/HF ratio. To aid interpretation, data were back-transformed from the log scale for presentation in the results.

Adherence to the exercise program for both groups was expressed as the total number of training days that each participant completed of the prescribed number of training days and total exercise time during the 12-week supervised exercise program. Once it was confirmed that the sample data satisfied the normality assumption, statistical analyses relevant to our main research interest were conducted.
### Table 3. Partial correlation between physiologic characteristics and indices of heart rate variability after 12 wk of exercise training.

<table>
<thead>
<tr>
<th>Time domain</th>
<th>Frequency domain</th>
<th>LF</th>
<th>HF</th>
<th>LF/HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCT</td>
<td>SDNN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rMSSD</td>
<td>-0.611</td>
<td>0.326</td>
<td>0.159</td>
</tr>
<tr>
<td></td>
<td>LF</td>
<td>0.017</td>
<td>0.162</td>
<td>0.0049</td>
</tr>
<tr>
<td></td>
<td>LF/HF</td>
<td>0.264</td>
<td>0.264</td>
<td>0.017</td>
</tr>
<tr>
<td>HIT</td>
<td>SDNN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rMSSD</td>
<td>-0.467</td>
<td>0.363</td>
<td>0.167</td>
</tr>
<tr>
<td></td>
<td>LF</td>
<td>0.045</td>
<td>0.183</td>
<td>0.0049</td>
</tr>
<tr>
<td></td>
<td>LF/HF</td>
<td>0.264</td>
<td>0.264</td>
<td>0.017</td>
</tr>
</tbody>
</table>

Physiologic characteristics:
- Weight (kg)
- BMI (kg/m²)
- Waist circumference (cm)
- Body fat (%)
- Cardiorespiratory fitness
- VO₂peak (ml·kg⁻¹·min⁻¹)

*"rMSSD = root mean square successive difference of RR intervals; SDNN = SD of RR intervals; HF = high-frequency spectral power; LF = low-frequency spectral power; BMI = body mass index.*

Statistical analyses were conducted using SPSS version 22 (IBM, Armonk, NY, USA).
RESULTS

A total of 28 potential physically inactive subjects were assessed for eligibility, 7 of whom were excluded for not meeting the inclusion criteria. Ten participants were randomly allocated to the MCT group, and 11 participants were randomly allocated to the HIT group. After allocation, 1 participant in the MCT group withdrew from this investigation for reasons unrelated to this study (lack of time because of work schedule).

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

Table 2 and Figure 2 list the effects of the exercise interventions on HRV and physiological parameters. Difference between groups were observed on SDNN change, with 3.4 (8.9) milliseconds in the MCT group and 29.1 (7.6) milliseconds in the HIT group (difference between groups 32.6 [95% CI, 24.9 to 40.4; \(p = 0.01\); \(d = 1.14\ [95\% \text{ CI}, 0.19 to 2.00]) and in the LF/HF ratio, with a change of 0.5 (0.9) milliseconds in the MCT group and 0.5 (0.4) milliseconds in HIT group \((p\text{ between groups} = 0.016; d = 0.01\ [95\% \text{ CI}, −0.88 to 0.88])\). In addition, the percentage of body fat did not change in the MCT group.
(0.8); \( d = 0.01 \) (95% CI, −0.92 to 0.92), whereas it decreased by 1.1 percent in the HIT group (−1.1 (1.5); \( d = 0.10 \) (95% CI, −0.73 to 0.93), (difference between groups 1.2 [95% CI, 0.1 to 2.4; \( p = 0.04 \)]; \( d = −0.88 \) [95% CI, −1.81 to 0.03]). There were no significant treatment effects on other parameters.

Spearman correlation (\( r_s \)) characteristics for various physiologic variables and HRV indices after 12 weeks of program training are presented in Table 3. We observed a moderate negative correlation between BMI and rMSSD in the MCT group (\( r_s = −0.667; p ≤ 0.05 \)). When analyzing the HRV change from baseline to 12-week follow-up, we observed negative correlations between rMSSD and WC (\( r_s = −0.747; p < 0.001 \)), changes in SDNN (\( r_s = −0.720; p ≤ 0.05 \)) and HF25 (\( r_s = −0.700; p ≤ 0.05 \)), in the HIT group. Finally, we observed a stronger correlation between Ln rMSSD and R-R interval in the HIT group (\( r_s = 0.834; p ≤ 0.05 \)) Figure 3A. We also observed a nonsignificant correlation between Ln rMSSD and R-R interval in the MCT group (\( r_s = 0.396; p = 0.290 \), Figure 3B.

No adverse events were reported during this investigation. The average exercise-training days and total exercise time during the program were 35.5 days (SD 1.3) and 1,100 minutes (SD 258) in MCT group; and 35.4 days (SD 0.9) and 1,031 minutes (SD 147) in HIT group (\( p = 0.043 \)). As expected, self-reported physical activity increased as a result of training (\( F_{1,5,135,0.3} = 4.37; p < 0.001 \)). Pairwise comparison analyses showed that the participants sustained levels of vigorous or moderate physical activity at the 12-weeks follow-up (data not shown).

Figure 4 shows differences on prevalence of “responders” and “non-responders” based on relevant HRV indices after the 12-week supervised exercise. However, no significant ES difference was found between “responders” and “non-responders” prevalence for the HRV variables: \( \Delta \) SDNN (milliseconds) 33.3% vs. 63.6% (\( d = 0.35 \) [CI 95%, −0.53 to 1.24] \( p = 0.206 \)), \( \Delta \) rMSSD (milliseconds) 44.4% vs. 63.6% (\( d = 0.19 \) [CI 95%, −0.63 to 1.03] \( p = 0.180 \)), and \( \Delta \) R-R interval length (milliseconds) 66.6% vs. 63.6% (\( d = 0.10 \) [CI 95%, −0.79 to 0.74] \( p = 0.155 \)) in the MCT and HIT groups, respectively.

**DISCUSSION**

To our knowledge, this is the first randomized clinical trial on the effect of exercise-training intensity on HRV in physically inactive adults from the Latin-American population. Our findings suggest that HIT was a more effective medium-term strategy to increase HRV, specifically SDNN and LF/HF ratio, than MCT was. Collectively, the magnitude of the change for both training groups was not significantly different in the remaining parameters, suggesting that either training protocol may provide similar medium-term benefits in cardiovascular health. In addition, we did not find differences in the “responder” prevalence in relation to improvements HRV in any of these or secondary outcomes.

Increasing attention is being focused on the role of the autonomic nervous system in health and disease (51). Exercise has been reported to be effective in improving HRV because exercise serves to reduce the activity of sympathetic nervous system while increasing the activity of the parasympathetic nervous system (10). Specifically, aerobic exercise training increases cardiac vagal modulation via functional and structural adaptations in cardiovascular system (e.g., stroke volume) (38,45). Our study showed an increase in SDNN in the HIT group compared with the MCT group. SDNN reflects the cyclic components responsible for variability in the period of recording and is reflective of both sympathetic and parasympathetic tone (27). In this sense, studies in adults with type 2 diabetes also did not report improvements in HRV after 12 (32) or 16 weeks (16) of aerobic exercise programs, although improvements did occur in programs of 24 weeks (43). Thus, HRV improvement may be affected by the length of the exercise period. Therefore, HIT seems to favor a greater impact on neuro-cardiac activity than MCT in the medium term (44). The mechanism by which HIT has greater effects on the markers of cardiac autonomic outflow compared with MCT is not clear. It may be that supramaximal exercise generates higher catecholamine concentrations compared with lower-intensity exercise (31). This finding may contribute to the observed autonomic modulation (1). Furthermore, the higher catecholamine levels could explain major reductions in percentage of body fat in the HIT group, as catecholamines would stimulate lipolysis, which is primarily responsible for fat release from adipose tissue fat stores (57). However, the observed increase in HRV is consistent with a study in middle-aged men after 2 weeks of HIT (4–6 × 30 seconds of all-out cycling efforts with 4-minute recovery) compared with aerobic training (40–60 minutes at 60% of peak workload) (33).

Our findings also indicate differences between groups in LF/HF ratio changes. Although the mechanisms are not clear, this result could be explained by a larger increase in vagal- or baroreflex-mediated modulation of the sinoatrial node with HIT compared with MCT (33). In addition, differences in the hemodynamic oscillations experienced during the exercise sessions could be involved as could alterations, according to several authors, of intrinsic HR, S-A node sensitivity (7) and/or alterations of myocardial phenotype (2). High-intensity training might be an efficient short-term strategy to improve cardiac autonomic function and may have an important antiarrhythmic effect (22). Therefore, the results of this study indicate that the decrease in sympathetic activity after HIT is smaller than the increase in parasympathetic activity.

Another finding of this study is our demonstration of variability in the individual responses following different training protocols (MCT and HIT). Several reports have recommended that before individuals are classified as responders or nonresponders, it is important to determine...
if variability in the individual responses within the experimental condition are greater than within-subject variation (7,59,60). Specifically, our results demonstrated that intervention protocols which differ in intensity, time, and metabolic demand, such as MCT and HIT, can induce different adaptive responses in HRV indices, blood pressure, and cardiopulmonary fitness within a given individual (7). This indicates that after the same stimulus, some subjects may achieve positive benefits (i.e., responders – “R”), whereas other subjects may experience a worsened or unchanged response after training (i.e., nonresponders – “NR”). Environmental and genetic factors have been described as the main reasons for this phenomenon (7). Thus, it is relevant to understand the unexplored environmental factors that may be related to eliciting an increased or decreased NR incidence to plan future well-designed genetic studies.

In addition, we observed a stronger correlation between Ln rMSSD and R-R interval in the HIT group (r = 0.834; p ≤ 0.05, i.e., positive training adaptation). However, for the first time in this study, we demonstrate how these variables can also change during positive adaptation to HIT. In this context, parasympathetic tone is likely maintained and/or increased. As such, the expected increases in Ln rMSSD (a measure of vagal modulation) were blunted by the high levels of vagal tone and parasympathetic saturation in the case of the HIT group. In this case, vagal saturation and decreases in cardiac parasympathetic indices of HRV after regular training can be related to positive, healthy outcomes (15,22,33,34,46). The main changes observed in vagal-related indices with additional HIT training may well be due to the greater training intensity needed for HRV change in healthy participants (22).

Finally, the impact of HIT on body composition compared with MCT is controversial. Cycling protocols showed that HIT interventions are superior to MCT in inducing FM loss (19), or generate similar improvements (20). Contrasting our results, studies using treadmill protocols have not shown any difference in body weight and composition between these isocaloric programs (7). Our results support that HIT interventions are superior in terms of enhancing fat oxidation than MCT (19,20). Therefore, difference between fat reductions after HIT compared with MCT could suggest that obesity is a key contributing factor to vascular dysfunction; which has been corroborated in obese (37,50) and type 2 diabetic subjects (32). Considering the sedentary lifestyle of the population, obesity and the risk for noncommunicable diseases are increasing; the knowledge of a more effective mode of training (i.e., training modes as endurance, HIT, or other protocols that achieve a reduced amount of responders—“R” prevalence after training interventions), in accordance with the profile of individuals (i.e., physically inactive, unhealthy individuals, or athletes) and achievement of improvements in their risk factors may be useful information for practitioners, public health exercise programs, and populations with/at risk of CAD. This may positively affect disease morbidity, mortality, and health care expenditures (35).

The strengths of this study included state-of-the-art measures of HRV, physical fitness, and supervised exercise training in a nonclinical setting. In addition, adherence to the intervention was approximately 98%. All subjects completed 32 of the 36 exercise sessions, and research technicians supervised each session while HR was being monitored. A primary limitation of this study was the lack of a true nonexercising control group. Thus, we are unable to determine causality in our interpretation of the observed exercise-induced improvements in cardiovascular health parameters. However, among studies comparing HIT and MCT that included a control group, no changes in autonomic function were observed in the control group (44). Second, BIA was used in this study as a common tool to assess body weight and the relevant parameters of body composition. However, BIA is not the “gold standard” in body composition measurement. Future studies may consider tighter control of these factors such that the effects of these different factors may be isolated and identified in a relatively longer intervention.

**Practical Applications**

These data underline the importance of a multidisciplinary approach aiming at promoting HIT exercise program in physically inactive adults. For the practitioners/clinicians or trainer working with inactive populations should promote HIT exercise longer than 12 weeks to improve outcomes in cardiovascular health, due to HRV is a direct predictor of cardiovascular risk and all-cause mortality. Additional randomized controlled trials are required to elucidate the mechanisms responsible for these results in physically inactive adults and other populations, such as metabolic syndrome, obese, or insulin-resistant adults.

**Acknowledgments**

The authors declare that they have no competing interests. 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 de Rosario (Code No FIUR DN-BG001). The results of this study do not constitute endorsement of the product by the authors or the NSCA. All the authors contributed to the study design, data collection, and article preparation. The authors disclose funding received for this work from any of the following organizations: National Institutes of Health (NIH); Welcome Trust; Howard Hughes Medical Institute (HHMI); and other(s). This manuscript contains material that is original and not previously published in text or on the Internet, nor is it being considered elsewhere until a decision is made as to its acceptability by the *Journal of Strength and Conditioning Research* Editorial Review Board.


Exercise and postprandial lipemia: effects on vascular health in inactive adults

Robinson Ramírez-Vélez1*, María Correa-Rodríguez2, Alejandra Tordecilla-Sanders1, Viviana Aya-Aldana1, Mikel Izquierdo3, Jorge Enrique Correa-Bautista1, Cristian Álvarez4,5 and Antonio García-Hermoso6

Abstract

Background: There is evidence to suggest that postprandial lipemia are linked to the impairment of endothelial function, which is characterized by an imbalance between the actions of vasodilators and vasoconstrictors. The aim of this study was to determine the effects of a 12-week high-intensity training (HIT) and moderate continuous training (MCT) protocol on postprandial lipemia, vascular function and arterial stiffness in inactive adults after high-fat meal (HFM) ingestion.

Methods: A randomized clinical trial was conducted in 20 healthy, inactive adults (31.6 ± 7.1 years). Participants followed the two exercise protocols for 12 weeks. To induce a state of postprandial lipemia (PPL), all subjects received a HFM. Endothelial function was measured using flow-mediated vasodilation (FMD), normalized brachial artery FMD (nFMD), aortic pulse wave velocity (PWV) and augmentation index (AIX). Plasma total cholesterol, high-density lipoprotein cholesterol (HDL-c), triglycerides and glucose were also measured.

Results: The effects of a HFM were evaluated in a fasted state and 60, 120, 180, and 240 min postprandially. A significant decrease in serum glucose between 0 min (fasted state) and 120 min postprandially was found in the HIT group \((P = 0.035)\). Likewise, FMD (%) was significantly different between the fasted state and 60 min after a HFM in the HIT group \((P = 0.042)\). The total cholesterol response expressed as area under curve (AUC\(_{0-240}\)) was lower following HIT than following MCT, but no significant differences were observed \((8\%, P > 0.05)\). Similarly, triglycerides AUC\(_{0-240}\) was also lower after HIT compared with MCT, which trended towards significance \((24\%, P = 0.076)\). The AUC\(_{0-240}\) for the glucose response was significantly lower following HIT than MCT \((10\%, P = 0.008)\). FMD and nFMD AUC\(_{0-240}\) were significantly higher following HIT than following MCT \((46.9\%, P = 0.021\) and 67.3\%, \(P = 0.009\), respectively). PWV AUC\(_{0-240}\) did not differ following between the two exercise groups \((2.3\%, P > 0.05)\).

Conclusions: Supervised exercise training mitigates endothelial dysfunction and glucose response induced by PPL. Exercise intensity plays an important role in these protective effects, and medium-term HIT may be more effective than MCT in reducing postprandial glucose levels and attenuating vascular impairment.

Trial registration: ClinicalTrials.gov ID: NCT02738385 Date of registration: April 14, 2016.

Keywords: Postprandial lipemia, Endothelial function, Exercise intensity, High-intensity exercise, Moderate continuous training

* Correspondence: robin640@hotmail.com; robinson.ramirez@urosario.edu.co
1Centro de Estudios en Medición de la Actividad Física (CEMA), Escuela de Medicina y Ciencias de la Salud, Universidad del Rosario, Bogotá, D.C, Colombia
Full list of author information is available at the end of the article

© The Author(s). 2018 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Background

Postprandial lipemia (PPL) is defined as the elevation of circulating triglyceride-rich lipoproteins after high-fat meal (HFM) consumption. There is evidence to suggest that these exaggerated elevations in triglycerides are linked to impairment of endothelial function, characterized by an imbalance between the actions of vasodilators and vasoconstrictors [1]. Although the pathophysiology of endothelial dysfunction has not been fully clarified, reduced nitric oxide (NO) and increased oxidative stress are important contributors to the reduction of the vasodilatory response [2].

Endothelial dysfunction induced by PPL is considered an early and reversible predictor of atherosclerotic disease and cardiac events [3, 4]. As humans spend a considerable part of the day in a postprandial state, interventions that can reduce the magnitude and duration of this metabolic state may be beneficial in the prevention of cardiovascular disease (CVD).

Exercise training prior to high-fat meal ingestion has been shown to have an attenuating effect on postprandial metabolism [5, 6]. In the same line, studies have reported that energy expenditure through prior exercise is related to the magnitude of this effect [7, 8]. Mestek et al. reported, however, that isocaloric sessions before a meal mitigate PPL independently of the intensity of the exercise session [9]. By contrast, other studies showed that the magnitude of PPL was influenced by prior exercise intensity [5, 10]. Thus, the effects of the intensity of the exercise undertaken on postprandial response remain contentious.

With regard to postprandial endothelial function, evidence has shown that a single bout of exercise prior to HFM consumption improves fasting and postprandial endothelial function compared with a resting control condition [11–13]. Accordingly, it has been reported that acute moderate- and high-intensity exercise has transient benefits for macrovascular endothelial function in both fasting and postprandial states, and that these effects may be due to the improvement in antioxidant status [14, 15]. Nevertheless, the limited prior studies carried out to investigate the effects of exercise intensity have produced inconsistent findings [15–17].

The aforementioned studies investigating the protective effects of exercise performed a few hours before consumption of an HFM on postprandial metabolism and endothelial function have focused on the acute effects of exercise. Thus, although a 12-week training program has been established as a protocol to assess the chronic effects of exercise [18], the potential impact on postprandial metabolism and vascular function after HFM have not been previously investigated. A recent narrative review summarized the current literature on the possible contributions of medium- to long-term physical training to the reduction of the postprandial response, concluding that the data are inconclusive [18]. Interestingly, a recent systematic review and meta-analysis on cardiometabolic health showed that performing even a short period (~4 min) of high-intensity exercise has greater benefits than moderate-intensity exercise in terms of cardiometabolic risk factors [19].

Considering that most adults do not meet the public health recommendations of at least 150 min per week of moderate-intensity exercise and also that habitual physical activity declines during middle age [20], it is of special interest to identify how much high-intensity exercise is needed to optimize vascular function in adulthood.

Thus, we Given the above, we hypothesized that medium-term exercise could attenuate the postprandial decrement in metabolism and endothelial function and that this effect would differ according to exercise intensity. On this basis, we aimed to determine the effects of a 12-week high-intensity training (HIT) or a moderate continuous training (MCT) program on postprandial metabolism and vascular function and arterial stiffness after HFM ingestion in healthy, inactive Latin-American adults.

Methods

Study design and setting

Details of the study design and methods of the primary HIT-Heart Study trial have been described elsewhere (ClinicalTrials.gov ID: NCT02738385; April 14th, 2016) [21]. 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, Colombia (ID 27–0500–2015). Postprandial biochemical and vascular function responses were assessed at baseline and over 12 weeks of training. We have previously provided an overview of the methods as per the Consolidated Standards of Reporting Trials (CONSORT) checklist [22].

Participants

Participants (n = 20) were recruited at the University of Rosario (Bogota, Colombia) from February 2015 to May 2016. Subjects were eligible to participate if they were located in the metropolitan region, with available time (1 h per day) to support the trial. Inclusion criteria were individuals aged 18–45 years who were inactive (<150 min-wk.−1 of moderate-intensity activity or 75 min-wk.−1 of vigorous-intensity activity), had a body mass index (BMI) ≥18 and ≤30 kg/m² and identified as being willing and having almost immediate availability. Individuals with a history of any medical condition identified by the American Heart Association as an absolute contraindication to exercise testing were excluded from the study [23]. 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 mmHg or diastolic blood pressure 95 mmHg), gastrointestinal disease (including self-reported chronic hepatitis or cirrhosis, any episode of alcoholic hepatitis or alcoholic pancreatitis within the 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 ≥118 mg·dl⁻¹), use of any prescribed drugs, any active use of illegal or illicit drugs, or inability to participate because of a physical impairment. In addition, two exercise physiologists tested whether subjects had alterations in ventricular function and/or cardiomyopathy, measured by standard 12-lead electrocardiography (ECG) at rest and every 3 min during a maximum treadmill exercise test. All subjects remained under usual medical care and clinical follow-up (i.e., regular appointments with a physician) throughout the protocol. All participants provided written informed consent before participating in the study.

Blinding and randomization
The coordinating Research Center for Physical Activity Measurement (CEMA) in Bogotá randomized the procedures with software using randomly permuted blocks. Group allocation was conducted via an online system in which the details of eligible participants were entered to obtain group assignments (i.e., 3:2 or 2:3). Assessors were blinded to study group assignments.

Interventions
After inclusion, patients performed a maximal cardiopulmonary exercise test on a maximum treadmill exercise test (Pecor TRM 885, Pecor Corp., Rome, Italy) following the modified Balke protocol [24]. Physiological parameters (maximal O₂ consumption [VO₂max], heart rate [HR] and Borg ratings) from the test were used to establish the exercise intensity. Based on averaged maximum HR (HRmax) and VO₂peak, the participants were classified according to normative values, referenced to age and sex. MCT and HIT interventions lasted 12 weeks, with 3 sessions per week consisting of fast walking or running on a treadmill with the deck inclined to reach the desired intensity. HR was recorded each session using an HR monitor (Polar Pacer, Polar Electro, Kempele, Finland). In addition, rating of perceived exertion (RPE) was also measured in each exercise session.

Moderate continuous training (MCT) group
Each preparatory period started with an exercise dose of 6 kcal·kg⁻¹·week⁻¹, which was increased progressively by 2 kcal·kg⁻¹·week⁻¹ until week 4 and was then maintained at 12 kcal·kg⁻¹·week⁻¹ for weeks 5 to 12, which was equivalent to 300 kcal of energy expended by the end of the training and cool-down (3 min) periods with total exercise time ranging from 45 to 55 min. Exercise training sessions were designed to elicit a response in the acceptable moderate-to-vigorous range, that is, 60–75% of heart rate reserve (HRR), and were adjusted according to ratings on the Borg scale [25]. During the supervised intervention, HR was recorded using an HR monitor (Polar Pacer) to ensure compliance with the exercise stimulus at the predetermined target HR zone.

High-intensity training (HIT) group
We calculated training energy expenditures according to participants’ age ranges and set the target energy expenditures to meet the consensus public health recommendations from the Cardiometabolic HIT-RT Study [25]. Each preparatory period started with an exercise dose of 6 kcal·kg⁻¹·week⁻¹, which was increased progressively by 2 kcal·kg⁻¹·week⁻¹ until week 4 and was then maintained at 12 kcal·kg⁻¹·week⁻¹ for weeks 5 to 12. The overall goal for the HIT group was to perform exercise sessions in 4 × 4-min intervals at 85–95% of HRR (with the target zone maintained for at least 2 min), interspersed with a 4-min recovery period at 75–85% of HRR. During each exercise session, participants adhered to the 12 kcal·kg⁻¹·week⁻¹ energy expenditure format, which was equivalent to 300 kcal of energy expended by the end of the training and cool-down (3 min) periods, with total exercise time ranging from 32 to 45 min. During the supervised intervention, HR and Borg ratings were measured as described for the MCT group.

Both groups were instructed to refrain from exercise training and to avoid changing their physical activity levels outside the study. All participants reported adhering to these instructions. Although diet was not controlled, participants met with the study dietician for nutrition assessment and counseling at baseline, and an individualized iso-energetic nutrition intervention plan was developed from the baseline food intake assessment according to participant preferences. This plan was standardized at 1300–1500 kcal·day⁻¹ (50–55% carbohydrates, 30–35% total fat, < 7% saturated fat and 15–22% protein), distributed across 3–4 meals per day.

Data collection and outcome measures
Experimental procedure
All measurements were performed at baseline and at the 12-week follow-up by personnel who were blinded to
the treatment allocation. To control for confounding variables, we instructed the subjects to: i) fast for 10–12 h, ii) abstain from exercise for 24 h, iii) abstain from caffeine, tobacco, and vitamin supplements for 12 h, and iv) be awake between 0600 and 0700 h, all prior to each testing session. The HFM, which has been previously reported [26], consisted of a breakfast containing 1049 cal: 79 g of fat, 31 g of saturated fat, 4.5 g of trans fat, 666 mg of cholesterol, 69 g of carbohydrates, 31 g of protein, and 2.22 mg of sodium, adjusted by individual body weight. The effects of the HFM were measured in a fasted state and 60, 120, 180, and 240 min postprandially. Figure 1 represents the schedule of experimental events for each subject.

Biochemical parameters
Blood was drawn from an antecubital vein. The biochemical profile included plasma total cholesterol, high-density lipoprotein cholesterol (HDL-c), triglycerides and glucose (measured by enzymatic colorimetric methods). Inter-assay reproducibility (coefficients of variation) was determined via ten replicate analyses of five plasma pools over 15 days and was shown to be 2.0, 3.2, 2.6 and 1.5% for total cholesterol, HDL-c, triglycerides and serum glucose, respectively. All determinations were analyzed in serum using a Cardiocheck® and A1CNow +® system.

Vascular function and arterial stiffness measures
All subjects were tested at the same time of day and after consumption of a low nitrate diet for 48 h. Vascular function and arterial stiffness, as measured by flow-mediated vasodilation (FMD), aortic pulse wave velocity (PWV) and the augmentation index (AIx) were measured. FMD was measured as described in previous studies from our group in the Colombian population [26] using the guidelines reported by Atkinson et al. [27]. The same operator performed all Doppler ultrasound (Mindray M-9® DS USA; Mahwah, NJ) examinations using a 7.5-MHz linear array probe. Ultrasound imaging of the brachial artery was performed with the subjects in the supine position after 15 min of rest, with the arm abducted approximately 80° from the body and the forearm supinated. The ultrasound probe was positioned with a 60° insonation angle in a longitudinal plane at a site 1–3 cm proximal to the antecubital fossa to visualize the anterior and posterior lumen-intima interfaces, to measure diameter and central flow velocity (pulsed Doppler). After the baseline images were recorded, a blood pressure cuff, positioned on the arm, was inflated to 200 mmHg for 5 min; to assess FMD, images were acquired continuously for 3 min after cuff deflation, during the reactive hyperemia period. Brachial artery diameter recording was restarted at least 30 s before cuff deflation and continued for 3 min thereafter. The peak artery diameter and the time to reach this peak after cuff deflation were recorded. Images were recorded on a DVD for subsequent measurements by one observer blinded to the study design. FMD was calculated as the percent rise of peak diameter from the preceding baseline diameter and was measured every 1 min after deflation for 3 min. Normalized brachial artery FMD (FMDn) was calculated according to the allometric relationship between the baseline artery diameter and the peak diameter [27]. The intra-session coefficient of variation was ≤1% for the baseline diameter. Reliability was estimated by intra-class correlation coefficients based on four baseline measurements (n = 8 subjects), yielding values of 0.91 for baseline diameter and 0.83 for FMD (our own data). The technical error of measurement was 1.23% for baseline diameter, 1.77% for maximum diameter and 20% for %FMD.

The PWV was measured by analyzing the oscillometric pressure curves registered from the upper arm. Patient data and the measured distance between the jugulum and the symphysis were registered in an arteriographic computer program (Arteriograph Software v.1.9.9.2; TensioMed, Budapest, Hungary). A tape measure was used to measure the distance between the jugulum and the symphysis, 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 previously validated [28]. PWV was calculated as the jugulum-to-symphysis distance (m) divided by one-half of the return time (return time/2) (s). For PWV measurements, the two recordings with

![Fig. 1 Schedule of experimental events for each subject. HIT, high-intensity interval training; MCT, moderate-intensity continuous training; HFM, high-fat meal. Discontinuous arrows represent capillary blood samples and assessment of endothelial function](image-url)
the lowest standard deviations were chosen. The standard deviation was calculated on the basis of all heartbeats during a period of 8 s.

The AIx was calculated as the ratio of the difference between the systolic peaks of the first pulse [1] and second pulse [2] relative to the central pulse pressure, expressed as a percentage [(pulse 2 - pulse 1/central pulse pressure) × 100]. Thus, it provides the brachial/aortic AIx without applying a transfer function. The R value, used as an estimate of the measurement errors for the repeated measurements between two sessions, was low for the arteriograph (1.18 m·s⁻¹).

Statistical analysis
To retain the data of all randomly allocated participants, we performed an intention-to-treat analysis (all randomly assigned patients). The Shapiro-Wilk test was used to verify data distribution normality. Once it was confirmed that the sample data satisfied the normality assumption, statistical analyses relevant to our main research interests were conducted. T-tests for continuous variables and chi-squared tests for categorical variables were used to investigate any possible differences in baseline characteristics between the two conditions (HIT and MCT). We used a generalized linear model to analyze the influence of the different training protocols on biochemical and vascular function outcomes with repeated measures [2 (group) × 2 (test time)]. The area under the curve (AUC), expressed in arbitrary units (au) via the trapezoidal method, was calculated and used to analyze the response to the training protocols. The effect of training on AUC measures was analyzed by two-way analysis of variance. Significant differences in AUC from 0 to 240 min after the HFM following 12 weeks of HIT or MCT were analyzed using two-way analysis of variance. A criterion alpha level of \( P \leq 0.05 \) was used to determine statistical significance. All data are reported as the mean ± standard deviation. Statistical analyses were conducted using PASW Statistics 17 for Windows (SPSS, Inc., Chicago, IL).

Results
Study participants
Additional file 1: Figure S1 (Supplemental Digital Content) shows the flowchart of this randomized clinical trial. A total of 28 physically inactive subjects were assessed for eligibility, of which seven were excluded for not meeting the inclusion criteria. Of the 21 participants who started the study, 20 finished and one participant in the MCT group withdrew for reasons unrelated to the study (lack of time due to work schedule). Ten participants were randomly allocated to the MCT group, and 11 were randomly allocated to the HIT group.

The demographic features of the HIT and MCT groups, as well as their biochemical and endothelial function variables in the fasted state at baseline, are outlined in Table 1. No statistically significant differences (\( P > 0.05 \)) in baseline characteristics between the exercise training protocols were found (t-test), confirming that participants in both groups began the trial under similar conditions.

Biochemistry and endothelial response
Postprandial biochemical and endothelial function responses with summary measures of these responses after 12 weeks of HIT or MCT are shown in Table 2. A significant difference in glucose between 0 min (fasted state) and 120 min postprandially in the HIT group was found (\( P = 0.035 \)). Likewise, %FMD was significantly different between the fasted state and 60 min after HFM in the HIT group (\( P = 0.042 \)).

Figure 2 shows the effects of HIT and MCT on total cholesterol, triglycerides and glucose postprandial responses, with summary measures of these responses. The total cholesterol response expressed as AUC\(_{(0–240)}\) was lower following HIT than following MCT, but no significant differences were observed (8%, \( P > 0.05 \)). Similarly, triglycerides AUC\(_{(0–240)}\) was also lower following HIT than following MCT, with a trend toward significance (24%, \( P = 0.076 \)). AUC\(_{(0–240)}\) for the glucose response was significantly lower following HIT than MCT (10%, \( P = 0.008 \)).

Figure 3 shows the effects of HIT and MCT on FMD, nFMD and PWV postprandial responses, with summary measures of these responses. FMD and nFMD AUC\(_{(0–240)}\) were significantly higher following HIT than following MCT (46.9%, \( P = 0.021 \) and 67.3%, \( P = 0.009 \), respectively). PWV AUC\(_{(0–240)}\) did not differ between HIT and MCT (2.3%, \( P > 0.05 \)).

No adverse events were reported during this study. As we have previously reported, the average exercise-training days and total exercise time during the program were 35.5 days (SD 1.3) and 1100 min (SD 258) in the MCT group and 35.4 days (SD 0.9) and 1031 min (SD 147) in the HIT group (\( P = 0.043 \)) [21].

Discussion
The aim of this study was to investigate the effects of chronic MCT and HIT on postprandial lipemia and vascular function and arterial stiffness after HFM consumption in inactive adults. The main finding of this study is that a 12-week regimen of HIT reduces glucose concentrations and exerts greater post-HFM endothelial function than MCT, supporting the idea that the effect of chronic exercise on postprandial response is dependent on exercise intensity [29].
Recent evidence has shown that acute exercise increases FMD following HFM consumption [12, 30]. To the best of our knowledge, the present study is the first to demonstrate that medium-term exercise training prevents the decline in FMD induced by PPL, supporting the protective effect of regular exercise on vascular function. This is clinically relevant since endothelial function is an independent risk factor of CVD [3]. Interestingly, the higher AUC values of FMD in the HIT group after HFM (P = 0.009) indicates that this regimen may provide major vascular benefits in inactive adults.

In agreement with our results, previous studies focusing on the effect of acute exercise demonstrated that FMD remained greater after HFM consumption following a single bout of HIT as compared with MCT [15, 31]. However, although it is of special interest to calculate incremental AUC values drawn from hourly measurements up to 4 h using the trapezoid rule [12, 32], most previous studies have not included these data. In contrast to our present findings, a recent study conducted in 11 physically active young men reported that FMD response did not differ between the two conditions [16]. The differences in training status between this study population (physically active) and our study cohort (inactive) might explain the inconsistent findings, since it has been shown that FMD responses after HFM consumption may differ between active and inactive subjects [33]. Thus, based on our results and previous research, it can be hypothesized that exercise attenuates the negative effects of HFM consumption on endothelial cell function in an inactive population. Further studies investigating the mecha-sensory mechanisms contributing to the effect of exercise on vascular function as well as possible interactions among molecular pathways are required [34].

The mechanism by which chronic exercise training can modulate postprandial endothelial function is unclear. Regular exercise has been proposed to decrease PPL and therefore reduce postprandial oxidative stress by maintaining low lipoprotein levels [35]. An alternative mechanistic explanation is that regular exercise might increase antioxidant capacity, leading to maintenance of endothelial function [15]. Indeed, a substantial increase

| Table 1 Demographic, biochemical and endothelial function variables in the fasted state across HIT and MCT groups at baseline |
|------------------|------------------|------------------|
| Characteristics  | HIT (n = 11)     | MCT (n = 9)      | P value |
| Sex, n (%)       | 8 (40.0)         | 5 (55.6)         | 0.898   |
| Male             | 3 (60.0)         | 4 (44.4)         | 0.916   |
| Female           | 31.8 (7.8)       | 31.4 (6.4)       | 0.928   |
| Age, mean (sd), y | 159.4 (47.4)     | 170.1 (41.8)     | 0.301   |
| Total cholesterol (mg/dL) | 46.9 (9.6) | 43.0 (14.1) | 0.236   |
| High-density lipoprotein (mg/dL) | 100.4 (36.8) | 134.1 (82.2) | 0.118   |
| Glucose (mg/dL)  | 78.3 (5.6)       | 82.3 (13.7)      | 0.190   |
| Vascular function parameters, mean (SD) |
| Dbase, mm        | 3.0 (0.6)        | 2.7 (0.4)        | 0.157   |
| FMD, %           | 7.2 (3.3)        | 7.3 (5.6)        | 0.487   |
| Dpeak, mm        | 3.2 (0.5)        | 3.0 (0.5)        | 0.140   |
| DFiff             | 0.2 (0.5)        | 0.3 (0.4)        | 0.496   |
| FMDn, %          | 6.5 (2.9)        | 7.4 (5.7)        | 0.433   |
| PWV, m·s\(^{-1}\) | 6.7 (0.8)        | 7.1 (1.2)        | 0.204   |
| Aix (aortic), %  | 41.7 (10.4)      | 24.5 (32.7)      | 0.148   |
| Aix (brachial), % | 16.5 (5.2)       | 25.1 (16.5)      | 0.152   |
| Pulse Pressure (mmHg) | 45.3 (5.2) | 44.6 (4.1) | 0.931   |
| Central systolic blood pressure (mmHg) | 99.6 (43.7) | 108.0 (5.8) | 0.493   |
| Pulmonary artery occlusion pressure (mmHg) | 40.6 (6.8) | 35.6 (3.9) | 0.087   |
| Systolic blood pressure (mmHg) | 116.2 (6.5) | 116.8 (5.1) | 0.184   |
| Diastolic blood pressure (mmHg) | 710.8 (7.2) | 723.7 (7.0) | 0.278   |
| Mean blood pressure (mmHg) | 860.0 (7.6) | 873.7 (6.0) | 0.482   |

HIT 4 × 4-min high-intensity interval training, MCT moderate-intensity continuous training, D diameter, FMD flow-mediated vasodilation, nFMD normalized flow-mediated vasodilation, PWV pulse wave velocity, Aix augmentation index
in exercise intensity has been linked to greater protection of vascular function against oxidative stress, supporting the possibility that HIT might trigger larger vascular effects at the cellular and molecular levels [15]. Likewise, exercise might exert a positive effect on endothelial function by stimulating the production and bioavailability of NO, as physical activity induces the activity of endothelial NO synthase (eNOS), increases the capacity of the cellular antioxidant system and diminishes the formation of reactive oxygen species (ROS) [36]. In addition, it has been demonstrated that a single session of exercise increased circulating and intramuscular free radical levels [37], which may lead to inactivation of NO with consequences for endothelium-mediated vasodilation [38]. It seems that acute exercise mediates the oxidant-antioxidant balance in favor of antioxidants, resulting in the maintenance of vascular function, and a similar effect is observed from the co-ingestion of antioxidants [39]. Thus, it is tempting to speculate that the effects of different intensities of exercise on postprandial FMD are related to changes in antioxidant status.

We also found that medium-term HIT decreased the glucose response over the postprandial observation period by 10% compared with MCT (P = 0.008), indicating that the 7magnitude of postprandial glucose response was dependent on exercise intensity. This result contrasts with previous reports that failed to find differences

### Table 2 Intent-to-treat analysis of the effect of 12 weeks of HIT or MCT on postprandial lipemia biochemical and vascular function response after HFM ingestion

<table>
<thead>
<tr>
<th></th>
<th>HIT</th>
<th>MCT</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biochemical parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>151.3 (21.0)</td>
<td>153.1 (29.0)</td>
<td>0.008</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>76.4 (11.0)</td>
<td>85.9 (6.3)</td>
<td>0.0008</td>
</tr>
<tr>
<td>High-density lipoprotein (mg/dL)</td>
<td>46.0 (14.1)</td>
<td>42.1 (9.5)</td>
<td>0.0008</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>108.1 (35.8)</td>
<td>117.7 (33.1)</td>
<td>0.0008</td>
</tr>
<tr>
<td><strong>Vascular function parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dba (mm)</td>
<td>2.7 (0.4)</td>
<td>3.1 (0.5)</td>
<td>0.008</td>
</tr>
<tr>
<td>FMD (aortic), %</td>
<td>6.3 (7.3)</td>
<td>3.2 (0.4)</td>
<td>0.008</td>
</tr>
<tr>
<td>PWV, m·s⁻¹</td>
<td>6.6 (1.5)</td>
<td>6.7 (0.9)</td>
<td>0.008</td>
</tr>
<tr>
<td>AIx (aortic), %</td>
<td>26.3 (14.6)</td>
<td>38.7 (69.0)</td>
<td>0.008</td>
</tr>
<tr>
<td>Alx (brachial), %</td>
<td>22.3 (28.9)</td>
<td>19.1 (9.2)</td>
<td>0.008</td>
</tr>
<tr>
<td>PPao (mmHg)</td>
<td>45.5 (7.7)</td>
<td>47.4 (7.4)</td>
<td>0.008</td>
</tr>
<tr>
<td>SBPao (mmHg)</td>
<td>107.6 (14.2)</td>
<td>106.3 (9.6)</td>
<td>0.008</td>
</tr>
<tr>
<td>PPa (mmHg)</td>
<td>40.6 (6.6)</td>
<td>30.7 (12.9)</td>
<td>0.008</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>112.5 (9.1)</td>
<td>113.0 (7.6)</td>
<td>0.008</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>67.0 (10.3)</td>
<td>67.0 (8.0)</td>
<td>0.008</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>82.0 (9.2)</td>
<td>82.8 (7.8)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Values are participant characteristics at baseline, mean (SD). HIT4×4-min high-intensity interval training, MCT moderate-intensity continuous training, D diameter, FMD flow-mediated vasodilation, FMDn normalized flow-mediated vasodilation, PWV P-wave velocity, Alx augmentation index, PP pulse pressure, SBPao central systolic blood pressure. PPao, pulmonary artery occlusion pressure. Whole group repeated measures ANOVA, a = 0 min vs 60 min; b = 0 min 120 min.
between the two training protocols regarding postprandial glucose levels [10, 15, 16]. However, it should be noted that these studies only examined the effect of postprandial glucose level after acute exercise. Thus, it is possible that only medium or long-term training has a significant effect on postprandial glucose response.

We found similar total cholesterol, HDL-c and triglyceride responses after HFM consumption in both training regimens, suggesting that medium-term exercise training might not play an important role in the postprandial decrement in lipid responses. In previous studies focusing on acute exercise, significant differences between HIT and MCT were found for triglycerides, but not for total cholesterol or HDL levels [10, 15, 16]. In this context, results from preliminary studies have suggested that the positive effect of exercise training on PPL might be short lived, demonstrating variations in the effect sizes for exercise training performed within 24 h prior to HFM ingestion and for exercise training performed more than 24 h pre-prandial [8, 40]. Thus, we hypothesize that postprandial triglyceride response might be short lived, showing a relevant effect only after acute exercise.

**Study limitations**

This study has some limitations. Due to the high sensitivity of endothelium to nutritional changes, it would be ideal to administer isocaloric meals to participants at least three days before the measurement of endothelial function. In this study, although diet was not controlled, a dietician provided an individualized iso-energetic nutrition intervention plan. Second, since endothelial function is well known to be affected by age and training status and our study cohort comprised healthy, inactive mature adults, our findings may not be generalizable to other populations with different characteristics. A final possible limitation is that we did not examine other factors such as antioxidant status, NO, IL-6 and TNF-α levels that might ameliorate postprandial response, and this should be studied in future research.
The main strength of our study is that, to our knowledge, this is the first randomized clinical trial on the effect of exercise-training intensity on biochemical parameters and endothelial functional responses to HFM consumption in inactive adults from the Latin-American population. In addition, we provide measurements of these PPL responses at multiple time points to better describe their time course after chronic exercise.

**Conclusion**

In summary, the novel finding of this study was that medium-term supervised physical training may mitigate endothelial dysfunction and glucose response induced by PPL. Exercise intensity seems to play an important role in these protective effects, suggesting that HIT might be the more effective in reducing postprandial glucose levels and attenuating vascular impairments. Therefore, medium-term HIT is an effective strategy to reduce CVD.

**Additional file**

**Additional file 1: Figure S1.** CONSORT guidelines flow diagram for enrolment and randomization. (TIFF 1515 kb)

**Acknowledgements**

We would like to thank and acknowledge the enthusiastic group of test participants who made this study possible.

**Funding**

This study as funded in part by the Center for Studies on Measurement of Physical Activity, School of Medicine and Health Sciences, Universidad del Rosario (Code N° FIUR DN-BG001). We declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or appropriate data manipulation.

**Availability of data and materials**

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

**Authors’ contributions**

RR-V conceived and designed the project. RR-V, and JEC-B, reviewed the literature studies and conducted data extraction. RR-V, JEC-B and VA-A...
conducted data analyses. RR-V, MC-R, and MI were responsible for data interpretation. AT-S, MC-R, and RR-V drafted the manuscript, and MI revised it critically for intellectual contributions. MI and RR-V coordinated the study development. All authors reviewed and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Details of the study design and methods of the primary HIT-Heart Study trial have been described elsewhere (ClinicalTrials.gov ID: NCT02738385; April 14th, 2016). 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, Colombia (ID 27–0500-2015). All participants provided written informed consent before participating in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details
1Centre de Estudios en Medición de la Actividad Física (CEMA), Escuela de Medicina y Ciencias de la Salud, Universidad del Rosario, Bogotá, D.C., Colombia. 2Faculty of Health Sciences, University of Granada, Granada, Spain. 3Department of Health Sciences, Public University of Navarre, CIBER de Fragilidad y Envejecimiento Saludable (CIBERfes), Tudela, Navarre, Spain. 4Department of Physical Activity Sciences, Universidad de Los Lagos, Osorno, Chile. 5Research Nucleus in Health, Physical Activity and Sports, Universidad de Los Lagos, Osorno, Chile. 6Laboratorio de Ciencias de la Actividad Física, el Deporte y la Salud, Facultad de Ciencias Médicas, Universidad de Santiago de Chile, USACH, Santiago, Chile.

Received: 1 February 2018 Accepted: 25 March 2018
Published online: 03 April 2018

References


Introduction: We aimed to investigate whether 12 weeks of high-intensity interval training (HIIT), resistance training (RT), concurrent training (CT=HIIT+RT) or nutritional guidance (NG) induced improvements in metabolic syndrome (MetS) risk factors, vascular function parameters and ideal cardiovascular health (CVH) in sedentary and overweight adults, and to compare the training adaptations between intervention groups. Methods and results: A randomized controlled clinical trial was conducted in subjects aged 30–50 years. Parameters linked to CVH such as anthropometric and body composition, cardiovascular, metabolic and performance indices were assessed. After the intervention, a significant reduction in total body fat and trunk fat mass was identified in the HIIT and RT groups (P<0.001). A significant improvement was observed for flow-mediated vasodilation (FMD) after the three exercise programs, but only RT was found to improve aortic pulse wave velocity (PWV) and aortic augmentation index (AIX) (P<0.001). RT induced an increase in aortic AIX when compared with CT (P=0.021). Moreover, HIIT, RT and CT improved maximal oxygen uptake (VO2max) and muscular strength, but HIIT elicited the largest improvements for VO2max (effect size=0.579) and RT for muscular strength (effect size=0.625). There was an increase of 33.4% and 41.6% in CVH metrics in HIIT and RT groups, respectively. Conclusion: A 12-week HIIT training program resulted in greater cardiorespiratory fitness than nutritional guidance alone, whereas an RT program improved arterial stiffness over CT. Both HIIT and RT programs increase ideal CVH metrics, supporting the positive effect of both exercise training programs on CVH in sedentary and overweight adults.
Submission Files Included in this PDF

File Name [File Type]
coverletter.pdf [Cover Letter]
Highlights.docx [Highlights]
Titlepage_IJC.docx [Title Page (with Author Details)]
VersionIJC.docx [Manuscript File]
Consort_F1.tif [Figure]
Body Composition_F2.tif [Figure]
Metabolic Parameters_F3.tif [Figure]
Vascular parameters_F4.tif [Figure]
Fitness_F5.tif [Figure]
AHA all_F6.tif [Figure]
All metrics by Groups_F7.tif [Figure]
Table 1.docx [Table]
Author_Agreement_Form_IJC.pdf [Author Agreement]
Table S1.docx [Supplementary Material]
Table S2.docx [Supplementary Material]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
The data that has been used is confidential
Highlights

Regular physical activity and nutritional guidance are among the therapeutic actions used to reduce metabolic syndrome in adults.

The effect of different exercise intervention programs on sedentary and overweight males has been scarcely investigated.

A 12-week HIIT training program resulted in greater cardiorespiratory fitness than nutritional guidance alone, whereas an RT program improved arterial stiffness over CT.
Effects of Exercise Training Type and Intensity or Nutritional Guidance on Metabolic Syndrome Risk Factors, Ideal Cardiovascular Health Parameters, Endothelial Function and Arterial Stiffness in Overweight Adults: Cardiometabolic HIIT-RT Study, A Randomized Controlled Trial

Robinson Ramírez-Vélez\(^1\) (E-mail: robinson.ramirez@urosario.edu.co)
Alejandra Hernandez\(^1\) (E-mail: hernandezalejandra40@gmail.com)
Karem Castro\(^1\) (E-mail: gerencia@karemcastro.com)
Alejandra Tordecilla-Sanders\(^1\) (E-mail: alesanders_0615@hotmail.com)
Katherine González-Ruíz\(^1,2\) (E-mail: katherine.gonzalez@docentes.umb.edu.co)
Jorge Enrique Correa-Bautista\(^1\) (E-mail: jorge.correa@urosario.edu.co)
Mikel Izquierdo\(^1,3\) (E-mail: mikel.izquierdo@gmail.com)
Antonio García-Hermoso\(^4\) (E-mail: antonio.garcia.h@usach.cl)
Cristian Álvarez\(^5\) (E-mail: cristian.alvarez@ulagos.cl)
Rodrigo Ramírez-Campillo\(^6\) (E-mail: r.ramirez@ulagos.cl)
Maria Correa-Rodríguez\(^7\) (E-mail: macoro@ugr.es)

\(^1\) Centro de Estudios en Medición de la Actividad Física (CEMA), Escuela de Medicina y Ciencias de la Salud, Universidad del Rosario, Bogotá, D.C, Colombia. "This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation".

\(^2\) Grupo de Ejercicio Físico y Deportes, Facultad de Salud, Programa de Fisioterapia, Universidad Manuela Beltrán, Bogotá, D.C, Colombia. "This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation".

\(^3\) Department of Health Sciences, Public University of Navarra, Navarrabiomed, CIBER of Frailty and Healthy Aging (CIBERFES), Instituto de Salud Carlos III, Pamplona, Navarra, Spain. "This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation".

\(^4\) Laboratorio de Ciencias de la Actividad Física, el Deporte y la Salud, Universidad de Santiago de Chile, USACH, Santiago, Chile. "This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation".

\(^5\) Department of Physical Activity Sciences, Universidad de Los Lagos, Osorno, Chile.

\(^6\) Research Nucleus in Health, Physical Activity and Sports, Universidad de Los Lagos, Osorno, Chile. "This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation".

\(^7\) Departament of Nursing. Faculty of Health Sciences, University of Granada, Avda. De la Ilustración, 60, Granada, Spain. "This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation".

*Corresponding Author:

Robinson Ramírez-Vélez
Centro de Estudios en Medición de la Actividad Física (CEMA)
Escuela de Medicina y Ciencias de la Salud
Acknowledgments: The study was supported by Universidad del Rosario Grant Number FIUR DN-BG001.

Conflict of interest: All authors state that they have no conflicts of interest.

Keywords: High-intensity interval training, Resistance training, Concurrent training, Cardiovascular health, Vascular function, Metabolic syndrome.
Abstract

Introduction: We aimed to investigate whether 12 weeks of high-intensity interval training (HIIT), resistance training (RT), concurrent training (CT=HIIT+RT) or nutritional guidance (NG) induced improvements in metabolic syndrome (MetS) risk factors, vascular function parameters and ideal cardiovascular health (CVH) in sedentary and overweight adults, and to compare the training adaptations between intervention groups.

Methods and results: A randomized controlled clinical trial was conducted in subjects aged 30–50 years. Parameters linked to CVH such as anthropometric and body composition, cardiovascular, metabolic and performance indices were assessed. After the intervention, a significant reduction in total body fat and trunk fat mass was identified in the HIIT and RT groups (P<0.001). A significant improvement was observed for flow-mediated vasodilation (FMD) after the three exercise programs, but only RT was found to improve aortic pulse wave velocity (PWV) and aortic augmentation index (AIx) (P<0.001). RT induced an increase in aortic AIx when compared with CT (P=0.021). Moreover, HIIT, RT and CT improved maximal oxygen uptake (VO\textsubscript{2 max}) and muscular strength, but HIIT elicited the largest improvements for VO\textsubscript{2 max} (effect size=0.579) and RT for muscular strength (effect size=0.625). There was an increase of 33.4% and 41.6% in CVH metrics in HIIT and RT groups, respectively.

Conclusion: A 12-week HIIT training program resulted in greater cardiorespiratory fitness than nutritional guidance alone, whereas an RT program improved arterial stiffness over CT. Both HIIT and RT programs increase ideal CVH metrics, supporting the positive effect of both exercise training programs on CVH in sedentary and overweight adults. ClinicalTrials.gov NCT02715063 (Date: March 8, 2016)
1. Introduction

Obesity is a major public health problem worldwide [1]. The prevalence of overweight/obesity is rapidly increasing and, in the Latin-American population, it is estimated that more than 19% of adults are obese [2]. Excess body weight is an independent risk predictor for cardiometabolic disease, including heart disease, diabetes, hypertension, chronic kidney disease or stroke [1]. In addition, excessive accumulation of body fat has been related to the metabolic syndrome (MetS), a clustering of central obesity, hyperglycemia or hyperinsulinemia, dyslipidemia, and elevated blood pressure [3], thus increasing cardiovascular risk [4]. A recent estimation of the prevalence of MetS among U.S. adults is 34.3% [5].

Regular physical activity and nutritional guidance are among the therapeutic actions used to reduce MetS in adults [6]. According to the World Health Organization (WHO) and the American College of Sports Medicine, the recommended exercise prescription for improving and maintaining health is at least 150 min of moderate-intensity physical activity (40–60% maximum oxygen uptake, VO$_2$max) or 75 min of vigorous-intensity physical activity (60–85% VO$_2$max) per week for healthy adults [7]. Despite the importance of engaging in regular exercise, most adults fail to meet these recommendations, reporting lack of time as a major barrier for being physically active [8]. Accordingly, identifying more time-efficient modes of exercise training is a major area of interest.

High-intensity interval training (HIIT), characterized by brief, intermittent bursts of vigorous activity interspersed by periods of low-intensity exercise, has become a popular and more time-efficient alternative to traditional exercise strategies [9]. The impact of HIIT on cardiometabolic risk factors such as body composition, metabolic parameters, vascular
function and physical fitness has been investigated extensively in overweight-obese populations [10–16]. Compared with moderate-intensity continuous exercise, HIIT might result in a superior or equal improvement in body composition, cardiovascular health (CVH) and cardiopulmonary fitness [17–21]. Resistance training (RT), a type of strength training, has also been shown to be effective in improving several cardiometabolic risk factors including insulin resistance/hyperglycemia, dyslipidemia, hypertension and obesity [22–24].

Previous studies investigating the effects of concurrent aerobic and RT on cardiometabolic health have produced inconclusive findings [14, 25–28]. For example, Ho et al. showed that 12 weeks of combination exercise training yielded greater benefits for body composition and cardiopulmonary fitness than moderate-intensity aerobic and RT modalities in overweight/obese adults [14]. By contrast, Willis et al. reported that a program of combined aerobic training and RT in middle-aged overweight/obese adults failed to significantly reduce fat or body mass over aerobic training alone, suggesting that aerobic training is the optimal mode of exercise for reducing these parameters. They also found that a program including RT was needed to increase lean mass in this group [25]. Interestingly, Kemmler et al. reported that a high-intensity aerobic and resistance exercise program affected MetS risk factors and significantly lowered the severity of MetS in thirty-two elderly females (69.5 ± 4.3 years), supporting that high intensity is more strongly inversely related to the MetS than low-intensity exercise [29]. However, the potential greater benefits of specific concurrent HIIT and RT across cardiometabolic markers over these modalities alone has not been widely investigated and thus the optimization of HIIT adaptations with RT remains an important goal for clinical research [30].

Nutritional guidance has been recently postulated as an effective intervention for promoting cardiometabolic health [31]. Indeed, nutritional guidance with adequate nutrition
has been shown to improve insulin resistance, reduce oxidative stress and lipid profile and prevent excess weight, hypertension, type 2 diabetes, and low grade chronic inflammation [32]. Nevertheless, the potential effects of nutritional guidance on cardiometabolic health when directly compared with different training modalities remain to be determined.

To the best of our knowledge, the effect of different exercise intervention programs on sedentary and overweight males has been scarcely investigated. This is understandable, because such investigations require a multidisciplinary team and long-term study to detect the changes occurring in this population. Against this background, the aim of the present study was to investigate whether 12 weeks of HIIT, RT, CT or a nutritional guidance plan induced improvements in MetS risk factors, vascular function and ideal CVH in sedentary and overweight males, and to compare the training adaptations between intervention groups.

2. Methods

2.1 Study design

The Cardiometabolic HIIT-RT study is a single blind, randomized controlled 2 × 2 factorial trial (ClinicalTrials.gov ID: NCT02715063) conducted from March 2016 to June 2017 in Bogotá, Colombia. The study was approved by the Research Ethics Committee of The University of Manuela Beltran (ID 06-1006-2014) and complied with the revised ethical guidelines of the Declaration of Helsinki (revision of 2013). Randomization was performed by a third party using variable permuted block sizes with computer-generated random numbers. Details of sample calculation, randomization, characteristics of participants, design, methods and measurements of the Cardiometabolic HIIT-RT study have been published elsewhere [33]; however, the most relevant information is briefly described below.

2.2 Participants
The study included a total of 51 sedentary subjects (no participation in exercise more than once a week for the previous six months), aged 30–50 years, with abdominal obesity (waist circumference [WC] ≥90 cm [men] ≥80 cm [women]) or excess weight, body mass index ≥ 25 and ≤ 30 kg/m², identified as being willing and with almost immediate availability was enrolled. Subjects were recruited from a private health care institution (Clinica Rangel Pereira, IPS) and the Rosario University in Bogotá. Before being enrolled in the study, all participants were informed of the purpose and risk of the study and signed an informed consent form.

2.3 Exercise training intervention

1. Nutritional guidance (NG): Without exercise training. Participants received counseling about goals for CVH, as well as monitoring CVH over time in the Colombia population, key signs and symptoms, diet and screening for cardiometabolic risk factors. All participants received NG four times during the program: twice in individual sessions (baseline and after 12 weeks) and twice in groups (fourth and eighth weeks). Diets were monitored by means of three-day dietary recall, in accordance with the standards of the American Dietetic Association. The prescribed NG was based on an exchange list, by reducing 250 kilocalories (kcal) from the calorie total in the diet, in order to promote a 250 g reduction per week in body mass (0.5 to 1.0 kg per month). Total energy intake in kilocalories and the amounts of each nutrient (carbohydrates, fat, and protein in grams) were assessed at baseline (0, 4 and 12 week) using a 24-h weighed dietary record method. The assessment was carried out by trained registered clinical dietitians (A.H), and the scoring was controlled by one researcher (R.R.V).

2. High-intensity interval training (HIIT) group: All HIIT sessions were preceded with a 5-min warm-up and ended with a 4-min cool-down at 65% heart rate maximum (HRmax) until
the subject expended between 400 and 500 kcal. The HIIT protocol consisted of four bouts of 4-min intervals at 85–95% HRmax interspersed with 4 min of active recovery at 75–85% HRmax. Participants were instructed to reach their target HR for each interval within the first 2 min of the 4-min interval. We calculated the training energy expenditure with the consensus public health recommendations from WHO [34] and the US Department of Health and Human Services [35]. HR monitors (A3, Polar Elector OY, Finland) were used to adjust workload to achieve the target HR. In addition, a rating of perceived exertion was also measured during each exercise session (15–17 during high intensity and 11–13 during recovery).

3. Resistance training (RT) group: The RT session was initiated with ~12–15 repetitions per set of six exercises that targeted all the major muscle groups at high intensity. A 60-s recovery was permitted as many times as needed according to the subject’s weight until the subject expended between 400 and 500 kcal at 50 to 70% of one-repetition maximum (1RM).

4. Concurrent training (CT = HIIT+RT) group: The CT group did the 50% aerobic training program plus the 50% RT program during each session. The energy expenditure associated with the physical training prescribed for the CT group was therefore ~400 to 500 kcal/session [33].

2.4 Training intensity and energy expenditure during the exercise session

In terms of exercise intensity, the actual intensity values were reported as the mean of HR measured in the HIIT and CT groups and as the average value of workload and repetitions determined in the acute session in the RT group. The intensity of the HIIT or CT group was based on the percentage of each individual’s HRmax derived from a maximal treadmill test. Research staff monitored and recorded compliance with target HR and energy expenditure during the sessions.
2.5 Blood draws and analysis

Participants arrived at the Rosario University CEMA-Laboratory between 6:00 and 9:00 following a 10- to 12-h overnight fast. Participants were reminded to maintain standardized conditions (i.e., a hydrated state and abstaining from caffeine and alcohol consumption for 36 h). The following blood parameters were measured: (i) high-density lipoprotein cholesterol (HDL-C), (ii) triglycerides, (iii) low-density lipoprotein cholesterol (LDL-C), (iv) total cholesterol, (v) fasting glucose, and (vi) the metabolic regulators glucose and hemoglobin A1C (HbA1c) (by enzymatic colourimetric methods). All determinations we measured by using Cardiocheck® (Polymer Technology Systems, PTS, Indianapolis, IN, USA) and A1CNow+® (Bayer Diabetes care, Sunnyvale, CA, USA)

2.6 Blood pressure and heart rate

Systolic and diastolic blood pressure were recorded using an automatic monitor (Omron HEM® 7114TM, Omron Healthcare Co. Ltd.) in duplicate after 15 minutes of rest, with the subjects in a seated position and with both feet resting on the floor [36]. The mean arterial pressure (MAP) was calculated using the following formula: MAP = (systolic blood pressure + (2 x diastolic blood pressure)) / 3. Resting HR was measured using a chest monitor (V-800®, Polar Electro Inc., Kempele, Finland) after subjects had rested in the supine position for at least 15 min.

2.7 Anthropometry and body composition

Body mass (Tanita® BC-418, Tokyo, Japan) and height (Seca® 274, Hamburg, Germany) were measured in duplicate using standard protocols. BMI was calculated with the following formula: BMI = body weight (kg)/height squared (m²). WC was measured to the nearest 1 mm with a flexible steel tape measure (Lufkin W606PM®, Parsippany, NJ, USA) placed midway between the lowest rib and the iliac crest while participants were in a standing
position at the end of an exhalation, in accordance with the International Society for the Advancement of Kinanthropometry guidelines [37]. The technical error of measurement values was less than 2% for all anthropometric variables. Whole body fat and lean mass, trunk fat mass index, muscle index and appendicular muscle mass were measured by dual-energy X-ray absorptiometry (QDR-1500, Hologic Corp., Software version 7.10, Waltham, MA).

2.8 Cardiorespiratory fitness and muscular strength

At 48 h after the start of the training period, the VO$_2$max of inactive subjects was determined 24 h before the acute intervention using a maximum treadmill exercise test (Precor TRM 885, Italy). Exercise capacity was evaluated according to treadmill exercise test duration, which was used to estimate aerobic consumption expressed in metabolic equivalents (METs), based on well-characterized regression equations recommended by the American College of Sports Medicine. In addition, previous studies demonstrated that treadmill test time correlates well ($r=0.92$) with VO$_2$max [38, 39]. Blood pressure was recorded at rest, at each stage change, at peak exercise, and during recovery using a standardized cuff sphygmomanometer.

Regarding muscular strength, 1RM was measured for six different exercises: bicep screw curl, triceps extension, dumbbell side lateral raise, military press, dumbbell squat and dumbbell front lunge, which were implemented based on similar procedures [33]. The 1RM was performed in six resistance exercises and was conducted between 09:00 and 11:00 a.m.; the highest load of three attempts per exercise was reported. The 50–70% value of the 1RM was used to determine the workload during the sessions for the RT and CT groups.

2.9 Endothelial function measures
Endothelial function was measured by flow mediated-dilation (FMD), aortic pulse wave velocity (PWV) and the augmentation index (AIx). FMD was measured as described previously by our group [40] using the guidelines reported by Corretti et al. [41]. The intra-session coefficient of variations was ≤1% for the baseline diameter. The technical error of measurement was 1.23% for baseline diameter, 1.77% for maximum diameter and 20% for %FMD. Images were recorded on a DVD player for subsequent measurements by an observer blinded to the study design. FMD was expressed as % change=[(maximum – baseline diameter) / baseline diameter] × 100. Normalized brachial artery FMD (FMDn) was calculated according to the allometric relationship between base diameter (Dbase) and peak diameter (Dpeak) [42]. PWV was measured by analyzing the oscillometric pressure curves registered from the upper arm with an arteriographic computer program (Arteriograph Software v.1.9.9.2; TensioMed, Budapest, Hungary). The algorithm measuring blood pressure in the arteriography device has been previously validated [43]. PWV was calculated as the jugulum-to-symphysis distance (m) divided by the return time (return time/2) (s). The arteriograph calculates the AIx on the basis of the formula (AIx% pulse 2 - pulse 1/central pulse pressure) ×100 and thus provides the brachial/aortic AIx without applying a transfer function. The R value as an estimate of the measurement errors for the repeat measurements between two sessions (n=6) was low for the arteriograph (1.18 m·s⁻¹).

2.10 Ideal CVH behaviors and CVH risk factors

The metrics for ideal CVH in children and adolescents defined by the American Heart Association (AHA) were followed as precisely as possible (Supplemental Table S1).

Data on smoking were collected via self-reported questionnaires (number of cigarettes smoked per day). Ideal smoking status was determined as non-smoker or quit smoking ≥12 months. Although the AHA relies on physical activity to determine active habits, we used
estimated cardiorespiratory fitness (CRF), due to its robust association with cardiovascular risk factors and ideal CVH in this population [44]. Exercise capacity was dichotomized to high (Ideal CVH) versus low (non-ideal CVH) based on estimated CRF (cut-off point of $\dot{V}O_2$max > 35 mL/kg/min in women or >40 mL/kg/min in men).

BMI was classified using WHO criteria (normal: 18.5 to 24.9 kg/m²; overweight: 25.0 to 29.9 kg/m²; and obese: ≥30 kg/m²) [45]. A seven-day recall was the dietary assessment tool used to assessed the Mediterranean diet (MetDiet) quality. As suggested by Thanapoulou et al., the total score was divided into two categories of Mediterranean diet quality: (1) ≤8 points = poor diet quality; and ≥9 points = good diet quality (optimal Mediterranean diet style) [46]. Participants who had at least ≥9 points were categorized as having an ideal healthy diet, whereas adults with 8 points were classified as having a non-ideal healthy diet. Glucose fasting, total cholesterol and blood pressure were included as CVH risk factors.

2.11 AHA criteria

The AHA guidelines [47] were used to construct an ideal CVH index based on 7 metrics and using the cut-off points for adults, with participants receiving one point for the presence of each ideal metric. The ideal behaviors defined by the AHA were as follows: BMI <25 kg/m², CRF ($\dot{V}O_2$max >35 mL/kg/min in women or >40 mL/kg/min in men), non-smoking status (either never having smoked), and consumption of a dietary pattern that promotes ideal CVH. The factors were classified as an untreated systolic blood pressure <120 mmHg and diastolic blood pressure <80 mmHg, untreated total cholesterol ≤200 mg/dL, and untreated fasting blood glucose <100 mg/dL or HbA1c <5.7%.

Finally, the participants were categorized into 1 of 3 health levels based on the number of CVH metrics in the ideal range that they exhibited; the healthiest level (favorable ideal CVH score) was defined as having between 5 and 7 metrics in the ideal range; the intermediate level,
3 to 4 metrics; and the unfavorable level, 0 to 2 metrics. These cut-off points have been used in previous international studies [48, 49].

2. Cardiometabolic parameters

We calculated a composite cardiometabolic z-score that reflects a continuous score of the five metabolic abnormalities. The cardiometabolic z-score was calculated from subjects’ data, based on the International Diabetes Federation [50], and standard deviations using data from the entire subject cohort at baseline. The equation used was: MetScore = ([HDL-C: ♂≤40 or ♀≤50 mg/dL]/SD*[-1]) + ([TG: 150 mg/dL]/SD) + ([fasting glucose: 100 mg/dL]/SD) + ([WC: ♂≥94 or ♀≥80 cm]/SD) + ([MAP: 100 mmHg]/SD). The mean of this continuously distributed cardiometabolic z-score was therefore zero by definition.

2.13 Statistical analyses

Baseline demographics were summarized as means and standard deviations, and between group differences were examined using ANOVA for continuous data. Categorical data were summarized as frequencies and percentages, and group differences at baseline were examined using lineal \(\chi^2\) tests. The mean change in each group was reported as the estimated margin of the mean, as assessed by 95% confidence intervals (CI) with adjustment for kcal for diet, sex and baseline values as covariates using an unstructured covariance matrix for the repeated measures. Within-group differences were considered significant when the 95% CI did not include zero. In the per-protocol mixed model analyses, we used 95% CI and \(p\) values (<0.05) for the intergroup comparisons, for each outcome measure across group \(\times\) time interaction factors. Cohen's \(d\) for effect size was also calculated to determine the magnitude of the group differences. The effect size was classified as small, medium, and medium-to-large effects (<0.20, 0.2–0.6 and 0.6–1.2, respectively), and partial \(\eta^2\) was considered small if \(\eta^2<0.04\), and large if \(\eta^2>0.36\) in interaction effect analysis [51].
To examine the cumulative effects of the 7 CVH metrics, we created a dichotomized variable for each component of the health metrics: “ideal” was coded as 1, and “poor” was coded as 0. The total ideal CVH metrics score of each individual ranged from 0 to 7. Changes in CVH metrics were calculated by subtracting the total score for the metrics obtained in pre-from the total score obtained in post-intervention. Participants were divided into three categories based on the changes in the 7 CVH metrics. Categorical CVH metrics were described as percentages and were compared using $\chi^2$ tests. The significance level adopted to reject the null hypothesis was $P < 0.05$. All analyses were performed using the SPSS software package (Version 24, IBM, New York, USA).

3. Results

3.1 Characteristics of the subjects

Figure 1 shows the CONSORT flow diagram of study progression. A total of 80 participants were eligible after assessment. Reasons for eligible subjects declining to participate included ‘lack of time’ (n=5), and ‘personal reasons’ (n=3). Of the remaining 72 participants, 18 were randomized into each of the following intervention groups: i) NG; ii) HIIT; iii) RT; and iv) CT (HIIT and RT protocol).

*** Figure 1 ***

Baseline parameters are shown in Table 1. In total, 9 patients had abnormalities in total cholesterol levels, 86.2% had low HDL-C levels, 43.1% had high triglyceride levels, and 39.7% had HbA1c $>5.6\%$. In total, 72.4% of participants were not currently smoking, 5.2% had normal weight, 46.6% had a healthy VO$_{2}$max, and 32.8% had a healthy diet by MetDiet score. Most study participants reached ideal health status for the following cardiovascular factor metrics: total cholesterol (84.5%), fasting glucose (87.9%), and blood
pressure (89.7%). Other details of vascular function, exercise and diet parameters are shown in Table 1.

### Table 1

#### 3.2 Training compliance

Training compliance (% of total sessions completed; mean) for each training group was the following: HIIT, 95%; RT, 96%; CT, 88%. There were no differences in training compliance between intervention groups (P = 0.671).

#### 3.3 Changes in body composition parameters

The results of the intention to treatment (ITT) analysis in body composition are shown in Supplemental Table S2 and Figure 2. We found a decrease in total body fat (%) from PRE to POST intervention both in HIIT (-3.082, CI95% = -4.20 to -1.95; ES= 0.663; P < 0.001) and RT (-3.273, CI95% = -5.35 to -1.18; ES = 0.392; P < 0.001), and for trunk fat mass (g) both in HIIT (-2.004, CI95% = -3.27 to -0.72; ES = 0.392; P < 0.001) and RT (-2.007, CI95% = -3.74 to -0.26; ES = 0.258; P < 0.001). In the per-protocol analyses, none of the interventions significantly changed body fat (%) (F(interaction)=1.628; P = 0.198) or trunk fat mass (F(interaction)=1.217; P = 0.313).

*** Figure 2 ***

#### 3.4 Changes in metabolic parameters

With regards to metabolic parameters (Figure 3), we found a decrease in cholesterol (-16.833, CI95% = -29.51 to -4.15; ES = 0.289; P < 0.001) and LDL-c (-25.727, CI95% = -44.52 to -6.92; ES=0.329; P < 0.001) levels from PRE to POST in HIIT and in triglyceride levels in the NG group (-45.250, CI95% = -72.91 to -17.58; ES = 0.294; P < 0.001). There were statistically significant decreases for cardiometabolic z-score in the four groups after the intervention (time effect F (49.12); ES = range 0.365–0.468; all P values < 0.001);
however, the training response (mean changes) difference between the four groups was not statistically significant (interaction effect F (0.261); P = 0.853).

*** Figure 3 ***

3.5 Changes in vascular function parameters

The exercise effort test results for the four intervention groups are shown in Figure 4. After 12 weeks of supervised training, all three exercise programs significantly increased FMD (%), in the following increasing order: HIIT group (5.442, CI95% = 3.234 to 7.649; ES = 0.584; P < 0.001); RT group (6.427, CI95% = 2.340 to 10.510; ES = 0.393; P < 0.001); and CT group (7.450, CI95% = 4.032 to 10.860; ES = 0.590; P < 0.001), (time effect F (47.57); P < 0.001). In the RT group, there was a medium effect on PWV (m·s⁻¹) (-0.382, CI95% = -0.620 to -0.138; ES = 0.391; P < 0.001) and brachial AIx (%) (15.209, CI95% = 3.260 to 27.150; ES = 0.298; P < 0.001, interaction effect F (3.505); P = 0.021), indicating positive adaptations in the RT group compared with the CT group. There were no significant treatment effects on other vascular parameters.

*** Figure 4 ***

3.6 Changes in exercise parameters

Figure 5 shows the results for HRrest (bpm), VO₂max, (mL/kg/min) and handgrip strength (kg) for the four groups. With regards to the HRrest after the intervention, the only significant result was observed in the RT group (PRE 62.3 (11.2) vs. POST 57.0 (8.3); mean difference -5.364, CI95% = -10.21 to -0.51; ES = 0.243; P < 0.0001). When adjusted for kcal diet, sex and baseline values, three exercise modalities improved VO₂max: HIIT +8.375; ES =0.579, RT +4.145; ES =0.579, and CT +6.370; ES =0.579; all P < 0.001. The improvement difference between the groups was statistically significant between the HIIT and NG groups (P = 0.028). There were also significant differences in handgrip strength between PRE and
POST training measures: HIIT +3.158; ES =0.209, RT +6.336; ES =0.625, and CT +3.160; ES =0.254; all P < 0.001. The improvement difference between the groups was not statistically significant, interaction effect F (0.344) P = 0.794.

*** Figure 5 ***

3.7 Change in CVH score (behaviors and factors) metrics according to AHA criteria

We compared the baseline characteristics of the 7 categories cardiovascular behaviours and factors metrics in Figure 6. Only the NG group had an increase in the percentage of the pre-value in the healthy diet metric, whereas HIIT and CT groups had an improvement in VO$_2$max (P < 0.05). The estimated changes of 3 health categories based on the number of CVH metrics are shown as a percentage of the pre-value in Figure 7. The HIIT and RT groups had a 33.4% (P = 0.032) and 41.6% (P = 0.020) increase in >5 metrics, respectively.

*** Figure 6 ***

*** Figure 7 ***

4. Discussion

Our aim was to compare the effects of 12 weeks of HIIT, RT, CT (HIIT+RT) or NG on MetS risk factors, ideal CVH parameters and vascular function in a cohort of sedentary and overweight adults. The main findings of this study are that 12 weeks of HIIT leads to significant improvements in VO$_2$max as compared with NG, and also that RT stimulates a greater increase in arterial stiffness than CT. Moreover, both HIIT and RT modalities increase ideal CVH metrics, supporting the positive effect of both training programs for CVH in sedentary and overweight males.

Exercise training is a well established means of enhancing vascular health [52]. Consistent with previous studies [15, 53, 54], our data reveal that all three exercise regimens
improves FMD. Furthermore, RT was found to improve PWV and aortic AIx, supporting the concept that different types of exercise training might produce diverse adaptations of arterial stiffness in obese and sedentary adults [55]. Indeed, we observed that aortic AIx was higher in the RT than in the CT group. This is of particular interest since high AIx, a measure of pulse wave reflections influencing the central blood pressure, predicts mortality and cardiovascular events [56]. Although RT programs have shown beneficial effect on arterial stiffness [57, 53, 58], a recent study found that a 8-week period of RT did not change arterial stiffness in individuals with MetS or healthy controls [59]. Considering that the aforementioned study used a shorter training period, it could be hypothesized that only longer RT interventions have a substantial effect on arterial stiffness. It has been suggested that the role of different exercise training interventions on vascular function might be mediated by the synthesis of molecular mediators, changes in neurohormonal release and/or oxidant/antioxidant balance [52]. Nevertheless, the specific role of RT in arterial stiffness in overweight and sedentary adults is unclear and warrants further investigation.

With regards to body composition parameters, although no significant difference in training response was found between HIIT, RT, CT and NG, we found decrease in total body fat and trunk fat mass after the HIIT and RT interventions. The HIIT program yielded the largest improvements in total body fat (ES=0.663) and trunk fat mass (ES=0.392), indicating that HIIT is an optimal mode of exercise for fat loss in sedentary and overweight adults. Also, HIIT has been reported to be the more time-efficient approach to achieve the beneficial effects of exercise on body composition [13].

All four regimens led to reductions in WC (NG=-1.69 cm, HIIT=-4.39 cm, RT=-3.95 cm, and CT=-2.86 cm). It has been suggested that WC reduction is an important component that influences adult MetS, as it reflects abdominal fat excess and is closely related to
cardiovascular diseases. The potential mechanisms underlying the HIIT-induced fat loss effect are unknown but might include increased exercise and post-exercise fatty acid oxidation and suppressed appetite [60]. Therefore, the data of the present study strongly suggest that the response of overweight adults to a program of exercise training consists of a change in visceral obesity and a decrease in the risk factors for MetS.

No changes in metabolic parameters were observed between the four intervention groups; however, we found significant decreases in total cholesterol and LDL-c from baseline to post-exercise in the HIIT group. Previous studies examining HIIT protocols for blood lipids have also demonstrated positive changes [10–13, 60]. In agreement with previous research [10, 61], we failed to find any favorable change in HDL-c levels in overweight and sedentary adults. By contrast, Tjonna et al. reported that HDL-c increased in middle-aged adults in response to 16 weeks of aerobic interval training [62]. It is relevant to consider, however, that this study was conducted in a cohort of adults with MetS and very low baseline HDL-c values. The lack of consistent results might also be explained by differences in HIIT programs, and it is possible that only long-term training has a substantial effect on HDL-c response.

Regarding cardiometabolic health, significant decreases in the cardiometabolic z-score were identified in the four groups after the intervention, although the training response difference between the groups was not statistically significant. Considering that HIIT yielded the largest improvement in cardiometabolic z-score (ES=0.468), it seems that HIIT could be a more effective intervention for improving cardiometabolic risk in overweight and sedentary adults. Existing research has indeed shown that high intensity exercise is more strongly inversely related with MetS when compared with low-intensity exercise [29, 63].
Despite the similarity in volume and duration of the HIIT and RT interventions with the CT program, we saw a greater VO\textsubscript{2}max, (ES=0.579) improvement with HIIT than with RT (ES=0.263), CT (ES=0.326) or NG (ES=0.001). Nevertheless, the improvement difference between the groups was statistically significant only between HIIT and NG groups (P = 0.028). This is consistent with previous studies [11, 18, 21, 22]. Moreover, our results overall are in line with other studies [11,12,19,25,28] in that all groups responded positively to exercise and presented an increase in VO\textsubscript{2}max or METs and a decrease in HR\textsubscript{rest}. Improvements in CRF parameters were also demonstrated through a decrease in HR\textsubscript{rest} and the use of more intense workloads. Better heart and muscle function likely played a role in this improved performance [63].

We found an inverse relationship between aerobic fitness and fat content, and both were significantly related to the lipid profile [62,70]. This reinforces the importance of supervised exercise training as a non-pharmacologic strategy for reversing the adverse effects of lack of exercise among overweight adults, thereby preserving fat-free mass in sedentary and overweight males.

Ideal CVH metrics are inversely associated with cardiovascular events, supporting the use of these metrics as a useful tool to predict cardiovascular disease risk [64]. To the best of our knowledge, this study is the first to investigate the effect of different exercise training modalities and NG on CVH metrics in a population of middle-age overweight adults. We found an increase in healthy diet metrics after 12-weeks of NG and an improvement in VO\textsubscript{2}max after HIIT and CT. Indeed, there was an increase of 33. 4% and 41.6% in CVH metrics in HIIT and RT groups, respectively. In line with our findings, recent systematic reviews and meta-analyses concluded that HIIT can improve some cardiometabolic risk factors in overweight/obese populations [13, 19]. Similarly, available data indicate that an
RT program is also an effective exercise modality for reducing the risk of cardiovascular disease [65]. Both exercise protocols and NG were effective in promoting important changes to a number of health-related parameters. Accordingly, a 12-week training program comprising HIIT or RT should be recommended for overweight and sedentary adults in order to improve their CVH.

Limitations

This study had some limitations. First, since endothelial function is well known to be affected by age and training status, and our study cohort comprised overweight and sedentary middle-aged adults, this could imply that our findings may not be generalizable to other populations with different characteristics. Another limitation is the lack of dietary control during the course of the intervention. However, we continually reminded subjects of their commitment to maintain their current dietary habits in order to minimize the influence of diet.

The main strength of our study is that it is the first RCT, to our knowledge, on the effect of 12 weeks of HIIT, RT, CT or NG on MetS risk factors, ideal CVH and vascular function in adults from the Latin-American population. Secondly, there was high exercise compliance and we used state-of-the-art measures of cardiovascular fitness and metabolic/endothelial function. Moreover, body composition parameters were assessed by dual-energy X-ray absorptiometry, considered the current “gold standard” for body composition measurement.

6. Conclusions

A 12-week training program of HIIT resulted in greater cardiorespiratory fitness than NG, whereas an RT program improved arterial stiffness over CT, supporting the notion that different exercise training regimens might produce different adaptations of arterial stiffness.
in obese and sedentary adults. Both HIIT and RT programs increase ideal CVH metrics, supporting the positive effect of both training programs on CVH in sedentary and overweight males.

References


Wilkins Health, Philadelphia


42. Atkinson G (2014) Shear rate normalization is not essential for removing the dependency of flow-mediated dilation on baseline artery diameter: past research revisited. Physiol Meas 35:1825–1835.


asymptomatic humans. Exp Physiol 96:57–70.


Metrics and Associations With All-Cause and CVD Mortality Among US Adults. JAMA 307:1273.

**Figure caption**

**Figure 1.** Consolidated Standards of Reporting Trials (CONSORT) flow diagram.

**Figure 2.** Training response in body composition parameters between intervention groups. NG, nutritional guidance; HIIT, high-intensity interval training; RT, resistance training; Concurrent training (HIIT+RT) group; Data shown are mean changes and individual participant responses. ES, effect size.

**Figure 3.** Training response in metabolic parameters between intervention groups. NG, nutritional guidance; HIIT, high-intensity interval training; RT, resistance training; Concurrent training (HIIT+RT) group; Data shown are mean changes and individual participant responses. ES, effect size.

**Figure 4.** Training response in vascular function parameters between intervention groups. NG, nutritional guidance; HIIT, high-intensity interval training; RT, resistance training; Concurrent training (HIIT+RT) group; Data shown are mean changes and individual participant responses. ES, effect size.

**Figure 5.** Training response in exercise parameters between intervention groups. NG, nutritional guidance; HIIT, high-intensity interval training; RT, resistance training; Concurrent training (HIIT+RT) group; Data shown are mean changes and individual participant responses. ES, effect size.

**Figure 6.** Changes after the 12-wk follow-up in ideal cardiovascular health metrics criteria according to AHA by intervention groups. NG, nutritional guidance; HIIT, high-intensity interval training; RT, resistance training; Concurrent training (HIIT+RT) group; Data shown are unadjusted prevalence (%).

**Figure 7.** Changes after the 12-wk follow-up in categorical ideal cardiovascular health metrics criteria according to AHA by intervention groups. NG, nutritional guidance; HIIT, high-intensity interval training; RT, resistance training; Concurrent training (HIIT+RT) group; Data shown are unadjusted prevalence (%).
Table 1. Characteristics of the subjects (n=58)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex, men n (%)</strong></td>
<td>23 (39.7)</td>
</tr>
<tr>
<td><strong>Morphological parameters, mean (SD)</strong></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>40.78 (7.06)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>79.55 (12.30)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>162.51 (7.94)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>30.04 (3.49)</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>92.68 (9.49)</td>
</tr>
<tr>
<td>Fat mass index, kg/m²</td>
<td>11.61 (2.90)</td>
</tr>
<tr>
<td>Trunk fat mass, g</td>
<td>16.42 (4.36)</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>46.01 (8.28)</td>
</tr>
<tr>
<td>Fat-free mass, kg/m²</td>
<td>18.02 (1.84)</td>
</tr>
<tr>
<td>Appendicular skeletal muscle mass, kg/m²</td>
<td>7.82 (1.07)</td>
</tr>
<tr>
<td><strong>Cardiometabolic risk factors parameters, mean (SD)</strong></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>167.29 (34.51)</td>
</tr>
<tr>
<td>High total cholesterol, n (%)</td>
<td>9 (14.5)</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>37.15 (9.53)</td>
</tr>
<tr>
<td>Low HDL-C, n (%)</td>
<td>50 (86.2)</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>99.42 (31.30)</td>
</tr>
<tr>
<td>High LDL-C, n (%)</td>
<td>25 (43.9)</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>172.37 (108.65)</td>
</tr>
<tr>
<td>High triglycerides, n (%)</td>
<td>25 (43.1)</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>89.68 (7.92)</td>
</tr>
<tr>
<td>High glucose, n (%)</td>
<td>7 (12.1)</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.45 (0.47)</td>
</tr>
<tr>
<td>High HbA1c, n (%)</td>
<td>23 (39.7)</td>
</tr>
<tr>
<td>Cardiometabolic z-score</td>
<td>0.767 (2.98)</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>116.12 (10.30)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>73.01 (9.87)</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>87.37 (9.66)</td>
</tr>
<tr>
<td><strong>Vascular function parameters, mean (SD)</strong></td>
<td></td>
</tr>
<tr>
<td>D&lt;sub&gt;base&lt;/sub&gt;, mm</td>
<td>32.0 (4.98)</td>
</tr>
<tr>
<td>FMD, %</td>
<td>9.81 (6.62)</td>
</tr>
<tr>
<td>D&lt;sub&gt;peak&lt;/sub&gt;, mm</td>
<td>35.08 (5.36)</td>
</tr>
<tr>
<td>D&lt;sub&gt;diff&lt;/sub&gt;</td>
<td>5.58 (5.64)</td>
</tr>
<tr>
<td>FMDn, %</td>
<td>2.68 (1.77)</td>
</tr>
<tr>
<td>PWV, m·s&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>7.33 (1.06)</td>
</tr>
<tr>
<td>AIx (aortic), %</td>
<td>24.52 (18.57)</td>
</tr>
<tr>
<td>AIx (brachial), %</td>
<td>-19.89 (27.03)</td>
</tr>
<tr>
<td><strong>AHA Ideal CVH criteria, n (%)</strong></td>
<td></td>
</tr>
</tbody>
</table>

Behavior metrics
<table>
<thead>
<tr>
<th>Not currently smoking</th>
<th>42 (72.4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI &lt;25 kg/m²</td>
<td>3 (5.2)</td>
</tr>
<tr>
<td>VO(<em>2)(</em>{\text{max}}), (men &gt;40, women &gt;35 mL/kg/min)</td>
<td>27 (46.6)</td>
</tr>
<tr>
<td>Healthy diet by MetDiet &gt;8 points</td>
<td>19 (32.8)</td>
</tr>
<tr>
<td><strong>Factors metrics</strong></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol &lt;200 mg/dL</td>
<td>49 (84.5)</td>
</tr>
<tr>
<td>Glucose &lt;100 mg/dL or HbA1c &lt; 5.7%</td>
<td>51 (87.9)</td>
</tr>
<tr>
<td>Blood pressure &lt;100 mmHg MAP</td>
<td>52 (89.7)</td>
</tr>
<tr>
<td><strong>Exercise parameters</strong></td>
<td></td>
</tr>
<tr>
<td>Rest HR, bpm</td>
<td>59.53 (9.34)</td>
</tr>
<tr>
<td>Maximal HR, bpm</td>
<td>170.70 (15.65)</td>
</tr>
<tr>
<td>Mets</td>
<td>10.82 (2.40)</td>
</tr>
<tr>
<td>VO(<em>2)(</em>{\text{max}}), mL/kg/min</td>
<td>37.88 (8.40)</td>
</tr>
<tr>
<td><strong>Diet parameters</strong></td>
<td></td>
</tr>
<tr>
<td>Baseline daily caloric intake, mean (SD)</td>
<td>1666 (460.0)</td>
</tr>
<tr>
<td>Protein, %</td>
<td>18 (4.6)</td>
</tr>
<tr>
<td>Fat, %</td>
<td>36.7 (6.0)</td>
</tr>
<tr>
<td>Saturated fat, %</td>
<td>10.1 (2.9)</td>
</tr>
<tr>
<td>Carbohydrate, %</td>
<td>45.3 (7.8)</td>
</tr>
</tbody>
</table>
Author Agreement Form – International Journal of Cardiology

Manuscript Title: Effects of Exercise Training Type and Intensity or Nutritional Guidance on Metabolic Syndrome Risk Factors, Ideal Cardiovascular Health Parameters, Endothelial Function and Arterial Stiffness in Overweight Adults: Cardiometabolic HIIT-RT Study, A Randomized Controlled Trial

List of all Authors: Robinson Ramírez-Vélez, Alejandra Hernandez, Karem Castro, Alejandra Tordecilla-Sanders, Katherine González-Ruíz, Jorge Enrique Correa-Bautista, Mikel Izquierdo, Antonio García-Hermoso, Cristian Álvarez, Ramírez-Campillo, María Correa-Rodriguez.

Corresponding Author: Robinson Ramírez-Vélez

This statement is to certify that all authors have seen and approved the manuscript being submitted, have contributed significantly to the work, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission to the International Journal of Cardiology.

We attest that the article is the Authors' original work, has not received prior publication and is not under consideration for publication elsewhere. We adhere to the statement of ethical publishing as appears in the International of Cardiology (citable as: Shewan LG, Rosano GMC, Henein MY, Coats AJS. A statement on ethical standards in publishing scientific articles in the International Journal of Cardiology family of journals. Int. J. Cardiol. 170 (2014) 253-254 DOI:10.1016/j.ijcard.2013.11).

On behalf of all Co-Authors, the corresponding Author shall bear full responsibility for the submission. Any changes to the list of authors, including changes in order, additions or removals will require the submission of a new author agreement form approved and signed by all the original and added submitting authors.

All authors are requested to disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work. If there are no conflicts of interest, the COI should read: “The authors report no relationships that could be construed as a conflict of interest”.

Centro de Estudios en Medición de la Actividad Física (CEMA)
Escuela de Medicina y Ciencias de la Salud
Universidad del Rosario
Bogotá, D.C, Colombia
Address: Cra. 24 No. 63C - 69
Phone: +57 (1) 2970200 ext. 3428
E-mail: robin640@hotmail.com // robinson.ramirez@urosario.edu.co
## Supplement Table 1. Definition of the Ideal Cardiovascular Health Metrics (>20 Years of Age) as Defined by the American Heart Association and the Criteria Used in this Study.

<table>
<thead>
<tr>
<th><strong>Health behaviors</strong></th>
<th><strong>Ideal Metric, AHA Definition</strong></th>
<th><strong>Ideal Metric, Definition in this Study</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Smoking</strong></td>
<td>Never tried; never smoked whole cigarette</td>
<td>Never smoked a cigarette</td>
</tr>
<tr>
<td><strong>Body mass index</strong></td>
<td>&lt; 25kg/m²</td>
<td>&lt; 25kg/m²</td>
</tr>
<tr>
<td><strong>Physical activity</strong></td>
<td>≥60 min of moderate- or vigorous-intensity activity every day</td>
<td>Exercise capacity was dichotomized to high vs. low based on estimated aerobic consumption (cut-off point of VO₂max &gt; 35 mL/kg/min in women or &gt; 40 mL/kg/min in men)</td>
</tr>
<tr>
<td><strong>Diet</strong></td>
<td>4–5 components:</td>
<td>Mediterranean diet quality</td>
</tr>
<tr>
<td></td>
<td>Fruit and vegetables: ≥4.5 cups/d</td>
<td>Participants who had at least ≥8 points were categorized as having an ideal healthy diet</td>
</tr>
<tr>
<td></td>
<td>Fish: 2 or more 3.5-oz servings/wk</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fiber-rich whole grains: 3 or more 1-oz-equivalent servings/d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium: &lt;1500 mg/d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sugar-sweetened beverages: ≤450 kcal (36 oz)/wk</td>
<td></td>
</tr>
<tr>
<td><strong>Health factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total cholesterol</strong></td>
<td>&lt;170 mg/dL (&lt;4.40 mmol/L)</td>
<td>&lt;170 mg/dL</td>
</tr>
<tr>
<td><strong>Blood pressure</strong></td>
<td>&lt;120/&lt;80 mmHg</td>
<td>&lt;120/&lt;80 mmHg</td>
</tr>
<tr>
<td><strong>Plasma glucose</strong></td>
<td>&lt;100 mg/dL (&lt;5.6 mmol/L)</td>
<td>&lt;100 mg/dL or HbA1c &lt; 5.7%</td>
</tr>
</tbody>
</table>
Supplement Table 2. Characteristics of the subjects before and after the 12-wk follow-up

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control (n=12)</th>
<th>HIIT (n=14)</th>
<th>RT (n=12)</th>
<th>CT (n=13)</th>
<th>Time (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
<td>Baseline</td>
<td>Follow-up</td>
<td>Baseline</td>
</tr>
<tr>
<td>Morphological parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>82.4 (18.6)</td>
<td>81.3 (18.6)</td>
<td>76.3 (11.4)</td>
<td>71.4 (12.2)</td>
<td>85.6 (10.9)</td>
</tr>
<tr>
<td>Mean difference</td>
<td>-1.13</td>
<td>-4.98</td>
<td>-4.98</td>
<td>-1.60</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>-2.79 to 0.54</td>
<td>-8.11 to -1.85</td>
<td>-7.44 to -1.53</td>
<td>-3.69 to 0.49</td>
<td></td>
</tr>
<tr>
<td>ƞ²</td>
<td>0.066</td>
<td>0.369</td>
<td>0.532</td>
<td>0.151</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166.9 (7.0)</td>
<td>167.4 (7.0)</td>
<td>159.4 (7.3)</td>
<td>159.3 (7.4)</td>
<td>164.1 (7.5)</td>
</tr>
<tr>
<td>Mean difference</td>
<td>0.481</td>
<td>-0.108</td>
<td>0.009</td>
<td>0.490</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>-0.16 to 1.12</td>
<td>-0.70 to 0.48</td>
<td>-0.23 to 0.25</td>
<td>-0.04 to 1.02</td>
<td></td>
</tr>
<tr>
<td>ƞ²</td>
<td>0.080</td>
<td>0.008</td>
<td>0.001</td>
<td>0.202</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.3 (3.8)</td>
<td>28.7 (4.5)</td>
<td>29.8 (2.5)</td>
<td>27.9 (2.8)</td>
<td>31.7 (3.3)</td>
</tr>
<tr>
<td>Mean difference</td>
<td>-0.62</td>
<td>-1.98</td>
<td>-1.90</td>
<td>-0.83</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>-1.25 to 0.01</td>
<td>-3.17 to -0.79</td>
<td>-3.23 to -0.56</td>
<td>-1.7 to 0.029</td>
<td></td>
</tr>
<tr>
<td>ƞ²</td>
<td>0.130</td>
<td>0.389</td>
<td>0.348</td>
<td>0.220</td>
<td></td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>95.1 (12.3)</td>
<td>93.4 (13.4)</td>
<td>90.433 (8.6)</td>
<td>86.0 (9.1)</td>
<td>96.2 (6.9)</td>
</tr>
<tr>
<td>Mean difference</td>
<td>-1.69</td>
<td>-4.39</td>
<td>-3.95</td>
<td>-2.86</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>-3.03 to -0.357</td>
<td>-6.85 to -1.92</td>
<td>-6.51 to -1.39</td>
<td>-4.94 to -0.790</td>
<td></td>
</tr>
<tr>
<td>ƞ²</td>
<td>0.200</td>
<td>0.422</td>
<td>0.384</td>
<td>0.366</td>
<td></td>
</tr>
<tr>
<td>Fat mass index, kg/m²</td>
<td>10.2 (2.1)</td>
<td>9.3 (2.0)</td>
<td>11.1 (2.1)</td>
<td>9.8 (2.3)</td>
<td>11.9 (3.1)</td>
</tr>
<tr>
<td>Mean difference</td>
<td>-0.528</td>
<td>-1.219</td>
<td>-1.422</td>
<td>-0.569</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>-1.01 to -0.037</td>
<td>-2.00 to -0.43</td>
<td>-2.47 to -0.36</td>
<td>-1.28 to 0.14</td>
<td></td>
</tr>
<tr>
<td>ƞ²</td>
<td>0.158</td>
<td>0.386</td>
<td>0.322</td>
<td>0.161</td>
<td></td>
</tr>
<tr>
<td>Fat mass, %</td>
<td>36.6 (4.1)</td>
<td>35.2 (5.1)</td>
<td>39.6 (5.0)</td>
<td>36.6 (5.8)</td>
<td>39.4 (7.4)</td>
</tr>
<tr>
<td></td>
<td>Mean difference</td>
<td>95% CI</td>
<td>( \eta^2 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-----------------</td>
<td>----------------------</td>
<td>-------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Trunk fat mass, g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean difference</td>
<td>-0.973</td>
<td>-2.02 to 0.07</td>
<td>0.122</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>-3.082</td>
<td>-4.20 to -1.95</td>
<td>0.663</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \eta^2 )</td>
<td>-3.273</td>
<td>-5.35 to -1.18</td>
<td>0.392</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-1.080</td>
<td>-2.45 to 0.29</td>
<td>0.157</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>19.917 ((-0.001))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lean body mass, kg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean difference</td>
<td>-0.518</td>
<td>-1.20 to 0.17</td>
<td>0.091</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>-2.004</td>
<td>-3.27 to -0.72</td>
<td>0.392</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \eta^2 )</td>
<td>-2.007</td>
<td>-3.74 to -0.26</td>
<td>0.258</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.804</td>
<td>-1.90 to 0.30</td>
<td>0.138</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fat-free mass, kg/m^2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean difference</td>
<td>-0.94</td>
<td>-0.76 to 1.08</td>
<td>0.040</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>0.159</td>
<td>0.041</td>
<td>0.008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \eta^2 )</td>
<td>1.213</td>
<td>0.043</td>
<td>0.043</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Appendicular skeletal muscle mass, kg/m^2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean difference</td>
<td>0.129</td>
<td>0.117</td>
<td>0.016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>0.093</td>
<td>0.014</td>
<td>0.017</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \eta^2 )</td>
<td>0.091</td>
<td>0.021</td>
<td>0.014</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Metabolic parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total cholesterol, mg/dL</strong></td>
<td>169.0 (29.4)</td>
<td>162.3 (23.0)</td>
<td>164.3 (43.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean difference</td>
<td>-6.625</td>
<td>-16.833</td>
<td>0.129</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>-17.73 to 4.48</td>
<td>-29.51 to -4.15</td>
<td>-0.053</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \eta^2 )</td>
<td>-8.818</td>
<td>-8.74 to 26.37</td>
<td>-0.063</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-4.500</td>
<td>-18.29 to 9.29</td>
<td>4.675</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HDL-C, mg/dL</strong></td>
<td>37.1 (11.4)</td>
<td>37.0 (10.7)</td>
<td>34.6 (6.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean difference</td>
<td>-0.063</td>
<td>1.083</td>
<td>0.0289</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>-2.22 to 2.09</td>
<td>-2.04 to 4.21</td>
<td>0.062</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \eta^2 )</td>
<td>-0.176</td>
<td>-0.49 to 9.77</td>
<td>0.031</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.132</td>
<td>-1.23 to 2.22</td>
<td>3.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>97.1</td>
<td>102.8</td>
<td>104.7</td>
<td>79.0</td>
<td>106.2</td>
</tr>
<tr>
<td></td>
<td>(27.4)</td>
<td>(27.9)</td>
<td>(38.4)</td>
<td>(23.2)</td>
<td>(33.6)</td>
</tr>
<tr>
<td>Mean difference</td>
<td>3.969</td>
<td>-25.727</td>
<td>0.273</td>
<td>1.153</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>-6.93 to 14.86</td>
<td>-44.52 to -6.92</td>
<td>-17.01 to 17.56</td>
<td>-14.40 to 16.70</td>
<td></td>
</tr>
<tr>
<td>$\eta^2$</td>
<td>0.021</td>
<td>0.329</td>
<td>0.000</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>173.5</td>
<td>128.3</td>
<td>185.4</td>
<td>175.5</td>
<td>129.1</td>
</tr>
<tr>
<td></td>
<td>(83.7)</td>
<td>(40.8)</td>
<td>(128.8)</td>
<td>(104.1)</td>
<td>(66.7)</td>
</tr>
<tr>
<td>Mean difference</td>
<td>-45.250</td>
<td>-9.917</td>
<td>12.18</td>
<td>-34.200</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>-72.91 to -17.58</td>
<td>-44.03 to 24.19</td>
<td>-11.69 to 36.05</td>
<td>-100.05 to 31.65</td>
<td></td>
</tr>
<tr>
<td>$\eta^2$</td>
<td>0.294</td>
<td>0.019</td>
<td>0.064</td>
<td>0.076</td>
<td></td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>88.2</td>
<td>86.3</td>
<td>93.5</td>
<td>91.3</td>
<td>88.7</td>
</tr>
<tr>
<td></td>
<td>(5.4)</td>
<td>(7.3)</td>
<td>(9.9)</td>
<td>(5.7)</td>
<td>(5.6)</td>
</tr>
<tr>
<td>Mean difference</td>
<td>-1.875</td>
<td>-2.167</td>
<td>1.364</td>
<td>-0.500</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>-5.32 to 1.57</td>
<td>-7.43 to 7.43</td>
<td>-3.64 to 6.37</td>
<td>-6.15 to 5.15</td>
<td></td>
</tr>
<tr>
<td>$\eta^2$</td>
<td>0.044</td>
<td>0.038</td>
<td>0.019</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.6</td>
<td>5.4</td>
<td>5.3</td>
<td>5.3</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>(0.3)</td>
<td>(0.5)</td>
<td>(0.6)</td>
<td>(0.5)</td>
<td>(0.5)</td>
</tr>
<tr>
<td>Mean difference</td>
<td>-0.169</td>
<td>-0.075</td>
<td>0.019</td>
<td>-0.090</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>-0.41 to 0.07</td>
<td>-0.21 to 0.066</td>
<td>-0.63 to -0.00</td>
<td>-0.20 to 0.02</td>
<td></td>
</tr>
<tr>
<td>$\eta^2$</td>
<td>0.068</td>
<td>0.061</td>
<td>0.213</td>
<td>0.150</td>
<td></td>
</tr>
<tr>
<td>Cardiometabolic z-score</td>
<td>0.58 (0.32)</td>
<td>-1.20 (0.32)</td>
<td>1.63 (0.39)</td>
<td>-0.64 (0.39)</td>
<td>1.17 (0.56)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean difference</td>
<td>-1.796</td>
<td>-2.271</td>
<td>-2.729</td>
<td>-2.504</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>-2.732 to -0.860</td>
<td>-3.435 to -1.107</td>
<td>-4.426 to -1.033</td>
<td>-4.239 to -0.770</td>
<td></td>
</tr>
<tr>
<td>$\eta^2$</td>
<td>0.365</td>
<td>0.468</td>
<td>0.404</td>
<td>0.387</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>119.5</td>
<td>118.1</td>
<td>114.9</td>
<td>109.5</td>
<td>118.1</td>
</tr>
<tr>
<td></td>
<td>(12.5)</td>
<td>(12.2)</td>
<td>(10.6)</td>
<td>(10.7)</td>
<td>(8.0)</td>
</tr>
<tr>
<td>Mean difference</td>
<td>-1.313</td>
<td>-5.417</td>
<td>-4.000</td>
<td>-0.500</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>-6.178 to 3.55</td>
<td>-10.2 to -0.63</td>
<td>-8.54 to 0.54</td>
<td>-5.03 to 4.03</td>
<td></td>
</tr>
<tr>
<td>$\eta^2$</td>
<td>0.011</td>
<td>0.228</td>
<td>0.169</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>74.6</td>
<td>73.6</td>
<td>71.9</td>
<td>67.9</td>
<td>75.5</td>
</tr>
<tr>
<td></td>
<td>(12.5)</td>
<td>(11.4)</td>
<td>(8.3)</td>
<td>(6.0)</td>
<td>(7.6)</td>
</tr>
<tr>
<td>Mean difference</td>
<td>-1.000</td>
<td>-4.000</td>
<td>-7.273</td>
<td>-3.000</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>-5.35 to 3.35</td>
<td>-9.14 to 1.14</td>
<td>-10.46 to -4.08</td>
<td>-7.00 to 1.00</td>
<td></td>
</tr>
<tr>
<td>Parameter</td>
<td>Mean arterial pressure, mmHg</td>
<td>Mean difference</td>
<td>Mean difference</td>
<td>95% CI</td>
<td>$\eta^2$</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>------------------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>--------------</td>
<td>----------</td>
</tr>
<tr>
<td></td>
<td>89.5 (12.3)  88.5 (11.2)  86.1 (7.2)  89.8 (7.6)  83.5 (8.4)  86.3 (9.6)  84.1 (8.7)</td>
<td>-1.063  -4.500  -6.273  -2.200</td>
<td>-5.11 to 2.99  -9.26 to 0.26  -9.33 to -3.20  -6.09 to 1.69</td>
<td></td>
<td>0.008  0.122  0.577  0.145</td>
</tr>
<tr>
<td>Vascular function parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FMD, %</td>
<td>10.6 (0.74)  12.4 (0.74)  9.85 (0.74)  15.3 (0.74)  7.75 (1.37)  7.96 (1.13)</td>
<td>1.881  5.442  6.427</td>
<td>-0.276 to 4.03  3.234 to 7.649  2.340 to 10.51  4.032 to 10.86</td>
<td>-0.276 to 4.03  3.234 to 7.649  2.340 to 10.51  4.032 to 10.86</td>
<td>0.011  0.171  0.523  0.088</td>
</tr>
<tr>
<td>PWV, m·s⁻¹</td>
<td>7.2 (0.9)  7.5 (0.9)  7.5 (1.1)  7.3 (1.0)  7.0 (0.6)  6.6 (0.3)  7.0 (0.7)  6.7 (0.9)</td>
<td>0.269  0.561  0.393</td>
<td>-0.02 to 1.09  0.785 to 1.945  0.621 to 2.705  1.03 to 2.89</td>
<td>-0.02 to 1.09  0.785 to 1.945  0.621 to 2.705  1.03 to 2.89</td>
<td>0.162  0.305  0.229  0.126</td>
</tr>
<tr>
<td>AIx (aortic), %</td>
<td>24.8 (13.4)  23.8 (14.4)  27.0 (12.0)  20.2 (13.8)  9.2 (27.5)  24.4 (8.0)  25.6 (11.4)  24.5 (12.6)</td>
<td>-1.056  2.250  15.209</td>
<td>-0.00 to 0.54  0.785 to 1.945  0.621 to 2.705  1.03 to 2.89</td>
<td>-0.00 to 0.54  0.785 to 1.945  0.621 to 2.705  1.03 to 2.89</td>
<td>0.008  0.033  0.298  0.008</td>
</tr>
<tr>
<td>AIx (brachial), %</td>
<td>-25.2 (26.6)  -27.3 (28.5)  -20.9 (23.8)  -16.4 (27.3)  -24.3 (25.8)  -25.9 (15.8)  -23.6 (22.6)  -25.8 (24.9)</td>
<td>-2.081  4.433  -1.618</td>
<td>-5.73 to 3.62  -3.61 to 8.11  3.26 to 27.15  -5.50 to 3.32</td>
<td>-5.73 to 3.62  -3.61 to 8.11  3.26 to 27.15  -5.50 to 3.32</td>
<td>0.008  0.033  0.004  0.008</td>
</tr>
<tr>
<td>Exercise parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest HR, bpm</td>
<td>57.2 (9.7)  59.2 (6.9)  60.7 (9.3)  57.0 (7.9)  62.3 (11.2)  57.0 (8.3)  59.6 (8.2)  59.4 (7.9)</td>
<td>2.023 (0.158)</td>
<td>-11.30 to 7.14  -7.13 to 16.0  -15.58 to 12.34  -10.89 to 6.55</td>
<td>-11.30 to 7.14  -7.13 to 16.0  -15.58 to 12.34  -10.89 to 6.55</td>
<td>0.008  0.033  0.004  0.008</td>
</tr>
<tr>
<td></td>
<td>Mean difference</td>
<td>95% CI</td>
<td>$\eta^2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>----------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO2max, mL/kg/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.000</td>
<td>-3.750</td>
<td>-5.364</td>
<td>-0.200</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-1.59 to 5.59</td>
<td>-9.48 to 1.98</td>
<td>-10.21 to -0.51</td>
<td>-1.93 to 1.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.046</td>
<td>0.090</td>
<td>0.243</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40.3 (8.6)</td>
<td>40.6 (10.1)</td>
<td>37.4 (8.0)</td>
<td>45.7 (8.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40.0 (9.8)</td>
<td>44.1 (9.9)</td>
<td>33.8 (8.6)</td>
<td>40.2 (10.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17.023 (0.001)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Handgrip, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.279</td>
<td>8.375</td>
<td>4.145</td>
<td>6.370</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-3.96 to 3.40</td>
<td>4.94 to 11.80</td>
<td>0.59 to 7.69</td>
<td>1.32 to 11.41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>0.579</td>
<td>0.263</td>
<td>0.326</td>
<td></td>
</tr>
<tr>
<td></td>
<td>31.8 (6.9)</td>
<td>31.1 (9.3)</td>
<td>23.0 (7.2)</td>
<td>26.1 (7.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27.7 (7.8)</td>
<td>21.6 (5.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.023 (0.158)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Data are mean (standard deviation), and results are expressed as estimated margins of the mean (EMM) and 95% confidence intervals (CI), $\eta^2$ = eta partial