# Effects of high-intensity interval training on endothelial function, lipid profile, body composition and physical fitness in normal-weight and overweight-obese adolescents: A clinical trial

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ABSTRACT

Endothelium-aggressive factors are associated with the development of atherosclerosis. Exercise training can either prevent or attenuate this process, but little is known about the effects of high-intensity interval training (HIIT) in adolescents. Thus, we assessed the effects of HIIT on endothelial function, lipid profile, body composition and physical fitness in normal-weight and overweight-obese adolescents. Thirty-eight participants aged 14–17 years who were physically inactive (IPAq) were divided in two groups: normal weight (NW, $n = 13$) and overweight-obese (OW, $n = 25$). Body composition, lipid profile, physical fitness and endothelial function (flow-mediated dilation, FMD) were assessed before and after undergoing the study protocol consisting of 12-week HIIT ($\sim 15$ min) + sport activities (30 min, $3 \times$/week) + no diet. The differences were tested by GEE, Bonferroni post-hoc, $p < 0.05$. There were no changes in body composition after training period, but the OW group showed a reduction in waist (4.8 cm; $p = 0.044$) and abdominal circumference (3.7 cm; $p = 0.049$). We found improved physical fitness (cardiorespiratory endurance, explosive strength, abdominal muscle endurance and flexibility) in both groups. Lower endothelial function was found in the OW compared to NW ($p = 0.042$) at baseline. FMD increased ($p < 0.001$) in both groups from baseline (NW Δ4.1%; Cohen's effect size 0.64; OW Δ4.5%; Cohen's effect size 0.73) with no significant difference between the groups. In conclusion, a HIIT program even without any dietary changes can improve physical fitness and endothelial function among adolescents. These findings are clinically relevant because they support a reduction in endothelial damage that precedes the development of atherosclerosis.

## 1. Introduction

Cardiovascular events account for nearly 30% of deaths in Brazil [1]. Primary prevention of major risk factors for cardiovascular diseases including physical inactivity could mitigate this scenario [2]. There is evidence showing an increase of 0.68 years in life expectancy when physically inactive individuals engage in regular physical activities [3]. Furthermore, obesity is strongly associated with physical inactivity [4–6] and it potentiates the harmful impact of other health conditions including dyslipidemia, systemic arterial hypertension and diabetes mellitus among others.

The endothelium plays a central role in the regulation of blood flow and blood pressure through vascular tone modulation determined by a balance between vasodilator and vasoconstrictor agents [7]. Persistent endothelial damage may lead to endothelial dysfunction and, when there are high levels of low-density cholesterol (LDL), it may precede the atherosclerotic process [8].

Prevention strategies such as physical exercise during childhood and adolescence could reduce cardiovascular risk factors in adult life [9]. In Brazil, however, only 38.6% of boys and 20.1% of girls show an adequate level of physical activity [10]. Regular physical exercise bring direct benefits on the endothelium either by preventing or even attenuating endothelial dysfunction [11,12]. It is also associated with lower obesity rates [13] and healthier lipid profile [14].

Several strategies have been devised to make physical exercise more attractive; however, few training protocols have been effective to increase adherence among adolescents [15]. Adolescents do not frequently follow the recommended guidelines of regularly engaging in

moderate-intensity training for 30–60 min a day [15–17]. Hence, offering a training program with challenging attainable goals could be an effective strategy to improve adherence and high-intensity interval training (HIIT) has showed good adherence among adolescents [5,15].

HIIT has well-established beneficial effects on endothelial function [18] and LDL levels [19]. However, most evidence was gathered from studies with adults [20]. Thus, the primary objective of our study was to compare the effects of HIIT on endothelial function among normal-weight (NW) and overweight-obese (OW) adolescents. Our secondary objective was to assess and compare body composition, lipid profile and physical fitness in NW and OW participants in response to a HIIT program.

## 2. Methods

We conducted an evaluator-blinded clinical trial. The study followed the Consolidated Standards of Reporting Trials (CONSORT) Statement [20] as well as the Declaration of Helsinki ethical principles. It was also approved by the Research Ethics Committee of Instituto de Cardiologia do Rio Grande do Sul. All participants and/or their caregivers signed a consent form agreeing to participate in the study.

### 2.1. Study participants

Participation in the study was voluntary and all participants were recruited from a sports program for public school adolescents (*Projeto Forças no Esporte*, PROFESP) developed as a partnership between Brazil's Ministry of Education and the Ministry of Defense Armed Forces. A total of 100 adolescents were included in the sports program. Of these, 62 adolescents said they were willing to participate in the study's HIIT intervention protocol. We administered the International Physical Activity *Questionnaire* (*IPAQ*) [21] to assess physical activity in this population and they all were considered to be physically inactive. These adolescents had undergone prior medical evaluation before entering the public sports program and they did not have any history of cardiovascular, endocrine or neuromuscular conditions or orthopedic limitations or any other limitations that prevented physical exercise.

Of the 62 adolescents pre-selected, 10 dropped out of the sports program due to personal issues and five did not meet the study's inclusion criteria. Thus, a total of 47 adolescents entered our HIIT protocol. During the study, seven participants were excluded due to frequent no-shows and two dropped out, totaling 38 adolescents (girls, $n = 18$ and boys, $n = 20$) who completed our intervention (Fig. 1).

In addition to the intervention protocol, they also engaged in sports initiation activities (30-min sessions, three times a week) as part of the public sports program. The study participants were advised not to make any diet changes.

### 2.2. Body composition

We used a scientific plicometer (Cescorf, Porto Alegre, Brazil), a pachymeter (Cescorf, Porto Alegre, Brazil) and a stadiometer (MedSize, Porto Alegre, Brazil) for body measurements. Skinfold thicknesses measurements (triceps, subscapular, biceps, iliac crest, supra spinale, abdominal, front thigh, medial calf, axillary) were taken for body composition by a single evaluator (GW) certified level III by the International Society for the Advancement of Kinanthropometry (ISAK). The body fat (%BF) was estimated by Slaughter equations [22], using the sum of triceps and calf skinfolds (ΣDOC) (Boys: 0.610 x ΣDOC + 5.1 and Girls: 0.735 x ΣDOC + 1.0). Also, the sum of skinfold thicknesses measurements (in millimeters) was also used to express total body adiposity and totaling 9 skinfold thicknesses.

### 2.3. Biochemical analyses

We collected blood samples (total cholesterol, triglycerides, HDL, LDL and fasting glycemia) from all participants and they were sent for analysis in a conventional clinical laboratory. Blood samples were drawn following a 12-h fasting, and post-test samples were collected 72 h after the last activity of the training protocol. Blood was taken from an antecubital fossa vein in the forearm by a skilled nurse. An enzymatic method was used for the analyses of the biological material.

### 2.4. Endothelial function

Flow-mediated dilation (FMD) was the noninvasive method used to assess arterial endothelial function. The Philips LS-12 (4–12 MHz) linear array transducer was used to capture longitudinal images of the brachial artery walls. Images of arterial diameter were frozen along with electrocardiographic tracing. The pressure cuff was placed around the participant's arm and inflated to a pressure about 50 mmHg higher the systolic pressure for 5 min. FMD in the brachial artery occurs around 1 min and 20 s after cuff release. New images were then taken for comparison against the baseline images. Offline imaging analyses were carried out to measure the brachial artery diameter at the end of diastole (at the peak of electrocardiographic R wave). Brachial artery imaging was taken at three different sites (an average of 3 images for pre- and post-reactive hyperemia). Flow-dependent vasodilation responses were expressed as a percent change in diameter [23]. All assessments were carried out in a temperature-controlled environment and participants received recommendations on sleep, use of stimulants and physical exercise on the day preceding the test. Duplicate measurements were taken by a single blinded evaluator (BE).

### 2.5. Physical fitness assessment (Brazil's sports project [PROESP-BR] indicators)

A 6-minute run test was used to assess cardiorespiratory fitness (CRF) [25]. In brief, the participants were asked to run for 6 min around a 400-meter track; the total distance covered was recorded. Explosive strength of upper extremities was measured by medicine-ball throw distance (using a 2-kg ball and a measuring line attached perpendicular to a wall) [24]. In turn, explosive strength of lower extremities was measured by horizontal jumps across a line on the ground (and using a measuring line) [24]. A sit-and-reach test was used to assess muscle flexibility (using an adhesive tape and a measuring line stretched out on the floor). With their arms stretched and on top of each other, they reached forward along the measuring line as far as possible; they made two attempts and the farthest distance was recorded [24]. An abdominal endurance test was performed with the participants in dorsal decubitus, knees at 45° and arms crossed over their chest; they performed repeated abdominal curls as much as possible in one minute [24].

### 2.6. Exercise training

The participants were allowed one week to get some practice of the activities of the HIIT protocol. Total training duration was 12 weeks with 3 sessions held per week. It consisted of 100-meter running sprints interspersed with a variable period of active recovery time (Table 1).

### 2.7. Statistical analyses

The study results are described as means ± standard deviation. Levene's test was used to test for homogeneity and Shapiro-Wilk test was used to check for normality. The generalized estimating equations method followed by Bonferroni post-hoc was used for comparisons between BMI (normal-weight and overweight-obese) and time point (baseline and post-intervention), as well as their interactions. For variables with different baseline values for girls and boys, Student's t-test (independent samples) was conducted to assess gender differences. Since the convenience sample, we calculated the power analysis for FMD (primary outcome), resulting in 78%. Additionally, we calculated
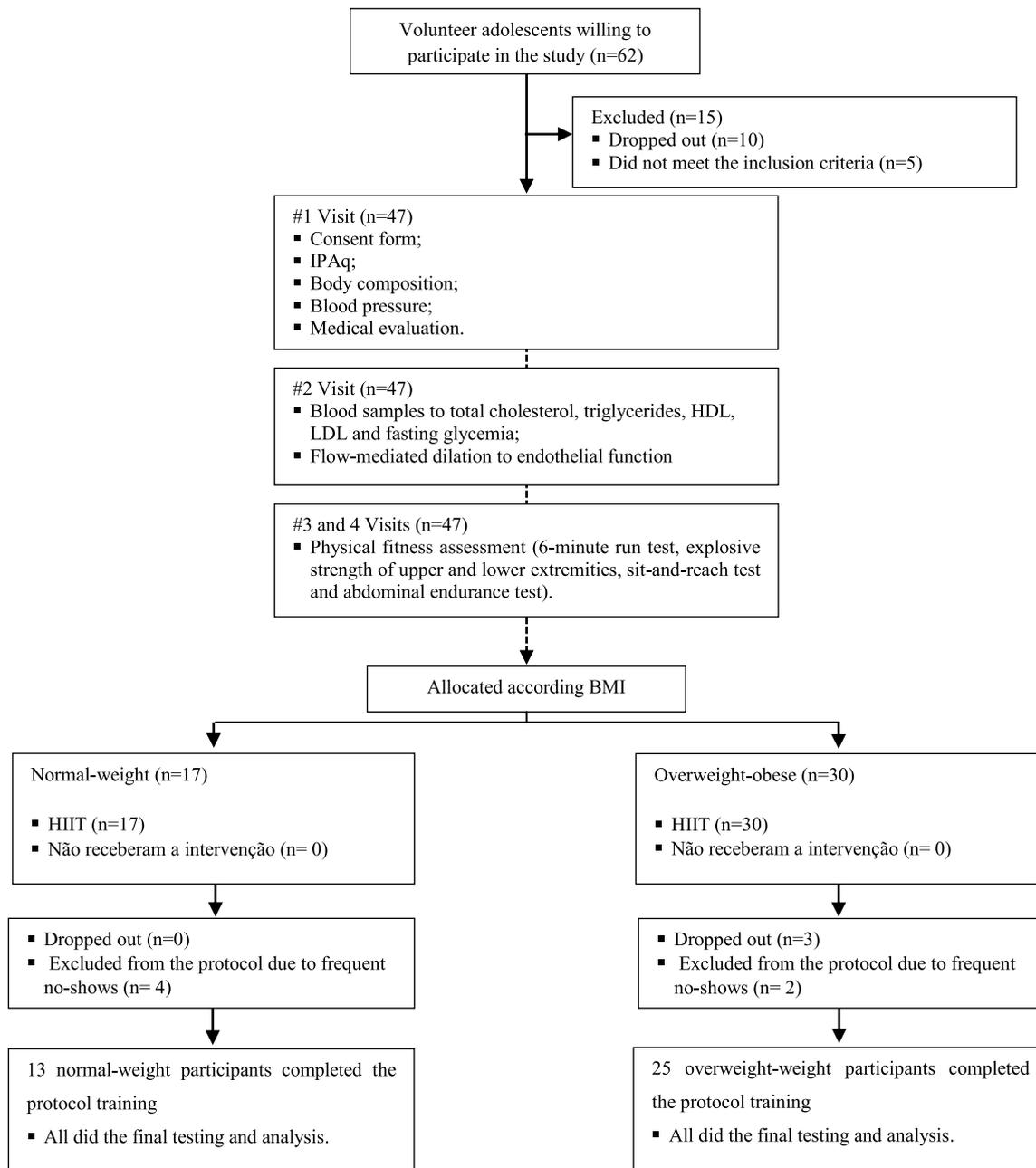
Fig. 1. Study design according CONSORT Statement.

**Table 1**
High-intensity interval training protocol.

| Week | 100-m sprints | Recovery time | Session duration |
|------|---------------|---------------|------------------|
| 1 | 2 sprints | Open | 10 min |
| 2 | 2 sprints | 5 min | 10 min |
| 3 | 3 sprints | 5 min | 11 min |
| 4 | 3 sprints | 3 min | 7 min |
| 5 | 4 sprints | 4 min | 13 min 20 s |
| 6 | 4 sprints | 2 min | 7 min 20 s |
| 7 | 5 sprints | 4 min | 17 min 40 s |
| 8 | 5 sprints | 2 min | 9 min 40 s |
| 9 | 6 sprints | 5 min | 27 min |
| 10 | 6 sprints | 4 min | 22 min |
| 11 | 6 sprints | 4 min | 22 min |
| 12 | 6 sprints | 4 min | 22 min |

HIIT: high-intensity interval training.

Cohen's effect size to FMD measurements (pre versus post exercise training); we considered that Cohen's effect from 0.20 to 0.49 are small, between 0.50 and 0.79 are medium, and effect sizes above 0.80 are large [25]. A 5% significance level was set for all statistical analyses.

## 3. Results

At the study entry, both normal-weight (NW) and overweight-obese (OW) groups had similar age ($15.0 \pm 0.9$ years vs. $15.1 \pm 1.0$ years; $p = 0.526$) and height ($166.2 \pm 9.2$ cm vs. $164.3 \pm 7.6$ cm; $p = 0.383$). At baseline, total body mass was $55.9 \pm 8.2$ kg and $76.7 \pm 14.7$ kg in the NW and OW groups and BMI was $21.1 \pm 3.3$ and $28.2 \pm 3.4$ kg/m$^2$, respectively ($p < 0.001$ for both comparisons). Table 2 shows body composition of all participants during the study and differences in body composition between boys and girls. As expected, in our sample, all baseline measures—body mass; sum of skinfold thicknesses; fat ratio; waist circumference and abdominal

**Table 2**
Body composition of participants.

| | NW | | OW | |
|---|---|---|---|---|
| | Baseline | Post-training | Baseline | Post-training |
| **Body mass (kg), _n_ = 38** | **55.9 ± 8.2** | **57.1 ± 7.8** | **76.7 ± 14.7\*** | **76.5 ± 14.2\*** |
| Girls (_n_ = 18) | 54.7 ± 7.3 | 56.4 ± 7.1 | 71.6 ± 15.1\* | 71.1 ± 15.4\* |
| Boys (_n_ = 20) | 56.6 ± 9.0 | 57.4 ± 8.7 | 82.9 ± 12.3\*,\*\*\* | 83.1 ± 10.7\*,\*\*\* |
| **Σ 9 SF (mm), _n_ = 38** | **83.7 ± 35.9** | **84.4 ± 38.1** | **236.0 ± 69.8\*** | **231.9 ± 74.7\*** |
| Girls (_n_ = 18) | 131.3 ± 20.8 | 133.9 ± 24.9 | 229.7 ± 74.1\* | 229.2 ± 80.8\* |
| Boys (_n_ = 20) | 63.4 ± 13.6\*\*\* | 63.3 ± 14.7\*\*\* | 243.6 ± 67.8\* | 235.3 ± 71.2\* |
| **% BF, _n_ = 38** | **15.5 ± 2.5** | **15.6 ± 2.6** | **34.4 ± 1.8\*** | **33.2 ± 1.9\*** |
| Girls (_n_ = 18) | 24.3 ± 3.4 | 24.8 ± 4.5 | 34.4 ± 9.5\* | 34.6 ± 10.1\* |
| Boys (_n_ = 20) | 11.7 ± 3.1\*\*\* | 11.6 ± 2.8\*\*\* | 33.0 ± 7.5\* | 31.4 ± 7.1\* |
| **WC (cm), _n_ = 38** | **69.9 ± 2.9** | **70.3 ± 2.6** | **88.9 ± 2.1\*** | **84.1 ± 1.8\*,\*\*** |
| Girls (_n_ = 18) | 68.5 ± 3.8 | 68.4 ± 3.0 | 82.5 ± 10.4\* | 78.7 ± 10.1\*,\*\* |
| Boys (_n_ = 20) | 70.5 ± 3.8 | 71.0 ± 3.0 | 92.2 ± 9.5\*,\*\*\* | 86.7 ± 5.5\*,\*\*,\*\*\* |
| **AC (cm), _n_ = 38** | **74.3 ± 3.1** | **74.2 ± 3.2** | **96.2 ± 2.2\*** | **92.5 ± 2.3\*,\*\*** |
| Girls (_n_ = 18) | 77.2 ± 3.7 | 76.9 ± 3.3 | 93.2 ± 12.5\* | 89.1 ± 12.9\*,\*\* |
| Boys (_n_ = 20) | 73.1 ± 4.8 | 73.1 ± 4.3 | 99.9 ± 9.9\*,\*\*\* | 94.2 ± 9.1\*,\*\*,\*\*\* |

NW: normal-weight group (girls, _n_ = 7; boys, _n_ = 6); OW: overweight-obese group (girls, _n_ = 13; boys, _n_ = 12). Σ 9 SF: sum of 9 skinfold measurements (triceps, subscapular, biceps, iliac crest, supra spinale, abdominal, front thigh, medial calf, axillary), % BF: body fat (%), WC: waist circumference. AC: abdominal circumference. The _generalized estimating_ equation (_GEE_) method followed by _Bonferroni's post-hoc_ was used.

\* $p < 0.05$ vs. NW at the same time point.

\*\* $p < 0.05$ vs. baseline within the same group.

\*\*\* $p < 0.05$ vs. girls.

circumference—were greater in the OW compared to the NW group (of any gender). In response to HIIT training, body mass, sum of skinfold thicknesses and fat ratio measures remained higher in the OW compared to the NW group showing with no intragroup differences (baseline vs. post-training). However, when compared to baseline, post-training waist and abdominal circumference measures were reduced by 4.8 cm ($p = 0.044$) and 3.7 cm ($p = 0.049$), respectively.

Table 3 shows hemoglobin, lipid profile and fasting glucose levels among the study participants. Both NW and OW groups had similar baseline levels of hemoglobin, total cholesterol, HDL, LDL and fasting glucose. Only triglyceride levels were greater in the OW compared to NW group ($p = 0.004$). In response to HIIT training, there was seen a reduction in hematocrit from baseline in both NW and OW groups (Δ 4.3 % vs. Δ 2.5%; $p < 0.001$ for both comparisons). There was an increase in triglyceride levels in both groups from baseline to post-training (NW Δ 25.6 mg/dL, $p < 0.001$; OW Δ 29.5 mg/dL, $p = 0.003$).

Table 4 shows the results of physical fitness assessed by PROESP-BR indicators. There were differences between boys and girls. Interestingly, both NW and OW groups showed at baseline similar results in the running test, explosive strength (upper and lower extremities) and flexibility. Only in the one-minute abdominal endurance test the NW group performed better than the OW group (36.0 vs. 26.8 repetitions,

$p = 0.002$). Post-training results for running, explosive strength (upper and lower extremities) and flexibility similarly improved in both groups (Table 4). In the abdominal endurance test, the participants in the OW group improved their absolute performance compared to the NW group (Δ 3.7 vs. Δ 5.0), but not enough to match NW performance.

Endothelial function assessed by FMD of the brachial artery was lower in the OW compared to NW group at baseline ($p = 0.042$). However, endothelial function improved after the 12-week HIIT in both groups (NW Δ 4.1%, $p < 0.001$, Cohen's effect sizes 0.64; OW Δ 4.5%, $p < 0.001$, Cohen's effect sizes 0.73). In addition, there was a slight improvement in endothelial function in the OW compared to NW group, which was enough to make it similar in both groups; that is, OW participants showed lower FMD values at baseline, but post-training values were similar in both groups.

## 4. Discussion

Excess weight among Brazilian adolescents is a concerning issue because it is associated with several health conditions including endothelial dysfunction, dyslipidemia, systemic arterial hypertension and diabetes mellitus among others. It is well-known that only 38.6% of boys and 20.1% of girls have an adequate level of physical activity [10],

**Table 3**
Biochemical parameters in the study participants.

| | NW | | OW | |
|---|---|---|---|---|
| | Baseline | Post-training | Baseline | Post-training |
| Red blood cells (million/uL) | 5.0 ± 0.3 | 4.8 ± 0.2 | 4.8 ± 0.5 | 4.7 ± 0.5 |
| Hemoglobin (g/dL) | 14.6 ± 1.2 | 14.0 ± 0.8 | 13.7 ± 1.1 | 13.6 ± 1.2 |
| Hematocrit (%) | 44.5 ± 3.1 | 40.2 ± 2.01\*\* | 42.2 ± 3.3 | 39.7 ± 3.2\*\* |
| White blood cells (cells/uL) | 8338 ± 2399 | 7483 ± 2141 | 7616 ± 1411 | 7807 ± 1290 |
| TC (mg/dL) | 149.9 ± 24.5 | 156.7 ± 28.4 | 143.2 ± 15.4 | 163.4 ± 31.0 |
| TRG (mg/dL) | 47.5 ± 28.1 | 73.1 ± 19.5\*\* | 76.2 ± 19.5\* | 105.7 ± 44.5\*,\*\*\* |
| HDL (mg/dL) | 53.5 ± 11.0 | 48.3 ± 10.5 | 44.4 ± 12.8 | 42.4 ± 8.5 |
| LDL (mg/dL) | 86.9 ± 19.3 | 93.7 ± 22.4 | 89.7 ± 22.7 | 99.3 ± 24.5 |
| Fasting glucose (mg/dL) | 94.1 ± 5.0 | 91.2 ± 6.3 | 95.1 ± 7.5 | 93.6 ± 5.8 |

NW: normal-weight group (girls, _n_ = 7; boys, _n_ = 6); OW: overweight-obese group (girls, _n_ = 13; boys, _n_ = 12). TC: total cholesterol; TRG: triglycerides; HDL: high-density cholesterol; LDL: low-density cholesterol. The _generalized estimating_ equation (_GEE_) method followed by _Bonferroni's post-hoc_ was used.

\* $p < 0.05$ vs. NW at the same time point.

\*\* $p < 0.05$ vs. baseline within the same group.

**Table 4**
Physical fitness assessment.

| | NW | | OW | |
|---|---|---|---|---|
| | Baseline | Post-training | Baseline | Post-training |
| **6-min run (m), *n* = 38** | **1022 ± 316** | **1202 ± 390**[**] | **921 ± 227** | **1037 ± 326**[*] |
| Girls (*n* = 18) | 786 ± 273 | 823 ± 303 | 815 ± 204 | 962 ± 239[**] |
| Boys (*n* = 20) | 1136 ± 289[***] | 1391 ± 300[**,***] | 1055 ± 186[***] | 1133 ± 403[***] |
| **Exp S UE (cm), *n* = 38** | **290 ± 100** | **349 ± 120**[**] | **310 ± 100** | **360 ± 110**[**] |
| Girls (*n* = 18) | 246 ± 62 | 267 ± 72 | 261 ± 73 | 291 ± 89 |
| Boys (*n* = 20) | 319 ± 112[***] | 383 ± 120[***] | 376 ± 83[***] | 429 ± 87[*,**,***] |
| **Exp S LE (cm), *n* = 38** | **160 ± 30** | **180 ± 40**[**] | **140 ± 20** | **160 ± 20**[**] |
| Girls (*n* = 18) | 120 ± 17 | 137 ± 27 | 138 ± 13 | 147 ± 14 |
| Boys (*n* = 20) | 172 ± 28[***] | 192 ± 27[***] | 152 ± 18 | 167 ± 22 |
| **Abdominal endurance (*n*), *n* = 38** | **36.0 ± 9.3** | **39.7 ± 10.5**[**] | **26.8 ± 8.2**[*] | **31.8 ± 10.4**[*,**] |
| Girls (*n* = 18) | 26.0 ± 5.3 | 29.3 ± 6.7 | 23.4 ± 7.7 | 29.5 ± 9.9[**] |
| Boys (*n* = 20) | 39.6 ± 8.4[***] | 44.8 ± 9.5[**,***] | 31.1 ± 6.8[*,***] | 36.1 ± 9.8[*,***] |
| **Flex (cm), *n* = 38** | **43.2 ± 8.1** | **46.5 ± 9.2**[**] | **43.2 ± 6.8** | **50.0 ± 8.2**[**] |
| Girls (*n* = 18) | 42.3 ± 7.6 | 43.0 ± 11.8 | 46.1 ± 6.2 | 53.1 ± 7.0 |
| Boys (*n* = 20) | 43.4 ± 8.7 | 47.6 ± 8.7 | 39.6 ± 5.9[***] | 43.7 ± 6.7 |

NW: normal-weight group (girls, *n* = 7 e meninos, *n* = 6); OW: grupo sobrepeso (girls, *n* = 13 e meninos, *n* = 12). 6-min run: 6-minute running test; Exp S: explosive strength; UE: upper extremities; LE: lower extremities; Flex: flexibility; *Generalized estimating* equations (*GEE*) followed by *Bonferroni's post-hoc* were used.

[*] *p* < 0.05 vs. NW at the same time point.

[**] *p* < 0.05 vs. baseline within the same group.

[***]‡ *p* < 0.05 vs. girls.

which further aggravates the impact of excess weight. Normal-weight and overweight-obese adolescents followed our 12-week HIIT protocol. The main finding of our study was consistent improvement of endothelial function assessed by FMD in both NW and OW groups, but the magnitude of improvement was greater in the latter group. Physical fitness improved in both groups, but waist and abdominal circumference measures were reduced only among those with excess weight. These are clinically relevant findings because they show cardiovascular risk reduction and particularly improved endothelial function at a young age.

The OW group showed at baseline reduced endothelial function compared to NW participants. Excess weight is a likely explanation [26] given that both groups were physically inactive with normal lipid profiles and there were no other factors known to be associated with endothelial dysfunction.

There has been considerable discussion in the literature about FMD cutoff values for endothelial dysfunction. Corretti (2002) recommended a cutoff of 8% as suggestive of endothelial dysfunction, i.e., FMD < 8% would indicate increased cardiovascular risk due to endothelial dysfunction in adults. However, there are no FMD cutoff values currently defined for "normal endothelial function" in children or adults. Yet, our findings are clinically relevant because endothelial function consistently improved in both NW (Δ 4.1%) and OW participants (Δ 4.5%). Moreover, it is worth noting that endothelial dysfunction precedes atherosclerotic changes [27] and there is increased risk when associated with excess body weight and/or sedentary lifestyle [28] as we found among many participants in our sample. No matter at which value the FMD cutoff is set, exercise training using HIIT improved endothelium function and proved widely beneficial. Comparatively, a study with a sample of obese Chinese adolescents found FMD values around 10% after reactive hyperemia [29], which corroborates baseline findings in our study. These authors demonstrated that BMI was strongly associated with increased mean carotid intima-media thickness (*r* = 0.58, *p* < 0.01) and reduced FMD (*r* = −0.42, *p* < 0.01). Furthermore, a meta-analysis found that the pooled relative risk of cardiovascular events per 1% increase in FMD, adjusted for confounding risk factors, was 0.87 (95% CI 0.83–0.91) [30]. Though our sample consisted of adolescents, our results are clinically significant.

Both normal-weight and overweight-obese participants who completed our training protocol showed significantly improved endothelial function, which is a finding of major clinical relevance. A beneficial

impact on endothelial function is directly associated with endothelial damage prevention. Several mechanisms may explain the effects of physical exercise on the endothelium, but the most likely one is increased blood flow during exercise with consequent increase in shear stress [31] and greater bioavailability of nitric oxide (NO), which is a major endothelial vasodilator, due to increased synthesis of an NO precursor—NO endothelial synthase (eNOS). Another likely explanation is a reduction of oxidative stress [32]. Although these mechanisms have been described in studies of continuous training [33, 34], HIIT benefits seems to be superior to those seen in response to continuous aerobic training [35]. In a meta-analysis, Ramos (2015) compared the effects of HIIT (four rounds of a 4-minute exercise series at 85–95% of maximal heart rate interspersed with 3 min of recovery at 60–70% of maximal heart rate, three times a week for 12–16 weeks) with continuous moderate training on FMD of the brachial artery. They found a superiority of HIIT of 2.26 (95% CI 0.92–3.59) over continuous training. Although a comparison between HIIT and continuous training was out of the scope of our study, the HIIT protocol described in the meta-analysis was very similar to ours, which reinforces the positive impact of this modality of training on endothelial function.

Studies of training protocols with less than 2 weeks of duration did not show satisfactory effects on the endothelium. Bond (2015) assessed 13 adolescents undergoing a 2-week HIIT (8–10 series of 1-minute cycling, 90% $VO_2$max) and found no endothelial function changes. Another study carried out with postmenopausal women following a 2-week HIIT protocol did not find any positive effects on endothelial function [36]. Thus, our findings are in agreement with those reported in the literature and they may be dependent on total duration of HIIT (3 times a week for 12 weeks).

The effectiveness of our HIIT protocol is evidenced by improved physical fitness (cardiorespiratory capacity, upper and lower extremity strength, abdominal endurance and muscle flexibility) among study participants. At baseline all adolescents were considered physically inactive and thus they had a risk factor for cardiovascular diseases in adult life [37,38]. Our exercise protocol lead to improved cardiorespiratory fitness in both NW and OW groups (Δ 180 m vs. Δ 116 m), being more effective among normal-weight participants. In their systematic review with meta-analysis, Costigan (2015) demonstrated that, compared to conventional moderate-intensity training, HIIT was more effective for improving cardiorespiratory fitness. The PROESP-BR sports project recommends the following "critical" cut-off values for the

running test: <995 m for girls and <1120 m for boys [24]. Among normal-weight participants, the girls were below average (823 m) and the boys improved their performance from "fairly good" (1136 m) to "very good" (1391 m). Among those with excess weight, both girls and boys improved their performance from "weak" to "fairly good."

With regard to explosive strength of upper and lower extremities, we found a similar improvement in both groups; and this improvement has a direct impact on functional capacity and daily activities [39]. As for upper extremity strength, normal-weight participants remained at the "critical" cut-off level for their age group both at baseline and post-training (<400 cm and <300 cm for boys and girls, respectively) [24]. The OW group started at a "critical" level (376 cm) and reached a "fairly good" performance post-training (429 cm). As for lower extremity strength, the NW group showed greater improvement (Δ 20 cm) from baseline with their performance improving from "weak" to "fairly good"; however, this response was seen among boys only (Table 4). Abdominal muscle exercises are widely used in training programs. Both NW and OW groups showed a fairly good performance in the abdominal endurance test based on the PROESP-BR recommended cut-off values [24], i.e., 23 (girls) and 35 (boys) repetitions for in a continuous 1-min timed effort. Strengthening of abdominal muscles reduces intervertebral disk and lumbar pressure and may reduce the risk of degenerative diseases in the lumbar spine [40]. The American College of Sports Medicine recommends including flexibility exercises as a component of health-related physical fitness programs [41]. Muscle flexibility can be defined as the physiologically normal range of motion in a joint [42] and without adequate *flexibility daily* activities become more difficult to perform. In addition, reduced flexibility may cause musculoskeletal damage [43]. Thus, maintaining (or increasing) flexibility is crucial as it allows adequate joint motion reducing the risk of injury [44]. In our study, although the training protocol did not focus on flexibility, both groups showed improved flexibility with direct beneficial effects in population of adolescents.

Improvement of endothelial function in the study participants seems more likely to have resulted from physiological adaptations to exercise rather than a reduction of correlated factors such as obesity and inadequate lipid profile among others because body mass, sum of skinfold thicknesses and body fat ratio did not change in response to HIIT in both groups studied. However, the OW group showed a reduction in waist and abdominal circumferences. Buchan (2012) evaluated 41 female and male adolescents divided into an HIIT group (maximum 20-meter sprints interspersed with 20–30 s of recovery) and a control group. These authors did not find any favorable changes in body composition and waist circumference in the intervention group. Kargarfard (2016) investigated 30 normal-weight and obese adolescents undergoing an 8-week HIIT (4 min at 70% $VO_2$max, followed by 2 min at 40% $VO_2$max for recovery) or continuous running training compared to a control group. They also did not report any changes in body composition. Both studies claimed that the lack of effect on body composition was due to short training period (duration of 7 and 8 weeks). We, on the other hand, believe that this result can be attributed to unchanged diet rather than total duration of training. Similarly, there were no consistent changes in lipid profile and fasting glucose in both NW and OW groups. Our findings are in agreement with those reported in the literature. Bond (2015) found similar results after evaluating a HIIT protocol. Buchan (2011) also tested a HIIT protocol in 47 boys and 10 girls and compared it against a continuous moderate-intensity training program and a control group. They did not report any changes in the levels of LDL, HDL and triglycerides. Yet, another study conducted by the same group of authors reported that participants undergoing a HIIT intervention without any diet changes even showed an increase in LDL and total cholesterol [45].

*4.1. Study limitations*

Our study has some limitations. The main limitation is that our

study lacks a control group. For our study we recruited at-risk adolescents from a social program and we do not think it would be ethically justified to select (though randomly) a different set of adolescents for a non-intervention group. Comparing pre- vs. post-intervention results and differences between overweight vs. obese groups can provide strong consistent evidence even in the absence of a control group, which are also supported by Cohen's effect size. Also, the study participants did not keep a food journal and they were offered two meals consisting of ultra-processed foods with unrestricted portion sizes as part of the PROFESP sports program. Another potential limitation is that most participants of the study were male, and it is reasonable to believe that boys engage more in high-intensity physical activities than girls and are more likely to show greater physical resistance in more vigorous activities than girls. Finally, the sample size has sufficient power to detect the primary outcome (FMD). Bearing in mind there may be changes in body mass, a larger sample size would be necessary for an acceptable statistical power. Moreover, a longer intervention (over 12 weeks of training) would allow to detect these changes. However, we stress that, for the primary outcome (FMD), a 12-week intervention is sufficient to promote beneficial effects on endothelial function as reported in the literature [46–49] and by our results.

## 5. Conclusions

Our study showed that a HIIT protocol as part of routine physical activities of normal-weight and overweight-obese adolescents can help improve overall physical fitness and endothelial function. These are clinically relevant findings because this exercise intervention may reduce endothelial damage preceding the atherosclerotic process. The finding of no changes in body composition and lipid profile of participants can be due unchanged diet. Further research should investigate HIIT protocols together with diet changes (Fig. 2).

**Supplementary materials**

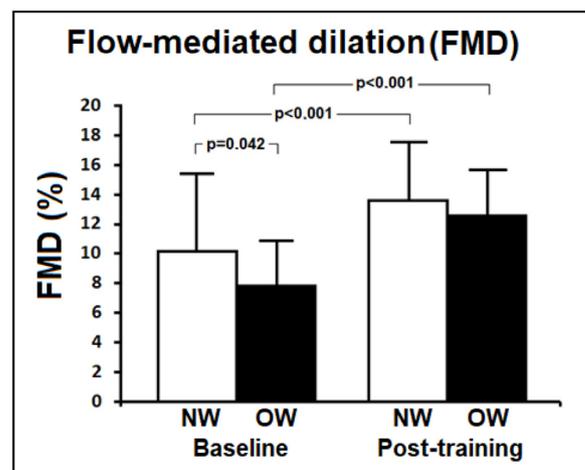Supplementary material associated with this article can be found, in



**Fig. 2.** Endothelial function assessed by flow-mediated dilation (FMD). NW: normal-weight group; OW: overweight-obese group. Generalized estimating equation (GEE) method followed by Bonferroni's post-hoc was used ($p < 0.05$).

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## References

[1] J.d.G. Jorge, M.A. Santos, J.A. Barreto Filho, J.L. Oliveira, E.V. Melo, N.A. Oliveira, G.B. Faro, A.C. Sousa, Level of physical activity and in-hospital course of patients with acute coronary syndrome, Arq. Bras. Cardiol. 106 (1) (2016) 33–40, https://doi.org/10.5935/abc.20160006.

[2] A.M. McManus, P.N. Ainslie, D.J. Green, R.G. Simair, K. Smith, N. Lewis, Impact of prolonged sitting on vascular function in young girls, Exp. Physiol. 100 (11) (2015) 1379–1387, https://doi.org/10.1113/EP085355.

[3] I.M. Lee, E.J. Shiroma, F. Lobelo, P. Puska, S.N. Blair, P.T. Katzmarzyk, Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy, Lancet 380 (9838) (2012) 219–229, https://doi.org/10.1016/S0140-6736(12)61031-9.

[4] B.Q. Farah, D.G. Christofaro, P.B. Balagopal, B.R. Cavalcante, M.V. de Barros, R.M. Ritti-Dias, Association between resting heart rate and cardiovascular risk factors in adolescents, Eur. J. Pediatr. 174 (12) (2015) 1621–1628, https://doi.org/10.1007/s00431-015-2580-y.

[5] D.S. Buchan, S. Ollis, J.D. Young, N.E. Thomas, S.M. Cooper, T.K. Tong, J. Nie, R.M. Malina, J.S. Baker, The effects of time and intensity of exercise on novel and established markers of CVD in adolescent youth, Am. J. Hum. Biol. 23 (4) (2011) 517–526, https://doi.org/10.1002/ajhb.21166.

[6] M. Kargarfard, E.T. Lam, A. Shariat, M. Asle Mohammadi, S. Afrasiabi, I. Shaw, B.S. Shaw, Effects of endurance and high intensity training on ICAM-1 and VCAM-1 levels and arterial pressure in obese and normal weight adolescents, Phys. Sportsmed. 44 (3) (2016) 208–216, https://doi.org/10.1080/00913847.2016.1200442.

[7] R.F. Furchgott, J.V. Zawadzki, The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine, Nature 288 (5789) (1980) 373–376.

[8] P. Libby, M. DiCarli, R. Weissleder, The vascular biology of atherosclerosis and imaging targets, J. Nucl. Med. 51 (Suppl 1) (2010) 33S–37S, https://doi.org/10.2967/jnumed.109.069633.

[9] E. Csongradi, M. Kaplar, B. Nagy Jr., C.A. Koch, A. Juhasz, L. Bajnok, Z. Varga, I. Seres, Z. Karanyi, M.T. Magyar, L. Olah, A. Facsko, J. Kappelmayer, G. Paragh, Adipokines as atherothrombotic risk factors in obese subjects: associations with haemostatic markers and common carotid wall thickness, Nutr. Metab. Cardiovasc. Dis. 27 (6) (2017) 571–580, https://doi.org/10.1016/j.numecd.2017.02.007.

[10] V.K. Matsudo, G.L. Ferrari, T.L. Araujo, L.C. Oliveira, E. Mire, T.V. Barreira, C. Tudor-Locke, P. Katzmarzyk, Socioeconomic status indicators, physical activity, and overweight/obesity in Brazilian children, Rev. Paul. Pediatr. (2016), https://doi.org/10.1016/j.rpped.2015.04.003.

[11] M.E. Francois, C. Durrer, K.J. Pistawka, F.A. Halperin, J.P. Little, Resistance-based interval exercise acutely improves endothelial function in type 2 diabetes, Am. J. Physiol. Heart Circ. Physiol. 311 (5) (2016) H1258–H1267, https://doi.org/10.1152/ajpheart.00398.2016.

[12] C.A. Dow, B.L. Stauffer, D.L. Brunjes, J.J. Greiner, C.A. DeSouza, Regular aerobic exercise reduces endothelin-1-mediated vasoconstrictor tone in overweight and obese adults, Exp. Physiol. 102 (9) (2017) 1133–1142, https://doi.org/10.1113/EP086454.

[13] R.J. Sigal, A.S. Alberga, G.S. Goldfield, D. Prud'homme, S. Hadjiyannakis, R. Gougeon, P. Phillips, H. Tulloch, J. Malcolm, S. Doucette, G.A. Wells, J. Ma, G.P. Kenny, Effects of aerobic training, resistance training, or both on percentage body fat and cardiometabolic risk markers in obese adolescents: the healthy eating aerobic and resistance training in youth randomized clinical trial, JAMA Pediatr. 168 (11) (2014) 1006–1014, https://doi.org/10.1001/jamapediatrics.2014.1392.

[14] A.S. Alberga, D. Prud'homme, R.J. Sigal, G.S. Goldfield, S. Hadjiyannakis, R. Gougeon, P. Phillips, J. Malcolm, G.A. Wells, S. Doucette, J. Ma, G.P. Kenny, Does exercise training affect resting metabolic rate in adolescents with obesity? Appl. Physiol. Nutr. Metab. 42 (1) (2017) 15–22, https://doi.org/10.1139/apnm-2016-0244.

[15] B. Bond, E.J. Cockcroft, C.A. Williams, S. Harris, P.E. Gates, S.R. Jackman, N. Armstrong, A.R. Barker, Two weeks of high-intensity interval training improves novel but not traditional cardiovascular disease risk factors in adolescents, Am. J. Physiol. Heart Circ. Physiol. 309 (6) (2015) H1039–H1047, https://doi.org/10.1152/ajpheart.00360.2015.

[16] M.J. Sedgwick, J.G. Morris, M.E. Nevill, L.A. Barrett, Effect of repeated sprints on postprandial endothelial function and triacylglycerol concentrations in adolescent boys, J Sports Sci. 33 (8) (2015) 806–816, https://doi.org/10.1080/02640414.2014.964741.

[17] S.A. Costigan, N. Eather, R.C. Plotnikoff, D.R. Taaffe, D.R. Lubans, High-intensity interval training for improving health-related fitness in adolescents: a systematic review and meta-analysis, Br. J. Sports Med. 49 (19) (2015) 1253–1261, https://doi.org/10.1136/bjsports-2014-094490.

[18] A.C. Corte de Araujo, H. Roschel, A.R. Picanco, D.M. do Prado, S.M. Villares, A.L. de Sa Pinto, B. Gualano, Similar health benefits of endurance and high-intensity interval training in obese children, PLoS ONE 7 (8) (2012) e42747, https://doi.org/10.1371/journal.pone.0042747.

[19] L. Bruyndonckx, V.Y. Hoymans, A. De Guchtenaere, M. Van Helvoirt, E.M. Van Craenenbroeck, G. Frederix, K. Lemmens, D.K. Vissers, C.J. Vrints, J. Ramet, V.M. Conraads, Diet, exercise, and endothelial function in obese adolescents, Pediatrics 135 (3) (2015) e653–e661, https://doi.org/10.1542/peds.2014-1577.

[20] K.F. Schulz, D.G. Altman, D. Moher, CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials, BMJ 340 (2010) c332, https://doi.org/10.1136/bmj.c332.

[21] L. Wadolowska, J. Kowalkowska, M. Lonnie, J. Czarnocinska, M. Jezewska-Zychowicz, E. Babicz-Zielinska, Associations between physical activity patterns and dietary patterns in a representative sample of Polish girls aged 13-21 years: a cross-sectional study (GEBaHealth project), BMC Publ. Health 16 (2016) 698, https://doi.org/10.1186/s12889-016-3367-4.

[22] M.H. Slaughter, T.G. Lohman, R.A. Boileau, C.A. Horswill, R.J. Stillman, M.D. Van Loan, D.A. Bemben, Skinfold equations for estimation of body fatness in children and youth, Hum. Biol. 60 (5) (1988) 709–723.

[23] M.C. Corretti, T.J. Anderson, E.J. Benjamin, D. Celermajer, F. Charbonneau, M.A. Creager, J. Deanfield, H. Drexler, M. Gerhard-Herman, D. Herrington, P. Vallance, J. Vita, R. Vogel, F. International Brachial Artery Reactivity Task, Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the international brachial artery reactivity task force, J. Am. Coll. Cardiol. 39 (2) (2002) 257–265.

[24] Gaya A., Gaya A.Projeto esporte Brasil: manual de testes e avaliação Porto Alegre: UFRGS; 2016. Available from:https://www.ufrgs.br/proesp/arquivos/manual-proesp-br-2016.pdf.

[25] J. Cohen, A power primer, Psychol. Bull. 112 (1) (1992) 155–159, https://doi.org/10.1037//0033-2909.112.1.155.

[26] N. Amabile, S. Cheng, J.M. Renard, M.G. Larson, A. Ghorbani, E. McCabe, G. Griffin, C. Guerin, J.E. Ho, S.Y. Shaw, K.S. Cohen, R.S. Vasan, A. Tedgui, C.M. Boulanger, T.J. Wang, Association of circulating endothelial microparticles with cardiometabolic risk factors in the framingham heart study, Eur. Heart. J. 35 (42) (2014) 2972–2979, https://doi.org/10.1093/eurheartj/ehu153.

[27] M.A. Gimbrone Jr., G. Garcia-Cardena, Endothelial cell dysfunction and the pathobiology of atherosclerosis, Circ. Res. 118 (4) (2016) 620–636, https://doi.org/10.1161/CIRCRESAHA.115.306301.

[28] U. Ekelund, J. Luan, L.B. Sherar, D.W. Esliger, P. Griew, A. Cooper, C. International Children's Accelerometry Database, Moderate to vigorous physical activity and sedentary time and cardiometabolic risk factors in children and adolescents, JAMA 307 (7) (2012) 704–712, https://doi.org/10.1001/jama.2012.156.

[29] W. Zhu, X. Huang, J. He, M. Li, H. Neubauer, Arterial intima-media thickening and endothelial dysfunction in obese Chinese children, Eur. J. Pediatr. 164 (6) (2005) 337–344, https://doi.org/10.1007/s00431-005-1642-y.

[30] Y. Inaba, J.A. Chen, S.R. Bergmann, Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis, Int. J. Cardiovasc. Imag. 26 (6) (2010) 631–640, https://doi.org/10.1007/s10554-010-9616-1.

[31] T.M. Tinken, D.H. Thijssen, N. Hopkins, E.A. Dawson, N.T. Cable, D.J. Green, Shear stress mediates endothelial adaptations to exercise training in humans, Hypertension 55 (2) (2010) 312–318, https://doi.org/10.1161/HYPERTENSIONAHA.109.146282.

[32] A.Z. Jamurtas, I.G. Fatouros, C.K. Deli, K. Georgakouli, A. Poulios, D. Draganidis, K. Papanikolaou, P. Tsimeas, A. Chatzinikolaou, A. Avloniti, A. Tsiokanos, Y. Koutedakis, The effects of acute low-volume HIIT and aerobic exercise on leukocyte count and redox status, J. Sports Sci. Med. 17 (3) (2018) 501–508.

[33] C.A. DeSouza, L.F. Shapiro, C.M. Clevenger, F.A. Dinenno, K.D. Monahan, H. Tanaka, D.R. Seals, Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men, Circulation 102 (12) (2000) 1351–1357.

[34] Y. Higashi, S. Sasaki, S. Kurisu, A. Yoshimizu, N. Sasaki, H. Matsuura, G. Kajiyama, T. Oshima, Regular aerobic exercise augments endothelium-dependent vascular relaxation in normotensive as well as hypertensive subjects: role of endothelium-derived nitric oxide, Circulation 100 (11) (1999) 1194–1202.

[35] J.S. Ramos, L.C. Dalleck, A.E. Tjonna, K.S. Beetham, J.S. Coombes, The impact of high-intensity interval training versus moderate-intensity continuous training on vascular function: a systematic review and meta-analysis, Sports Med. 45 (5) (2015) 679–692, https://doi.org/10.1007/s40279-015-0321-z.

[36] M. Klonizakis, J. Moss, S. Gilbert, D. Broom, J. Foster, G.A. Tew, Low-volume high-intensity interval training rapidly improves cardiopulmonary function in post-menopausal women, Menopause 21 (10) (2014) 1099–1105, https://doi.org/10.1097/GME.0000000000000208.

[37] G. Racil, J.B. Coquart, W. Elmontassar, M. Haddad, R. Goebel, A. Chaouachi, M. Amri, K. Chamari, Greater effects of high- compared with moderate-intensity interval training on cardio-metabolic variables, blood leptin concentration and ratings of perceived exertion in obese adolescent females, Biol. Sport. 33 (2) (2016) 145–152, https://doi.org/10.5604/20831862.1198633.

[38] C.A. Vella, K. Taylor, D. Drummer, High-intensity interval and moderate-intensity continuous training elicit similar enjoyment and adherence levels in overweight and obese adults, Eur. J. Sport Sci. 17 (9) (2017) 1203–1211, https://doi.org/10.1080/17461391.2017.1359679.

[39] A. Macaluso, G. De Vito, Comparison between young and older women in explosive power output and its determinants during a single leg-press action after optimisation of load, Eur. J. Appl. Physiol. 90 (5–6) (2003) 458–463, https://doi.org/10.1007/s00421-003-0866-7.

[40] C.E. Ladeira, L.W. Hess, B.M. Galin, S. Fradera, M.A. Harkness, Validation of an abdominal muscle strength test with dynamometry, J. Strength Cond. Res. 19 (4) (2005) 925–930, https://doi.org/10.1519/R-16664.1.

[41] ACSM, Manual do ACSM Para Avaliação da Aptidão Física Relacionada à Saúde, 3a. Ed., Rio de Janeiro, Guanabara Koogan, 2011.

[42] G.J. Holland, K. Tanaka, R. Shigematsu, M. Nakagaichi, Flexibility and physical functions of older adults: a review, J. Aging Phys. Act 10 (2) (2002) 169–206.

[43] E.M. Badley, S. Wagstaff, P.H. Wood, Measures of functional ability (disability) in arthritis in relation to impairment of range of joint movement, Ann. Rheum. Dis. 43 (4) (1984) 563–569.

[44] W. Spirduso, K. Francis, P. Francis, Physical Dimensions of Aging, Human Kinetics, Champaign, IL, 1995.

[45] D.S. Buchan, S. Ollis, J.D. Young, S.M. Cooper, J.P. Shield, J.S. Baker, High intensity interval running enhances measures of physical fitness but not metabolic measures of cardiovascular disease risk in healthy adolescents, BMC Public Health 13 (2013) 498, https://doi.org/10.1186/1471-2458-13-498.

[46] R. Kelishadi, M. Hashemi, N. Mohammadifard, S. Asgary, N. Khavarian, Association of changes in oxidative and proinflammatory states with changes in vascular function after a lifestyle modification trial among obese children, Clin. Chem. 54 (1) (2008) 147–153, https://doi.org/10.1373/clinchem.2007.089953.

[47] K.D. Sung, E.J. Pekas, S.D. Scott, W.M. Son, S.Y. Park, The effects of a 12-week jump rope exercise program on abdominal adiposity, vasoactive substances, inflammation, and vascular function in adolescent girls with prehypertension, Eur. J. Appl. Physiol. 119 (2) (2019) 577–585, https://doi.org/10.1007/s00421-018-4051-4.

[48] A.E. Tjonna, T.O. Stolen, A. Bye, M. Volden, S.A. Slordahl, R. Odegard, E. Skogvoll, U. Wisloff, Aerobic interval training reduces cardiovascular risk factors more than a multitreatment approach in overweight adolescents, Clin. Sci. (Lond.) 116 (4) (2009) 317–326, https://doi.org/10.1042/CS20080249.

[49] K. Watts, P. Beye, A. Siafarikas, E.A. Davis, T.W. Jones, G. O'Driscoll, D.J. Green, Exercise training normalizes vascular dysfunction and improves central adiposity in obese adolescents, J. Am. Coll. Cardiol. 43 (10) (2004) 1823–1827, https://doi.org/10.1016/j.jacc.2004.01.032.