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Exercise Dose on Hepatic Fat and Cardiovascular Health in Adolescents with Excess of Adiposity

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Abstract

Objective: The HEPAFIT study was aimed at examining the impact of a 6-month physical education intervention, considering various levels of exercise intensity, on hepatic fat and cardiometabolic health outcomes in adolescents with excess adiposity.

Methods: Adolescents (11 to 17 years with excess adiposity by body fat >30%), were randomly assigned to one of the following 4 groups for 6 months: (1) standard physical education lessons, control (CTRL); (2) high-intensity physical education(HIPE); (3) low-to-moderate intensity physical education(LIPE); (4) combined HIPE and LIPE(PLUS). The primary outcome was hepatic fat content measured by vibration-controlled transient elastography (controlled attenuation parameter [CAP]). Secondary outcomes were traditional cardiovascular health markers (body composition, serum lipids, aminotransferases, and health-related physical fitness components).

Results: Adjusted mixed effects linear models revealed a significant decrease in CAP levels in HIPE (−13.81 dB/m, p=0.030) (p=0.001 *vs* CTRL group) and PLUS (−22.41 dB/m, p=0.001) (p=0.002 *vs* CTRL group) groups. Body fat decreased in the HIPE, −1.47 %, p<0.001) and PLUS, −1.88 %, p=0.001) groups. The physical fitness components were increased in the HIPE and PLUS group relative to the baseline (p<0.05), and the HIPE group showed a reduction in the total cholesterol (p<0.05).

Conclusions: Implementation of a 6-month physical education exercise program, particularly high-intensity or combined high and low-intensity, improves hepatic fat storage and significantly reduces cardiometabolic markers in adolescents with excess of adiposity. Interventions involving supervised physical exercise may help to improve metabolism and fat deposition at the hepatic level, thus preventing the development of non-alcoholic fatty liver disease in adolescents.

Trial Registration clinicaltrials.gov Identifier: NCT02753231

Introduction

The epidemic obesity in adolescents is now recognized worldwide as a critical health issue. Obesity in adolescence is related to several comorbidities such as metabolic syndrome (1), non-alcoholic fatty liver disease (NAFLD) (2), and type 2 diabetes mellitus (3), once considered diseases of adulthood. Accordingly, adolescents with overweight/obesity are at high risk of developing serious medical complications, either when still young or later in life, and show a greater morbidity and mortality in adulthood.

A lack of physical activity, along with other factors such as poor diet and a sedentary lifestyle, are associated with the initiation and progression of obesity and obesity-associated metabolic comorbidities among adolescents (4). For this reason, both diet modifications and physical exercise/activity interventions are considered the first lines of approach to treat children and adolescents with obesity (4,5). In this context, we recently reported that increasing supervised exercise in adolescents with overweight and obesity, regardless of caloric restriction, is beneficial for reducing hepatic lipid composition, blood lipid profile, and visceral and subcutaneous adipose tissue (6), as well as for improving cardiorespiratory fitness (CRF) and the chronic low-grade inflammatory state associated with this disease (7).

In adolescent's with obesity, the utility of school-based exercise programs (with no caloric restriction) as a strategy for reducing obesity-related metabolic risk factors is unclear. A previous meta-analysis suggested a positive effect of school-based interventions on physical fitness (8); however, the optimal exercise regimen for reducing adiposity and its cardiometabolic risk factors, hepatic lipid composition and insulin resistance have not been fully clarified. As far as we are aware, no previous studies have examined the cardiometabolic response of adolescents at high risk of NAFLD to exercise interventions in the context of physical education instruction.

Since most adolescents spend the majority of their waking hours at school, and this setting is often equipped with playgrounds and sports facilities, numerous studies have emphasized the importance of schools in promoting exercise programs (9,10). Thus, the HEPAFIT study aimed at examining whether a 6-month physical education (PE) exercise program is beneficial with regards to hepatic fat content and inflammation as well as cardiovascular health outcomes among adolescents with excess of adiposity. The primary outcome was hepatic fat content measured using vibration-controlled transient elastography (VCTE). Secondary outcomes included total body fat, anthropometric indices of body fat distribution, physical fitness components, cardiometabolic risk factors, and hepatic metabolism parameters.

METHODS

Study design and participants

This was a randomized clinical trial with single blind, parallel-group trial, HEPAFIT (ClinicalTrials.gov ID: NCT02753231), conducted from September 2017 through December 2018. Details on the rationale, design, inclusion and exclusion criteria, and methods have been described elsewhere (11). The study protocol was reviewed and approved by the research ethics boards of the Medical Research Ethics Committee of The University of Rosario (Code UR-CEI-ABN026-000140) and complied with the revised ethical guidelines of the Declaration of Helsinki (revision of 2013). The description and characteristics of the study were given to parents or legal guardians, and written informed consent was provided from both guardians and adolescents. To qualify for randomization, participants needed to attend at least 54 of 72 prescribed exercise sessions (≥76.3% adherence) during the run-in period.

Participants (Table 1) were adolescents aged 11 to 17 years (Tanner stage II to V), 67% girls, with a body mass index (BMI) z-score at or above the 85th percentile, according to the International Obesity Task Force (12), and/or with excess of adiposity (body fat >30%). Briefly, exclusion criteria included habitual exercise more than twice weekly for more than 60 min per session, pregnancy, cardiovascular disease, diabetes mellitus (type 1 or 2) or any illness or disability rendering PE inadvisable or unfeasible. Additionally, participants with other causes of liver disease featuring elevated liver enzymes and/or hepatotropic viruses (i.e., positive for HBsAg/IgG, anti-HBc/anti-HCV/anti-HIV antibodies and/or others) were excluded. Routine school PE classes were not an exclusion criterion.

Randomization and blinding

Randomization was in permuted blocks to allocate all participants into the groups in a 1:1:1:1 ratio using a computer-generated random number sequence. The coding key was only known by the programmer, who was not working with the study staff or patients enrolled int his study. The study staff received electronic information of the actual randomization of the patient via email after logging into the electronic randomization tool with their own access codes. They received one unique number assigning the patient to either treatment group in a blinded fashion.

Intervention

Trained physiotherapist and physical educators supervised each training session. Participants entered a run-in period that included a school-based PE exercise program, 3 times weekly for 6 months (**Full interventions details in the Supplement eTable 1-2**). Risks were minimized by identifying contraindications to the testing and training protocols *via* a health history questionnaire and a thorough physician examination prior to the testing sessions. Details of the exercise programs are shown in (**eTable 3 in the Supplement**).

Primary Outcome

The VCTE measurements were obtained using the FibroScan® model 502 V2 Touch, equipped with a medium (M, 3.5 mHz, diameter 7 mm) probe (in 99% of tests). Liver tissue imaging was performed with the participant lying in supine position, with the right arm in maximum abduction. The controlled attenuation parameter (CAP, a measure of fat deposition measured in decibels/meter dB/m), and liver stiffness measurement (LSM, measured in kilopascals [kPa]) of each participant was obtained simultaneously in the examination. Results were included in the final analysis only if the following three criteria were met: fasting time of at least 3 hours, 10 or more complete measures, and an interquartile range less than 30% of the median value. The inter-rater reliability was 0.96 for CAP and 0.92 for LSM (n=10).

Secondary outcomes

Body mass, height and sitting height, waist circumference, and blood pressure were measured as previously described (11). BMI z-score was calculated using WHO Anthro-Plus 1.0.4 software. Somatic maturity was estimated by peak height velocity as proposed by Mirwald et al. (13). Body composition parameters (fat mass, skeletal mass muscle, and visceral adipose tissue), were measured by DEXA using the Hologic Horizon System® (Hologic, Inc., Bedford, Massachusetts) with Discovery software®, version 12.3.

CRF was assessed by the 20-m shuttle run test until volitional fatigue, with the total number of completed laps registered. Upper-limb strength was assessed by the maximum grip strength test using a standard adjustable handle digital dynamometer. Two trials were allowed for each limb and the average score recorded the peak grip strength (kg). Lower-limb strength was assessed using the standing long-jump test, which was performed three times and the longest jump was recorded in centimeters (cm). Speed-agility was assessed using the 4 × 10-m shuttle run test. The test was performed twice, and the fastest time was recorded in seconds. Lower scores indicated greater speed-agility levels (14).

Venous blood samples were obtained from the antecubital vein between 7:00 and 9:00 AM following a 10–12 h overnight fast. Samples were handled according to Clinical Laboratory standards (ANALIZAR, SAS, Bogotá, Colombia. Lipid panel, including high- (HDL-C), low-density (LDL-C) lipoprotein cholesterol, total cholesterol, total triglycerides, serum insulin, C-peptide, glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), creatinine, ureic nitrogen, serum iron, iron capacity, iron fixation capacity and ferritin levels, were determined by standard laboratory techniques used in clinical chemistry laboratories by duplicate (**eTable 4 in the Supplement**). The Homeostatic Model Assessment was used to calculate insulin resistance (HOMA-IR) calculator, released by the Diabetes Trial Unit, University of Oxford, and as described elsewhere (15). Cardiometabolic z-scores were calculated as the sum of the age-sex standardized scores of waist circumference, triglycerides, systolic blood pressure, HDL-C, and fasting glucose.

Blood pressure was measured by a well-trained physiotherapist using an automatic sphygmomanometer (Omron HEM 7130 Blood Pressure Monitor). We used the KIDMED questionnaire to determine the degree of adherence to the Mediterranean diet (16). The sum of all values from the administered questionnaire was categorized in two levels: (1) 0–7, poor/average adherence and (2) 8–12, good adherence. This questionnaire has high consistency (Cronbach's alpha = 0.79) and comprises 16 questions with an affirmative (twelve items) or negative (four items) response (17).

Research staff reported all directly observed injuries or other adverse events and those spontaneously reported by the participants. Participants were also questioned about adverse events at each study visit by physician staff.

Statistical analysis

Baseline results are expressed as the mean (standard deviation, SD) for continuous variables and percentage for categorical variables, unless otherwise specified. Change in CAP was the primary outcome of the study. For hepatic fat measured by VCTE, a sample size of 100 (25 participants in each group) will have 80% power to detect a difference in hepatic fat of 5.0% assuming 4.1% SD and an α-value of 0.025, two-sided significance level (18). Incorporating an allowance for an anticipated 10– 20% loss to follow-up (e.g., lack of time, refusal to continue participation in the study and/or illness) resulted in a required sample size of 120 patients (30 per group). This sample size provided a power of >99% to detect a 5% (~15 dB/m) difference in CAP (hepatic fat) and a power of >88% to detect a 3% (~10 dB/m) difference in CAP. We examined the effect of the intervention using an intention-to-treat analysis for only randomized subjects with baseline data. Missing follow-up data values were estimated using the multiple-imputations procedure with 10 iterations and 5 imputations. Adjusted mixed effects linear models was used to determine treatment change differences for each variable, using the imputed data with adjustment for baseline measures of each variable of interest (n=120). Because the HEPAFIT study was designed as an efficacy study, we also examined the effect of the exercise intervention using per-protocol analyses in participants who had complete baseline and follow-up data (n=99), with adjustment for baseline measures of each variable of interest. Sidack's *post hoc* test was used for multiple comparisons. All results are presented as least-squares means with 95% confidence intervals (CIs). The null hypothesis was rejected at a level of significance of p<0.05, and all statistical tests were two-tailed. Statistical analyses were performed using SPSS version 25 (IBM Corp, Chicago, IL) software, SAS, version 9.2 (SAS Institute, Cary, NC) commercially available software, and the R environment (version 3.6.2).

Results

A total of 160 adolescents were screened, with 33 subjects (20.6%) ineligible after applying the exclusion and inclusion criteria. A total of 127 participants (79.3%) with informed consent were scheduled for baseline clinical measurements; 120 (75.0%) had complete VCTE data at baseline and were randomized; 99 (82.5%) completed the study and were included in the secondary analyses. Reasons for withdrawal are shown in **Figure 1**.

*****Figure 1 near here*****

The descriptive characteristics of the study sample are shown in **Table 1**. Baseline characteristics were similar among participants assigned to the four interventions and between those assigned to an intervention and those who completed the study. Participant characteristics were generally in accord with those observed in population studies, with predominantly girls (*n*=82, 68%). The mean (SD) age of the participants was 13.25 (1.62) years, BMI z-score was 1.80 (0.57), and body fat was 39.73% (1.64). The mean baseline CAP was 225.93 dB/m (41.60). Adherence to exercise was 74.0%, 76.9%, 76.7%, and 72.1% for the CTRL, HIPE, LIPE, and PLUS groups, respectively. (**eTable 5 in the Supplement**), describes the exercise prescription in detail and measurements obtained during the intervention period.

*****Table 1 near here*****

Primary Outcome

Liver Fat

Adjusted mixed effects linear models revealed a significant decrease in CAP levels in HIPE (−13.81 dB/m, 95% confidence interval [CI], −26.37 to −1.24, $p<0.001$) ($p=0.001$ *vs* CTRL group) and PLUS (−22.41 dB/m, 95% CI, −34.82 to −10.00, $p=0.005$) ($p=0.002$ *vs* CTRL group) groups, Table 2. In per-protocol analyses, the HIPE group had greater changes in CAP levels (−26.12, 95% CI, −39.90 to −12.35, $p=0.003$) vs the CTRL group ($p<0.001$), and PLUS

−22.79 dB/m (95% CI, −36.14 to −9.424, *p*=0.025) (*p*=0.007 *vs* CTRL group) groups (**eTable 6 in the Supplement**).

Secondary Outcomes

Significant changes in secondary endpoints included a decrease in insulin, −4.07 (95% CI, −6.90 to −1.24, *p*=0.003) in the HIPE group, and increased +5.64 (95% CI, 2.79 to 8.50) in the CTRL group. Also, body fat decreased in the HIPE group −1.47 % (95% CI, −2.57 to −0.38, *p*<0.001), and the PLUS group −1.88 % (95% CI, −2.98 to −0.79, *p*=0.022), and cardiometabolic z-score decreased in the HIPE group −0.65 % (95% CI, −1.25 to −0.06, *p*=0.046). Of note, all groups showed improvements in skeletal muscle mass (*p*<0.05) after the 6-month intervention with the exception of the PLUS group. Mean arterial pressure and heart rate decreased in the HIPE group (*p*<0.01). With regards to the effects of the exercise interventions on physical fitness components, CRF (laps), upper-limb strength, and lower-limb strength improved in the HIPE and PLUS intervention groups (*p*<0.001). Similarly, the HIPE group showed improvements in speed-agility after the 6-months intervention (*p*<0.05). The findings from the intention-to-treat analysis closely matched the per-protocol analysis except in speed-agility and lower-limb absolute strength outcomes (**eTable 6 in the Supplement**).

*****Table 2 near here*****

Table 3 shows the effects of the interventions on blood cardiometabolic parameters. At follow-up, participants in the HIPE group showed reduced total cholesterol −9.06 mg/dl (95% CI, −15.88 to −2.25, *p*<0.001) and insulin −4.07 mg/dl (95% CI, −6.90 to −1.24, *p*=0.014) (*p*=0.007 *vs* CTRL group). Also, cardiometabolic z-score levels decreased in the HIPE group (*p*<0.05), whereas ATS/ALT ratio decreased in the LIPE group (*p*<0.05). Similarly, triglyceride levels decreased in the PLUS group −21.14 mg/dl (95% CI, −38.37 to −3.91, *p*=0.009) (*p*=0.008 *vs* CTRL group).

Of note, glucose, insulin, ALT and HOMA-IR levels significantly increased in the CTRL group ($p<0.01$). No significant between- or within-group differences were observed in the HDL-C, LDL-C, AST, GGT, TG/HDL ratio or ferritin levels. In both analyses the results of the intention-to-treat and per-protocol were different (**eTable 7 in the Supplement**).

*****Table 3 near here*****

Details of adverse events are listed in (**eTable 8 in the Supplement**). Overall, unrelated adverse events occurred in 2 of 21 HIPE group participants (10%), in 1 of 23 PLUS group participants (4%), and in 1 of 23 CTRL (4%). Almost all related adverse events involved musculoskeletal injury or discomfort, upper/lower body injury (minor injury or falls) such as bruises (contusions). No medical adverse events were related to the intervention.

Discussion

The main finding from this randomized controlled exercise trial involving adolescents with overweight/obesity is that both high intensity and PLUS PE exercise interventions are associated with significant reductions in intrahepatic fat measured as CAP levels. Intention-to-treat analysis also showed greater CAP decreases in the HIPE and PLUS group than in the standard physical education lessons (CTRL) group. Furthermore, the cumulative benefit across all cardiometabolic outcomes was greater in the HIPE and PLUS groups than in the LIPE and/or CTRL alone groups. To our knowledge, this is the first randomized trial involving adolescents with excess of adiposity to compare the liver fat content and cardiometabolic markers, as well as fitness health related components, after 6 months of four different school-based PE training regimens.

Accumulating clinical and epidemiological evidence shows that regular exercise training with or without diet can independently impact liver metabolism (19). According to the mean changes observed in primary endpoints, the CAP significantly increased in the CTRL group and decreased in the HIPE and PLUS groups. The modest decreases in liver fat in the HEPAFIT trial are comparable to those reported by Larson-Meyer et al. (20), who examined the effects of 25% caloric restriction (CR) of baseline energy requirements against, 12.5% CR + 12.5% increase in total energy expenditure through structured exercise (CR+EX), low-calorie diet (LCD) until 15% reduction in body weight, and a control intervention with weight maintenance by a healthy diet, during a 6-month period. That study showed a decreased treatment effect for liver lipid levels from baseline in the CR, CR+EX, and LCD groups ($p<0.01$). No differences in the response between treatments were observed, but the response in each treatment was significantly different ($p<0.05$) from that in the control group. This finding is consistent with two recently published systematic reviews and meta-analyses (4-7) showing that exercise significantly reduces liver fat content in youth. In the same line, a study by Medrano et al. (20) revealed that both aerobic and resistance supervised exercise training at vigorous and moderate-to-vigorous intensities, in sessions of ≥60 min and a frequency ≥3 sessions per week, are more effective at reducing hepatic fat content in children and adolescents. These results support the American Academy of Pediatrics (21) recommendation of physical activity programs consisting of moderate-vigorous intensity aerobic physical activity combined with resistance training in children/adolescents aged 6–17 years. These guidelines recommend 60 min of aerobic and muscle strengthening supervised-exercise training at moderate and vigorous intensity every day or at least 3 days per week in this population. Exercise is a powerful regulator of systemic insulin sensitivity, which exerts its effects on hepatic metabolism at multiple levels, such as reducing intrahepatic fat by limiting hepatic exposure to substrates used to synthesize lipids, and changes to the transcriptional machinery to synthesize the lipids (22-25). In this sense, our results indicate that exercise training might be an effective way to counteract the development of NAFLD.

With regards to anthropometric and body composition parameters, we found that the HIPE and PLUS groups showed a significant decrease in body fat percentage. This is consistent with a study by van der Heijden et al. (26), who reported that a 12-week aerobic exercise program (4 × 30 min/week at 70% of peak oxygen consumption) without calorie restriction was associated with reductions in body fat (−3%) and visceral adipose tissue (−9%) in a mixed sample Hispanic boys and girls with obesity (*n*=15).

Similarly, Sigal et al. (27) reported that a 22-week program of aerobic training, resistance training, combined aerobic and resistance training, or non-exercising control, all with a daily energy deficit of 250 kcal (4 × 20 to 45 min/week) without weight loss, reduced body fat among previously inactive postpubertal adolescents aged 14 to 18 years with BMI at or above the 95th percentile for age and sex or at or above the 85th percentile plus an additional diabetes mellitus or cardiovascular risk factor. Attenuating increases in body fat during adolescence could confer important cardiometabolic protection because each additional year of abdominal obesity is associated with a 4% greater risk of developing diabetes mellitus, independent of the degree of abdominal adiposity (28). According to Johnson et al. (29), these changes in adolescents may help by reducing the flow of fatty acids to the liver and, consequently, decreasing the hepatic fat deposit, thus preventing the development of NAFLD at young ages.

We found a decrease of –2.82 mmHg for resting mean blood pressure, and –3.21 mmHg for diastolic blood pressure in the participants in the HIPE plus, which agrees with the observations of Wong et al. (30), who found a significant reduction in mean or diastolic blood pressure in their study of 24 adolescents with obesity who underwent a 12-week intervention involving combined training. A meta-analysis on this topic concluded that there was little evidence that exercise reduces resting blood pressure in normotensive adolescents, whereas aerobic exercise consistently reduces resting blood pressure in hypertensive adolescents (31).

In terms of the effects of exercise interventions on fitness components, CRF, upper and lower-limb strength, increased in the HIPE and PLUS groups. These results are consistent with previous studies (32) on adolescents with obesity (33), and show the need to implement interventions involving aerobic and resistance training as a stimulus to enable the early development of cardiovascular and musculoskeletal adaptations similar to those generated by traditional continuous aerobic exercise (9).

Additionally, the interventions implemented in our study have beneficial effects on several biochemical parameters related to cardiometabolic risk. Compared with the baseline values, triglycerides levels decreased significantly in the PLUS group, whereas insulin levels and total cholesterol decreased significantly in HIPE group. Gibson et al. (34) previously conducted a randomized controlled trial to examine the role of regular exercise by itself (i.e., no calorie restriction) in reducing obesity-related risk factors in previously sedentary adolescents with overweight/obesity, in which the exercise intervention resulted in an increase on insulin sensitivity and oxidative metabolic activity, and reduced hepatic fat and cardiometabolic risk in a young population. These results are consistent with several studies such as by Mezghanni et al. (35) on teenage girls with obesity, which showed that high-intensity aerobic training improves cardiometabolic risk such as insulin levels and lipid profile. Lee et al. (36) found that three exercise modalities (i.e., aerobic, resistance and concurrent exercise) are beneficial in reducing total fat and skeletal muscle lipid and improving risk factors for type 2 diabetes mellitus (insulin sensitivity and oral glucose tolerance test 2-hour glucose level) in adolescents with overweight and obesity. Our results also showed that cardiometabolic z-score in the HIPE group decreased when compared with the CTRL group (p=0.019), whereas the LIPE group showed a significant reduction in the AST/ALT ratio. The effectiveness of regular exercise in reducing cardiometabolic parameters in adolescents with overweight/obesity has been addressed in a meta-analysis, demonstrating beneficial effects in reducing blood lipid parameters (7-9). Other studies (37,38) have shown significant decreases in BMI, but not in reducing the levels of aminotransferases.

No significant between-group differences were observed in the levels in any of the three primary liver enzymes with exercise training. A recent systematic review by Smart et al. (40) in adults (>18 years) with overweight/obesity and/or fatty liver disease (21 trials, n=1530 participants) showed that an exercise intervention, with or without dietary intervention, did not significantly change the liver enzymes ALT, AST and GGT. Our study confirms the previous findings that liver enzymes have limited sensitivity in predicting metabolic dysregulation, the hepatic manifestation of which is NAFLD (40). And it appears that liver enzymes are inaccurate markers for assessing change in liver function after exercise training in early ages. In addition, we found no changes in cardiometabolic z-score or ferritin levels.

This study has some limitations and strengths that should be noted. Since the research was conducted on a population of adolescents with excess adiposity, the findings may not be generalizable to non-obese subjects. The gold standard in the diagnosis of NAFLD is liver biopsy, but this procedure is difficult to justify in adolescents with obesity considered “healthy”. This was not possible as it was contraindicated in some subjects due to its invasiveness, cost, and the possibility of causing potentially serious complications in the participants. Instead, we used transient elastography, a highly sensitive and non-invasive technique that allowed us to accurately evaluate hepatic lipid accumulation. Finally, physical activity levels with respect to the time, intensity and duration were not determined. By contrast, the main strength of our study is that it is the first to compare the differential expression of several cytokine levels measured using an antibody-based membrane array after 6 months of different physical activity regimens.

Additionally, highly standardized procedures have been developed within the HEPAFIT study to avoid measurement bias.

In summary, the HEPAFIT study showed that implementing a 6-month school-based PE exercise program for adolescents with overweight/obesity decreases hepatic fat storage and significantly reduces cardiometabolic markers. Adolescence is a critical period with respect to adopting health maintenance behaviors. Supervised physical exercise programs in the school setting could help improve metabolism and liver fat deposition, thus preventing the development of NAFLD in adolescents with excess of adiposity.

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Data available on request from the authors

DATA AVAILABILITY STATEMENT

Data availability statement:

Data available on request from the authors.

Legend to figures

Figure 1. HEPAFIT Study Flow Diagram

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Table 1. Baseline characteristics of participants.

Characteristics	CTRL group (*n* = 30)		HIPE group (*n* = 30)		LIPE group (*n* = 30)		PLUS group (*n* = 30)		All participants (*n* = 120)		Participants who completed the study (*n* = 99)	
Sex												
Boys/girls, n	21/9		18/12		23/7		20/10		82/38		68/31	
Anthropometric outcomes												
Chronological age, years	13.36	1.62	13.13	1.83	12.73	1.50	13.80	1.47	13.25	1.64	13,31	1,67
Peak height velocity	0.79	1.27	0.79	1.20	0.50	1.13	1.07	1.37	0.79	1.25	0,84	1,24
Body mass, kg	23.99	3.19	23.91	3.57	23.66	4.79	23.76	3.56	23.83	3.78	23,59	2,97
BMI, z-score	1.36	0.38	1.48	0.49	1.35	0.32	1.34	0.32	1.38	0.34	24,08	3,91
Waist circumference, cm	77.86	7.49	76.16	9.39	74.41	6.54	74.71	7.18	75.79	7.75	76,29	7,57
Body composition												
Body fat, %	39.79	4.48	39.84	4.51	39.83	4.64	39.89	4.29	39.84	4.42	39,83	4,63
Skeletal muscle mass, kg	17.66	3.81	15.90	3.74	16.29	3.37	17.55	2.73	16.85	3.48	17,07	3,60
Visceral adipose tissue, cm^3	361.03	110.33	337.78	106.79	328.10	93.43	339.43	95.53	341.59	101.21	346,03	103,94
Transient elastography												
CAP, dB/m	239.90	47.02	230.05	41.66	220.53	43.80	213.23	28.64	225.93	41.60	226,59	42,37
Liver stiffness, kPa	3.75	0.73	3.84	0.99	3.79	0.78	3.83	0.74	3.80	0.81	3,84	0,83
Blood pressure												
Systolic blood pressure, mmHg	108.13	8.65	108.57	10.49	107.50	7.25	111.57	7.91	108.94	8.69	108,63	8,91
Diastolic blood pressure, mmHg	66.13	6.66	68.73	6.57	66.30	4.65	65.53	5.86	66.68	6.04	66,04	5,81
Mean blood pressure, mmHg	79.99	6.62	81.89	6.94	79.90	4.46	80.72	5.23	80.63	5.88	80,10	5,83
Heart rate, bpm	80.47	16.76	77.83	9.26	79.57	9.93	80.00	11.47	79.46	12.14	78,78	11,66
Physical fitness components												
CRF, laps	16.17	8.12	18.25	11.29	18.43	11.42	23.20	13.42	19.01	11.38	19,49	11,74
Upper-limb absolute strength, kg	20.95	5.11	20.22	5.94	19.69	5.18	22.75	4.64	20.90	5.30	21,12	5,50
Lower-limb absolute strength, cm	120.44	23.67	116.57	18.50	112.55	21.15	123.52	21.76	118.27	21.48	118,36	22,98
Speed-agility, sec[b]	14.44	1.20	14.62	1.54	14.23	1.15	13.84	1.31	14.28	1.33	9,49	0,71
Biochemical profile												
Total cholesterol, mg/dl	155,69	24.11	159.58	19.35	165.47	30.54	154.58	24.82	158.83	25.07	159.08	25,77
HDL-C, mg/dl	40,98	7.89	44.19	8.36	44.28	7.59	44.80	8.93	43.56	8.25	43.44	8,59
LDL-C, mg/dl	99,21	20.00	101.79	17.54	108.41	27.47	96.85	20.99	101.57	21.97	101.60	22,84
Triglycerides, mg/dl	128,20	50.22	122.20	51.82	136.47	54.93	114.94	50.65	125.45	51.89	125.03	51,34
Glucose, mg/dl	96,53	17.85	98.53	14.06	99.77	14.64	88.90	15.44	95.93	15.94	95.97	15,75
Insulin, uU/ml	20,22	11.50	22.25	16.36	14.21	5.13	13.89	4.72	17.64	11.09	17.68	11,83
Cardiometabolic z-score	0,61	2.72	0.39	3.38	-0.64	2.21	-0.36	2.09	0.01	2.66	0.01	2,72
C-peptide, ng/ml	2,55	0.91	2.64	1.16	2.36	0.79	2.24	0.76	2.45	0.92	2.47	0,96
Alanine aminotransferase, U/l	23,36	26.04	17.98	7.33	16.38	4.93	15.88	5.15	18.40	14.13	18.98	15,29
Aspartate aminotransferase, U/l	22,98	10.70	21.65	6.15	20.84	3.11	19.78	4.04	21.31	6.70	21.18	6,69
γ-glutamyl transpeptidase, U/l	20,57	14.52	19.81	15.34	20.32	14.67	16.11	6.18	19.20	13.18	19.41	14,01
Creatinine, mg/dl	0,61	0.08	0.60	0.13	0.58	0.11	0.64	0.10	0.61	0.11	0.61	0,11
Ureic nitrogen, mg/dl	10,92	2.66	10.09	2.66	10.90	2.48	10.37	2.60	10.57	2.59	10.70	2,67
Serum iron, ug/dl	87,83	23.02	86.52	31.33	90.82	30.67	105.57	32.39	92.68	30.19	94.28	28,86
Iron capacity, ug/dl	258,88	43.95	281.12	56.63	260.50	49.29	246.26	48.64	261.69	50.78	258.65	49,33
Iron fixation capacity, ug/dl	346,77	38.43	367.82	43.52	351.33	44.10	351.87	37.40	354.45	41.24	352.95	41,01
Ferritin, ng/ml	80,22	57.37	65.46	57.51	66.58	43.39	71.62	33.76	70.97	48.78	71.80	49,94
TG/HDL-C ratio	3,40	1.80	3.01	1.77	3.24	1.53	2.76	1.51	3.10	1.66	3.12	1,70
AST/ALT ratio	1,29	0.43	1.39	0.89	1.35	0.33	1.32	0.34	1.34	0.54	1.29	0,36
Insulin resistance (HOMA-IR)	4,70	2.44	5.55	4.91	3.47	1.25	3.07	1.20	4.20	3.00	4.21	3,23
Dietary habits												
KIDMED score, points	7.40	1.81	7.93 (2.70)		6.50	2.39	6.93	2.32	7.19	2.36	7.12	2.27
KIDMED score (8–12 points), %[a]	15 (50.0)		19 (36.7)		18 (40.0)		17 (43.5)		69 (42.5)		54 (56.8)	

Results are shown as mean and standard deviation (SD) for continuous variables, and percentages for categorical variables[a]. Cardiometabolic z-score was calculated as the sum of the age-sex standardized scores of waist circumference, triglycerides (TG), systolic blood pressure, HDL-C, and fasting glucose. Upper-limb absolute strength was measured by the handgrip strength test. Lower-limb absolute strength was measured by the standing long-jump test. Speed-agility was measured by the 4×10-meter shuttle run test. Cardiorespiratory fitness (CRF) was measured by a 20-meter shuttle run test (20m SRT). [b]Lower values indicate better performance.

Table 2. Change in controlled attenuation parameter, body composition and fitness variables

Variable	From Baseline to 6 months, least square mean (95% confidence interval)						
	Within-Group Changes			Between-Group Comparations vs. Control Group Changes			η² (P value for interaction)
Primary endpoint							
Controlled attenuation parameter, dB/m							
CTRL group	6.18	−6.34	18.72				
HIPE group	−13.81	−26.37	−1.24	19.99	2.01	37.98	0.087 (0.015)
LIPE group	−8.21	−20.60	4.18	14.39	-3.30	32.09	
PLUS group	−22.41	−34.82	−10.00	28.60	11.05	46.14	
Secondary endpoints							
Body mass, kg							
CTRL group	0.46	−0.33	1.27				
HIPE group	0.10	−0.69	0.91	0.36	−0.77	1.495	0.042 (0.103)
LIPE group	0.96	0.15	1.76	−0.49	−1.62	0.64	
PLUS group	−0.43	−1.23	0.36	0.90	−0.23	2.03	
Body mass index z-score							
CTRL group	0.20	0.03	0.38				
HIPE group	0.07	−0.10	0.24	0.13	−0.10	0.38	0.033 (0.282)
LIPE group	0.24	0.06	0.41	−0.03	−0.27	0.21	
PLUS group	0.03	−0.13	0.21	0.17	−0.07	0.41	
Waist circumference, cm							
CTRL group	1.72	0.30	3.14				
HIPE group	0.89	−0.53	2.32	0.83	−1.19	2.86	0.030 (0.317)
LIPE group	2.71	1.30	4.13	−0.99	−3.00	1.01	
PLUS group	1.31	−0.10	2.74	0.40	−1.59	2.41	
Body fat, %							
CTRL group	−0.69	−1.78	0.39				
HIPE group	−1.47	−2.57	−0.38	0.78	−0.76	2.32	0.049 (0.121)
LIPE group	−0.15	−1.25	0.93	−0.54	−2.08	1.00	
PLUS group	−1.88	−2.98	−0.79	1.19	−0.35	2.73	
Visceral adipose tissue, cm³							
CTRL group	−3.88	−33.29	25.53				
HIPE group	−4.90	−34.45	24.64	1.02	−40.69	42.74	0.025 (0.408)
LIPE group	13.80	−15.61	43.22	−17.68	−59.28	23.91	
PLUS group	−22.0	−51.54	7.41	18.18	−23.44	59.81	
Skeletal muscle mass, kg							
CTRL group	1.54	0.88	2.20				
HIPE group	1.38	0.72	2.04	0.16	−0.76	1.09	0.060 (0.068)
LIPE group	1.72	1.05	2.38	−0.17	−1.11	0.77	
PLUS group	0.55	−0.10	1.21	0.99	0.06	1.93	
Systolic blood pressure, mmHg							
CTRL group	−3.04	−7.00	0.91				
HIPE group	−2.42	−6.34	1.49	−0.62	−6.21	4.96	0.004 (0.919)
LIPE group	−1.31	−5.22	2.59	−1.73	−7.30	3.84	
PLUS group	−2.99	−6.92	0.92	−0.04	−5.65	5.56	
Diastolic blood pressure, mmHg							
CTRL group	−2.53	−5.04	−0.03				
HIPE group	−3.21	−5.70	−0.72	0.67	−2.84	4.20	0.014 (0.661)
LIPE group	−1.46	−3.99	1.06	−1.07	−4.66	2.51	
PLUS group	−1.25	−3.74	1.24	−1.28	−4.81	2.24	
Mean arterial pressure, mmHg							
CTRL group	−2.49	−4.91	−0.07				
HIPE group	−2.82	−5.25	−0.40	0.33	−3.09	3.76	0.006 (0.880)
LIPE group	−1.63	−4.07	0.80	−0.85	−4.29	2.57	
PLUS group	−1.74	−4.17	0.68	−0.75	−4.18	2.67	
Heart rate, bpm							
CTRL group	−5.96	−10.27	−1.66				0.021 (0.499)
HIPE group	−7.74	−11.75	−3.73	1.78	−4.10	7.65	

LIPE group	−4.77	−8.85	−0.69	−1.19	−7.13	4.74	
PLUS group	−3.49	−7.50	0.51	−2.47	−8.35	3.40	
CRF, laps							
CTRL group	5.30	1.18	9.41				
HIPE group	7.64	3.57	11.71	−2.34	−8.19	3.51	0.032
LIPE group	2.16	−1.87	6.20	3.13	−2.64	8.91	(0.287)
PLUS group	6.07	2.03	10.11	−0.77	−6.55	5.00	
Upper-limb absolute strength, kg							
CTRL group	1.04	−0.02	2.11				
HIPE group	2.29	1.23	3.34	−1.25	−2.75	0.25	0.036
LIPE group	0.84	−0.21	1.90	0.19	−1.31	1.70	(0.232)
PLUS group	1.44	0.38	2.50	−0.40	−1.92	1.11	
Lower-limb absolute strength, cm							
CTRL group	3.18	−3.00	9.38				
HIPE group	9.96	3.80	16.11	−6.77	−15.47	1.93	0.034
LIPE group	2.50	−3.65	8.65	0.68	−8.06	9.44	(0.266)
PLUS group	7.70	1.49	13.90	−4.51	−13.35	4.32	
Speed-agility, sec							
CTRL group	0.32	−0.19	0.83				
HIPE group	0.55	0.04	1.06	−0.23	−0.96	0.49	0.033
LIPE group	−0.10	−0.61	0.39	0.42	−0.29	1.14	(0.269)
PLUS group	0.05	−0.45	0.55	0.26	−0.45	0.98	

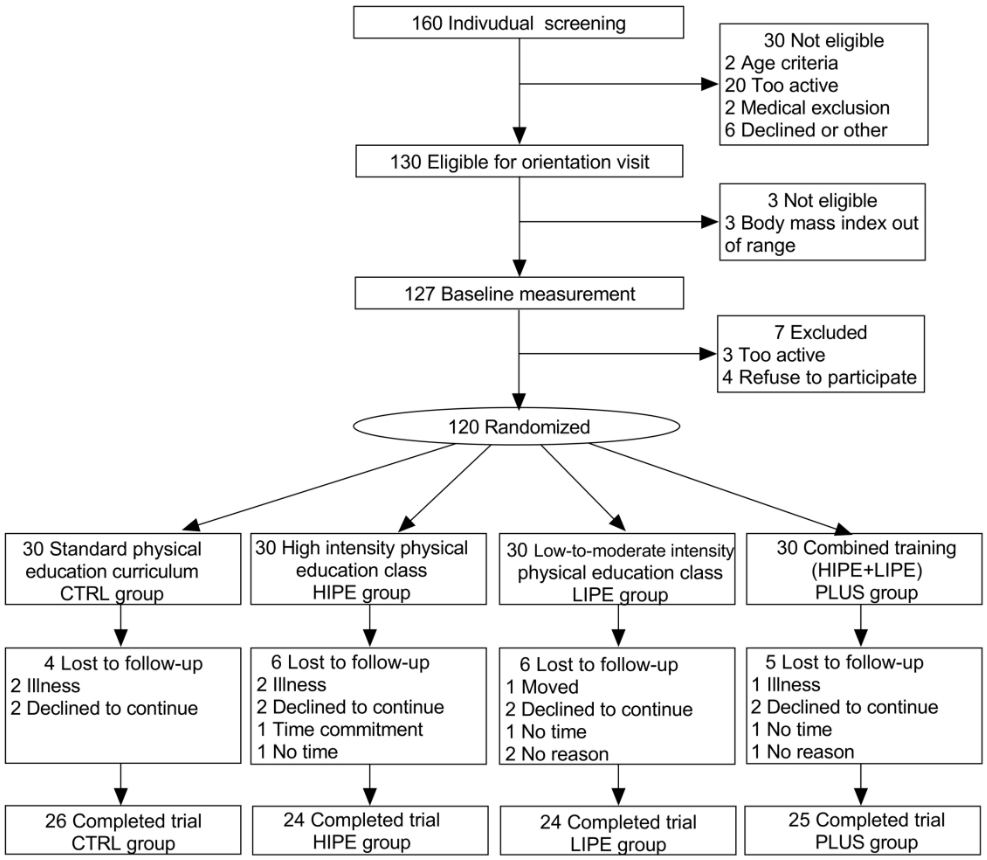
Values are least square mean differences and 95% confidence intervals as derived from ANCOVA models, adjusted baseline measures of each variable. Mean arterial pressure was estimated as the product of the average diastolic pressure plus one-third of the average pulse pressure. Eta partial squared (η^2) was assessed by η^2 obtained from the ANCOVA with small ($\eta^2 = 0.01$), medium ($\eta^2 = 0.06$), and large ($\eta^2 = 0.14$) effects defined according to Cohen.

Table 3. Change in cardiometabolic parameters in blood

Variable	From Baseline to 6 months, least square mean (95% confidence interval)						
	Within-Group Changes			**Between-Group Comparations vs. Control Group Changes**			**η^2 (P value for interaction)**
Total cholesterol, mg/dl							
CTRL group	−0.23	−7.06	6.58				
HIPE group	−9.06	−15.88	−2.25	8.83	−0.78	18.44	0.056 (0.082)
LIPE group	3.08	−3.71	9.88	−3.32	−12.96	6.31	
PLUS group	−0.34	−7.20	6.52	0.10	−9.62	9.83	
HDL-C, mg/dl							
CTRL group	0.37	−1.79	2.53				
HIPE group	1.27	−0.91	3.45	−0.89	−3.99	2.19	0.006 (0.887)
LIPE group	0.13	−2.02	2.30	0.23	−2.82	3.29	
PLUS group	0.87	−1.28	3.03	−0.50	−3.56	2.55	
LDL-C, mg/dl							
CTRL group	1.81	−4.31	7.93				
HIPE group	−0.40	−6.50	5.69	2.21	−6.40	10.83	0.022 (0.457)
LIPE group	6.49	0.40	12.59	−4.68	−13.33	3.95	
PLUS group	2.53	−3.63	8.70	−0.72	−9.49	8.04	
Triglycerides, mg/dl							
CTRL group	16.74	−0.47	33.97				
HIPE group	−11.01	−28.15	6.12	27.76	3.43	52.09	0.119 (0.002)
LIPE group	18.41	1.27	35.55	−1.66	−25.92	22.59	
PLUS group	−21.14	−38.37	−3.91	37.89	13.39	62.39	
Glucose, mg/dl							
CTRL group	12.78	7.48	18.08				
HIPE group	9.69	4.53	14.86	3.09	−4.32	10.50	0.011 (0.737)
LIPE group	11.43	6.24	16.61	1.35	−6.13	8.83	
PLUS group	13.56	8.35	18.76	−0.77	−8.30	6.75	
Insulin, uU/ml							
CTRL group	5.64	2.79	8.50				
HIPE group	−4.07	−6.90	−1.24	9.72	5.66	13.78	0.166 (<0.001)
LIPE group	1.17	−1.69	4.05	4.47	0.34	8.59	
PLUS group	0.56	−2.27	3.41	5.08	1.11	9.05	
Cardiometabolic z-score, SD							
CTRL group	0.35	−0.23	0.94				
HIPE group	−0.65	−1.25	−0.06	1.01	0.16	1.85	0.082 (0.019)
LIPE group	0.54	−0.04	1.14	−0.19	−1.03	0.64	
PLUS group	−0.24	−0.84	0.35	0.60	−0.23	1.43	
Hemoglobin A1C, %							
CTRL group	0.29	0.17	0.40				
HIPE group	0.21	0.10	0.33	0.07	-0.08	0.23	0.029 (0.339)
LIPE group	0.26	0.15	0.38	0.02	-0.13	0.18	
PLUS group	0.15	0.04	0.26	0.13	-0.02	0.30	
C-peptide, ng/ml							
CTRL group	0.73	0.36					
HIPE group	0.15	−0.21	0.52	0.58	0.06	1.10	0.086 (0.015)
LIPE group	0.75	0.38	1.12	−0.01	−0.54	0.50	
PLUS group	0.11	−0.25	0.48	0.62	0.10	1.14	
Alanine aminotransferase, U/l							
CTRL group	3.56	0.23	6.90				
HIPE group	−2.69	−6.06	0.68	6.26	1.47	11.04	0.050 (0.118)
LIPE group	2.58	−0.73	5.90	0.98	−3.72	5.68	
PLUS group	−2.14	−5.47	1.18	5.71	1.02	10.41	
Aspartate aminotransferase, U/l							
CTRL group	1.63	−0.42	3.69				
HIPE group	−1.15	−3.22	0.90	2.79	−0.14	5.73	0.086 (0.016)
LIPE group	0.06	−1.98	2.11	1.57	−1.33	4.48	
PLUS group	−1.65	−3.70	0.39	3.29	0.39	6.19	
γ-glutamyl transpeptidase, U/l							

CTRL group	0.60	−2.65	3.86				
HIPE group	−0.51	−3.75	2.72	1.12	−3.48	5.73	0.037
LIPE group	1.95	−1.28	5.19	−1.34	−5.94	3.25	(0.227)
PLUS group	−2.73	−5.97	0.50	3.33	−1.26	7.94	
Ferritin, ng/ml							
CTRL group	1.87	−11.01	14.77				
HIPE group	0.11	−12.84	13.06	1.76	−16.50	20.04	0.005
LIPE group	0.58	−12.32	13.50	1.29	−16.96	19.54	(0.892)
PLUS group	−4.86	−17.76	8.04	6.74	−11.50	24.98	
TG/HDL ratio							
CTRL group	0.56	0.02	1.09				
HIPE group	−0.26	−0.80	0.26	0.83	0.06	1.59	0.083
LIPE group	0.48	−0.05	1.01	0.08	−0.67	0.83	(0.018)
PLUS group	−0.42	−0.96	0.10	0.99	0.23	1.74	
AST/ALT ratio							
CTRL group	−0.05	−0.18	0.08				
HIPE group	0.08	−0.04	0.22	−0.13	−0.32	0.05	0.063
LIPE group	−0.16	−0.30	−0.03	0.11	−0.07	0.30	(0.056)
PLUS group	0.01	−0.11	0.15	−0.07	−0.26	0.11	
Insulin resistance (HOMA-IR)							
CTRL group	2.33	1.48	3.18				
HIPE group	−0.60	−1.44	0.23	2.93	1.73	4.13	0.171
LIPE group	0.52	−0.32	1.38	1.80	0.57	3.03	(<0.001)
PLUS group	0.64	−0.19	1.48	1.69	0.51	2.87	

Values are least square mean differences and 95% confidence intervals as derived from ANCOVA models, adjusted for baseline measures of each variable. Eta partial squared (η^2) was assessed by η^2 obtained from the ANCOVA with small ($\eta^2 = 0.01$), medium ($\eta^2 = 0.06$), and large ($\eta^2 = 0.14$) effects defined according to Cohen.



Flowchart hepafit trial

217x190mm (300 x 300 DPI)

Supplementary Online Content

González-Ruíz K, et al. Effects of Different Doses of Exercise on Hepatic Fat and Cardiovascular Health in Adolescents with Excess of Adiposity: A Randomized Controlled Trial (The HEPAFIT Study).

eMethods.

1. Full interventions details.

eTable 1. Example of playground games ranked by oxygen consumption and energy expenditure with accelerometer counts and heart rate data for the MOVI program/the HEPAFIT Study

eTable 2. Example of playground games by oxygen consumption, metabolic equivalents and energy expenditure with heart rate data for the HEPAFIT Study

eTable 3. Example of resistance training by for the HEPAFIT Study

eTable 4. Listing of cardiovascular biomarkers and metabolic proteins detected from the targeted panel

eTable 5. Exercise prescription in detail and measurements obtained during the intervention period

eTable 6. Change in controlled attenuation parameter, body composition and fitness variables (per-protocol analysis)

eTable 7. Change in cardiometabolic parameters in blood (per-protocol analysis)

eTable 8. Adverse events by study group related and unrelated to participation in the HEPAFIT Study

This supplementary material has been provided by the authors to give readers additional information about their work.

1. Full interventions details:

Participants were randomly assigned to the following groups[1]:

1. Standard physical education curriculum (Control group). These subjects were not carry out any intervention different from the physical education class prescribed by the Colombian education legislation [Legislation 115 of 1994 – decree 1860 of 1994][2], which is recognized as a fundamental area and, is consequently obligatory in all educational institutions in the country. This session includes 60 min per week of physical activity at low-to-moderate intensity. However, during this period, education was offered in lifestyles and hygienic–dietary management at no cost to participants, and nutritional counselling was offered as well[3].

2. High-intensity training plus physical education class (HIPE group). Standard physical education curriculum plus "the physical activity intervention on the obesity of schoolchildren (*in Spanish* MOVI-2 program)", which is based on a socio-ecological model and, targets the school, parents, teachers and children, with a focus on increasing physical activity[4]. MOVI-2 includes basic sports games, traditional games, and other outdoor activities such as running, gymkhanas (which consist of performing different types of competitions where contestants overcome several tests with obstacles that make the task difficult), lifting, pushing, wrestling or hauling (http://www.movidavida.org/), for 60 min sessions three times per week for 6 months with an energy expenditure goal of 300 to 500 kcal/session at 75–85% HRmax. For example: prescribed exercise energy expenditure during month 1= 300 kcal session^{-1}, exercise energy expenditure in Conveyors game (1) = 9.2 kcal min^{-1}, exercise duration = 100 kcal session^{-1} divided by 9.2 kcal min^{-1} = 11 min session^{-1}; The 4 corners game (2) = 10.8 kcal min^{-1}, exercise duration = 100 kcal session^{-1} divided by 10.8 kcal min^{-1} = 9 min session^{-1}; and Stealing handball game (3) = 9.4 kcal min^{-1}, exercise duration = 100 kcal session^{-1} divided by 9.4 kcal min^{-1} = 10 min session^{-1}. All playground games were preceded by a 5-min warm up with endurance or resistance games (i.e. The fastest, The clock, Iceman, Kangaroo race, etc). The duration and intensity of all playground games sessions will be verified by a downloadable HR monitor (RS 400; Polar Electro Inc., Woodbury, NY) to achieve the prescribed target HR.

All activities were implemented by monitors with technical qualifications in physical activity and sports, or by sport science graduates, specifically engaged and adequately trained for the program. This protocol is applicable for school-aged boys and girls, independent of the level of skill or their physical aptitude, and is a health-oriented program aimed at balanced physical and psychological development. The MOVI program uses attractive alternative materials not often used in the subjects´ daily lives or in the standard physical education classes. It is accessible, given that it occurs at the end of the classroom activities in the school's facilities or nearby; it is free of charge and does not require the provision of any specific equipment. In its first edition, the MOVI program showed a moderate effect on reducing the adiposity and improving the lipid profile, but did not significantly improve global cardiometabolic risk or lowered insulinaemia[3]. The second edition (MOVI-2) increased the duration and intensity of the sessions and further enhanced the development of muscle strength. Likewise, analyses showed reduced adiposity in both sexes, and an improvement in the girls' cardiovascular risk profile (LDL-c and insulin) was observed[5].

The characteristics of the MOVI-2 program's sessions that were used in this work are the following:

Frequency	3-days per week excluding holidays and vacation periods
Duration	60 min, structured into 5 min of warm-up, 45 min dedicated to the main training curriculum, and 10 min dedicated to returning to a rest state
Intensity	Each of the sessions were work on activities based on health-related physical fitness (cardiovascular/muscle endurance and flexibility) at an intensity of between 8.0 and 12 metabolic equivalents (METs). All the games have been carried out previously with schoolchildren during sessions of the MOVI-2 program while indirect calorimetry (through a portable gas analyzer), cardiac frequency and accelerometry were evaluated for each game. In addition, we have previously monitored the implementation of all the games with overweight/obese adolescents from Bogotá, Colombia (See eTable 1-2).

3. Low-to-moderate intensity training plus physical education class (LIPE group): Standard physical education curriculum plus endurance and resistance games from the MOVI-2 programme will be used with a goal to achieve expenditure of 1500 kcal/week (60 min session, three times per week for 6 months for 300 to 500 kcal energy expenditure per session at 55–75% maximum heart rate (HRmax), as shown by the following:

Frequency	3-days per week excluding holidays and vacation periods
Duration	60 min, structured into 5 min of warm-up, 45 min dedicated to the main training curriculum, and 10 min dedicated to returning to a rest state
Intensity	Each of the sessions were work on activities based on health-related physical fitness (cardiovascular/muscle endurance and flexibility) at an intensity of between 4.0 and 7.0 MET (See eTable 1-2).

4. *Combined or plus group:* The combined group performed the 30 min aerobic training program and the 30 min resistance training (3 days), that involve training program used in both HIPE and LIPE. The combined energy expenditure of standard PE plus the additional exercise intervention did not exceed an average of 1500 kcal/week. In ended each session (10–15 min), all participants were performed resistance training or "fitness fusion workout" (~ 1 min per exercise), with dumbbells (lateral raise, shoulder press, biceps curl, triceps press, front raise, squat, lunge) or by using one's own body weight as resistance (planks, sit-ups and abdominal crunches).

Participants were asked to rest for ~ 1 min between sets and were instructed on proper breathing techniques. The research staff monitored attendance and appropriate progression by reviewing sign-in sheets, exercise plan cards and completion of exercise logs. All the study participants receive a hygienic–dietary management plan that includes educational and nutritional support throughout the study.

Training intensity was assessed via mean HR measured in the HIPE, LIPE or PLUS groups in a sub-sample (25-30 participants). Energy expenditure was estimated during exercise via indirect calorimetry using a Cosmed K-5 portable metabolic system (Rome, Italy), assuming a non-protein respiratory exchange ratio. This was assessed in 30% of participants and helped to maintain compliance, as well as to provide a detailed description of each playground games session. All study participants received a hygiene–dietary management plan that included educational and nutritional support throughout the study. HR monitors (Polar FS1; Polar Electro Oy) were used when adjusting workloads to achieve target HR. Compliance with the exercise protocol, an essential element of an efficacy study, was defined as successfully completing >70 % of scheduled exercise sessions, maintaining the target exercise HR ± 4 beats·min−1 for the prescribed duration of the playground games session. Details of the exercise programs are shown in (eTable 1 and eTable 2 in the Supplement).

eTable 1. Example of playground games ranked by oxygen consumption and energy expenditure with accelerometer counts and heart rate data for the MOVI program/the HEPAFIT Study

Games type	Playground for group	VO_2 (mL/kg/min)	Energy expenditure kcal kg/h	Accelerometer (counts/min)	Heart rate (beats/min)
Endurance games					
The fastest	Warm-up	17.9 (5.8)	5.2 (0.8)	868 (312)	150 (10)
The clock		18.8 (8.8)	5.5 (2.6)	1772 (380)	147 (10)
Iceman		19.0 (7.9)	5.5 (1.1)	2263 (700)	151 (24)
Toll	LIPE group or Combined group	20.3 (5.6)	5.9 (1.4)	1794 (457)	171 (21)
Shooting		20.8 (5.6)	6.1 (1.5)	2690 (773)	162 (16)
The broom		20.9 (4.5)	6.1 (0.7)	910 (552)	135 (16)
10 passes		22.5 (4.3)	6.6 (1.1)	2225 (1295)	152 (18)
The rocks		22.9 (8.7)	6.7 (1.8)	2460 (1377)	153 (20)
Balloon hunter		26.7 (5.8)	7.8 (1.1)	1874 (938)	151 (9)
Hop relay		26.7 (8.9)	7.8 (2.1)	5123 (2139)	157 (12)
Get in the car		27.7 (4.1)	8.1 (0.7)	2239 (444)	151 (15)
Countries		28.1 (10.1)	8.2 (0.5)	1514 (1260)	145 (47)
Rats and mice		28.5 (5.1)	8.3 (1.0)	2129 (1338)	142 (12)
Dodge		28.5 (10.5)	8.4 (1.4)	2444 (1314)	167 (22)
Football w/o ball		28.7 (11.3)	8.9 (2.0)	1898 (853)	142 (23)
Conveyors	HIPE group	31.3 (7.8)	9.2 (1.8)	3412 (569)	184 (10)
Steal the treasure		32.2 (7.7)	9.4 (0.9)	2640 (1107)	162 (10)
Stealing handball		32.4 (10.6)	9.4(0.5)	3001 (1336)	161 (48)
Stealing football		33.9 (10.2)	9.9 (0.7)	2877 (1398)	169 (52)
The 4 corners		37.3 (6.6)	10.8 (1.7)	3115 (505)	176 (14)
Mobile basket		40.2 (9.9)	11.7 (1.1)	3008 (625)	167 (14)
Resistance games					
Foot pulse	Warm-up	15.8 (3.5)	3.1 (1.1)	958 (204)	131 (13)
Cockfight		17.5 (4.5)	3.4 (1.1)	2067 (1409)	153 (25)
Kangaroo race		19.4 (6.1)	3.5 (1.6)	2192 (1087)	153 (21)
Gipsy wrestling	LIPE group or Combined group	25.0 (4.4)	4.1 (0.6)	1916 (1464)	146 (6)
Great squat		27.1 (5.5)	5.4 (2.0)	3346 (1359)	157 (13)
Strongman team		27.4 (9.0)	4.1 (0.9)	1044 (391)	149 (23)
Handling	HIPE group	30.5 (4.6)	6.1 (1.7)	1501 (645)	146 (15)
The annoying		36.6 (2.1)	6.4 (2.2)	2695 (1586)	165 (13)
The wounded		41.3 (8.2)	6.1 (0.4)	1785 (399)	163 (12)

Each game is an average for 5±1 subjects (SE). Games with VO_2 up to 20 mL/kg/min; Games with VO_2 between 20 and 30 mL/kg/min; Games with VO_2 above 30 mL/kg/min. Full description for the preparation and administration of these games can be found at www.movidavida.org.

eTable 2. Example of playground games by oxygen consumption, metabolic equivalents, and energy expenditure with heart rate data for the HEPAFIT Study

ID	Playground for group (*Spanish*)	Playground for group	VO_2 (mL/kg/min)	Metabolic equivalents Units	Energy expenditure kcal kg/h	Heart rate (beats/min)
	Warm-up					
1	Relevos con balón de pilates	Pilates ball relay	6	2	1.6	160
2	El pase más largo	The longest pass	8	2	2.4	163
4	Palomitas de maíz	Popcorn	9	2	2.5	152
5	Los 10 pases	The 10 passes	10	3	3.0	150
6	Balones y números	Balls and numbers	11	3	3.3	150
7	¡Que no te atrapen!	Do not get caught!	12	3	3.4	156
8	Zig zag Encesta	Zig zag Basket	12	3	3.5	150
	LIPE playground					
10	¿Cómo me lo llevo?*	How do I take it? *	15	4	4.3	122
14	Pisa zapatillas	Tread slippers	16	5	4.8	138
16	Hale Hoop	Hale hoop	17	5	4.7	167
17	Balón tiro circular	Circular shot ball	17	5	4.9	147
18	El transportador	The transporter	17	5	4.9	149
19	Los ladrones	Thieves	17	5	4.8	155
20	Jimmy el rápido	Jimmy the fast	19	5	5.4	146
22	La isla	The island	19	5	5.3	132
23	Pulso con los pies*	Pulse with the feet *	19	5	5.3	126
26	Fuera de mi zona*	Outside my area *	20	6	5.6	123
29	relevos con globos	balloon relays	20	6	5.9	132
31	Con mi pareja	With my partner	20	6	5.7	137
35	El pelotón	The platoon	21	6	5.9	147
39	El equipo forzudo*	The strong team *	22	6	6.2	144
	HIPE playground					
43	Los dragones	The Dragons	23	7	6.6	175
44	Pelea de gallos*	Cockfight*	23	7	6.6	154

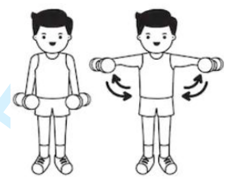
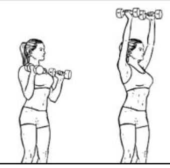
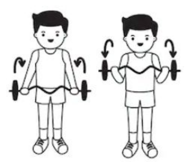
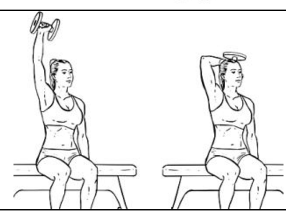
46	Los tres lobos	The three wolves	24	7	7.3	182
47	Juego limpio	Fair play	24	7	6.8	184
50	Apunta al cono	Aim for the cone	25	7	7.2	189
51	Tiro al aro	Hoop shot	25	7	7.1	161
54	El barrido *	The sweep *	26	7	7.2	167
55	Balón rápido	Fast ball	26	7	7.8	173
56	Toma la delantera	Take the lead	26	7	7.6	167
57	La cuerda pirata	The pirate rope	26	7	7.7	178
58	Cara y cruz variante de rodillas	Heads and tails variant of knees	26	7	7.4	180
60	Cambios de lugar	Changes of place	27	8	7.4	189
61	Voleibol tenis	Volleyball tennis	27	8	7.7	164
63	El albañil	The bricklayer	27	8	8.2	176
66	Atrapar al león	Catch the lion	28	8	8.4	174
67	Las nueve esquinas	The nine corners	28	8	8.1	160
68	Pollos zorros y víboras	Chicken foxes and snakes	28	8	8.4	161
69	El tiburón	The shark	28	8	8.1	179
70	Mano a mano	Hand to hand	29	8	8.5	185
71	Los acumuladores	Accumulators	29	8	8.8	181
74	Los lémures	Lemurs	30	8	8.7	195
75	Pasa y gana con aros	Pass and win with hoops	30	8	8.8	180
76	La conquista de los balones	The conquest of the balls	30	9	8.7	186
78	Multiporterias	Multi goals	30	9	8.7	179
79	Vóley trapos	Rags volleyball	30	9	8.1	165
80	La cadena cortada	The cut chain	30	9	9.0	168
81	El que llegue primero	Whoever comes first	31	9	8.7	179
85	La tela de araña	Spider web	31	9	9.3	183
86	Escalera humana + abdominal	Human + abdominal ladder	31	9	9.3	172
87	El correcaminos	Roadrunners	31	9	9.4	166
88	Triqui cono	Triqui cone	34	10	9.1	180
89	Capicúa	Capicúa	34	10	9.3	179
90	A robar trapos	To steal rags	31	9	9.0	153

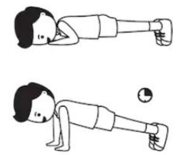
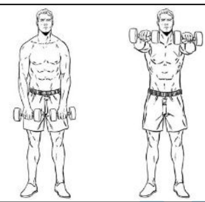
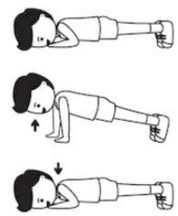
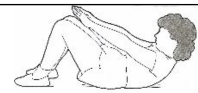
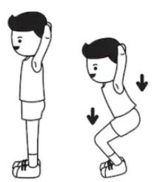
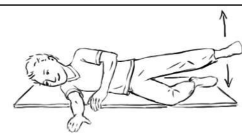
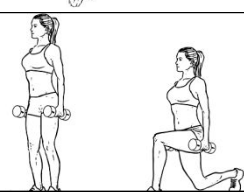
91	Toma y dame	Take and give me	32	9	8.9	165
92	Atraco al banco	Bank robbery	32	9	9.5	175
94	Aro y gol	Hoop and goal	33	9	9.4	177
95	Números y columnas	Numbers and columns	33	9	9.7	192
96	Objetivo móvil amigo	Mobile target friend	33	9	9.9	167
97	La rueda loca	The crazy wheel	33	9	9.3	179
99	Balón prestado	Borrowed ball	33	10	10.4	181
100	Policías y ladrones	Cops and robbers	33	10	10.2	173
101	Quien la hace la paga	Who makes it pay	34	10	9.8	185
102	De mano en mano	From hand to hand	34	10	10.1	162
103	Desconexión peligrosa	Dangerous disconnection	34	10	9.6	170
104	Los esquinaos	The corners	34	10	9.8	175
105	Los cuatro lados	All four sides	34	10	10.4	173
106	El caza tornados	The tornado hunter	34	10	10.0	155
107	Inténtalo otra vez Sam	Try again sam	34	10	10.6	175
108	Pega al cono	Glue to the cone	35	10	9.9	156
109	A por el mío	To mine	35	10	10.0	176
110	El zorro y los polluelos	The fox and the chicks	35	10	10.1	169
111	El lobo y las ovejas	The wolf and the sheep	35	10	11.1	178
112	Las rocas	The rocks	35	10	10.1	197
113	Fútbol por parejas	Soccer in pairs	35	10	10.0	194
114	El molino y el molinero	The mill and the miller	36	10	11.0	160
115	Balón speed	Speed ball	36	10	11.0	176
117	Pasando el testigo	Passing the baton	36	10	10.7	197
118	La estrella	The star	36	10	10.7	172
119	Caza de rodillas	Hunting on your knees	36	10	10.6	192
120	Ojo que voy	Eye I'm going	37	11	10.7	183
121	Las cuatro esquinas	The four corners	37	11	10.6	199
122	Cangrejos hacia atrás	Crabs back	37	11	11.8	164
124	Todos en orden	All in order	37	11	10.6	171
125	Las piedras y el rio	The stones and the river	38	11	11.0	180

126	Relevos con banderas	Relay with flags	38	11	11.3	171
127	Atrapa la bolsa	Catch the bag	38	11	10.8	171
128	Carreras de banderas	Racing flags	38	11	11.3	163
129	Amarillo o azul	Yellow or blue	39	11	11.1	180
130	El más veloz	The fastest	39	11	11.1	182
131	Copia el camino	Copy the path	39	11	11.2	189
132	Los zapateros	The shoemakers	39	11	11.7	184
133	Persecución de tres	Chase three	40	11	12.7	192
134	No entres al aro	Do not enter the hoop	40	11	11.4	181
137	Recorre y vence	Walk and win	40	11	11.7	183
138	Carrera de sacos	Sack-race	40	11	11.4	192
139	Enjaulados	Caged	41	12	11.9	185
142	Balón sprint	Sprint ball	41	12	12.0	174
143	En busca del tesoro misterioso	In search of the mysterious treasure	41	12	12.3	182
144	Saltamontes goleador	Scorer grasshopper	42	12	12.1	185
145	Cambios de lugar	Changes of place	42	12	12.2	161
146	Cesta móvil	Mobile basket	42	12	11.9	184
147	Zigzag encesta	Zigzag scores	43	12	12.5	171
148	El oso hormiguero	The anteater	43	12	12.6	182
151	Carrera de cuerdas	Ropes race	44	13	12.9	183
152	Tierra mar y aire	Land sea and air	45	13	13.2	182
153	Botas o te botan	Boots or you get booted	46	13	14.3	174
154	Transmilenio	Transmilenio	46	13	13.9	187
155	Circulo azofaito	Azofaito circle	47	13	13.9	189
156	Balones sobre conos	Balls on cones	47	13	13.9	167

* Playground games with resistance strength

eTable 3. Example of resistance training “fitness fusion workout” for the HEPAFIT Study

Resistance training exercise		Week no.	Frequency (days/week)	Work-load (kg)	Time for exercise per set (min)
Lateral raise		0-20	2-3	1-3	1
Shoulder press		0-20	2-3	1-3	1
Bicep curl		20-40	2-3	2-5	1
Triceps press		20-40	2-3	1-3	1
Abdominal crunches		0-72	2-3	N.A	1

Plank		30-72	2-3	N.A	1
Front raise		40-60	2-3	1-3	1
Push-up		0-72	2-3	N.A	1
Sit-ups		0-72	2-3	N.A	1
Squat		0-72	2-3	2-10	1
Lying side leg lifts		50-72	2-3	1-3	1
Lunge		0-72	2-3	2-10	1

Superman		30-72	2-3	N.A	1

N.A= No applicable, use one's own body weight as resistance.

eTable 4. Listing of cardiovascular biomarkers and liver proteins detected for each assay

Biomarker					
Full name	**Cluster**	**Resource**	**Reagent/source (ID)**	**Range measures assay**	**CV%**
Total cholesterol, mg/dl	Cardiovascular biomarkers	Roche Diagnostics®	Cholesterol Gen.2 # 03039773-190	3.86 to 800 mg/dl	0.59 to 0.78 %
HDL-C, mg/dl	Cardiovascular biomarkers	Roche Diagnostics®	HDL-Cholesterol Gen.4 #07528566-190	3.09 to 150 mg/dl	0.93 to 1.95 %
LDL-C, mg/dl	Cardiovascular biomarkers	Roche Diagnostics®	LDL-Cholesterol Gen.3 # 07005717-190	3.87 to 549 mg/dl	0.66 to 0.94 %
Triglycerides, mg/dl	Cardiovascular biomarkers	Roche Diagnostics®	Triglycerides # 20767107-322	8.85 to 885 mg/dl	0.89 to 1.17 %
Glucose, mg/dl	Cardiovascular biomarkers	Roche Diagnostics®	Glucose HK # 04404483-190	2.0 to 750 mg/dl	1.06 to 1.21 %
Hemoglobin A1C, %	Cardiovascular biomarkers	Roche Diagnostics®	Tina-quant® HbA1c Gen. 3	4 to14 %	0.54 to 0.86 %
Insulin, µU/ml	Cardiovascular biomarkers	Roche Diagnostics®	Elecsys Insulin # 12017547-122	0.2 to 1000 µU/ml	1.67 to 2.26 %
Peptide C, ng/ml	Cardiovascular biomarkers	LIAISON®	LIAISON DiaSorin # 316171	0.01 to 30 ng/ml	4.90 % to 6.82 %
Alanine aminotransferase, U/l	Liver metabolism	Roche Diagnostics®	Alanine Aminotransferase acc. to IFCC with pyridoxal phosphate activation # 04467388-190	5.0 to 700 U/l	1.84 to 3.08 %
Aspartate aminotransferase, U/l	Liver metabolism	Roche Diagnostics®	Aspartate Aminotransferase acc. to IFCC without pyridoxal phosphate activation # 20764949-322	5.0 to 700 U/l	1.14 to 1.23 %
G-glutamyl transferase, U/l	Liver metabolism	Roche Diagnostics®	γ-Glutamyltransferase, ver.2 standard versus to IFCC/Szasz # 03002721-122	3.0 to 120 U/l	1.28 to 1.39 %
Creatinine, mg/dl	Cardiovascular biomarkers	Roche Diagnostics®	Creatinine plus ver.2 # 03263991-190	0.06 to 30.5 mg/dl	0.72 to 0.81 %
Ureic Nitrogen, mg/dl	Cardiovascular biomarkers	Roche Diagnostics®	UREAL # 04460715-190	1.4 to 112 mg/dl	0.24 to 0.75 %
Serum Iron, µg/dl	Cardiovascular biomarkers	Roche Diagnostics®	IRON Gen.2 # 03183696-122	5.00 to 1000 µg/dl	1.51 to 2.76 %
Unsaturated Iron-Binding Capacity, µg/dl	Cardiovascular biomarkers	Roche Diagnostics®	UIBC # 04536355-190	16.8 to 700 µg/dl	5.68 % to 7.28 %
Ferritin, ng/ml	Cardiovascular biomarkers	Roche Diagnostics®	Ferritin # 03737551-190	0.50 to 2000 ng/ml	1.60 % to 1.82 %
Plasma Reagins IVD (Treponema pallidum)	Serologic markers	SPINREACT,S.A./S.A.U	SPINREACT # 1200401	RPR-carbon (Treponema pallidum) By examine macroscopically the presence or absence of visible agglutination	Diagnostic sensitivity: 100 % Diagnostic specificity: 100 %

eTable 5. Exercise prescription in detail and measurements obtained during the intervention period[a]

Measurements	Groups			
	CTRL group (n=26)	**HIPE group (n=24)**	**LIPE group (n=25)**	**PLUS group (n=25)**
Attendance, %	74.0	76.9	76.7	71.1
Daily average heart rate, beats/min	N.A	163 (27)	140 (22)	156 (25)
Intensity, metabolic equivalents[b]	N.A	9.8 (1.7)	5.3 (1.6)	6.8 (1.8)
Daily energy expenditure, kcal/d[b]	N.A	536 (10)	479 (10)	476 (11)
Total energy expenditure, kcal (min/max)[b]	N.A	30011-38585	26222-34285	26034-34043

[a]Data are expressed as mean (SD) unless otherwise noted.

[b]Intensity and energy expenditure were estimated from daily attendance and heart rate measures using the mean of oxygen consumption from each child's game (sub-sample 50%). Calculated by per-protocol analysis. N.A= No applicable

eTable 6. Change in controlled attenuation parameter, body composition and fitness variables (per-protocol analysis)

Variable	From Baseline to 6 months, least square (LS) mean (95% CI)						
	Within-Group Changes			Between-Group Comparations vs. Control Group Changes			η^2 (P value for interaction)
Primary endpoint							
Controlled attenuation parameter, dB/m							
CTRL group	8.87	−4.39	22.14				
HIPE group	−26.12	−39.90	−12.35	35.00	8.59	61.41	0.153 (0.002)
LIPE group	−16.13	−30.76	−1.51	25.01	−2.13	52.16	
PLUS group	−22.79	−36.14	−9.44	31.66	6.36	56.97	
Secondary endpoints							
Body mass, kg							
CTRL group	3.11	1.75	4.47				
HIPE group	2.42	0.93	3.91	0.68	−2.03	3.41	0.071 (0.084)
LIPE group	2.59	1.11	4.06	0.52	−2.20	3.25	
PLUS group	0.64	−0.76	2.05	2.46	−0.21	5.14	
Body mass index, z-score							
CTRL group	0.21	0.02	0.40				
HIPE group	0.12	−0.07	0.32	0.08	−0.28	0.46	0.040 (0.270)
LIPE group	0.24	0.04	0.43	−0.02	−0.40	0.34	
PLUS group	−0.01	−0.21	0.18	0.22	−0.14	0.59	
Waist circumference, cm							
CTRL group	1.65	0.48	2.81				
HIPE group	0.81	−0.43	2.07	0.83	−1.50	3.17	0.047 (0.209)
LIPE group	2.18	0.99	3.37	−0.53	−2.79	1.73	
PLUS group	0.57	−0.63	1.77	1.08	−1.17	3.33	
Body fat, %							
CTRL group	−0.70	−1.77	0.36				
HIPE group	−1.55	−2.66	−0.43	0.84	−1.25	2.94	0.032 (0.394)
LIPE group	−0.96	−2.07	0.15	0.25	−1.84	2.35	
PLUS group	−1.90	−2.97	−0.83	1.19	−0.86	3.25	
Visceral adipose tissue, cm^3							
CTRL group	0.98	−25.65	27.62				
HIPE group	−3.09	−31.22	25.03	4.08	−48.69	56.87	0.032 (0.400)
LIPE group	−5.81	−33.57	21.93	6.80	−45.45	59.06	
PLUS group	−29.10	−55.96	−2.24	30.09	−21.12	81.32	
Skeletal muscle mass, kg							
CTRL group	1.39	0.86	1.93				
HIPE group	1.40	0.82	1.98	−0.00	−1.06	1.05	0.068 (0.076)
LIPE group	1.14	0.56	1.72	0.25	−0.82	1.32	
PLUS group	0.47	−0.07	1.01	0.92	−0.11	1.96	
Systolic blood pressure, mmHg							
CTRL group	−3.46	−8.07	1.13				
HIPE group	−0.60	−5.49	4.27	−2.86	−12.01	6.29	0.016 (0.693)
LIPE group	−0.00	−4.56	4.55	−3.46	−12.26	5.34	
PLUS group	−0.16	−4.84	4.51	−3.30	−12.27	5.67	
Diastolic blood pressure, mmHg							
CTRL group	−1.75	−4.81	1.30				
HIPE group	−1.56	−4.83	1.69	−0.18	−6.22	5.84	0. 009 (0.852)
LIPE group	−0.17	−3.27	2.92	−1.58	−7.57	4.41	
PLUS group	−0.33	−3.43	2.76	−1.42	−7.34	4.50	
Mean blood pressure, mmHg							
CTRL group	−2.08	−4.93	0.75				
HIPE group	−1.08	−4.13	1.97	−1.00	−6.67	4.66	0.011 (0.818)
LIPE group	−0.45	−3.32	2.42	−1.66	−7.13	3.86	
PLUS group	−0.33	−3.24	2.57	−1.75	−7.27	3.77	
Heart rate, bpm							
CTRL group	−5.39	−10.46	0.32				0.029

HIPE group	−4.91	−9.84	0.02	−0.48	−10.13	9.16	(0.516)
LIPE group	−4.84	−10.23	0.54	−0.55	−10.69	9.58	
PLUS group	−0.76	−5.60	4.07	−4.63	−14.11	4.84	
CRF, laps							
CTRL group	5.86	1.15	10.57				
HIPE group	8.62	3.73	13.51	−2.76	−12.11	6.59	0.020 (0.621)
LIPE group	3.98	−1.07	9.03	1.88	−7.52	11.29	
PLUS group	6.63	1.91	11.36	−0.77	−9.83	8.28	
Upper-limb absolute strength, kg							
CTRL group	0.94	−0.15	2.04				
HIPE group	2.23	1.08	3.37	−1.28	−3.43	0.86	0.031 (0.400)
LIPE group	1.24	0.12	2.36	−0.29	−2.44	1.84	
PLUS group	1.73	0.61	2.84	−0.78	−2.94	1.37	
Lower-limb absolute strength, cm							
CTRL group	1.92	−5.57	9.41				
HIPE group	11.71	4.27	19.16	−9.79	−24.10	4.51	0.050 (0.220)
LIPE group	2.40	−4.86	9.68	−0.48	−14.70	13.73	
PLUS group	6.73	−0.44	13.92	−4.81	−19.03	9.40	
Speed-agility, sec							
CTRL group	0.35	−0.13	0.84				
HIPE group	0.61	0.10	1.12	−0.25	−1.23	0.71	0.032 (0.413)
LIPE group	0.32	−0.18	0.84	0.02	−0.94	1.00	
PLUS group	0.00	−0.48	0.50	0.34	−0.59	1.28	

Values are least square mean differences and 95% confidence intervals as derived from ANCOVA models, adjusted baseline measures of each variable. Mean arterial pressure was estimated as the product of the average diastolic pressure plus one-third of the average pulse pressure. Eta partial squared (η^2) was assessed by η^2 obtained from the ANCOVA with small ($\eta^2 = 0.01$), medium ($\eta^2 = 0.06$), and large ($\eta^2 = 0.14$) effects defined according to Cohen.

eTable 7. Change in cardiometabolic parameters in blood (<u>per-protocol analysis</u>)

Variable	From Baseline to 6 months, least square (LS) mean (95% CI)						
	Within-Group Changes			Between-Group Comparations vs. Control Group Changes			η^2 (P value for interaction)
Total cholesterol, mg/dl							
CTRL group	0.95	−6.52	8.43				
HIPE group	−6.29	−14.13	1.54	−7.24	−18.01	3.51	0.038 (0.315)
LIPE group	3.45	−4.84	11.74	−9.74	−21.18	1.69	
PLUS group	−3.41	−11.17	4.34	−2.87	−14.07	8.32	
HDL-C, mg/dl							
CTRL group	−0.61	−2.86	1.64				
HIPE group	1.10	−1.20	3.41	−1.71	−6.13	2.71	0.015 (0.703)
LIPE group	0.87	−1.40	3.15	−1.48	−5.83	2.87	
PLUS group	0.87	−1.37	3.10	−1.48	−5.77	2.82	
LDL-C, mg/dl							
CTRL group	3.59	−2.89	10.08				
HIPE group	0.09	−6.64	6.82	3.50	−9.12	16.12	0.021 (0.568)
LIPE group	6.33	−0.37	13.04	−2.74	−15.42	9.94	
PLUS group	1.20	−5.48	7.89	2.39	−10.39	15.16	
Triglycerides, mg/dl							
CTRL group	18.33	−0.45	37.12				
HIPE group	−10.81	−30.27	8.64	29.14	−7.60	65.89	0.117 (0.008)
LIPE group	22.39	2.93	41.85	−4.06	−40.80	32.68	
PLUS group	−16.27	−35.38	2.84	34.60	−1.91	71.11	
Glucose, mg/dl							
CTRL group	10.97	5.46	16.47				
HIPE group	11.64	6.10	17.18	−0.67	−11.24	9.89	0.09 (0.848)
LIPE group	9.11	3.42	14.80	1.86	−9.21	12.92	
PLUS group	12.53	7.09	17.97	−1.56	−12.16	9.04	
Insulin, uU/ml							
CTRL group	6.10	2.86	9.33				
HIPE group	−4.19	−7.54	−0.83	10.28	3.86	16.70	0.168 (0.001)
LIPE group	1.12	−2.28	4.52	4.98	−1.52	11.47	
PLUS group	0.56	−2.74	3.87	5.53	−0.64	11.70	
Hemoglobin A1C, %							
CTRL group	0.29	0.16	0.42				0.024 (0.515)
HIPE group	0.24	0.10	0.37	0.05	−0.21	0.30	
LIPE group	0.27	0.14	0.41	0.02	−0.24	0.27	
PLUS group	0.16	0.02	0.29	0.13	−0.12	0.38	
Cardiometabolic Z-Score, SD							
CTRL group	0.16	−0.49	0.81				
HIPE group	−0.57	−1.27	0.13	0.73	−0.58	2.03	0.068 (0.118)
LIPE group	0.58	−0.13	1.30	−0.42	−1.75	0.90	
PLUS group	−0.24	−0.91	0.43	0.40	−0.86	1.66	
Peptide C, ng/ml							
CTRL group	0.81	0.38	1.25				
HIPE group	0.18	−0.27	0.63	0.63	−0.22	1.48	0. 093 (0.032)
LIPE group	0.80	0.34	1.26	0.01	−0.85	0.88	
PLUS group	0.09	−0.35	0.53	0.72	−0.11	1.55	
Alanine aminotransferase, U/l							
CTRL group	1.56	−0.71	3.83				
HIPE group	−0.69	−3.07	1.69	2.25	−2.27	6.77	0.068 (0.084)
LIPE group	0.26	−2.08	2.60	1.30	−3.12	5.72	
PLUS group	−1.49	−3.78	0.81	3.05	−1.33	7.42	
Aspartate aminotransferase, U/l							
CTRL group	3.17	−0.44	6.77				
HIPE group	−1.95	−5.76	1.87	5.11	−2.10	12.32	0.039 (0.283)
LIPE group	2.12	−1.60	5.85	1.04	−5.99	8.07	
PLUS group	−2.33	−5.99	1.33	5.50	−1.44	12.44	

G-glutamyl transferase, U/l							
CTRL group	0.73	−2.85	4.31				
HIPE group	0.08	−3.60	3.76	0.65	−6.35	7.65	0.045
LIPE group	2.32	−1.36	6.00	−1.59	−8.59	5.42	(0.205)
PLUS group	−2.98	−6.59	0.62	3.72	−3.21	10.65	
Ferritin, ng/ml							
CTRL group	2.80	−10.73	16.33				
HIPE group	2.80	−11.43	17.02	0.00	−26.69	26.69	0.007
LIPE group	−4.30	−18.38	9.78	7.10	−19.41	33.60	(0.887)
PLUS group	−0.35	−14.21	13.52	3.15	−23.13	29.42	
TG/HDL ratio							
CTRL group	0.61	0.01	1.20				
HIPE group	−0.22	−0.84	0.40	0.82	−0.35	2.00	0.081
LIPE group	0.54	−0.08	1.16	0.07	−1.10	1.24	(0.052)
PLUS group	−0.29	−0.89	0.32	0.89	−0.26	2.05	
AST/ALT ratio							
CTRL group	−0.07	−0.18	0.04				
HIPE group	0.05	−0.07	0.17	−0.12	−0.34	0.10	0.044
LIPE group	−0.09	−0.21	0.03	0.02	−0.20	0.24	(0.233)
PLUS group	0.03	−0.09	0.14	−0.10	−0.31	0.12	
Insulin resistance (HOMA-IR)							
CTRL group	2.34	1.42	3.26				
HIPE group	−0.52	−1.47	0.43	2.86	1.05	4.67	0.170
LIPE group	0.18	−0.79	1.16	2.16	0.28	4.03	(<0.001)
PLUS group	0.73	−0.21	1.66	1.61	−0.14	3.37	

Values are least square mean differences and 95% confidence intervals as derived from ANCOVA models, adjusted for baseline measures of each variable. Eta partial squared (η^2) was assessed by η^2 obtained from the ANCOVA with small ($\eta^2 = 0.01$), medium ($\eta^2 = 0.06$), and large ($\eta^2 = 0.14$) effects defined according to Cohen.

eTable 8. Adverse events by study group related and unrelated to participation in the HEPAFIT Study[a]

Adverse Events [n (percent)]	CTRL group (n=26)		HIPE group (n=24)		LIPE group (n=24)		PLUS group (n=25)	
Relationship to exercise intervention	**related**	**unrelated**	**related**	**unrelated**	**related**	**unrelated**	**related**	**unrelated**
Serious adverse events & Hospitalizations (Illness)	0 (0)	2 (8)	0 (0)	2 (10)	0 (0)	0 (0)	0 (0)	1 (4)
Any injury or musculoskeletal discomfort	0 (0)	0 (0)	3 (14)	0 (0)	4 (16)	0 (0)	6 (26)	0 (0)
Upper body injury (neck, shoulders, upper back, arms)	1 (4)	1 (4)	1 (5)	0 (0)	2 (8)	0 (0)	1 (4)	0 (0)
Lower body injury (lower back, hips, knees, ankle, feet)	1 (4)	1 (4)	2 (10)	0 (0)	2 (8)	0 (0)	2 (9)	0 (0)
Injury required modification of exercise program or restriction of activity	0 (0)	0 (0)	1 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Other (epistaxis)	0 (0)	0 (0)	1 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

[a]Data are the number (percentage) of participants. Percentages are rounded to the nearest 1%. Calculated by per-protocol analysis

References

1 González-Ruíz K, Correa-Bautista JE, Izquierdo M, García-Hermoso A, Dominguez-Sanchez MA, Bustos-Cruz RH, García-Prieto JC, Martínez-Vizcaíno V, Lobelo F, González-Jiménez E, Prieto-Benavides DH, Tordecilla-Sanders A, Schmidt-RioValle J, Perez G, Ramírez-Vélez R. Effects of an exercise program on hepatic metabolism, hepatic fat, and cardiovascular health in overweight/obese adolescents from Bogotá, Colombia (the HEPAFIT study): study protocol for a randomized controlled trial. Trials. 2018;19(1):330. doi: 10.1186/s13063-018-2721-5

2 Ministerio de Educación Nacional. Reglamentos y aspectos pedagógicos y organizativos generales de la Educación Física en Colombia. Ley 1860 de 1994. [Access 11-04-2017], Available URL: http://www.mineducacion.gov.co/1621/articles-86240_archivo_pdf.pdf.

3 Steckler A, Allegrante JP, Altman D. Health education intervention strategies. Health Educ Q 1995; 22:307-28. doi: 10.1177/109019819402200305

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5 Martínez Vizcaíno V, Salcedo Aguilar F, Franquelo Gutiérrez R, Solera Martínez M, Sánchez López M, Serrano Martínez S, López García E, Rodríguez Artalejo F. Assessment of an after-school physical activity program to prevent obesity among 9- to 10-year-old children: a cluster randomized trial. Int J Obes (Lond). 2008;32(1):12-22. doi: 10.1038/sj.ijo.0803738

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4-5
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	Supl file
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6, Supl file
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6-8
	6b	Any changes to trial outcomes after the trial commenced, with reasons	-
Sample size	7a	How sample size was determined	8,9
	7b	When applicable, explanation of any interim analyses and stopping guidelines	-
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	5
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	5
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	5

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	-
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	7-8
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	8
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Page 9, Figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	Page 9, Figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	5
	14b	Why the trial ended or was stopped	-
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Table 1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	10-11
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	10-11
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	10-11
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	12
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	16-17
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	17
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	16-17
Other information			
Registration	23	Registration number and name of trial registry	1
Protocol	24	Where the full trial protocol can be accessed, if available	Ref 11
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	2

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.