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Diabetes risk and oxidative and proinflammatory status in obese adolescents according to exercise dose: a randomized controlled trial

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DIABETES RISK AND OXIDATIVE AND PROINFLAMMATORY STATUS IN OBESE ADOLESCENTS ACCORDING TO EXERCISE DOSE : A RANDOMIZED CONTROLLED TRIAL

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To my dear parents

Cândida e José Neves

and my brother Miguel

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LIST OF ABBREVIATIONS AND ACRONYMS

BMI - Body mass index

BMI z-sc - Age- and sex-standardized Body Mass Index

BFM – Body Fat Mass

CRF - Cardiorespiratory fitness

CRP - C-reactive protein

CVD - Cardiovascular disease

FFM – Fat Free Mass

FPG - Fasting plasma glucose

FPI - Fasting plasma insulin

HDL-c - High-density lipoprotein cholesterol

HOMA-IR - Homeostatic model assessment for insulin resistance

IR - Insulin Resistance

LDL-c - Low-density lipoprotein cholesterol

MS - Metabolic Syndrome

MVPA - Moderate-to-vigorous physical activity

ox-LDL - oxidized-Low-density lipoprotein

PA -Physical Activity

T2DM - Type 2 Diabetes Mellitus

TC – Total cholesterol

TG - Triglycerides

WC – Waist circumference

WtHR – Waist to Height Ratio

TITLE

Diabetes risk and oxidative and proinflammatory status in obese adolescents according to exercise dose: a randomized controlled trial

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KEY-WORDS: PHYSICAL ACTIVITY, OBESE YOUTH, DIABETES RISK, OXIDATIVE STRESS

ABSTRACT

BACKGROUND Beside physical activity (PA) is crucial on prevention and treatment of pediatric obesity, there was a lack of consensus regarding the amount needed to improve body composition as also to reduce obesity related comorbidities.

AIM To test dose-response of an exercise intervention program with moderate-to-vigorous PA (MVPA) on several features: body composition, insulin resistance, oxidative stress, proinflammatory status and cardiorespiratory fitness (CRF).

SUBJECTS AND METHODS Obese adolescents (10-12 y) were recruited from a school (IG; n=34) and perform 3 times-week soccer and other activities; a control group (CG; n=10) performed no exercise other than curricular physical education. Baseline and post-intervention (after 6 months) measures were made including anthropometry, body composition, lipid profile, glucose and insulin metabolism, oxidative stress and pro-inflammatory status and CRF.

RESULTS MVPA performed by IG showed significate positive effects in BMI-z-score, waist circumference, WtHR, body fat mass and free-fat mass. Besides, there was also an improvement in lipid profile (p<0.05), oxidized low-density lipoprotein (p<0.001), adiponectin (p<0.001) and CRF (p<0.001). There were no significant differences after intervention in C-Reactive Protein and HOMA-IR. Increased CRF was correlated with decreased adiposity markers and as also Low- density lipoprotein cholesterol, oxidized low-density lipoprotein and increased adiponectin.

CONCLUSION An extra-curricular PA program with at least three times a week is effective in order to reduce co-morbidities related to obesity in youth population, principally cardiometabolic risk and oxidative and proinfllamatory status.

INTRODUCTION

Childhood obesity is considered a growing global epidemic (Wang, 2004). The prevalence has rapidly increased worldwide and about 110 million children are classified as being overweight or obese (Cali and Caprio, 2008). In the European region, prevalence of pediatric obesity is also increasing, being Portugal on the top 5 of the highest prevalence (Livingstone, 2001, Wijnhoven et al., 2014). Moreover, a recent study reported that 19.7% of portuguese children are classified as being overweight and 8.2% as being obese, which is an alarming situation for this population in Portugal (Bingham et al., 2013).

Overweight and obese children are expected to remain obese in adult life and are at great risk of developing cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) (DeBoer, 2013, Zimmet et al., 2007). The most involved co-morbidities in obese adolescents are the metabolic syndrome (MS), hypertension, dyslipidemia, prediabetes, ovarian hyperandrogenism and non-alcoholic fatty liver disease/non-alcoholic steato-hepatitis (Sypniewska, 2015, Morandi and Maffeis, 2014). It is important to identify metabolic complications in obese adolescents because these are at greater risk of developing a worse cardiometabolic prognosis compared to obese adolescents without metabolic impairments. However, if either obesity or MS resolves before adulthood this prognosis is no longer established (Morandi and Maffeis, 2014).

Insulin Resistance (IR) is commonly observed on obesity and is deeply associated with the etiology of hypertension, coronary heart disease and T2DM (Lee and Kim, 2013). Diabetes risk and CVD are associated with prediabetes, which comprises impaired fasting glucose and/or impaired glucose tolerance, measures of IR (Li et al., 2009). This condition is associated with an increased lipid accumulation in visceral compartments, liver and muscle tissues and by a gradual fail in β-cell function with loss of sensibility to insulin

secretion. Epidemiology shows a parallel increased prevalence of prediabetes and T2DM and the progression of one to another has proven to be faster in obese children comparatively to adults (D'Adamo and Caprio, 2011). This progression represents a gradual deterioration in glucose-stimulated insulin response (Cali and Caprio, 2008). As what concerns to MS, also called IR syndrome, a real consensus about its definition in the adolescent population does not exist but it is known that the principal component of MS, waist measurement, is an independent predictor of IR (Morandi and Maffeis, 2014, Zimmet et al., 2007). Systemic inflammation is linked to obesity and it was demonstrated that the visceral adipocytes play an important role in the inflammatory process. The excess of visceral adipose tissue will determine a consistent low-grade degree of inflammation, which worsens when central obesity increases. Moreover, it is important to underline that science and clinical evidence has shown an independent association between obesity-related inflammation and IR as well as the risk of developing T2DM (DeBoer, 2013).

There is a complex process linking IR to proinflammatory status and it appears to involve increases in oxidative stress in target tissues (DeBoer, 2013). Oxidative stress occurs when there is an unbalance between the production of reactive oxygen species and their clearance by endogenous antioxidants. ROS cause mitochondrial DNA damage which is importantly connected with obesity-related IR and the early pathophysiology of T2DM (Balagopal et al., 2011, D'Adamo and Caprio, 2011).

Regular physical activity (PA) is an important weapon in the treatment of obesity because it decreases body adiposity, increases lean mass, improves cardiovascular fitness, blood lipid profile and enhances psychosocial well-being (Riddell and Iscoe, 2006). Moreover, physical exercise is known to exert independent positive effects on both inflammatory and

oxidative homeostasis (Oliver et al., 2010). Besides those benefits, PA also has an “insulin-like” effect, facilitating the transport of glucose from the blood to the muscle through the increase in GLUT-4 concentration and thus managing a control in blood glucose levels. It is important that the PA recommended is regular, as the insulin sensitivity decreases at the end of the exercise (Lee and Kim, 2013). However, it is not well known if exercise alone results in improving insulin sensibility (Lee and Kim, 2013). Presently, there are few randomized controlled trials that relate the impact of exercise dose on glucose tolerance in obese adolescents. Furthermore, many studies fail to contemplate the pubertal stage, which is very important considering that IR occurs with puberty (Lee and Kim, 2013).

The purpose of this trial was to test the dose response effect of an exercise intervention program on IR, oxidative stress and proinflammatory status in obese adolescents. A secondary objective was to access the level of cardiorespiratory fitness and to correlate this variable with the aforementioned.

METHODS

This trial involved adolescents recruited for a soccer intervention program named "*Soccer as a novel therapeutic approach to pediatric obesity. A randomized controlled trial and its effects on fitness, body composition, cardiometabolic and oxidative markers"* sponsored by UEFA. It was a prospective controlled randomized trial, conducted from September 2013 to March 2014.

Inclusion criteria were age from 10 to 12 years old (including children who turned 10 years in 2014), male gender and body-mass index (BMI) ≥ 85th percentile for age and gender - according to WHO cut-off values (de Onis et al., 2007). Exclusion criteria were being on a weight loss program with or without exercise in the last year, being under medication or having any severe medical condition - CVD, type I diabetes mellitus, endocrine or hepatic disease.

Studied population were invited from a school in Perafita. A total of 65 adolescents were enrolled and 45 (69.2%) accepted to participate (Intervention Group - IG). The control group (CG) were 20 obese adolescents, matched for age and gender, which were alleatory selected from a pediatric obesity clinic in Porto city. The selection criteria were the absence of participation in any extra-school PA.

Agreement and written informed consent were obtained by all children and parents. The participants were treated with full respect and privacy, and the study coordinators were trained before the start of the activities. The study was previously approved by the Faculty of Sport of Porto University Ethics Committee and followed the Declaration of Helsinki (2000) principles.

Procedure and exercise interventions

IG was randomly separated into two different training groups: a soccer program group (SG) and a traditional activity program group (AG). Both groups had training sessions 3 days per week, coordinated by teachers from Faculty of Sport of Porto University. SG consisted in a warm-up (10-20 min), different technical exercises and small-sided games (40-60 min) and a cool-down (10 min). AG included a warm-up (10-20 min), different activities such as strength and coordination exercises, walking and running (40-50 min) and a cool-down (10 min). Training intensities for the IG were designed to maintain heart rates at approximately 70-80% of maximum confirmed by monitoring (Polar Team 2 Pro System, Polar Electro, Kempele, Finland). All intervention sessions were conducted by two physical education teachers under the guidance of the principal investigator.

Intensity of PA in SG and AG was measured during the first month of the trial, twice a week, using an ActiGraph accelerometer, model GT3X (Pensacola, FL USA). Percentage of Moderate-to-vigorous physical activity (MVPA) showed no significant differences between AG and SG (36.20% and 35.20% respectively). It was used a t Test in this statistical analyses with a *p* value of 0.667. Therefore, both groups were studied as one - IG.

The CG consisted in adolescents who had no exercise interventions but the normal school mandatory physical activities (2 classes per week, 45-90 min each).

Outcomes

All measurements were made at baseline (before randomization) and 6 months after (at the end of the intervention). Assessments were done under similar conditions and at approximately the same time of the day to minimize potential diurnal variation in measured

variables. From the study protocol which included several variables, it was selected for this study the following: anthropometric and nutritional status, body composition, pubertal stage, biochemical analyses and cardiorespiratory fitness.

1 - Anthropometric and nutritional status

All anthropometric measurements were accessed according to both methodology and techniques internationally suggested (Jelliffe, 1989) and were made with every participant being on underwear and barefoot.

Body mass evaluation was achieved using a physician's digital scale (Tanita®, BC-418MA, USA) with results expressed in kilograms, and height values were obtained through a fixed stadiometer (Holtain Ltd., UK), with results expressed in meters. BMI values were calculated and expressed in kilograms per square meter and z-score values for BMI (BMI z-sc) were obtained with WHO AnthroPlus® software (WHO anthro). Excess weight and obesity were defined as a z-score value equal or higher than 1.0364 (85^{th} percentile) and 1.6449 (95^{th} percentile) respectively.

Waist circumference (WC) was measured with a metallic tape (Holtain, Lda) on bare skin at the umbilical level and the ratio between WC and height (WtHR) was calculated. WtHR greater than 0.50 was defined as a predictor of increased cardiometabolic risk (McCarthy and Ashwell, 2006).

2 - Body composition

Body composition was accessed by dual-energy X-ray absorptiometry (DXA) (Hologic QDR 4500A, Hologic Inc., Waltham, MA, USA). The unit was calibrated according to manufacturer instructions and a trained technician did all exams. Percentage for body fat mass (%BFM) and fat-free mass (%FFM) were measured.

3 - Pubertal stage

Pubertal stage, an indicator of biological maturity, was characterized through a clinical evaluation of the secondary sex characteristics by an experienced pediatrician. The adolescents were stratified as: Tanner 1 - prepubertal, Tanner 2 and 3 - medium puberty, and Tanner 4 and 5 - final puberty, according to Tanner scale (Tanner, 1986).

4 - Biochemical analyses

Blood samples were collected by venipuncture in EDTA containing tubes after an overnight fasting (8-10h) and processed within 2 h of collection. This procedure was made to all the participants at baseline and after 6 months. Aliquots of plasma were stored at -80^{o}C until assayed.

The variables determined from the blood samples were total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), triglycerides (TG), fasting plasma glucose (FPG), fasting plasma insulin (FPI), oxidized low-density lipoprotein (ox-LDL), adiponectin and plasma levels of C-reactive protein (CRP).

TC, TG and HDL-c were determined by enzymatic colorimetric tests CHOD-PAP, GPO-PAP and Direct HDL cholesterol methods, respectively. Low-density lipoprotein

cholesterol (LDL-c) was calculated with the Friedewald formula (Friedewald et al., 1972). High cardiometabolic risk was defined as TC ≥ 200 mg/dL, TG ≥ 150 mg/dL, LDL-c ≥ 130 mg/dL and HDL-c ≤ 35 mg/dL, this score corresponds to the 95th percentile score for TC, TG and LDL-c and the 5th percentile for the HDL-c (Gidding et al., 2005).

FPG and FPI were obtained through routine automated technology (Roche). Insulin resistance was measured by Homeostatic model assessment for insulin resistance (HOMA-IR), calculated as the product of the FPI (µU/ml) and the FPG (mg/dL) divided by 405 (Matthews et al., 1985). IR was defined for HOMA-IR values equal or greater than 2.67 and 5.22 for pre- and pubertal adolescents, respectively (Kurtoglu et al., 2010). Pre-diabetes risk was determined by FPG ≥ 100 and < 126 mg/dL (Lee and Kim, 2013).

ox-LDL (Mercodia) and adiponectin (eBioscience) were evaluated by commercial enzyme-linked immunoassays (ELISA). CRP was evaluated by immunoturbidimetry using commercially kits (CRP latex High-Sensitivity, Roche Diagnostics).

5 - Cardiorrespiratory fitness (CRF)

A progressive treadmill exercise test was used to estimate maximal oxygen uptake (VO_2max). Boys walked/ran until exhaustion following a standardized protocol (Eiberg et al., 2005a, Eiberg et al., 2005b). The test started at 4 km/h without inclination and was maintained for 3 min to permit adaptation to the treadmill. After 3 min, the speed was increased to 8 km/h. After 5 min, the inclination was raised to 3% and then to 6% and 9% after 7 and 9 min, respectively. If children were able to endure more, the speed was increased to 9 km/h after 10 min and then 10 km/h after 13 min. Criteria for VO_2max included two of the following: (1) maximum heart rate >200 beats/min; (2) respiratory

exchange ratio (ratio of maximum carbon dioxide to VO_2) >1.0; and (3) a plateau in oxygen consumption. The cut-off value used to define low CRF was 43.6 mlO_2/min/Kg (Adegboye et al., 2011).

Statistical Analyses

Descriptive data are presented as mean and standard deviations for CG and IG at baseline and 6 months post-intervention, except for the metabolic variables FPI, HOMA-IR and CRP which were presented as median and interquartile range as these showed deviation from normal distribution (Kolmogorov-Smirnov normality test). It was used a paired-samples Student's t-test (for variables with a normal distribution) and a Wilcoxon signed-rank test (for variables without normal distribution) for longitudinal analysis. Correlations between studied variables were established by a Pearson's or Spearman's rank test as appropriate.

Statistical analyses were conducted using SPSS version 22.0. Statistical significance was accepted at *p* less than 0.05.

RESULTS

Characteristics of the study sample are presented in Table I. Only 10 out of 20 controls in CG and 34 out of 45 participants in the IG have completed both measurements (baseline and after 6 months). Subjects with only one measurement (baseline or post-intervention) were included as there was no difference in the various outcomes when comparing to the remaining subjects (data not shown).

All of them were male, aged between 10 and 12 years old (mean of 10.7±1.3 years) and more than half were classified as being at a medium-phase of puberty (61.4% in Tanner stage 2-3).

As what concerns to nutritional status and body composition, all of them were obese (BMI z-sc of 2.5±0.7; min: 1.8, max: 3.2) with no statistical difference between groups at baseline. The IG showed a significant decrease in BMI z-sc, WC, WtHR, %BFM and an increase in %FFM after intervention. Differently, in the CG presented a significant increase in %BFM ($p=0.047$) but no changes in %FFM. Besides the high cardiovascular risk according to WtHR observed in both groups even after intervention, the IG showed a significant decrease (Table I).

Regarding metabolic variables, a significant improvement was observed on TC, TG, LDL-c in the IG, after 6 months-intervention. Considering glucose and insulin metabolism, a decrease in HOMA-IR in IG and an increase in CG were observed after 6 months intervention, although without statistical significance. When referring to oxidative and proinflammatory status, it was observed a significant improvement in adiponectin and a reduction in ox-LDL in IG but no significant differences in CRP.

As for the CRF, there was an improvement of VO_2max in both groups, but a statistically significant value in IG (Table I).

Prevalence of “at risk” adolescents regarding each cardiometabolic marker can be observed in Table II.

Correlations between changes in BMI and VO_2max and changes in body composition, metabolic variables, IR and oxidative and proinflammatory status are presented in Table III.

It is noteworthy the significantly correlation between positive variations in BMI and increased WC, as also cardiometabolic risk factors (LDL-c and HOMA-IR) and a negative correlation with CRF (VO_2max) and adiponectin. Furthermore, an inverse association was observed for changes in VO_2max and adiposity markers (BMI, WC, LDL-c and ox-LDL).

DISCUSSION

A major contributor to childhood obesity epidemic is lack of sufficient PA and that is why the engagement in PA and sport is a very important weapon in obesity prevention (Janssen et al., 2005, Hills et al., 2011). Physical education in schools is an effective public health initiative for promoting PA in children and youth but extra-school PA may be important for increasing PA and improving cardiometabolic risk factors and body composition in this population (Klakk et al., 2013, Cordova et al., 2012). A large proportion of adolescents do not follow the recommended PA guidelines and, although these are essential to avoid excess of BFM, besides MVPA, vigorous PA or extra recreational programs might have additional benefits in preventing obesity (Hills et al., 2011, Martinez-Gomez et al., 2010, Calcaterra et al., 2013).

The objective of this trial was to test the relation between exercise dose and IR, oxidative and proinflammatory status on obese adolescents while accessing anthropometric, metabolic variables and CRF. Initially there were 3 groups: CG, SG and AG. However, no significant difference in exercise dose was observed between SG and AG (different exercise groups), these were evaluated as an IG and compared to a CG (only performed school PA), corresponding to a moderate/intense exercise dose group and a low exercise dose group, respectively.

All the participants in this trial were obese at baseline with no anthropometrics differences between groups (Table I). BMI and %BFM are widely used to access adiposity (Weber et al., 2013), but BMI alone is unable to make comparisons between overweight and obese children among groups of children. Alternatively, BMI z-sc was used to measure adiposity as it is age and gender specific and provides a continuous variable (Bell et al., 2007).

Exercise improves BMI z-sc in overweight and obese children and adolescents (Kelley et al., 2014). In agreement with this results, a significant reduction in BMI-zs was observed in IG, although adolescents maintained obese (BMI z-sc = 2.2±0.7) (Table I). BMI is a poor predictor of body fat as it does not differentiate between fat mass and fat-free mass (Wan et al., 2014). However, this can be executable with DXA that evaluates body composition. In this study, PA in IG was significantly associated with a decrease in %BFM and an increase of %FFM which is compatible with other similar studies that involve PA in obese adolescents (Farias et al., 2009).

WC and WtHR are anthropometric predictors of central obesity associated with cardiometabolic risk (Mushtaq et al., 2011). It was observed a positive influence of PA on WC and WtHR in IG (Table I) with no statistically difference in CG. WC in adolescents is a well-known risk factor for MS and obese children with WC above the 90th percentile are at greater risk for developing dyslipidemia and IR than obese children without WC above this limit (Bassali et al., 2010). Although a significant reduction of WC occurred in IG, all participants maintained a WC above the 90th percentile, according to age and gender (Sardinha et al., 2012). The optimum cut-off for WtHR as a predictor of high cardiometabolic risk is still controversial in adolescent population (Mehta, 2014). In this trial it was used a cut-off of 0.50 and all sample at baseline was classified as having a high cardiometabolic risk (mean WtHR = 0.59), and after 6 months both groups maintained that high risk (mean WtHR CG = 0.59; mean WtHR IG = 0.55).

Dyslipidemia is one of the most important co-morbidities in obese youth and it is characterized by low levels of HDL-c and high levels of TC, TG and LDL-c. Obesity plus an unfavorable lipid profile during pediatric age are the best predictors of risk of CVD

worldwide. Portuguese population has a high prevalence of obese progenitors as well as a high score of familiar dyslipidemia and therefore a possible genetic cause of dyslipidemia must be taken into account, other than obesity-related dyslipidemia (Rêgo, 2008). In this study, after intervention, there was a significant improvement in TC, TG and LDL-c in IG. HDL-c increased at the end of intervention in both groups, being statistically different in the CG (Table I).

Regular PA is considered a powerful weapon for glycemic control in obese population as well as reduction of co-morbidities associated to alterations of glucose metabolism and T2DM (Riddell and Iscoe, 2006). In this study, regarding the metabolism of glucose and insulin, no influence of PA on IR was observed in this trial. The only significant result was an increase in FPG in IG post-intervention. This result may be explained by the fact that the major puberty status in this group (T2-3 = 68.8%) is a stage that curses with physiologic IR. Even thus, it should be highlighted that HOMA-IR increased in CG whereas a slight reduction occurred in IG. There was no pre-diabetes risk as the baseline value of FPG of all sample was inferior to 100 mg/dL.

Levels of oxidative stress and proinflammatory status have been found to be raised in children with obesity and features of MS, and are associated with the development of atherosclerosis and T2DM (Dennis et al., 2013, Buchan et al., 2014). It has been demonstrated that obese adolescents have higher levels of ox-LDL than normal weight peers and that ox-LDL is associated with IR after adjustment for body fatness (Norris et al., 2011). Other explored variable is adiponectin which was an adipose-derived protein with insulin-sensitizing and anti-inflammatory properties, being a protective hormone in obesity. Several studies showed that raised levels of CRP correlate with CVD risk factors,

including adiposity and blood pressure (Balagopal et al., 2011). One of the links between obesity and associated co-morbidities already mentioned is chronic low-grade systemic inflammation which was observed in the studied population at baseline (CRP between 0.3-2.4). PA had a significant positive influence on ox-LDL and adiponectin in IG. This last adipocytokine also had a significant increase in CG suggesting a physiologic increment with age. Surprisingly, influence of PA on CRP was not established in this study. It is possible that longer intervention periods are necessary to achieve improvements in this inflammatory variable.

CRF is the estimation of the global capability of the cardiovascular and respiratory systems to carry out sustained exercises (Calcaterra et al., 2013). Changes in CRF are largely influenced by PA, as it can be seen by a significant VO_2 increase in IG post-intervention. Obesity and low CRF have been shown to independently rise the risk of cardiovascular mortality, however in this trial all VO2 measures presented were above the cut-off value for low CRF.

Low PA levels and low CRF have been associated with an elevated cluster of metabolic risk factors, not only in adults but also in youth. Recently, influence of PA on both fatness and fitness has been shown (Rizzo et al., 2007). This study, which involved a 6 month controlled recreational activity program with 3 times-weak of MVPA, demonstrated a significant inverse relation between the decrease of CRF and increasing BMI values (Table III). A better CRF was positively correlated with improvement of metabolic variables and oxidative and anti-inflammatory variables, as shown, respectively, by a decreased LDL-c, ox-LDL and increased adiponectin. Another important finding was the positive

correlation between changes in BMI and in IR (Table III). The absence of correlation between CRF and a decreased risk of IR may be due to the small sample size.

One of the most important limitations of this study are the small sample size (n=44), but also the fact that family history of CVD was not accessed and that exercise dose was not robustly classified in the CG. Other limitation in accessing diabetes risk was the fact that IR was not measured by the gold standard method.

Positive aspects rely on having a non-exercising obese CG, being a longitudinal study of considerable time (6 months) and the fact that various important variables linked to obesity were evaluated.

CONCLUSION

Moderate-to-vigorous PA improved cardiovascular risk, oxidative and proinflammatory status and CRF in obese adolescents, but not diabetes risk. In Portugal, school-based PA programs show to be ineffective in reducing obesity and, an extra-curricular PA program, at least three times a week with MVPA, is mandatory as more effective in improving the aforementioned aspects that are extremely linked with obesity and that traduces a risk of development of CVD and T2DM.

Declaration of interest

The authors report no declarations of interest.

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Table I:

Characteristics of the study sample at baseline and post-intervention: control (n=10) and intervention (n=34) groups

	All Sample	**Control Group (n=10)**			**Intervention Group (n=34)**		
	Baseline	Baseline	Post-intervention	*p*-Value	Baseline	Post-intervention	*p*-Value
CA (years)	10.7 (1.3)	10.6 (1.4)	11.2 (1.4)	**0.004**	10.8 (1.3)	11.3 (1.2)	**<0.001**
Height (z-score)	1.1 (1.1)	2.0 (1.3)	1.7 (1.3)	**0.003**	0.9 (0.9)	0.7 (0.9)	**<0.001**
BMI (z-score)	2.5 (0.7)	2.9 (0.6)	2.8 (0.6)	0.167	2.4 (0.7)	2.2 (0.7)	**<0.001**
WC	88.2 (10.4)	93.1 (10.2)	92.5 (10.7)	0.690	86.7 (10.1)	82.7 (9.8)	**<0.001**
WtHR	0.59 (0.06)	0.60 (0.06)	0.59 (0.06)	0.343	0.58 (0.06)	0.55 (0.06)	**<0.001**
BFM (%)	35.3 (7.3)	34.6 (6.6)	39.1 (10.6)	**0.047**	35.4 (7.5)	32.2 (8.5)	**<0.001**
FFM (%)	36.9 (8.3)	43.6 (9.2)	41.4 (9.5)	0.220	35.0 (7.0)	36.9 (7.6)	**<0.001**
Tanner Stage [n(%)]	**T1** 13 (29.5) **T2-3** 27 (61.4) **T4-5** 4 (9.1)	**T1** 2 (20) **T2-3** 7 (70) **T4-5** 1 (10)	**T1** 0 (0) **T2-3** 9 (90)) **T4-5** 1 (10)	0.168	**T1** 11 (32.4) **T2-3** 20 (58.8**)** **T4-5** 3 (8.8)	**T1** 9 (26.5) **T2-3** 21 (61.7) **T4-5** 4 (11.8)	**0.003**
TC (mg/dL)	167.2 (34.3)	170.3 (21.1)	171.3 (25.7)	0.854	166.3 (37.5)	155.2 (29.7)	**0.002**
TG (mg/dL)	80.6 (43.6)	81.0 (45.0)	72.3 (34.1)	0.440	80.5 (43.9)	60.9 (22.0)	**0.001**
LDL-c (mg/dL)	100.9 (33.2)	107.6 (19.6)	106.4 (27.5)	0.800	98.9 (36.2)	88.5 (29.6)	**0.004**
HDL-c (mg/dL)	50.5 (10.7)	46.5 (8.9)	50.4 (9.4)	**0.010**	51.6 (11.1)	54.5 (13.0)	0.064
FPG (mg/dL)	82.8 (6.2)	85.6 (8.0)	83.1 (9.3)	0.330	82.0 (5.4)	85.9 (6.8)	**0.002**
FPI (µU/mL)	10.3 (5.7-11.5)	9.3 (5.9-12.1)	11.5 (9.2-15.9)	0.285	10.3 (5.5-11.2)	7.6 (5.8-11.3)	0.739
HOMA-IR	1.9 (1.1-2.4)	1.8 (1.3-2.7)	2.4 (2.0-3.2)	0.285	1.9 (1.0-2.4)	1.8 (1.2-2.9)	0.326
ox-LDL (U/L)	58.5 (19.6)	65.1 (16.4)	61.9 (15.4)	0.296	56.5 (20.3)	50.5 (16.3)	**<0.001**
Adiponectin (µg/mL)	9.8 (4.1)	9.0 (2.2)	10.6 (2.7)	0.057	10.5 (4.7)	14.4 (6.4)	**<0.001**
CRP (mg/L)	0.7 (0.3-2.4)	0.9 (0.6-1.4)	0.8 (0.3-1.9)	0.878	0.6 (0.3-3.1)	0.7 (0.4-2.4)	0.521
VO_2max (mlO_2/min/Kg)	44.3 (9.5)	45.0 (12.9)	46.6 (18.8))	0.933	44.1 (8.7)	49.6 (7.4)	**<0.001**

Results are presented as mean (standard deviation) or median (interquartile range), unless otherwise indicated

CA: Chronological age; BMI: Body mass Index; WC: waist circumference; WtHR: Waist to height ratio; BFM: Body fat mass; FFM: Fat-free Mass; TC: Total cholesterol; TG: Triglycerides; LDL-c: Low-density lipoprotein cholesterol; HDL-c: High density lipoprotein cholesterol; FPG: Fasting plasma glucose; FPI: Fasting plasma insulin; HOMA-IR: Homeostatic model assessment for insulin resistance; ox-LDL: oxidized low-density lipoprotein; CRP: C-reactive protein; VO_2max: maximal oxygen uptake

p-values refer to longitudinal analyses (baseline vs post-intervention) within each group

Table II:

Prevalence of “at risk” adolescents, regarding cardiometabolic risk factors within control and intervention groups

	Control Group (n=10)		Intervention Group (n=34)	
	Baseline	Post-intervention	Baseline	Post-intervention
TC	1 (10)	1 (10)	5 (15)	3 (9)
TG	1 (10)	0 (0)	2 (6)	0 (0)
LDL-c	1 (10)	2 (20)	4 (12)	3 (9)
HDL-c	1 (10)	0 (0)	3 (9)	1 (3)
HOMA-IR	1 (10)	1 (10)	1 (3)	1 (3)

Values presented as n (%)

“At risk” was defined as: TC≥ 200 mg/dl or LDL-c ≥ 130 mg/dl or TG≥ 150 mg/dl or HDL-c ≤ 35 mg/dl or HOMA IR ≥ 2.67 and 5.22 for pre- and pubertal adolescents

TC: Total cholesterol; TG: Triglycerides; LDL-c: Low-density lipoprotein cholesterol; HDL-c: High density lipoprotein cholesterol; HOMA-IR: Homeostatic model assessment for insulin resistance

Table III:

Correlations between changes in BMI and VO_2max and changes in body composition, metabolic variables and oxidative and proinflammatory status

	Δ BMI		**Δ VO_2 max**	
	r	*p*-value	r	*p*-value
Δ BMI	---	---	-0.339	**0.032**
Δ WC	0.588	**<0.001**	-0.327	**0.040**
Δ LDL-c	0.337	**0.025**	-0.317	**0.046**
Δ HOMA-IR	0.335	**0.026**	-0.063	0.700
Δ ox-LDL	0.253	0.098	-0.321	**0.004**
Δ adiponectin	-0.432	**0.003**	0.392	**0.012**

Δ were calculated as the difference between post-intervention and pre-intervention measures for all sample (n=44)

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Authors are asked to take into account the diverse audience of the journal. Please avoid the use of terms that might be meaningful only to a local or national audience, or provide a clear explanation where this is unavoidable. Some specific points on style follow:

1. Authors should write in clear, concise English. If this is not your native language please ensure the manuscript has been reviewed by a native speaker. Please note: extensive rewriting of the text will not be undertaken by the editorial staff.
2. Acronyms for protein and gene names should in all cases be explained the first time they appear. In articles where acronyms are numerous, authors should include a table that lists all acronyms, each acronym's meaning or origin, and a short description of the function of each gene or protein.
3. Latin terminology, including microbiological and species nomenclature, should be italicized.
4. Use standard convention for human and animal genes and proteins: italics for genes and regular font for proteins, and upper case for human products and lower case for animal products.
5. "US" is preferred to "American", "USA" to "United States", and "UK" to "United Kingdom".
6. Double quotation marks rather than single are to be used unless the "quotation is 'within' another".
7. Punctuation of common abbreviations should adhere to the following conventions: "e.g."; "i.e."; "cf.". Note that such abbreviations should not generally be followed by a comma or a (double) point/period.

8. Upper case characters in headings and references should be used sparingly, e.g. only the first word of paper titles, subheadings and any proper nouns begin upper case; similarly for the titles of papers from journals in the references and elsewhere.
9. Apostrophes should be used sparingly. Thus, decades should be referred to as follows: “The 1980s [not the 1980’s] saw …”. Possessives associated with acronyms (e.g. APU), should be written as follows: “The APU’s findings that …” but note that the plural is “APUs”.
10. All acronyms for national agencies, examinations, etc., should be spelled out the first time they are introduced in text or references. Thereafter the acronym can be used if appropriate, e.g. “The work of the Assessment of Performance Unit (APU) in the early 1980s …” and subsequently, “The APU studies of achievement …”, in a reference “(Department of Education and Science [DES] 1989a)”.
11. Brief biographical details of significant national figures should be outlined in the text unless it is quite clear that the person concerned would be known internationally. Some suggested editorial comments in a typical text are indicated in the following with square brackets: “From the time of H. E. Armstrong [in the 19th century] to the curriculum development work associated with the Nuffield Foundation [in the 1960s], there has been a shift from constructivism to heurism in the design of [British] science courses”.
12. Non-discriminatory language is mandatory. Sexist or racist terms should not be used.
13. The referred local (national) usage for ethnic and other minorities should be used in all papers. For the USA, “African-American”, “Hispanic” and “Native American” are used, e.g. “The African-American presidential candidate, Jesse Jackson …”; for the UK, “Afro-Caribbean” (not “West Indian”), etc.
14. Material to be emphasised by italicisation in the printed version should be italicised in the typescript rather than underlined. Please use such emphasis sparingly.
15. Numbers in text should take the following forms: 300, 3000, 30 000 (not 30,000). Spell out numbers under 10 unless used with a unit of measure, e.g. nine pupils but 9 mm (do not use full stops (periods) within units). For decimals, use the form 0.05 (not .05, × 05 or 0× 05). “%” (not “per cent”) should be used in typescripts.
16. Authors must adhere to SI Units
17. Appendices should appear before the references section and after any acknowledgments section. The style of the title is shown by the following example:
    “Appendix C: The random network generator”.
    Figures and tables within appendices should continue the sequence of numbering from the main body of the text. Sections within appendices should be numbered, for example, C.1, C.2. Equations in appendices should be numbered, for example, (C 1), (C 2). If there is only one appendix, it is referred to as “the appendix” and not called “Appendix A”.

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For abbreviations and nomenclature, authors should consult the most recent edition of the *CSE Style Manual* available from the Council of Science Editors, 60 Revue Drive, Suite 500 Northbrook, IL, 60062, USA.

Mathematics
Please click here for more information on the presentation of mathematical text.

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Footnotes to tables should be typed directly below the table and are indicated by the following symbols: * (asterisk or star), † (dagger), ‡ (double dagger), ¶ (paragraph mark), § (section mark), || (parallels), # (number sign). Reinitialize symbol sequence within tables.

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ETHICS OPINION

Process CEFADE 12/2013

The Ethics Committee of Faculty of Sport from the University of Porto analyzed the project entitled "Soccer as a novel therapeutic approach to pediatric obesity. A randomized controlled trial and its effects on fitness, body composition, cardiometabolic and oxidative marker". This project was presented by André Filipe Teixeira Seabra, Ph.D, and we decided to address a positive opinion. It shows respect for ethical principles that govern this type of scientific work.

Porto and Faculty of Sport, March 13 from 2013

The chairman of the ethics committee,

Rui Manuel Proença de Campos Garcia