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Effect of a high-intensity exercise program in the number of circulation endothelial progenitor cells in overweight/obese adolescents

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Abstract

Background: Obesity has been increasing in the young population, consequently all its associated pathologies. This is currently associated with endothelial dysfunction, as well as with the number of progenitor cells and their regenerative endothelial capacity. The purpose of this study was to determine the effects of a high-intensity exercise training program in the circulating number of hematopoietic stem cells and endothelial progenitor cells in overweight/obese adolescents.

Methods: A total of 20 (mean age: 16.7 ± 1.0 years old, weight: 76.9 ± 13.3 kg, body mass index: 28.4 ± 3.9 kg/m^2) overweight/obese adolescents completed a 10-week high intensity training plan with 3 different workouts per week, each session lasting about 60 minutes, composed of warm up; high intensity functional exercises in circuit and cool down. The exercises were performed at maximum intensities, with a heart rate above 90% of the theoretical heart rate. Body composition, number of endothelial progenitor cells and hematopoietic stem cells were measured at baseline and after the 10-week intervention using flow cytometry.

Results: After the intervention, an increase in the circulating number of endothelial progenitor cells (0.0060 ± 0.0021 to 0.0081 ± 0.0020%, p=0.006) was observed; the number of hematopoietic stem cells did not change (0.0319 ± 0.0070 to 0.0321 ± 0.0041%, p=0.920). A significant decrease in body weight (76.91 ± 13.29 to 75.6 ± 12.55 kg, p=0.006), BMI (28.39 ± 3.86 to 27.96 ± 3.79 kg/m^2, p=0.011), waist circumference (94.05 ± 11.26 to 90.21 ± 12.00 cm, p<0.001), the amount of fat mass (27.53 ± 6.66 to 24.25 ± 6.19 kg, p<0.001) and a significantly increased in the amount of fat free mass (49.40 ± 9.57 to 51.35 ± 9.53 kg, p<0.001) was observed after the intervention.

Conclusions: A 10-week high-intensity exercise program improved several indexes of obesity and increased the circulation levels of EPCs.

Key-words: high intensity exercise; EPCs; obesity; prevention.

Resumo

Introdução: A obesidade tem aumentado entre a população jovem, e em consequência todas as suas patologias associadas. Isto está atualmente associado à disfunção endotelial, bem como ao número de células progenitoras e à sua capacidade endotelial regenerativa. O objetivo deste estudo foi determinar os efeitos de um programa de treino com exercícios de alta intensidade no número circulante de células tronco hematopoiéticas e células progenitoras endoteliais em adolescentes com sobrepeso/obesos.

Métodos: Um total de 20 (média de idade: 16.7 ± 1.0 anos de idade, peso: 76.9 ± 13.3 kg, índice de massa corporal: 28.4 ± 3.9 kg/m^2) adolescentes com sobrepeso/obesos completaram um plano de treino de alta intensidade de 10 semanas com 3 sessões diferentes por semana, cada sessão com a duração de cerca de 60 minutos, composta por aquecimento; exercícios funcionais de alta intensidade em circuito e retorno à calma. Os exercícios foram realizados em intensidades máximas, com frequência cardíaca acima de 90% da frequência cardíaca teórica. A composição corporal, o número de células progenitoras endoteliais e células tronco hematopoiéticas foram medidos na linha de base e após 10 semanas de intervenção usando citometria de fluxo.

Resultados: Após a intervenção, observou-se aumento do número de células progenitoras endoteliais (0.0060 ± 0.0021 to 0.0081 ± 0.0020%, p=0.006); o número de células tronco hematopoiéticas não sofreu alteração (0.0319 ± 0.0070 to 0.0321 ± 0.0041%, p=0.920). Uma diminuição significativa no peso corporal (76.91 ± 13.29 to 75.6 ± 12.55, p=0.006), IMC (28.39 ± 3.86 to 27.96 ± 3.79, p=0.011), circunferência abdominal (94.05 ± 11.26 to 90.21 ± 12.00, p<0.001), quantidade de massa gorda (27.53 ± 6.66 to 24.25 ± 6.19, p<0.001) e um aumento significativo de massa magra (49.40 ± 9.57 to 51.35 ± 9.53, p<0.001) foram observados após a intervenção.

Conclusão: Este programa de exercícios de alta intensidade de 10 semanas melhorou vários índices de obesidade e aumentou os níveis de circulação de EPCs.

Palavras-chave: exercício de alta intensidade; EPCs; obesidade; prevenção.

Introduction

Childhood and adolescent obesity has been considered one of the most serious public health challenges of the 21st century (WHO, 2017). In Portugal, recent studies determining the prevalence of obesity in childhood (Viveiro, Brito, & Moleiro, 2016) and adolescence (Camarinha, Graça, & Nogueira, 2016), showed a prevalence of 13,4% and 15,2%, respectively. Viveiro et al. (2016) assessed 6175 children and adolescents (52% female) and reported an overall overweight and obesity prevalence of 18.7% and 13.4%, respectively. Camarinha et al. (2016) showed a higher prevalence of overweight, 37.4%, in a sample of 8 974 pre and basic-school children. This high prevalence of overweight/obesity puts Portugal as the fourth country in Europe with the highest prevalence (WHO, 2017).

Childhood obesity has significant short term and long-term adverse medical and psychosocial effects extending into adulthood (Reilly, et al., 2003). The deleterious effects of obesity encompass type II diabetes mellitus, dyslipidemia, obstructive sleep apnea, certain cancers, and major cardiovascular diseases, such as heart failure, coronary heart disease, sudden cardiac death (Lavie, Milani, & Ventura, 2009). Strong evidence demonstrates that overweight and obesity in childhood and adolescence have adverse consequences on premature mortality and physical morbidity in adulthood (Reilly & Kelly, 2011).

Prolonged or repeated exposure to cardiovascular risk factors, such as obesity, can lead to a dysfunctional endothelium, or endothelial activation (Deanfield, Halcox, & Rabelink, 2007). In fact, this is not an irreversible process, and upon endothelial damage, the repair mechanisms are activated to mitigate the initiation/development of the atherosclerotic process (Bruyndonckx, et al., 2013).

Over the years, it has become clear that circulating endothelial progenitor cells (EPCs) are an important mechanism for endothelial integrity maintenance and repair. Asahara et al. (1997) were the first group to report the isolation and characterization of hematopoietic stem cells (HSC) from human blood, which possess the ability to differentiate into an endothelial phenotype. These were named EPCs. HSCs and EPCs can enhance angiogenesis, promote vascular repair, improve endothelial function, and induce neovascularization. In 2003, Hill et al. showed that EPCs derived from bone marrow have a role in the endothelial repair and that impaired mobilization or depletion of these cells contributes to endothelial dysfunction and cardiovascular disease progression. Werner and colleagues, in 2005, showed that the risk of death form cardiovascular causes is increased in patients with low EPCs levels, and their findings suggest that EPCs contribute to the restoration of the endothelial monolayer. The number of EPCs is decreased in obese individuals; MacEneaney et al. (2009) assessed whether adiposity influences EPCs number and colony-forming capacity in 63 adults, 24 obese, 18 overweight and 25 normal weight. They concluded that the number of circulating EPCs was lower in obese (0.0007 ± 0.0001%) compared with overweight (0.0016 ± 0.0004%) and normal weight (0.0015 ± 0.0003%); additionally, the EPC colony formation was significantly less in the obese and overweight compared with normal weight adults. This study showed that overweight/obese subjects have not only a reduced number of EPCs but the EPCS also express reduced functionality.

Physical exercise has been recommended as a key strategy to reduce the burden of obesity. For instance, the WHO guidelines recommend do at least 60 minutes of moderate to vigorous-intensity physical activity daily for children and adolescents (5-17 years old), and a minimum 150 minutes of moderate-intensity physical activity throughout the week, or at least 75 minutes of vigorous-intensity physical activity throughout the week, or an equivalent combination of moderate and vigorous-intensity activity to adults (WHO, 2017).

The participation in an exercise training program seems to increase the number of circulation EPCs, which could contribute to vascular regeneration/repair and angiogenesis (Ribeiro, et al., 2013). Watts et al. (2004) showed that exercise training reverses vascular dysfunction associated with obesity in adolescents. In 2004, Laufs and colleagues demonstrated in an animal model that physical activity, 28 weeks of running wheels, leads to an increased number of circulating EPCs. This increased occurred 7 days after training and was sustained for at least 4 weeks, providing evidence that EPC numbers can be increased by exercise training.

In the last decade, several studies, were done assessing the relationship between HSC, EPCs and endothelial repair in different populations and with different types of exercise, for instance in adults with cardiovascular risk factors (Hill, et al., 2003), in healthy adults after strenuous exercise (Möbius-Winkler, et al., 2009) or after an aerobic-endurance exercise in young and older healthy men (Hoetzer, et al., 2007). On the other hand, the number of studies assessing the effects of exercise on EPCs number in obese children and young adolescents is reduced. Previous studies showed i) a positive correlation between physical fitness and the number of EPCs in obese children/adolescents (Arnold, Wenta, Müller-Ehmsen, Sreeram, & Graf, 2010), ii) that body mass index and EPCs in children and adolescents with obesity (Pires, et al., 2015), ii) a marked increase in circulating EPC after a 10-month supervised diet and exercise program (Bruyndonckx, et al., 2015). Walther et al. (2009) examined whether additional school exercise lessons would result in improved EPCs number in 182 children, aged 11.1 ± 0.7 years, but only 12.8% of those participating in the exercise group were obese or overweight. They found a significant improvement in the EPCS number, but they did not observed changes in body mass variables.

To our best knowledge only Park et al. (2012) evaluated the effects of an exercise training intervention in the circulating levels of EPCs in overweight and obese children. In this study, they randomized 29 overweight/obese children (12.2 ± 0.1 years) into control (i.e. no after-school exercise, n = 14) and after-school exercise (n = 15) groups. The exercise group performed a 12-week exercise intervention consisted of 3 days of combined aerobic and resistance exercise per week. They showed that the exercise intervention increased the levels of EPCs and decrease the carotid intima-media thickness.

None of the above mentioned studies used a high intensity interval training program. The high intensity interval training (HIIT) includes circuits with a sequence of several exercises performed at 80% to 95% of an individual's estimated maximum heart rate (the objective is to do the maximum number of repetitions in each exercise) with brief periods of rest between each exercise. HIIT training has been shown to improve aerobic and anaerobic fitness, blood pressure, cardiovascular health, insulin sensitivity, cholesterol profiles, abdominal fat and body weight while maintaining muscle mass (ACSM, 2017).

Childhood and adolescent obesity is a global epidemic that should be fought back by families, communities and health professionals. Regarding physiotherapists, the World Confederation for Physical Therapy (WCPT) supports physiotherapists in their endeavours to counter the obesity epidemic; physiotherapists as exercise experts have a major role to play in the prevention and management of obesity (WCPT, 2017). The International Organisation of Physical Therapists in Paediatrics (IOPTP), a WCPT subgroup, advocates an active role of the physiotherapist in the implementation of comprehensive programmes that promote physical activity and reduce sedentary behaviours in children and adolescents, mainly because often key issues such as limitations to fundamental motor skill, underlying biomechanical issues or breathing difficulties are not considered when designing general programs (IOPTP, 2017).

Based on the limited information about the effects of an exercise intervention on the EPCs levels in young overweight/obese adolescents and the lack of a study assessing the impact of high intensity training on these indicators and in this population, we designed a study to determine the effects of a high-intensity exercise training program in the circulating number of hematopoietic stem cells and endothelial progenitor cells in overweight/obese adolescents.

Methods

Study design and participants

This is a quasi-experimental study, where the convenience sample was composed by students of the João Gonçalves Zarco School (Matosinhos). They participated, after authorization to perform the study has been granted by them, their parents and/or guardians and the school's director. The recruitment took place during the month of march and the intervention was promoted between the months of April and June of 2016. The following inclusion criteria were considered: 1) attending secondary education (10 to 12th year) in the João Gonçalves Zarco School; 2) have a BMI greater than or equal to 25 kg / m2. Regarding the exclusion criteria, the following were considered: 1) presence of contraindication for performing physical exercise; 2) non-delivery of informed consent and request for authorization, duly signed by their parents or guardians. All students with these characteristics were invited to join the physical exercise program, and 30 adolescents showed interest in participating. Of the 30 participants, 25 were female and 5 were male, but 4 participants were excluded because they did not meet the inclusion criteria, namely BMI <25 $kg.m^{-2}$. Then 26 were included in the intervention. Additionally, participants who failed to attend to at least 80 % of the exercise sessions were excluded from the statistical analysis.
After recruitment, participants completed questionnaires for general characterization, where information such as age, gender and general health status were collected to determine if there was any contraindication to physical exercise, in order to verify whether the inclusion / exclusion criteria were met. After that, they were submitted to body composition assessment and blood collection to analyze the study parameters before and at the end of the exercise program.
The study procedures were in accordance with the ethical standards on human experimentation and the requirements of the Declaration of Helsinki were observed. In addition to the consent of the participants, the written informed consent was obtained from

parents/ guardians. The Ethics Committee of the Faculty of Sport of the University of Porto approved this study.

Of the 26 adolescents recruited initially, only 20 completed at least 80% of the exercise sessions, attending all the proposed training sessions and attended the blood collection 48 hours after the last exercise session for post-program evaluation (Figure 1).

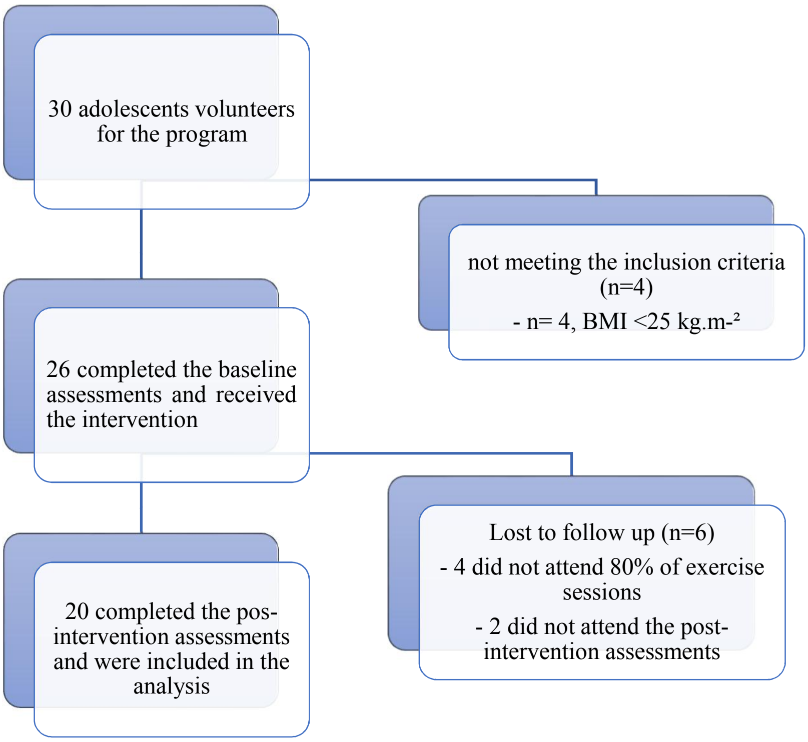


Figure 1: Flow diagram providing information concerning enrolment, intervention, follow-up, and analysis.

Anthropometry and Body Composition

The height of the adolescents was collected using a wall stadiometer, when the adolescents were wearing light clothing and no shoes. The waist circumference was collected using a tape-measure, no clothe, measurement made at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest, breathing normally (WHO, 2008). Weight and waist circumference were recorded to the nearest 0.1 cm.

The Tanita SC-240 foot-to-foot body composition analyzer (Tanita Cooperation, Tokyo, Japan) was used to assess bioelectrical impedance (Nyström, Henriksson, Alexandrou, & Löf, 2016). Measurements were collected at 50 Hz using the standard setting after manually imputing the measured height, gender, and age of the adolescents. They were bare foot and wore minimal clothing and were instructed to standstill with their feet touching all four metal plates. The measures of body index mass (BMI), fat mass, fat (percentage), fat free mass (FFM) and total body water (TBW) were obtained.

Measurement of endothelial progenitor cells and hematopoietic stem cells

Blood samples (3 mL) for EPC analysis were collected into EDTA tubes and treated with TransFix (Cytomark, Caltag Medsystems Ltd, Buckingham, UK) at a 1:5 ratio immediately after collection. These blood samples were stored in the dark at room temperature until flow cytometry analysis (at day three or day four after collection). It was demonstrated that it is possible to analyse TransFix stabilised blood cells up to seven days after blood collection (Hoymans, et al., 2012).

To evaluate EPCs in the peripheral blood by flow cytometry, whole blood samples were labelled with monoclonal antibodies against CD34 (APC, Miltenyi Biotec), CD309 (VEGFR-2/KDR; PE, Miltenyi Biotec), and CD45 (FITC, Miltenyi Biotec). After erythrocyte lysis, at least 250,000 $CD45^{+}$ cells were acquired on a FACS-Calibur flow cytometer (Becton Dickinson, San Jose, CA). Data were analyzed using Paint-a-Gate software (Becton Dickinson) and the identification of the EPCs was based on morphological properties and $CD45^{dim}/CD309^{+}/CD34^{+}$ profile, according to Schmidt-Lucke et al. sequential strategy (Schmidt-Lucke, et al., 2010). CD34+ cells were defined as hematopoietic stem cells. Leukocytes were identified using their characteristics in the forward-sideward scatter profile. The HSC and EPCs are reported as percentage of cells between the leukocytes.

Intervention: exercise training program

The protocol had a duration of 10 weeks (from April to June 2016), divided into 2 microcycles (each repeated over 4/5 weeks), composed of 3 different training sessions, performed on different days of the week. Each training session lasted 60 minutes, divided into three essential parts of the training: warm up, near 8 to 15 minutes, and general mobility exercises between 6 to 8 minutes; high intensity functional exercises in circuit between 30 to 35 minutes and cool down with stretching. The exercises that were used in the in circuit were adapted from the CrossFit. All the exercises were demonstrated to the participants, exemplifying the challenges, the numbers of repetitions and the times of each station foreseen for that session. All technical execution doubts were taken. The training plans are available in attachment (I to VI).

All exercises were performed at maximum intensities, the main objective being that the participant performed the whole circuit technically correctly and with a heart rate ideally above 90% of the theoretical heart rate. The target heart rate was continuously monitored using a heart rate monitor (Polar FT1, Kempele, Finland).

Data Analysis

Statistical analyses were performed using IBM SPSS statistics version 21.0 (IBM Corporation, Chicago, IL, USA). Normality of the data was tested with the Shapiro-Wilk test and all data were normally distributed. The differences between baseline and after intervention value were tested with paired sample test-t. Pearson correlation was used to test associations i) at baseline between the participants' characteristics, obesity indexes and circulating levels of EPCs, HSC and leukocytes; and ii) between the changes in EPCs, HSC and leukocytes with the changes in obesity indexes. The level of significance was set as $P \leq 0.05$.

Results

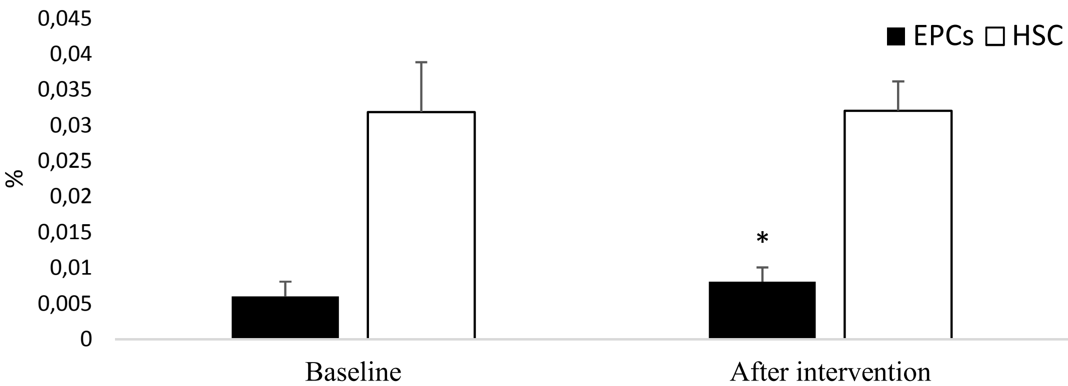
A total of 26 adolescents (4 boys and 22 girls, age 16.8 ± 1.0 years, height 164.5±9.6 cm, weight 76.3 ± 12.91 kg, BMI 28.16 ± 3.60 kg/m^2, EPCs 0.006 ± 0.002%) were recruited, but just 20 completed the study. The results of the 20 participants (4 boys and 16 girls, age 16.7±1.0 years, height 164.5±9.6 cm) that completed the program are shown in Table 1. A significant decrease in obesity indexes was observed; they decreased body weight, BMI, waist circumference, the amount of fat mass and increased significantly the amount of fat free mass (Table 1).

Table 1. Change in outcome measures from baseline to after intervention.

	Baseline	**After intervention**	**Mean difference (95% confidence interval)**	**P Value**
Weight (kg)	76.91±13.29	75.6±12.55	1.30 (0.43 to 2.17)	0.006
BMI (kg/cm^2)	28.39±3.86	27.96±3.79	0.42 (0.11 to 0.73)	0.011
WC (cm)	94.05±11.26	90.21±12.00	3.83 (2.70 to 4.96)	<0.001
Fat Mass (%)	35.75±5.42	32.06±5.65	3.68 (2.97 to 4.39)	<0.001
Fat Mass (kg)	27.53±6.66	24.25±6.19	3.27 (2.52 to 4.01)	<0.001
FFM (kg)	49.40±9.57	51.35±9.53	-1.96 (-2.69 to -1.22)	<0.001
TBW (kg)	36.17±7.01	37.60±6.98	-1.43 (-1.96 to -0.89)	<0.001

Data are mean ± SD; BMI: Body Mass Index; FFM: Fat Free Mass; TBW: Total Body Water; WC: Waist Circumference.

Regarding EPCs and HSC, the participants in the exercise training intervention increase significantly the circulating levels of EPCs (0.0060±0.0021 to 0.0081±0.0020, p=0.006) but the circulating levels of HSC did not change (0.0319±0.0070 to 0.0321±0.0041, p=0.920) (Figure 2).



* significantly different from baseline, p=0.006

Figure 2: Differences between EPCs and HSC in baseline and after intervention.

The statistical analyses of the leukocytes showed no changes in monocytes, lymphocytes and granulocytes from based to the after the intervention (Figure 3).

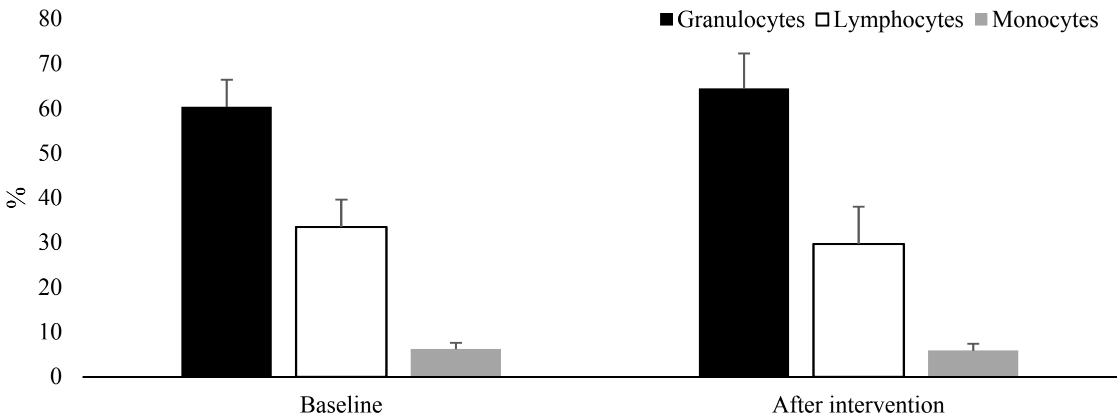


Figure 3: Leukocyte profile in baseline and after intervention.

Correlations

A significant and positive correlation was observed between HSC and body weight (r=0.524, p =0.018), waist circumference (r=0.465, p=0.039), and fat free mass (r=0.513, P=0.021) at baseline.

The changes induced by the intervention in body weight were correlated with changes in other obesity indexes; showing that the decrease in body weight was associated with the decrease in BMI (r=0.98; p<0.01); and fat mass (r=0.61; p=0.01). The decrease in body weight was also associated with an increase in FFM (r=0.59; p=0.01), showing that those with increasing change in body weight (lose) also showed and increased change in FFM (gain).

The changes in HSC (despite not significant) were correlated with changes in the percentage of fat mass (r=0.53; p=0.02) and kilograms (r=0.61; p<0.001) of fat mass. No further significant associations were observed between EPCs and HSC and the other variables.

Discussion

The main findings of the present study indicate that a 10-week exercise intervention improved several indexes of obesity and increased the circulating levels of EPCs, indicating that it has the potential to improve endothelial function and repair/regeneration.

After the intervention, several outcome measures such as weight, body mass index, waist circumference and fat mass decreased as well as increased the amount of fat-free mass. These findings show the effectiveness of the training program to reduce the indexes of obesity. This fact was at some extend expected, as a recent review of literature (Paes, Marins, & Andreazzi, 2015) demonstrated that physical exercise, regardless of type, promotes positive adaptations in several indexes of childhood obesity. Also, Bruyndonckx, et al. (2015) in a quasi-randomized study enrolling obese adolescents divided into an intervention group (N=33), treated with supervised diet and exercise (cardio and a resistance training) and a control group (N=28), showed that the intervention decreased BMI, weight, and body fat percentage. Similarly, Park, et al. (2012) investigated the effects of a high intensity exercise program (12-week, consisted of 3 days of combined aerobic and resistance exercise per week) in 29 overweight and obese children (15 in exercise group and 14 in control group), and also observed a decreased in body mass, BMI and waist circumference.

Regarding the level of EPCs, the results of the present study demonstrate a statistically significant increase in the circulating levels of EPCs after the intervention. Some previous studies showed a positive impact on EPCs of different types of exercise in obese individuals and young population (children and adolescents). One of these studies was conducted by Walther and colleagues in 2009 with 182 children (only 12% obese) divided into an intervention group (N = 109), who received 1 unit of exercise (45 minutes) with at least 15 minutes of resistance training, in addition to the two periods of 45 minutes per week included in the compulsory school plan, the latter was also performed by the control group (N = 73). They showed that the number of circulating progenitor cells increased in intervention group, and there was a positive trend in body mass index; however, the percentage of obesity used in their study was small. Park, et al. (2012), in the study described above, also observed a two-fold increase in EPCs in the exercise group. The differences between their study and this, are the type of training and the age of the subjects, but with similar results in relation to the number of EPCs. Also in the study of Bruyndonckx, et al. (2015), a marked increase in circulating EPC in exercise group was observed.

In 2010, Arnold, Wenta, Müller-Ehmsen, Sreeram, & Graf evaluated 24 obese children and adolescents, and concluded that a higher physical fitness, but not less body fat or body mass index, was associated with a higher number of endothelial progenitor cells, supporting the evidence of a positive association between physical fitness and the number of endothelial progenitor cells in obese children and adolescents. They did not find any correlation between the parameters of body composition and the number of EPCs, just like the results obtained in the present study.

The mechanisms of mobilization of EPCs from the bone marrow to circulation are not completely understood. However, an explanation for the exercise-induced mobilization of EPCs from the bone marrow is that exercise will activate endothelial nitric oxide synthase (eNOS), resulting in an increase of nitric oxide (NO), and stimulate the expression of circulating factors like VEGF, SDF-1, and EPO, subsequently, MMP-9 is activated leading to a mobilization of EPCs from the bone marrow (Lenk, Uhlemann, Schuler, & Adams, 2011). One of the main functions of NO is to promote relaxation of the smooth muscle, inducing vasodilation and regulating tissue perfusion, it also exerts central functions on vascular homeostasis, by inhibiting platelet and leukocyte adhesion and proliferation of vascular smooth muscle cells (Pires, Castela, Sena, & Seiça, 2015). Regular exercise also promotes the acute increase of blood flow and shear stress and, in turn, improves the NO bioavailability, hence increasing the endothelium-dependent vasodilatation (Ribeiro, Alves, & Oliveira, 2010)**.**

Concerning in the number of HSC, in the present study, no statistically significant differences were found. The improvement of the obesity index seems to be related to the increase in the number of HCS, although we did not find a relationship between changes in obesity variables and the EPCs levels. However, there may be no direct relationship between the decrease in obesity rates and the increase in EPCs, since the EPCs increase can be mediated by mechanisms independent of obesity. Also in the study described above, Arnold, Wenta, Müller-Ehmsen, Sreeram and Graf (2010) found no correlation between body composition parameters and number of EPCs.

At time when there are worrisome data on the prevalence of obesity in adolescence and childhood, these results reinforce the need to promote healthy habits early in life, such as physical exercise, to prevent obesity, improve endothelial function and consequently diminish the risk of atherosclerosis development and ultimately the risk of cardiovascular events.

Some limitations should be recognized. A major limitation of the present study is the lack of a control group. We cannot rule out the possibility, that other factors besides the high intensity exercise program have contributed to the increase of the EPCs levels. Another limitation is the sample size. However, previous studies enrolling children and adolescents, also have small sample sizes. Another limitation of this study is the fact that dietary intake was not assessed. This evaluation could be important to understand potential change in the dietary habits of the participants, in order to relate these data to the results obtained in the obesity indexes and ECPs.

In future studies, it would be interesting to evaluate for how long after the program ended the EPCs levels are maintained. Likewise, it would be important to assess dietary intake and to recruit a large sample to allow a stratified analysis, separating overweight and obese participants to determine if the response to the exercise intervention is similar among them. Future studies with a higher number of obese adolescents are needed to further examine the correlation between improvement in the values of physical performance/indexes of obesity, the number of circulating endothelial progenitor cells and in endothelial function.

Conclusion

A 10-week high-intensity exercise program improved several indexes of obesity and increased the circulation levels of EPCs, enhancing cardiovascular health and potentially improving endothelial function and repair/regeneration in overweight/obese adolescents.

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Bibliography

ACSM. (2017, Junho 27). *American College of Sports Medicine*. Retrieved from https://www.acsm.org/docs/.../high-intensity-interval-training.pdf

Arnold, C., Wenta, D., Müller-Ehmsen, J., Sreeram, N., & Graf, C. (2010). Progenitor cell number is correlated to physical performance in obese children and young adolescents. *Cardiology in the Young* , 20: 381–386.

Asahara, T., Murohara, T., Sullivan, A., Silver, M., Zee, R., Li, T., . . . Isner, J. (1997). Isolation of Putative Progenitor Endothelial Cells for Angiogenesis. *Science* , 275: 964.

Bruyndonckx, L., Hoymans, V., Craenenbroeck, A., Vissers, D., Vrints, C., Ramet, J., & Conraads, V. (2013). Assessment of Endothelial Dysfunction in Childhood Obesity and Clinical Use. *Oxidative Medicine and Cellular Longevity*, 1-20.

Bruyndonckx, L., Hoymans, V., De Guchtenaere, A., Helvoirt, M., Craenenbroeck, E., Frederix, G., . . . Conraads, V. (2015). Diet, Exercise, and Endothelial Function in Obese Adolescents. *PEDIATRICS*, 135: 3.

Camarinha, B., Graça, P., & Nogueira, P. (2016). A Prevalência de Pré-Obesidade/Obesidade nas Crianças do Ensino Pré-Escolar e Escolar na Autarquia de Vila Nova de Gaia, Portugal. *Acta Médica Portuguesa*, 29(1): 31-40.

Deanfield, J., Halcox, J., & Rabelink, T. (2007). Endothelial Function and Dysfunction Testing and Clinical Relevance . *Circulation: American Heart Association*, 115:1285-1295.

Hill, J., Zalos, G., Halcox, J., Schenke, W., Waclawiw, M., Quyyumi, A., & Toren Finkel, T. (2003). Circulating Endothelial Progenitor Cells, Vascular Function, and Cardiovascular Risk. *The New England Journal of Medicine*, 348: 593-600.

Hoetzer, G., Guilder, G., Irmiger, H., Keith, R., Stauffer, B., & DeSouza, C. (2007). Aging, exercise, and endothelial progenitor cell clonogenic and migratory capacity in men. *Journal of Applied Physiology*, 102: 847–852.

Hoymans, V., Van Craenenbroeck, A., Bruyndonckx, L., van Ierssel, S., Vrints, C., Conraads, V., & Van Craenenbroeck, E. (2012). TransFix(R) for delayed flow cytometry of endothelial progenitor cells and angiogenic T cells. *Microvascular Research*, 84: 384-386.

IOPTP, I. O. (2017). World Confederation of Physical Therapy/ International Organisation of Physical Therapists in Paediatrics. Retrieved from: http://www.wcpt.org/news/World-obesity-report-Apr16

Laufs, U., Werner, N., Link, A., Endres, M., Wassmann, S., Jürgens, K., . . . Nickenig, G. (2004). Physical Training Increases Endothelial Progenitor Cells, Inhibits Neointima Formation, and Enhances Angiogenesis. *Circulation*, 109: 220-226.

Lavie, C., Milani, R., & Ventura, H. (2009). Obesity and Cardiovascular Disease - Risk Factor, Paradox, and Impact of Weight Loss. *Journal of the American College of Cardiology*, 52(21): 1925-1932.

Lenk, K., Uhlemann, M., Schuler, G., & Adams, V. (2011). Role of endothelial progenitor cells in the beneficial effects of physical exercise on atherosclerosis and coronary artery disease. *J Appl Physiol*, 111: 321–328.

MacEneaney, O., Kushner, E., Guilder, G., Greiner, J., Stauffer, B. L., & DeSouza, C. A. (2009). Endothelial progenitor cell number and colonyforming capacity in overweight and obese adults. *Internacional Journal of Obesity*, 33(2): 219–225.

Möbius-Winkler, S., Hilberg, T., Menzel, K., Golla, E., Burman, A., Schuler, G., & Adams, V. (2009). Time-dependent mobilization of circulating progenitor cells during strenuous exercise in healthy individuals. *Journal of Applied Physiology*, 107: 1943–1950.

Nyström, C., Henriksson, P., Alexandrou, C., & Löf, M. (2016). The Tanita SC-240 to Assess Body Composition in Pre-School Children: An Evaluation against the Three Component Model. *Nutrients Journal*.

Paes, S., Marins, J., & Andreazzi, A. (2015). Efeitos metabólicos do exercício físico na obesidade infantil: uma visão atual. *Revista Paulista de Pediatria*, 33(1):122-129.

Park, J., Miyashita, M., Kwon, Y., Park, H., Kim, E., Park, J., . . . Park, S. (2012). A 12-week after-school physical activity programme improves endothelial cell function in overweight and obese children: a randomised controlled study. *BMC Pediatrics* , 1-9.

Pires, A., Castela, E., Sena, C., & Seiça, R. (2015). Obesidade: Paradigma da Disfunção Endotelial em Idade Pediátrica. *Acta Médica Portuguesa*, 28 (2) :233-239.

Pires, A., Martins, P., Paiva, A., Pereira, A. M., Marques, M., Castela, E., . . . Seiça, R. (2015). Circulating endothelial progenitor cells in obese children and adolescents. *Jornal de Pediatria* , 91 (6) 560-566.

Reilly, J., Methven, E., McDowell, Z., Hacking, B., Alexander, D., Stewart, L., & Kelnar, C. (2003). Health consequences of obesity. *Archives of Disease in Childhood*, 88: 748-752.

Reilly, J.J.; Kelly, J. (2011). Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: systematic review. *International Journal of Obesity*, 35: 891–898.

Ribeiro, F., Alves, A. J., & Oliveira, J. (2010). Is exercise training an effective therapy targeting endothelial dysfunction and vascular wall inflammation? . *International Journal of Cardiology*, 141:214-221.

Ribeiro, F., Ribeiro, I., Alves, A., Monteiro, M., Oliveira, N., Oliveira, J., . . . Duarte, J. (2013). Effects of Exercise Training on Endothelial Progenitor Cells in Cardiovascular Disease: A Systematic Review. *American Journal of Physical Medicine & Rehabilitation*, 1020-1020.

Schmidt-Lucke, C., Fichtlscherer, S., Aicher, A., Tschope, C., Schultheiss, H., Zeiher, A., & Dimmeler, S. (2010). Quantification of circulating endothelial progenitor cells using the modified ISHAGE protocol. *PLoS One*, 5: e13790.

Viveiro, C., Brito, S., & Moleiro, P. (2016). Sobrepeso e obesidade pediátrica: a realidade. *Revista Portuguesa de Saúde Pública*, 34(1): 30–37.

Walther, C., Gaede, L., Adams, V., Gelbrich, G., Leichtle, A., Erbs, S., . . . Schul, G. (2009). Effect of Increased Exercise in School Children on Physical Fitness and Endothelial Progenitor Cells - A Prospective Randomized Tria. *Pediatric Cardiology*, 120: 2251-2259.

Watts, K., Beye, P., Siafarikas, A., Davis, E., Jones, T., O'Driscoll, G., & Green, D. (2004). Exercise Training Normalizes Vascular Dysfunction and Improves Central Adiposity in Obese Adolescents. *Journal of the American College of Cardiology*, 43: 1823–7.

WCPT, (2017). World Confederation for Physical Therapy. Retrieved from http://www.wcpt.org/node/33341

Werner, N., Kosiol, S., Schiegl, T., Ahlers, P., Walenta, K., Link, A., . . . Nickenig, G. (2005). Circulating Endothelial Progenitor Cells and Cardiovascular Outcomes. *The New England Journal of Medicine*, 353: 999-1007.

WHO, W. H. (2008). Waist Circumference and Waist–Hip Ratio: Report of a WHO Expert Consultation. *World Health Organization*.

WHO, W. H. (2017). *Adolescent obesity and related behaviours: trends and inequalities in the WHO European Region, 2002–2014.* European Region: World Health Organization.

WHO, W. H. (2017). World Health Organization. Retrieved from http://www.who.int/mediacentre/factsheets/fs385/en/

Attachments

Attachment I

Warming: 8 minutes

Dynamic mobility exercises.

Mobility: 6 minutes

Static mobility of legs and shoulders.

Techniques: 12 minutes

Thruster

Burpee

Sit up

Jump rope

Push-ups walking

1) arm crunches with lateral displacement

2)

Challenge AMRAP 4´ (**A**s **M**any **R**ounds **A**s **P**ossible)

Three stations, with one minute of rest between each one.

- **Station 1:**

  50 rope jumps

  15 sit ups (variations: 1. support feet on the back; 2. trunk elevation at 45º)

- **Station 2:**

  10 thrusters with hand weight (variations: 1. performs two movements separately, squatting and then shoulder press)

  10 burpees (variations: 1. One leg at a time behind/front; 2. arms flexion with knee on the floor; 3) start in the squatting position and return to that position before lifting.)

- **Station 3:**

  10 push ups (variations: 1. rest your knees on the floor)

  4 x 15m shuttle run

Return to calm and stretching.

Attachment II

Warming: 15 minutes

Dynamic mobility exercises.

Mobility: 7 minutes

General static mobility.

Techniques: 10 minutes

Bear Crawl

Over Head Squat

Wall Climb

Walking Lunge

Challenge

- **Station 1** (4 repetitions or 6 minutes)

2x15 meters Bear Crawl

15 Over Head Squat (OHS) with stick (variations: 1. support the staff on the back**)**

- **Station 2** (4 repetitions or 6 minutes)

2 Wall climb (variations: 1. 5x: flexion + plank in the starting position of the wall climb)

20 walking lunge with load above head (variations: 1. without load; 2. do one step, stand up and then the other)

Return to calm and stretching.

Attachment III

Warming: 15 minutes

Dynamic mobility exercises.

Mobility: 7 minutes

General static mobility.

Techniques: 7 minutes

Up and down

Wall Ball

Challenge

- **Station 1** (30 minutes)

  In pairs, complete:

  100 rope jumps;
  50 up and down;
  100 rope jumps;
  50 wall ball;
  100 rope jumps;
  50 shuttle run;
  100 rope jumps.

(variations: 1. use 2kgs balls; 2. decrease the number of repetitions and do two complete laps in the circuit)

Return to calm and stretching.

Attachment IV

Warming: 5 minutes AMRAP

20 Jumping jacks

20 Mountain climbers

Mobility: 5 minutes

Static and dynamic mobility.

Techniques: Explain all the elements of WOD (workout of day)

Challenge: Each station has a maximum time of 8 minutes (except the race which are 6 minutes). One minute interval between each station.

- Station 1:

21-15-9 Sumo deadlift high pull

21-15-9 Push ups

7-5-3 Comes and goes

- Station 2:

15 Push Press with weight (<u>variations: flex the knees to propel)</u>

20 Walking lunges

25 Abdominal V-ups

push press

V-ups

- **Station 3:**

100 Jumping rope

20 Wall ball

20 Lumbar on the floor (Trunk lift)

lumbar

- **Station 4:**

Racing circuit – outdoor

Choose a course where 2 or 3 turns, or a time around 6 minutes.

As the activity is outside the work area, the athlete manages to have time to return to the room and prepare the next WOD.

Return to calm and stretching.

Attachment V

Warming: 6-8 minutes

Running

Mobility: 5 minutes

Static and dynamic mobility

Techniques: Explain all the elements of WOD

Challenge in doubles: Each station has a maximum time of 7 minutes or 3 *rounds* for each student. Each student makes one complete turn and then rests while the other student of the pair does

- **Station 1:**

  20 medicine ball clean

  20 hollow rocks (variation: flexion of legs)

Medicine ball clean

Hollow Rocks

- **Station 2:**

  15 thruster with weight (ask everyone to do with 5 kg because then go to rest)

  10 burpees

- **Station 3:**

  10 russian KTB swing (8kgs)

  10 globet squat KTB (8kgs)

Russian KTB Swing

Globet squat KTB

Return to calm and stretching.

Attachment VI

Warming part 1: 5 minutes

General mobility

Warming part 2: 7 minutes

3 rounds

50’’ abdominal isometry

10m wheelbarrow: go one and back the other

20 sit up in doubles: one plays in the other's hands

10 push up partner: make a push up and touch the colleague's opposite hand

Techniques: Explain all the elements of WOD (workout of day)

Challenge: 30’’on/15’’off (make the most repetitions)

Jump squats

Triceps on the chair

KTB swing

Abdominal with medicinal ball

Rowing with unilateral weight

Return to calm and stretching.