High-intensity high volume exercise on top of school exercise lessons reduces endothelial progenitor cells, inflammation and catabolism

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Physical exercise seems to increase circulating endothelial progenitor cells (EPCs) in healthy individuals and patients with cardiovascular diseases (CVDs) [1]. EPCs are circulating precursors of endothelial cells derived from the bone marrow, with the ability to enhance endothelial repair, neovascularization, and endothelial function [2].

In school children, daily school exercise lessons for 1 year, per opposition to the regular twice a week lessons, increased EPCs [3, 4]. High exercise levels seem to be favourable, but previous studies in swimmers showed airway epithelial damage and increased inflammation as a result of intensive training combined with exposure to by-products of chlorination [5]. Alterations in systemic immune parameters suggestive of suppressed immunity during and immediately after training sessions have also been reported, such as changes in the capacity of immune cells to produce inflammatory cytokines in response to an external stimulus [6].

In adolescents, the effects of the addition of high-intensity high volume exercise on top of school exercise in the circulating EPCs is poorly understood. Hence, this study aims to assess the effect of high-intensity high volume swimming in adolescent, in addition to regular school exercise lessons, on the circulating levels of EPCs, inflammatory and catabolic parameters.

Sixteen boys, eight elite swimmers from a swimming club and eight age-matched boys from a secondary school located on the same district, were invited to participate in the study. The inclusion criteria were: age between 13 and 16, participation in ≥5 training sessions/week in the last year (swimmers' group) or no regular exercise/sports practice, besides school exercise (age-matched group), in the 12 months preceding the study. Exclusion criteria: contraindications to exercise; cardiovascular, respiratory or metabolic disease; musculoskeletal injuries compromising regular exercise participation; any medication. The ethics committee approved the study (ref: 163/AD). Written informed consent was obtained from parents/guardians.

Clinical history, exercise participation, height, weight and body composition (Seca mBCA 514, Birmingham, UK) were recorded. Fasting blood samples were collected by venipuncture of the antecubital vein into serum separator or EDTA-coated tubes at least 24h after the last exercise session. EPCs were analysed by flow cytometry (FACS-Calibur flow cytometer, Becton Dickinson, San Jose, CA, USA) as described.[7] In brief, 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), according to manufacturer's instructions. After erythrocyte lysis, at least 250,000 CD45+ events were acquired and a minimum of 100 CD34+ cells were collected in each sample. Data were analysed using Paint-a-Gate software (Becton Dickinson) and the identification of the EPCs was based on morphological properties and CD45dim/CD309+/CD34+ profile, according to the modified ISHAGE (International Society for Hematotherapy and Graft Engineering) protocol gating strategy [8]. EPCs were reported as a percentage of leukocytes (CD45+ cells). C-reactive protein (CRP), interleukin (IL-) 6, tumour necrosis factor-like weaker inducer of apoptosis (TWEAK), myostatin, adiponectin, c-Kit, vascular endothelial growth factor (VEGF), matrix metalloproteinase-2 (MMP2), MMP9, tissue inhibitor of MMP1 (TIMP1), and TIMP2 were determined in serum by immunoblotting.

Data were analysed using IBM SPSS Statistics 20 (IBM Corporation, Chicago, IL, USA). Normality of data distribution was tested with the Shapiro–Wilk test. Data are reported as reported as mean ± SD. Independent t-tests or Mann-Whitney tests were performed to compare groups. P-value <0.05 was considered significant.

Table 1 summarizes the characteristics of the participants. The groups were well matched for age, body composition and weekly school exercise classes (2x50min/week). The swimmers had an average of 6 hours/week of swimming practice in addition to the school classes.

The swimmers showed significantly lower levels of EPCs (p=0.009), CRP (p=0.003), and myostatin (p=0.005); no differences between groups were observed for TWEAK, IL-6, c-Kit and VEGF (Figure 1). The swimmers also showed an upregulated MMP9/TIMP1 ratio (p=0.041), but no differences in MMP2/TIMP2 ratio (Figure 1).

Our results indicate that long-term high-intensity high volume swimming decreases EPCs level, inflammatory and catabolic status. Data regarding EPCs and adolescents are scarce. It was previously reported that obese children and adolescents showed increased levels of EPCs, which may suggest a response mechanism to counteract the endothelial activation/injury in order to repair the endothelial cell layer [9]. The lower level of EPCs in the swimmers' group may represent a healthier endothelium (lower endothelial activation/damage) and a reduced need to mobilize EPCs from bone marrow to circulation. This assumption is supported by the better low-grade vascular wall inflammation - lower CRP levels - observed in the swimmers. Nonetheless, contrary to the present findings, a study in healthy adolescents [3] and, particularly, studies in adults with CVDs showed that regular exercise increases the circulating levels of EPCs [1]. An effect that is expected to improve the prognosis, as the circulating number of EPCs is positively correlated with vascular function, inversely correlated with cardiovascular risk score, and predicts morbidity and mortality in adults with CVDs [10]. In this sense, our results should be considered as preliminary and read carefully; they may be used to inform future studies addressing this issue.

Myokines, such as myostatin, and MMPs have a regulatory role in the regeneration of the extracellular matrix, muscle remodelling, and immune modulation. Our results are in agreement with previous studies showing that myostatin was downregulated by endurance and resistance exercise in both humans and rodents. Exercise and physical activity are cornerstones of a healthy lifestyle. For instance, leisure-time physical activity shows a linear negative correlation with the risk of cardiovascular mortality regardless of age and gender [11];

high-intensity leisure-time physical activity has more pronounced cardiovascular benefits than moderate-intensity [11]. Interestingly, a meta-analysis investigating the effects of high-intensity interval versus moderate-intensity continuous training in patients with coronary artery disease showed that the superiority of the high-intensity to improve peak oxygen uptake disappears when the exercise training is isocaloric [12]. Taken together, our results suggest that high-intensity high volume exercise on top of school exercise lessons reduces inflammation and catabolism, with potential benefits in reducing the susceptibility to non-communicable diseases.

Some limitations should be acknowledged, such as the small sample size. Nonetheless, this work generated outcomes that can be used to inform larger studies aiming to clearly ascertain our findings. All participants were boys, so it is not clear if the same effect would be observed in females; future studies might determine if there are sex differences in the responses to exercise and include a measure of endothelial function (e.g. flow-mediated dilation).

In conclusion, high-intensity high volume exercise decreases CRP, myostatin and EPCs levels. Our findings highlight the anti-inflammatory and anti-catabolic effects of exercise in adolescents.

Author contributions

RS, RF, MF, and FR contributed to the conception or design of the work. MH, RF, ACG, IPR, MF, RF and FR contributed to the acquisition, analysis, or interpretation of data for the work. MH, RF, and RS drafted the manuscript. ACG, IPR, MF, RF and FR critically revised the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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Declaration of conflicting interests

The Authors declare that there is no conflict of interest.

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Table 1. Comparison between groups in age, body composition, and biochemical parameters (mean ± SD).

	Age-matched group	**Swimmers' group**	***p*-value**
Age (years)	14.3 ± 1.3	15.1 ± 0.7	0.125
Weight (kg)	52.3 ± 8.7	61.8 ± 12.9	0.117
Height (cm)	162.3 ± 9.4	166.3 ± 6.1	0.351
Body mass index (kg/m^2)	19.8 ± 2.0	19.5 ± 3.1	0.936
Fat mass (%)	11.0 ± 7.0	15.7 ± 6.6	0.223
Fat free mass (%)	88.9 ± 6.8	84.4 ± 6.6	0.234
High density lipoprotein (mg/dL)	43.0 ± 10.0	50.0 ± 13.0	0.250
Low density lipoprotein (mg/dL)	81.0 ± 18.0	77.0 ± 21.0	0.680
Aspartate transaminase (U/L)	27.0 ± 5.0	31.0 ± 4.0	0.080
Lactate dehydrogenase (U/L)	200.0 ± 18.0	203.0 ± 25.0	0.810
Creatinine kinase (U/L)	161.0 ± 62.0	222.0 ± 50.0	0.052
Erythrocytes (10^12/L)	5.1 ± 0.5	5.1 ± 0.3	0.890
Haemoglobin (g/dL)	14.6 ± 1.4	14.2 ± 1.6	0.800
Haematocrit (%)	42.2 ± 3.9	42.4 ± 4.1	0.960
Leukocytes (10^9/L)	6.7 ± 2.1	6.5 ± 0.9	0.810
Platelets (10^9/L)	231.5 ± 39.6	232.1 ± 35.7	0.970

Figure 1. Circulating levels of the CRP, IL-6 and TWEAK (panel A), myostatin, ratio MMP2/TIMP2 and ratio MMP9/TIMP1 (panel B), and EPCs, c-kit and VEGF (panel C).

*Significantly different from the age-matched group, $p<0.05$. Data reported as mean±SD. Au – arbitrary units; CRP – C-reactive protein; EPCs, endothelial progenitor cells; MMP2, matrix metalloproteinase-2; MMP9, matrix metalloproteinase-9; TIMP1, tissue inhibitor of metalloproteinase-1; TIMP2, tissue inhibitor of metalloproteinase-2; TWEAK, tumour necrosis factor-like weaker inducer of apoptosis; VEGF, vascular endothelial growth factor.