High-intensity, high-volume exercise in addition to school exercise classes reduces endothelial progenitor cells, inflammation and catabolism in adolescent boys

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Physical exercise seems to increase the number of circulating endothelial progenitor cells (EPCs) in healthy people and in patients with cardiovascular disease (CVD).¹ EPCs are circulating precursors of endothelial cells derived from the bone marrow and can enhance endothelial repair, neovascularization and endothelial function.²

Daily school exercise classes for one year increased the number of EPCs in schoolchildren relative to the standard twice-a-week exercise classes.³,⁴ High exercise levels seem to have advantages, but previous studies in swimmers have shown epithelial damage and increased inflammation of the airways as a result of intensive training combined with exposure to the 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

The effects of the addition of high-intensity, high-volume exercise in addition to school exercise classes on the levels of circulating EPCs is poorly understood in adolescents. We aimed to assess the effect of high-intensity, high-volume swimming in adolescents, in addition to regular school exercise classes, on the levels of circulating EPCs and other inflammatory and catabolic parameters.

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

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 24 h after the last training session. EPCs were measured by flow cytometry (FACS-Calibur flow cytometer, Becton Dickinson, San Jose, CA, USA) as described previously.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 the 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. The data were analysed using Paint-a-Gate software (Becton Dickinson) and the identification of the EPCs was based on morphological
properties and the CD45dim/CD309+/CD34+ profile according to the modified International Society for Hematotherapy and Graft Engineering protocol gating strategy. EPCs were reported as a percentage of leukocytes (CD45+ cells). C-reactive protein (CRP), interleukin 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 samples by immunoblotting.

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

Table 1 summarizes the characteristics of the participants. The groups were well matched for age, body composition and weekly school exercise classes (2 × 50 min/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 difference between groups was observed for TWEAK, interleukin 6, c-Kit nor VEGF (Figure 1). The swimmers also showed an upregulated MMP9/TIMP1 ratio (p = 0.041), but no difference in the MMP2/TIMP2 ratio (Figure 1).

Our results indicate that long-term, high-intensity, high-volume swimming decreases the level of EPCs and the inflammatory and catabolic status. Data on EPCs in 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 endothelial activation/injury to repair the endothelial cell layer. The lower level of EPCs in the swimmers’ may represent a healthier endothelium (lower endothelial activation/damage) and a reduced need to mobilize EPCs from bone marrow to the circulation. This assumption is supported by the better low-grade vascular wall inflammation (lower CRP levels) observed in the swimmers. Nonetheless, contrary to these findings, a study in healthy adolescents and, particularly, studies in adults with CVD, has shown that regular exercise increases the circulating levels of EPCs. This effect is expected to improve prognosis because the number of circulating EPCs is positively correlated with vascular function, inversely correlated with cardiovascular risk score, and predicts morbidity and mortality in adults with CVD. Our results should be considered as preliminary and be read carefully, although 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

|                | Age-matched group | Swimmers’ group | p     |
|----------------|------------------|----------------|-------|
| 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/m²) | 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 (10¹²/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 (10⁹/L) | 6.7 ± 2.1 | 6.5 ± 0.9 | 0.810 |
| Platelets (10⁹/L) | 231.5 ± 39.6 | 232.1 ± 35.7 | 0.970 |

Data presented as mean ± SD values.
rodents. Exercise and physical activity are the cornerstones of a healthy lifestyle. For instance, leisure time physical activity shows a negative linear correlation with the risk of cardiovascular mortality regardless of age and sex and high-intensity leisure time physical activity has more pronounced cardiovascular benefits than moderate intensity physical activity. 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 training in improving peak oxygen uptake disappears when the exercise training is isocaloric. Taken together, our results suggest that high-intensity, high-volume exercise in addition to school exercise classes reduces inflammation and catabolism, with potential benefits in reducing 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 the participants were boys, so it is not clear whether the same effect would be observed in girls; future studies may determine whether there are sex differences in the response 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

Figure 1. Circulating levels of: (a) CRP, interleukin 6 and TWEAK; (b) myostatin, MMP2/TIMP2 and MMP9/TIMP1; and (c) EPCs, c-kit and VEGF. Data reported as mean ± SD values. 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. *Significantly different from the age-matched group, p < 0.05.
findings highlight the anti-inflammatory and anti-catabolic effects of exercise in adolescents.

**Author contribution**

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 paper. ACG, IPR, MF, RF and FR critically revised the paper. All authors gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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