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The Effect of Brief Exercise on Circulating CD34$^+$ Stem Cells in Early and Late Pubertal Boys

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ABSTRACT: We tested the hypothesis that exercise could stimulate CD34$^+$ peripheral blood hematopoietic stem cells (PBSC) in children. Fourteen early pubertal boys (EP, age 10.3 ± 0.3 y) and 13 late pubertal boys (LP, age 16.5 ± 0.4 y) performed 20 min of moderate-to-vigorous cycle ergometer exercise. Blood was drawn before and after exercise. Cells were stained for surface CD34$^+$. Plasma granulocyte colony stimulating factor (G-CSF), Fms-like tyrosine kinase-3 (FLT-3), and stromal cell-derived factor-1 (SDF-1) were measured using ELISA. Exercise substantially increased PBSC (in EP from 112 ± 21 to 182 ± 30 cells/μL, $p = 0.0007$; in LP from 63 ± 8 to 152 ± 21, $p = 0.0008$), and to a smaller extent FLT-3 (in EP from 98 ± 5 to 110 ± 6 pg/mL, $p < 0.0001$; in LP from 73 ± 6 to 92 ± 6, $p < 0.0001$) and G-CSF (in EP from 26 ± 4 to 29 ± 4 pg/mL, $p < 0.0001$; in LP from 14 ± 1 to 18 ± 1, $p < 0.0001$). Baseline levels of PBSC, FLT-3, and G-CSF were significantly higher in EP. Exercise increased SDF-1α only in LP, and the FLT-3 increase was greater in LP than EP. Brief exercise affects PBSC and PBSC mediators in children. **(*Pediatr Res* 61: 491–495, 2007)**

It is now well established that brief bouts of exercise in healthy children and adolescents substantially increase the number of circulating neutrophils, monocytes, and lymphocytes and their subpopulations (1). In addition, exercise is associated with increases of inflammatory mediators such as IL-6, IL-1β, TNF-α and IL-1 receptor antagonist (IL-1ra) (2). While such responses have typically been considered to be a sign of stress and inflammation leading to catabolism, there is mounting evidence that the exercise associated stress response may also play a beneficial role in the healthy adaptation to physical activity (3). A focus of recent studies has been on the potential health benefits of CD34$^+$ PBSC mobilization because these pluripotential cells could play a role in key tissue repair mechanisms, like angiogenesis. The fact that brief exercise can increase circulating levels of G-CSF (4–6), a mediator used clinically to mobilize hematopoietic stem cells in children (7), supports the notion that exercise might lead to mobilization of stem cells which could, ultimately, play a role in beneficial tissue responses to exercise like muscle hypertrophy and new vessel formation.

Until now, the few studies that have examined the effects of exercise on stem cell mobilization have focused on adults and have invariably involved prolonged, continuous, and heavy exercise. Exercise protocols like these neither are well tolerated by children nor do they reflect natural patterns of physical activity (8). We hypothesized that exercise protocols designed to be feasible for children and more consistent with their typical, daily-life exercise, would lead to increased numbers of circulating stem cells. Moreover, we further hypothesized that key circulatory regulatory mediators known to mobilize stem cells from the bone marrow, namely, FLT-3, IL-3, SDF-1α, GM-CSF, and G-CSF would also increase with exercise. As noted, while heavy exercise in adults can increase circulating G-CSF, to our knowledge, the effect of brief exercise on FLT-3 and SDF-1 had never been measured previously in adults or children.

Although it is clear in general terms that the immune and hematopoietic systems change throughout childhood, including the leukocyte response to exercise (9,10), little is known about maturational aspects of possible stem cell mobilization and regulation in response to exercise. Suzuya *et al.* (11) recently did examine the factors that influence responsiveness of PBSC to G-CSF in 59 healthy donors ranging in age from 3 to 63 y old. Circulating CD34$^+$ cells were highest after treatment in younger subjects. The authors noted that the "most important factor" in predicting G-CSF induced PBSC yields was young age. Consequently, we further hypothesized that the pattern of stem cell appearance in the circulation would be influenced by maturational status when comparing early and late pubertal individuals.

METHODS

Participants. Twenty-seven healthy males (age range, 8–17 y) participated in this study (Table 1); 14 subjects were pre/early puberty and 13 subjects were late pubertal. Recently, Timmons *et al.* (10) demonstrated that the impact of exercise is not only significant but is very different in early and late pubertal children. Based on this study and preliminary data from our group, we chose to first focus on early and late pubertal males. We plan to study early and late pubertal females in future studies. Subjects were recruited using an information flyer distributed at a local recreation center and by word of mouth. Individuals participating in competitive sports and with a history of

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Abbreviations: EP, early pubertal; **FLT-3,** Fms-like tyrosine kinase-3; **G-CSF,** granulocyte colony stimulating factor; **GM-CSF,** granulocyte macrophage-colony stimulating factor; **LP,** late pubertal; **PBSC,** peripheral blood hematopoietic stem cells; **SDF-1α,** stromal cell-derived factor-1α; **$\dot{V}O_2$,** oxygen uptake

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Table 1. *Anthropometric and physiological characteristics*

	Prepubertal (n = 14)	Postpubertal (n = 13)
Age (y)	10.3 ± 0.3	16.5 ± 0.42*
Height (cm)	141.1 ± 2.4	174.8 ± 2.4*
Weight (kg)	35.8 ± 1.9	65.5 ± 2.9*
BMI (kg/Ht^2)	17.9 ± 0.6	21.4 ± 0.7*
BMI (percentile)	57.9 ± 6.3	50.6 ± 7.7
Peak $\dot{V}O_2$ (mL/kg/min)	42.1 ± 2.1	44.4 ± 1.9
Mean HR during exercise protocol (% peak HR)	92.1 ± 2.3	89.2 ± 2.2
Mean $\dot{V}O_2$ during exercise protocol (% peak $\dot{V}O_2$)	74.0 ± 0.05	79.0 ± 0.03
Mean work rate during exercise protocol (% peak work)	70.0 ± 0.02	73.0 ± 0.01
Increase in lactic acid (%) during exercise protocol	194 ± 50	464 ± 49†

Mean ± SEM. * $p < 0.001$; † $p < 0.0001$.

any chronic medical conditions or use of any medications were excluded from participation. The Institutional Review Board at the University of California, Irvine, approved the study, and written informed assent and consent were obtained from all participants and their parents upon enrollment.

Anthropometric measurements. Standard, calibrated scales and stadiometers were used to determine height, body mass, and body mass index (BMI = wt/ht^2). Because BMI changes with age, we also calculated BMI percentile for each child using the recently published standards from the Centers for Disease Control, National Center for Health Statistics (12). Pubertal status of the study participants was assessed by a validated self-administered questionnaire that has been widely used as a noninvasive indicator of pubertal status (13,14).

Measurement of fitness. Each subject performed a ramp-type progressive cycle ergometer exercise test to the limit of his tolerance [RER (respiratory exchange ratio) > 1.15]. In the ramp protocol, used often in children and adolescents to determine peak O_2 and the lactate or anaerobic threshold, the work rate increases linearly over time, and the sensation for the participant is one of riding up a hill that is becoming progressively steeper. Subjects were vigorously encouraged during the high-intensity phases of the exercise protocol, and the work rate load was reduced to 0-W upon the participant's signaling. Gas exchange was measured breath-by-breath, and the lactate/anaerobic threshold was calculated using a standard techniques based on changes in the patterns of oxygen uptake, carbon dioxide production, and minute ventilation (15).

Exercise protocol. High-intensity (above anaerobic/lactate threshold exercise) is very difficult for children to sustain. Consequently, we develop a protocol specifically for children using intermittent high-intensity exercise that has proven to be useful in this age group (15). The protocol consisted of 10 two-minute bouts of constant work rate cycle ergometry, with a one-minute rest interval between each bout of exercise. The work rate was individualized for each child and was calculated to be equivalent to the work rate corresponding roughly to 50% of the work rate between the anaerobic/lactate threshold and the peak oxygen uptake (as determined non-invasively from the ramp-type test). On occasion, we would adjust (increase or decrease) the work rate of the individual bouts based on the HR response and or subjective assessment of the individual participants. This resulted in a metabolic input that was roughly equivalent among study subjects (16).

Blood sampling and analysis. An indwelling catheter was inserted into the antecubital vein 30 min before the start of exercise. Venous blood was drawn before exercise and immediately following the exercise into a tube containing sodium heparin. Samples were used for flow cytometry (CD34$^+$), stem cell growth factors (FLT-3, IL-3, GM-CSF, G-CSF, and SDF-1α), and CBC. Blood samples for stem cells growth factors were spun at 3000 rpm, at 4°C for 20 min. The plasma was separated and stored at −80°C and thawed only once for analysis. Complete blood counts (CBC) for white blood cell analysis were obtained by standard methods from the clinical hematology laboratory at UCI. Cells were stained for CD34$^+$ using the International Society of Hematotherapy and Graft Engineering (ISHAGE) definition for identifying CD34$^+$, *i.e.* cells gated on CD45$^+$ dim/low side scatter CD34$^+$ positive cells (17). Samples were acquired using a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA) and analyzed using CellQuest Pro (BD Biosciences).

Stem cell growth factors. FLT-3/FLK-2 ligand plasma levels were determined by ELISA with the use of the R & D Systems Quantikine kit (R & D Systems, Minneapolis, MN): intra-assay CV was 1.4–2.7%, inter-assay CV was 6.2–11.1%, and the sensitivity is <7 pg/mL. IL-3 plasma levels were determined by ELISA with the use of the R & D Systems Quantikine kit: intra-assay CV was 3.2–5.7%, inter-assay CV was 5.6–10.7%, and the sensitivity was <7.4 pg/mL. G-CSF plasma levels were determined by ELISA with the use of the HS-Quantikine kit (R & D Systems): intra-assay CV was 3–8.3%, inter-assay CV was 8.5–15.8%, and the sensitivity is <0.4 pg/mL. GM-CSF plasma levels were determined by ELISA with the use of the HS-Quantikine kit (R & D Systems): intra-assay CV was 4.0–9.5%, inter-assay CV was 13.9–23.5%, and the sensitivity is <0.26 pg/mL. SDF-1α plasma levels were determined by ELISA with the use of the Quantikine kit (R & D Systems): intra-assay CV was 2.0–4.0%, inter-assay CV was 5.0–10.3%, and the sensitivity is <9.0 pg/m.

Statistical analysis. Two-sample t test was applied to assess baseline differences in anthropometric measurements, fitness, CD34$^+$, and stem cell growth factors levels between early and late pubertal boys. Paired t test was used to assess the change of CD34$^+$ and stem cell growth factors from pre-exercise to postexercise. The change from pre-exercise to postexercise was also compared between early and late pubertal boys with two-sample t test. Statistical significance was set at $p < 0.05$. Data are presented as mean ± SEM.

RESULTS

Anthropometric and physiologic characteristics. Anthropometric and physiologic characteristics of the study participants are described in Table 1. The ethnic make-up of the early pubertal males consisted of 90% Caucasian and 10% Hispanic; late pubertal males, 80% Caucasian and 20% Hispanic. As expected, late pubertal boys were older, taller, heavier, and had higher BMI levels. However, there were no significant differences between the early and late pubertal boys in BMI percentiles and peak O_2 normalized to body weight. In addition, during the exercise protocol, the average of heart rate, work rate (WR), and peak O_2 were calculated as a percentage using peak values from the ramp test; no differences were found between the early and late pubertal males.

Lactate levels increased significantly within both groups after the exercise protocol, however, the percentage change was significantly greater in the late pubertal males (194 ± 50% *versus* 464 ± 49%, early *versus* late pubertal males, respectively, $p = 0.0008$). The brief bout of vigorous exercise increased the total number of circulating white blood cells in both groups (early pubertal males: from 5671 ± 444 to 7371 ± 575 cells/μL, $p < 0.0001$; late pubertal males: from 4615 ± 232 to 7785 ± 385 cells/μL, $p < 0.0001$).

Effect of exercise nn CD34$^+$ cells and regulatory mediators. The effect of the a brief exercise bout on circulating levels of CD34$^+$ (PBSC) stem cells is shown in Figure 1 and on G-CSF and FLT-3 in Figures 2 and 3, respectively. Baseline level of CD34$^+$ (112 ± 21 and 63 ± 8 cells/μL; early and late pubertal, respectively), FLT-3 (98 ± 5 and 73 ± 6 pg/mL; early and late pubertal, respectively), and G-CSF (26 ± 4 and 14 ± 1 pg/mL; early and late pubertal, respectively) were significantly higher in the early compared with late pubertal males. No pubertal-related differences were found in baseline levels of IL-3 (0.8 ± 0.3 and 0.9 ± 0.2 pg/mL; early and late pubertal, respectively), GM-CSF (0.2 ± 0.04 and 0.4 ± 0.1 pg/mL; early and late pubertal, respectively) and SDF-1α (2270 ± 91 and 2406 ± 185 pg/mL; early and late pubertal, respectively). Moreover, while we found statistically significant change from pre- to postexercise in CD34$^+$ (70 ± 16 and 89 ± 20 cells/μL; early and late pubertal, respectively), FLT-3 (12 ± 2 and 19 ± 2 pg/mL; early and late pubertal, respectively), and G-CSF (3 ± 0.6 and 4 ± 0.9 pg/mL; early

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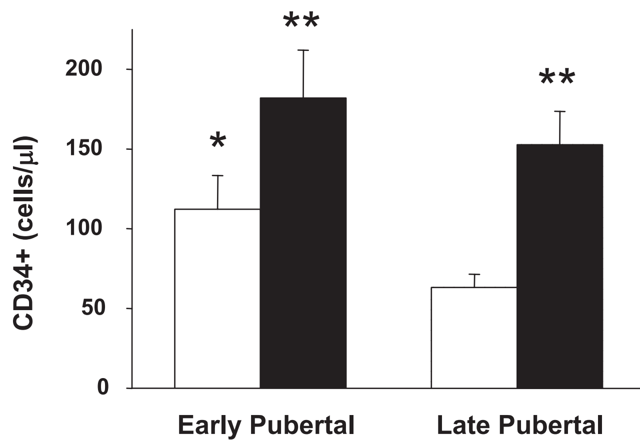

Figure 1. Effect of exercise on $CD34^+$ (PBSC) cells in early ($n = 14$) and late ($n = 13$) pubertal boys. Stem cell levels at baseline were significantly greater in the early compared with the late pubertal boys (*$p = 0.048$). Exercise increased $CD34^+$ cells in both groups (**$p < 0.001$). The increase was not significantly different between the two groups [pre-exercise (□) and postexercise (■)].

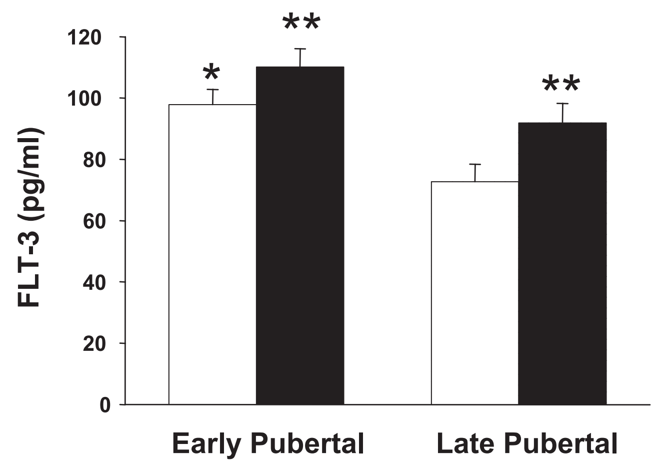

Figure 3. Effect of exercise on FLT-3 in early ($n = 14$) and late ($n = 13$) pubertal boys. FLT-3 levels at baseline were significantly greater in the early compared with the late pubertal boys (*$p = 0.003$). Exercise increased FLT-3 cells in both groups (**$p < 0.0001$). The increase was significantly higher in the late pubertal males ($p = 0.011$). Pre-exercise (□) and postexercise (■).

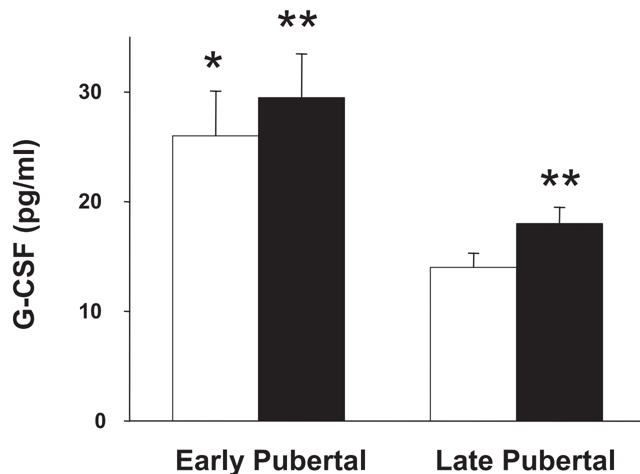

Figure 2. Effect of exercise on G-CSF in early ($n = 14$) and late ($n = 13$) pubertal boys. G-CSF levels at baseline were significantly greater in the early compared with the late pubertal boys (*$p = 0.01$). Exercise increased G-CSF cells in both groups (**$p < 0.0001$). The increase was not significantly different between the two groups [pre-exercise (□) and postexercise (■)].

and late pubertal, respectively) in both groups, no exercise effect was observed for IL-3 (1.1 ± 0.6 and 0.1 ± 0.2 pg/mL; early and late pubertal, respectively) and GM-CSF(-0.02 ± 0.02 and -0.03 ± 0.08 pg/mL; early and late pubertal, respectively). For SDF-1α, there was no change for early pubertal boys (30 ± 44 pg/mL) but a small but significant increase was noted in late pubertal boys following the exercise protocol (168 ± 60 pg/mL, $p = 0.02$). As noted, $CD34^+$ and G-CSF increased in both groups, but there was no between-group difference in the increase. The exercise-induced change in FLT-3 levels was significantly greater in the late pubertal males.

DISCUSSION

The present study is the first to examine in children the effect of exercise on $CD34^+$ PBSC and their key mediators. We found that a relatively brief bout of moderate-to-vigorous exercise resulted in a significant increase of $CD34^+$ cells (Fig. 1). Whereas IL-3 was unchanged by exercise, FLT-3, GCSF (Fig. 2 and 3), and, to a lesser extent, SDF-1α increased. Moreover, baseline levels of $CD34^+$ cells, FLT-3, and G-CSF were higher in the early compared with the late pubertal children, suggesting that pubertal status influences circulating levels of naturally occurring PBSC and their circulating mediators.

Small numbers of hematopoietic stem cells are found in the peripheral circulation in both adults and children, but their physiologic role remains the subject of much speculation. It has been recognized since at least the late1970s that physiologic conditions associated with stress [like the administration of endogenous endotoxin (18)] could mobilize stem cells [colony forming units-c (CFU-C)] into the peripheral circulation and, more recently, tissue ischemia and renal injury have been added to pathologic conditions leading to PBSC increase (19–21). There is mounting evidence that the PBSC may play beneficial roles in these conditions by enhancing angiogenesis and tissue repair. Angiogenesis and muscle hypertrophy are both key factors in the successful training adaptation to exercise (22,23); and the PBSC seem likely candidates to play a role in these processes. Our finding that PBSC numbers are elevated following exercise in children support the hypothesis that PBSC could play a role in tissue adaptation in response to exercise relatively early in life.

Heretofore, the few studies on exercise associated stem cell mobilization have been done only in adults, and the results have been mixed. In 1978, Barrett *et al.* (24) (probably the first report of this phenomenon) observed that heavy exercise (consisting of running up and down a set of stairs near the authors' laboratory) increased CFU-C in adults. In contrast, more recently, Bonsignore *et al.* (4) found that participation in a half-marathon or marathon (about 1–3 h of exercise) did not influence circulating stem cells immediately after exercise and even led to their decrease the following morning. The Bonsignore group did note that trained individuals had higher baseline levels of stem cells, suggesting an adaptive effect. In

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contrast, Rehman *et al.* (25) and Morici *et al.* (26) found that shorter bouts of maximal and supramaximal exercise, respectively, did increase circulating stem cells. Finally, Laufs *et al.* (27) showed that 10 min of exercise did not increase circulating stem cells and at least 30 min of moderate intensity exercise was required to increase stem cells in a group of adults (mean age, 28.4 ± 1.3 y).

Our findings now demonstrate that in both early and late pubertal boys, exercise of intensity and duration even less than might be encountered in a typical soccer, track, or football practice for children and/or adolescents, can substantially increase circulating PBSC (Fig. 1). The mechanisms that govern the mobilization of PBSC into the peripheral blood is complex and involves a number of steps ranging from maturation of progenitor cells to factors that facilitate the release of cells from the marrow stroma (28). The rapid increase in PBSC that we observed suggests that the mechanisms most likely associated with exercise involve the release of sufficiently mature stem cells from the marrow and/or other peripheral depots, rather than the much longer process of stimulating new stem cell formation or hastening their maturation within the marrow. One such depot for some stem cells might be the spleen (21). Indeed, in humans, as in other mammals, brief exercise and other stresses do lead to splenic contraction and release of red and white cells into the circulation (29,30).

Motivated by the need to harvest stem cells for a number of disease entities, much work has been done recently to identify exogenous cytokines and growth factors that can increase PBSC levels (28). Although many gaps remain in our understanding of how each of these agents work, there are growing data suggesting that combinations of these factors work better to increase PBSC than individual agents. Indeed, in the current study we found that the exercise-associated increase in $CD34^+$ cells was accompanied by significant increases in several of the PBSC mobilizing agents examined. Circulating G-CSF was higher with exercise and is known to increase PBSC by its ability to act on stem cells through several mechanisms ranging from hastening stem cell development in the bone marrow to their release into the circulation (31). Similarly, the more recently discovered PBSC mobilizing agent FLT-3 was elevated in both early and late pubertal boys and, like G-CSF, appears to enhance PBSC by acting at several points in the mobilization pathway. SDF-1 seems to function more in trafficking of PBSC (32), and it was elevated to a small but significant degree only in the late pubertal subjects. Finally, IL-3, a cytokine whose influence on PBSC mobilization derives from its ability to influence more primitive stem cells (28) was not changed by exercise. Precisely how exercise leads to increased levels of circulating PBSC mobilization mediators is not known in either children or adults.

Our data revealed potential maturational effects on PBSC in several areas. First, the baseline levels of $CD34^+$ cells were higher in the early pubertal children. Although previous workers have shown that PBSC levels were higher in newborns than in adults, we have found no studies that have examined basal levels of these cells throughout childhood. The higher levels of PBSC in the early pubertal children were accompanied, consistently, by higher circulating levels of G-CSF and FLT-3. Clearly, additional studies will be needed to corroborate our preliminary observations and to examine potential mechanisms for this maturational difference.

We also noted that the magnitude of the exercise increase in PBSC and their key mediators differed between the two groups in some respects. As noted, both early and late pubertal boys increased $CD34^+$ cells with exercise. Although not reaching statistical significance ($p = 0.067$), $CD34^+$ cells increased by 83 ± 19% in the early pubertal subjects, but by 170 ± 45 in the late pubertal boys. However, the exercise associated increase in G-CSF and FLT-3 (significant in both groups) was larger in the older compared with younger boys.

The mechanisms for these pubertal related differences may be related to the different physiologic patterns of response to exercise in younger and older boys. For example, although we designed the study so that the relative work rate was carefully measured to be equivalent in the two groups, one well-described key distinction between younger and older children is that younger subjects show smaller changes in pH (33) and lactate increases for a given amount of exercise. Consistent with this, the increase in lactate in the younger subjects in our study was substantially and significantly less than in the older [n.b., this is likely the result of lower levels of key muscle enzymes like lactate dehydrogenase in younger subjects (34)], even though the relative changes in the exercise intensity were the same. Since the degree of metabolic stress is highly sensitive to the magnitude of perturbations of acid-base balance, in this case, the change in lactate, it may be that the smaller lactate (and likely pH) changes in the younger children for a relative amount of exercise may be, in part, responsible for the subsequent change in PBSC.

In summary, we found a substantial and significant increase in $CD34^+$ PBSC and several key circulating stem cell mobilization factors following brief exercise in healthy early and late pubertal males. These responses appear to be influenced by puberty in boys at baseline, PBSC were higher in the early pubertal subjects, as were G-CSF and FLT-3. Moreover, in response to exercise, the increase in FLT-3 was significantly smaller in the younger children. Similar to pathologic stresses that lead to increased PBSC, we speculate that these cells might eventually play a role in the long-term adaptation to repeated bouts of exercise, particularly by contributing to new vessel formation in the working muscle.

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