RESEARCH ARTICLE

Kinetics of circulating progenitor cell mobilization during submaximal exercise

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Niemiro GM, Parel J, Beals J, van Vliet S, Paluska SA, Moore DR, Burd NA, De Lisio M. Kinetics of circulating progenitor cell mobilization during submaximal exercise. J Appl Physiol 122: 675–682, 2017. First published January 12, 2017; doi:10.1152/japplphysiol.00936.2016.—Circulating progenitor cells (CPCs) are a heterogeneous population of stem/progenitor cells in peripheral blood that includes hematopoietic stem and progenitor cells (HSPCs and HSCs), endothelial progenitor cells (EPCs), and mesenchymal stem cells (MSCs) that are involved in tissue repair and adaptation. CPC mobilization during exercise remains uncharacterized in young adults. The purpose of this study was to investigate the kinetics of CPC mobilization during and after submaximal treadmill running and their relationship to mobilization factors. Seven men [age = 25.3 ± 2.4 yr, body mass index = 23.5 ± 1.0 kg/m², peak O₂ uptake (V₀₂peak) = 60.9 ± 2.74 ml·kg⁻¹·min⁻¹] ran on a treadmill for 60 min at 70% V₀₂peak. Blood sampling occurred before (Pre), during [20 min (20e), 40 min (40e), 60 min (60e)], and after exercise [15 min (15p), 60 min (60p), 120 min (120p)] for quantification of CPCs (CD34⁺), HSPCs (CD34⁺/CD45low), HSCs (CD34⁺/CD45low/CD38⁻), CD34⁺MSCs (CD45⁻/CD34⁺/CD31⁻/CD105⁺), CD34⁻MSCs (CD45⁻/CD34⁻/CD31⁻/CD105⁺), and EPCs (CD45⁻/CD34⁻/CD31⁻) via flow cytometry. CPC concentration increased compared with Pre at 20e and 40e (2.7- and 2.4-fold, respectively, \( P < 0.05 \)). HSPCs and HSCs increased at 20e compared with 60p (2.7- and 2.8-fold, respectively, \( P < 0.05 \)), whereas EPCs and both MSC populations did not change. CXC chemokine ligand (CXCL) 12 (1.5-fold; \( P < 0.05 \)) and stem cell factor (1.3-fold; \( P < 0.05 \)) were increased at 40e and remained elevated postexercise. The peak increase in CPCs was positively correlated to concentration of endothelial cells during exercise with no relationship to CXCL12 and SCF. Our data show the kinetics of progenitor cell mobilization during exercise that could provide insight into cellular mediators of exercise-induced adaptations, and have implication for the use of exercise as an adjuvant therapy for CPC collection in hematopoietic stem cell transplant.

NEW & NOTEWORTHY Using a comprehensive evaluation of circulating progenitor cells (CPCs), we show that CPC mobilization during exercise is related to tissue damage, and not plasma concentrations of CXC chemokine ligand 12 and stem cell factor. These data have implications for the use of exercise interventions as adjuvant therapy for CPC mobilization in the context of hematopoietic stem cell transplant and also support the role of mobilized progenitor cells as cellular mediators of systemic adaptations to exercise.

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duration may be more feasible for both autologous and allogeneic donors. Determining the kinetics would inform clinical practice by defining the optimal time point to begin CPC collection. Additionally, running may produce different mobilization kinetics compared with cycling. This information would support further investigation into the use of exercise as an adjuvant therapy to enhance CPC mobilization efforts during transplantation.

The mechanisms underpinning exercise-induced CPC mobilization remain to be fully elucidated, but several growth factors, cytokines, and chemokines are likely involved (13, 17). Stem cell factor (SCF) and stromal derived factor-1α/ CXC chemokine ligand (CXCL) 12 are potent cytokines that are secreted by various cell types for maintenance and homing of progenitor cells in the bone marrow (25, 38). CPCs express CXCR4, the receptor for CXCL12, which is involved in CPC migration from and homing to the bone marrow (46). Disruption of the CXCR4-CXCL12 axis, either through elevated plasma CXCL2 levels or CXCR4 antagonists, causes release of CPCs into the peripheral blood (47). Similarly, SCF is a cytokine that is secreted by a variety of cell types, specifically, endothelial cells (ECs), fibroblasts, and MSCs (18, 27). CPCs express the cell surface receptor CD117, which interacts with SCF to stimulate CPC motility (10). Bone marrow progenitors express chemokine receptors that facilitate their homing and mobilization, particularly toward increasing concentrations of CXCL12 (31, 52). Exercise can increase production and secretion of known CPC mobilizing factors (23, 32); however, the kinetics of changes in plasma SCF and CXCL12 during and after exercise and their relationship to exercise-induced CPC mobilization have not been previously examined in vivo in humans. In addition to CPC chemoattractants, tissue damage and the resulting inflammatory response may also be a potent signal for CPC mobilization. Evidence in support of this notion is derived from pathological conditions that are accompanied by either acute or chronic tissue damage and inflammation, such as stroke (33), myocardial infarction (16), and obesity (2, 37). In this context, mobilized CPCs may contribute to tissue repair by participating in neovascularization (9, 39, 51, 55), differentiating into tissue-resident immune cells (31), or via the release of paracrine factors that stimulate local repair processes (35, 37). Developing a better understanding of the mechanisms responsible for CPC mobilization during and in recovery from exercise may provide new targets for increasing cell content in the context of HSCT and also provide novel insights into the role of CPCs in systemic adaptations to exercise.

As such, the purpose of this study was to examine the time course of CPC and CPC subpopulation mobilization induced by submaximal treadmill running for 1 h at 70% peak O2 uptake (Vo2peak) in physically fit, young adults. Additionally, to further investigate potential mechanisms responsible for exercise-induced CPC mobilization, we assessed the dynamics of SCF, CXCL12, and ECs, a marker of vascular damage (2, 3), in peripheral blood. We hypothesized that CPC content in peripheral blood would increase during exercise and remain transiently elevated during postexercise recovery. In addition, increases in CXCL12, SCF, and ECs both would be increased in response to exercise, and this response would be related to increases in CPC content.

METHODS

Participants. Eight healthy young men volunteered for this study. One participant was excluded from analysis due to technical difficulties during blood collection, thus final analyses were performed on n = 7 participants (age: 25.3 ± 2.4 yr, height: 173.2 ± 3.2 cm, weight: 71.2 ± 3.1 kg, body mass index: 23.5 ± 1.0 kg/m², body fat percentage: 14.5 ± 1.6%, and Vo2peak: 60.9 ± 2.7 ml kg⁻¹ min⁻¹). Participants were physically fit, regularly active, young adults with a Vo2peak > 50 ml kg⁻¹ min⁻¹ and exercised at least 3 times/wk. Each participant completed a routine medical screening questionnaire and was informed of the purpose of the study, experimental procedures, and all of its potential risks before providing written consent to participate. The study was approved by the University of Illinois Institutional Review Board and conformed to standards for the use of human participants in research as outlined in the sixth Declaration of Helsinki.

Anthropometric measures and body composition. Standing height and weight measurements were completed with participants wearing lightweight clothing and no shoes, using a stadiometer (Seca; model 240) and a Tanita WB-300 Plus digital scale (Tanita, Tokyo, Japan), respectively. Whole body and regional soft tissue composition were measured by dual-energy X-ray absorptiometry using a Hologic Discovery A bone densitometer (software version 12.7.3; Hologic, Bedford, MA). Precision for dual-energy X-ray absorptiometry measurements of interest are 1–1.5% in our laboratory.

Maximal exercise test. Participants completed a maximal exercise test on a motorized treadmill using a modified Balke protocol (40). The test involved participants running on a treadmill at a self-selected constant speed with incremental grade increases of 1% every minute until volitional exhaustion (40). Participants were fitted with a polar heart rate monitor (Polar Heart Rate Monitor Model T31; Lake Success, NY), and oxygen consumption was measured using an indirect calorimetry system (Parvomedics TrueOne 2400 Metabolic Measurement; Sandy, UT) every 15 s. Heart rate and ratings of perceived exertion were assessed every 2 min using the Borg scale (40).

Habituation trial. A habituation trial was conducted to familiarize participants with the exercise protocol and to calculate correct treadmill speed equal to 70% of their individual Vo2peak. Treadmill speeds were adjusted during the first 5 min to elicit 70% Vo2peak while participants ran at 1% incline for 60 min on a treadmill. Speed settings were recorded and used during the experimental trial.

Experimental trial. Participants reported to the laboratory at ~0700 after an overnight fast. Participants refrained from physical activity, alcohol, and caffeine for at least 2 days before the trial. Before beginning the exercise trial, participants were fitted with a heart rate monitor (Polar Heart Rate Monitor Model T31), and a Teflon catheter (BD, Franklin Lakes, NJ) was inserted into an antecubital vein of one arm and kept patent with 0.9% saline drip for repeated blood sampling. For all samples, the initial blood drawn (3 ml) was discarded to avoid any dilution effects from the saline. The exercise trial consisted of running on a motorized treadmill at 70% Vo2peak for 60 min. During the trial, ratings of perceived exertion were collected every 20 min using the Borg scale, and O2 uptake was monitored using an indirect calorimetry system (Parvomedics TrueOne 2400 Metabolic Measurement). The average rating of perceived exertion reported for our exercise protocol was 13.0 ± 2.9, suggesting our exercise stimulus was “somewhat hard” or “moderate,” according to the Borg scale (7). Immediately after the cessation of exercise, participants ingested a liquid meal (465 kcal; 18 g protein, 60 g carbohydrate, 17 g fat).

Blood analysis. Blood samples (~3 ml) were collected from an antecubital vein into EDTA-anticoagulant tubes (BD) at the following times: immediately before exercise (Pre); at 20 min (20e), 40 min (40e), and 60 min during exercise (60e); and at 15 min (15p), 60 min (60p), and 120 min postexercise (120p). Samples collected at Pre, 20e, 40e, 60e, and 15p were stored on ice and processed after collection of
was calculated as a two-factor (cell population in SigmaPlot 12.5 (Systat Software). Progenitor cell “responsiveness” analysis of ranks. Spearman’s normality were analyzed using Friedman’s repeated measures of Tukey’s post hoc test. Data that failed to meet the assumptions of ANOVA with repeated measures on time in SigmaPlot 12.5 (Systat

plasma protein concentrations were analyzed by repeated-measures ANOVA with repeated-measures ANOVA. Cells and proteins were enumerated using the commercially available Quantikine ELISA kit (R&D Systems, Minneapolis, MN) at the following time points: Pre, 20e, 40e, 60e, 15p, 60p, and 120p. Since there were no changes in CPC concentration during and after exercise, but are not related to changes in CPC content. CPC content was also significantly increased at 40e compared with 60p (Fig. 1; 60p: 373.0 ± 275.8 cells/mL vs. 60p: 281.0 ± 225.0 cells/mL, 120p: 432.4 ± 105.2 cells/mL; P < 0.05). Circulating HSPC quantity was significantly increased at 60e compared with both 60p and 120p (Fig. 1; 60p: 373.0 ± 104.1 cells/mL; 120p: 432.4 ± 105.2 cells/mL; P < 0.05). Circulating HSPC and CPC subpopulations to acute exercise. CD34+CPC quantity was significantly increased 2.7- and 2.4-fold, respectively, at 20 and 40 min into exercise compared with Pre values (Fig. 1; Pre: 342.2 ± 76.0 cells/mL, 20e: 908.3 ± 255.5 cells/mL, 40e: 831.8 ± 195.9 cells/mL; P < 0.05). CD34+CPC content was significantly increased at 20e compared with both 60p and 120p (Fig. 1; 60p: 373.0 ± 104.1 cells/mL; 120p: 432.4 ± 105.2 cells/mL; P < 0.05). CD34+CPC content was also significantly increased at 40e compared with 60p (Fig. 1; P < 0.05). Circulating HSPC quantity was significantly increased 2.7-fold at 20e compared with 60p (Fig. 2A; 20e: 762.8 ± 275.8 cells/mL vs. 60p: 281.0 ± 76.8 cells/mL; P < 0.05). Circulating HSPC quantity was significantly increased 2.8-fold at 20e compared with 60p (Fig. 2B; 20e: 613.8 ± 250.0 cells/mL vs. 60p: 222.7 ± 70.6 cells/mL; P < 0.05). Circulating HSPC and CPC content were not changed at any time point during or after exercise (Fig. 3). Both CD34− (reportedly bone marrow-derived; BM-MSCs) and CD34+ (reportedly adipose tissue-derived; AT-MSCs) MSCs were rare in circulation, and the quantity of either population was not altered by exercise (Fig. 4).

SCF and CXCL12 are increased during and after acute exercise, but are not related to changes in CPC content. The concentration of CXCL12 in plasma was significantly elevated 1.5-fold at 40e and remained significantly elevated until 15p
Fig. 3. Time course of EPC and EC accumulation in blood. EPCs (CD45−CD34+CD31−; A) and ECs (CD45−CD31−; B) were quantified from isolated PBMCs by flow cytometry at the reported time points during and after exercise. Values are means ± SE; n = 7.

DISCUSSION

The kinetics of CPC and CPC subpopulation mobilization during moderate-intensity exercise, as well as the mechanisms responsible for their release into circulation, have not been well described. A better understanding of these processes could provide further insight into the mechanisms responsible for exercise-induced adaptations throughout the body, as well as provide support for therapeutic strategies utilizing exercise for stem cell collection in the context of HSCT. The main findings from the present study are as follows: 1) CPCs are significantly increased compared with baseline at 20 and 40 min into submaximal treadmill exercise; 2) SCF and CXCL12 are significantly increased 40 min into submaximal treadmill exercise in humans; 3) increases in CPC quantity positively correlate to ECs during exercise; and 4) CPCs subpopulations are differentially responsive to exercise. These data suggest that submaximal treadmill exercise induces CPC mobilization during the exercise bout, which is more likely related to exercise-induced peripheral tissue damage rather than circulating CXCL12 and SCF.

The majority of previous studies have shown that CD34+ CPCs are transiently increased following acute exercise (4, 5, 34), although this effect may be dependent on exercise intensity and modality (5, 50). In the present study, we observed a significant increase in CD34+ CPCs during exercise that peaked at 20 min, remained elevated at 40 min, and exhibited a strong trend to remain elevated at 60 min (P = 0.068) into submaximal treadmill exercise in young men. Only one previous study has investigated the kinetics of CD34+ CPC mobilization during exercise and determined that CD34+ CPCs and EPCs were significantly increased at 180–210 min into a 4-h cycling protocol at 70% of the individual’s anaerobic threshold and remained elevated until cessation of exercise (32). Our data follow a slightly different time course, peaking at 20 min...
into exercise; however, they are similar to the findings of Möbius-Winkler and colleagues (32) in that CD34/CPC concentrations remained elevated throughout the rest of the exercise bout. The more rapid increase in CD34/CPCs observed in the present study could have been due to mechanical stimulation of bone, or the larger overall muscle mass engaged in the running exercise used in the present study provided an additional stimulus that accounted for an earlier increase in CPC mobilization.

A particularly novel aspect of the present investigation is the evaluation of two different populations of circulating MSCs: adipose tissue-derived CD34/MSCs (AT-MSCs), and bone marrow-derived CD34/MSCs (BM-MSCs) (49). In agreement with previous reports (2, 30), circulating MSCs were quite rare in our young, lean participants. A single previous report showed that, following moderate-intensity treadmill running, circulating MSC content was significantly decreased in young athletes (30). Our data indicate a numerical, but not statistically significant decrease in circulating MSC content postexercise. A slight difference between our study and the previous report was that Marycz and colleagues (30) used positive expression of CD51 as a phenotypic marker of MSCs, whereas we used positive expression of CD105, as suggested by the International Society for Cellular Therapy (15), which was previously established to identify MSCs in peripheral blood (28). CD51 is a cell-adhesion molecule; thus it is possible that the decrease in MSCs observed by Marycz and colleagues (30) could be due to a decreased cell-surface expression of CD51, which would be expected in mobilized cells, rather than a decrease in the MSC population.

To compare the relative responsiveness of each stem/progenitor cell population to exercise, we compared fold changes from Pre in each cell population across our time course. We found that, during exercise, CPCs increased relative to BM-MSCs and AT-MSCs. HSCs were increased vs. AT-MSCs at 20e and 60e and increased vs. BM-MSCs at 60e. Lastly, EPCs increased vs. both BM-MSCs and AT-MSCs at 60e. These data suggest differential responses to acute exercise among the different stem/progenitor cell populations. Acute exercise mobilized CPCs, HSCs, and EPCs into circulation, with CPCs and HSCs being mobilized more rapidly. Conversely, relative to other cell populations, MSCs are removed from circulation during exercise, perhaps indicating that these cells are more involved in early adaptive responses occurring during exercise.

Fig. 5. Time course of changes in CXCL12 and SCF concentrations in blood. Plasma was isolated via Ficoll density gradient for analysis of CXCL12 (A) and SCF (B) at the reported time points during and after exercise by ELISA. Values are means ± SE; n = 7. *P < 0.05 compared with Pre.

Fig. 6. Relationship between ECs and peak change in CPC subpopulations. The peak increase in CPC (A), HSPC (B), HSC (C), and EPC (D) concentrations were related to the increase in EC concentrations at the time point corresponding to the peak change in each respective subpopulation (i.e., 20e for CPCs, HSPCs, and HSCs, and 60e for EPCs). Coefficients and P values are presented on each graph.
Fig. 7. Progenitor cell “responsiveness” during and after exercise. CPCs increased relative to BM-MSCs (\( P < 0.01 \) for 20e and 40e, \( P < 0.001 \) for 60e) and AT-MSC (\( P < 0.001 \) for 20e, \( P < 0.05 \) for 40e, and \( P < 0.001 \) for 60e) during exercise. HSCs increased relative to AT-MSC (\( P < 0.05 \)) at 20e and 60e, and BM-MSCs (\( P < 0.05 \)) at 60e. EPCs increased relative to BM-MSCs (\( P < 0.01 \)) and AT-MSCs (\( P < 0.01 \)) at 60e. Values are presented as fold changes compared with Pre values of each respective progenitor cell population and are presented as means ± SE; \( n = 7 \).

In conclusion, our data demonstrate that CPCs are mobilized during, and shortly after, submaximal exercise and return to downhill running (28), in combination with our findings of positive relationships between CPC mobilization and EC content, suggest that other systemic factors and/or tissue damage are playing a role in exercise-induced CPC mobilization.

In the context of HSCT, it is possible that the increased inflammation and tissue damage response to running may result in collection of samples that are not ideal for transplantation. From a clinical perspective, it is important to note that granulocyte colony-stimulating factor, the most common clinical mobilizing agent, increases inflammatory cytokines, specifically IL-6, matrix metalloproteinase-9, and TNF-\( \alpha \) (29). Since administration of granulocyte colony-stimulating factor itself increases inflammatory cytokine levels, it is likely that CPCs mobilized by exercise may not be deemed unsafe for use. However, it will be important to fully characterize both phenotypically and functionally the heterogeneous CPC population mobilized by exercise before potentially implementing exercise protocols in HSCT donors. It is possible that the inflammatory conditions produced by exercise impairs the function of collected cells. To better understand these effects, it will be important to characterize the CPC population that is being mobilized by acute exercise to ensure that these cells not functionally impaired in their regenerative capacity. Along these lines, Krüger et al. (23) showed that acute exercise associated with varying degrees of muscle damage all increased CD34+/CD45− CPCs immediately postexercise. The increase in CPC content was positively correlated with markers of muscle damage. Additionally, colony-forming unit assays were significantly elevated immediately post-3 h post-, and 24 h postexercise, suggesting exercise increased the amount of CPCs in the blood capable of producing colonies. These data suggest that CPCs mobilized by exercise are safe in the context of HSCT. On the other hand, Kroepfl and colleagues (21) demonstrated that CPCs collected 10 min after exercise displayed decreased colony-forming capacity, suggesting decreased function. An important difference between these two studies is that the exercise protocol used by Kroepfl and colleagues (21) was a maximal exercise test to exhaustion. As such, there is likely a balance between enhancing mobilization vs. causing cellular damage to HSPCs. In the present study, we chose a continuous running protocol at 70% maximum \( O_2 \) uptake to attempt to optimize this balance based on previously published protocols.

A limitation of this study is the limited number of circulating factors that were evaluated that could be involved in CPC mobilization. Many previous studies have investigated the relationship between catecholamines (22), cortisol (45), and inflammatory and tissue damage markers (17, 20, 34, 54) to exercise-induced CPC mobilization. We focused on CXCL12, SCF, and endothelial damage as potential mechanisms responsible for CPC mobilization because these biomarkers are well-known to be involved in CPC mobilization, and we have shown CXCL12 and SCF to be increased in rodent muscle following exercise (17). However, these factors have not been well-characterized in the context of exercise. Now that we have established a time course for CPC mobilization, future studies will be able to perform multiplex analyses using specific time points to better understand the mechanisms responsible for exercise-induced CPC mobilization.
basal levels within 1 h after exercise. Additionally, mobilization of various CPC subpopulations is related to markers of tissue damage and not plasma levels of CXCL12 and SCF. These data suggest that CPCs may play a role in the physiological response to exercise, potentially acting as cellular mediators of exercise-induced adaptations. Furthermore, the increase in CPCs during submaximal exercise supports the potential for investigation of exercise interventions as an adjuvant therapy for stem cell mobilization in the context of bone marrow transplantation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

G.M.N., D.R.M., N.A.B., and M.D.L. conceived and designed research; G.M.N. and M.D.L. performed experiments; G.M.N. and M.D.L. analyzed data; G.M.N. and M.D.L. interpreted results of experiments; G.M.N. prepared figures; M.D.L. revised and approved final version of manuscript; G.M.N. and M.D.L. edited and revised manuscript.

REFERENCES


