

Acute exercise mobilizes hematopoietic stem and progenitor cells and alters the mesenchymal stromal cell secretome

Russell Emmons,¹ Grace M. Niemiro,¹ Olatomide Owolabi,¹ and Michael De Lisio¹

¹Department of Kinesiology and Community Health, University of Illinois at Urbana-Champaign, Urbana, Illinois

Submitted 2 November 2015; accepted in final form 2 January 2016

Emmons R, Niemiro GM, Owolabi O, De Lisio M. Acute exercise mobilizes hematopoietic stem and progenitor cells and alters the mesenchymal stromal cell secretome. *J Appl Physiol* 120: 624–632, 2016. First published January 7, 2016; doi:10.1152/jappphysiol.00925.2015.—Transplantation of hematopoietic stem and progenitor cells (HSPC), collected from peripheral blood, is the primary treatment for many hematological malignancies; however, variable collection efficacy with current protocols merits further examination into factors responsible for HSPC mobilization. HSPCs primarily reside within the bone marrow and are regulated by mesenchymal stromal cells (MSC). Exercise potently and transiently mobilizes HSPCs from the bone marrow into peripheral circulation. Thus the purpose of the present study was to evaluate potential factors in the bone marrow responsible for HSPC mobilization, investigate potential sites of HSPC homing, and assess changes in bone marrow cell populations following exercise. An acute exercise bout increased circulating HSPCs at 15 min (88%, $P < 0.001$) that returned to baseline at 60 min. Gene expression for HSPC homing factors (CXCL12, vascular endothelial growth factor- α , and angiopoietin-1) were increased at 15 min in skeletal muscle and HSPC content was increased in the spleen 48 h postexercise (45%, $P < 0.01$). Acute exercise did not alter HSPCs or MSCs quantity in the bone marrow; however, proliferation of HSPCs (40%, $P < 0.001$), multipotent progenitors (40%, $P < 0.001$), short-term hematopoietic stem cells (61%, $P < 0.001$), long-term hematopoietic stem cells (55%, $P = 0.002$), and MSCs (20%, $P = 0.01$) increased postexercise. Acute exercise increased the content of the mobilization agent granulocyte-colony stimulating factor, as well as stem cell factor, interleukin-3, and thrombopoietin in conditioned media collected from bone marrow stromal cells 15 min postexercise. These findings suggest that the MSC secretome is responsible for HSPC mobilization and proliferation; concurrently, HSPCs are homing to extramedullary sites following exercise.

hematopoietic stem cell; paracrine factors; secretome; mesenchymal stromal cells

HEMATOPOIETIC stem and progenitor cells (HSPCs) are a rare population of stem cells that are responsible for the maintenance and regeneration of the hematopoietic system across the life span (30). Their capacity for regeneration allows for their transplantation into patients to enhance hematopoietic recovery following myeloablative treatment regimens targeting certain cancers (4). HSPCs used for transplantation are obtained from the bone marrow, peripheral blood, or umbilical cord; however, additional focus has been placed into HSPC collection from peripheral blood due to decreased invasiveness of the procedure for obtaining circulating HSPCs (35), and increased engraftment compared with HSPCs obtained from bone marrow (41). Effective HSPC collection from peripheral blood relies on the use of pharmacological agents including granulocyte-colony stimulating factor (G-CSF) (15) and AMD3000 (37) to mobilize HSPCs and to enhance HSPCs concentration

in peripheral blood. However, limitations to pharmacological approaches range from long-dosing protocols to rare but negative drug side effects such as adult respiratory distress syndrome and pyoderma (19). Additionally, not all patients respond sufficiently to mobilizing agents and thus require multiple rounds of mobilization therapy (2). Thus further investigations into alternative or complementary interventions to current pharmacological treatments are necessary.

Exercise has been previously shown to mobilize HSPCs into peripheral blood. Intense bouts of acute exercise have been observed to transiently increase the content of HSPCs in peripheral blood in humans (24, 28). The exercise-induced increase in HSPC content is similar in magnitude to that induced by pharmacological interventions (7, 17, 28, 29). The primary limitation to the widespread adoption of exercise as an adjuvant therapy to pharmacological mobilization is the transient nature of the increase in HSPC quantity (17). It is most likely HSPCs are homing to sites of inflammation to contribute to repair/remodeling following exercise or migrating to other extramedullary sites. Developing a better understanding of the mechanisms responsible for HSPC mobilization into, and removal from peripheral blood in response to exercise may allow for the identification of key factors that can be targeted to increase the duration that HSPCs remain in circulation following exercise. To elucidate the mechanisms responsible for exercise-induced HSPC mobilization, Morici and colleagues (29) sought to evaluate changes in stem cell factor (SCF), G-CSF, and CXCL12 in plasma following an acute exercise bout. Although they observed an increase in mobilized HSPCs resulting from exercise, no changes were detected in HSPC mobilization or growth factors following exercise (29). This suggests that further investigations should be directed toward the primary sites where HSPCs reside, as local paracrine factors released in the bone marrow are likely responsible for mobilization and are not detected once HSPCs enter peripheral circulation.

HSPCs primarily reside within the bone marrow and are tightly regulated by their local microenvironment or niche (38). As such, evaluating factors within the HSPC niche in the bone marrow may provide more precise clues as to their regulation in response to exercise. In mice, HSPC content is enriched in the population of cells within the cell characterized phenotypically as Lineage⁻ Sca-1⁺ c-Kit⁺ (LSK) (14). The LSK population can be further enriched for long-term hematopoietic stem cells (LT-HSC: LSK-CD48⁻CD150⁺), short-term hematopoietic stem cells (ST-HSC: LSK-CD48⁻CD150⁻), and multipotent progenitor cells (MPP: LSK-CD48⁺CD150⁻) (9). These HSPC subpopulations are functionally divided by mitotic activity and reconstitution ability in bone marrow transplant assays (9). Mesenchymal stromal cells (MSCs) in the bone marrow are key regulators of HSPC quiescence, prolifer-

Address for reprint requests and other correspondence: M. De Lisio, 906 S. Goodwin Ave., Urbana, IL 61801 (e-mail: mdlisio@illinois.edu).

eration, homing, and mobilization (6, 20, 32). MSCs represent a heterogeneous population of cells; however, a purified population has been identified by the phenotypic marker platelet-derived growth factor receptor alpha (PDGFR α) (43), as well as a lack of lineage committed markers (13).

The purpose of the present study was to establish a time course of HSPC mobilization in mice, and characterize factors in the bone marrow and peripheral tissues that might be responsible for HSPC mobilization and removal from circulation. We hypothesized that an acute bout of exercise would transiently stimulate HSPC mobilization and proliferation, alter the secretome of MSCs in the bone marrow compartment, and increase expression of HSPC chemoattractants in mouse skeletal muscle.

MATERIALS AND METHODS

Mice. All protocols were approved by the Illinois Institutional Animal Care and Use Committee. Male and female C57Bl/6 (Jackson Laboratories, Bar Harbor, ME) mice were housed at 2 per cage and maintained in a 12:12-h light-dark schedule with food and water provided ad libitum. Mice were received at 10 wk of age and were 14–18 wk for experiments.

Exercise protocol. Male and female mice were equally and randomly divided between exercise (EX) and nonexercised control (CNT) groups. For the experiments, there were 8 mice (4 female and 4 male) per CNT and EX groups at 15 min, and 10 (5 female and 5 male) were assigned to the CNT and EX at 60 min. Two weeks before the exercise protocol, both EX and CNT mice were acclimated to the treadmill by a brief exposure 3 times every other day (M/W/F) consisting of 5 min at 8 m/min, 5 min at 10 m/min, and 5 min at 8 m/min. EX mice completed an intense acute exercise bout on a treadmill (Columbus Instruments, Columbus, OH) previously shown to induce stress on the bone marrow compartment (11). Briefly, the exercise protocol began at 8 m/min that increased 2 m/min every 10 min until a speed of 16 m/min was reached. Mice exercised at 16 m/min for 30 min, and then 18 m/min for 20 min. Mice were exercised at a 0% grade. CNT mice were placed into a control apparatus with the same lane specifications and placed on top of the treadmill to mimic the stress of handling and treadmill exposure.

HSPC quantification in peripheral blood. Peripheral blood was analyzed at 15 and 60 min postexercise for quantification of HSPCs in circulation. Peripheral blood was collected via submandibular facial bleed into 0.2 ml PBS/heparin solution. After red blood cells were lysed in lysing buffer (BD Biosciences, San Jose, CA), cell suspensions were incubated in the following antibodies: biotinylated lineage panel (BD Biosciences), anti-mouse Sca-1 PE (1:200, Life Technologies, Benicia, CA), anti-mouse c-Kit PE-CY7 (1:200, Life Technologies), and FITC streptavidin (1:800, BD Biosciences). Cells were analyzed with an Attune Acoustic Focusing Flow Cytometer (Life Technologies). Unstained and single stain controls were used for compensation and gating. Data were analyzed as percentages Sca-1 and c-Kit positive from lineage negative populations.

HSPC and PDGFR α quantification in bone marrow. Mouse bone marrow cells were analyzed at 48 h postexercise to detect changes in stem/progenitor cell quantity within the bone marrow compartment. Mice were euthanized via CO₂ asphyxiation followed by cervical dislocation. Both femurs and tibias were quickly removed and cleared of muscle and connective tissue. Marrow was flushed in 1 ml sterile PBS through a 22-gauge needle. Flushed bones were mechanically and enzymatically digested as previously described (1). Briefly, femurs and tibias were gently crushed using mortar and pestle in Dulbecco's modified Eagle's medium (DMEM). Bone fragments were further processed by cutting with scissors and suspended into a 0.2% collagenase solution for 1 h at 37°C. Cells obtained were recombined with cells previously flushed and placed through magnetic cell sorting

(MACS): EasySep Mouse Hematopoietic Progenitor Cell Enrichment Kit (StemCell Technologies, Vancouver, BC, Canada) per manufacturer's instruction. Cells were stained using the following antibodies for HSPC and MSC markers, respectively: Sca-1 PE (1:200, Life Technologies), c-Kit (1:200, Life Technologies), CD150 Brilliant Violet 421 (1:200, Life Technologies), CD 48 Brilliant Violet 521 (1:200, Life Technologies), and PDGFR α PE (1:200, BD Biosciences).

Spleen analysis. Spleens were obtained from mice 48 h postexercise following euthanasia and stored on ice until processing. Spleen cells were isolated by cutting the spleen on both ends and gently milking the cells out using forceps into PBS. Red blood cells were lysed and stained for HSPC markers as described above.

5'-Bromo-2' deoxyuridine (BrdU) incorporation. A subset of EX and CNT mice were used for detecting cell proliferation in the bone marrow. Ten mice from the CNT (5 male and 5 females) and EX (5 male and 5 female) were used. The average weights for each group were CNT 22.38 \pm 4.12 g and EX 23.95 \pm 2.78 g. Mice were injected (ip) with a sterile 2% BrdU solution (Sigma) at 0.5 mg/g body wt in PBS once a day for 10 days prior to the exercise bout. The final injection was given the morning of the exercise bout. The exercise bout was performed as described above. Cells obtained from the bone marrow were obtained as described above and processed using the BD Pharmingen BrdU Flow Kits (BD Bioscience, San Jose, CA) following the manufacturer's procedures. Briefly, cells were fixed, permeabilized, and stained using anti-BrdU FITC (1:50). Cells were analyzed with an Attune Acoustic Focusing Flow Cytometer (Life Technologies). Unstained and single stain controls were used for compensation and gating. Data were analyzed as percentages Sca-1 and c-Kit positive (HSPCs) and PDGFR α positive.

Fluorescent activated cell sorting (FACS) of bone marrow stromal cells. A subset of cells obtained following MACS/FACS was used to characterize changes in the bone marrow stromal cell secretome. Cells were stained with CD45 FITC (BD Biosciences) and separated using BD FACS Aria II Sorter (BD Biosciences). Following FACS, cells were placed into a 96-well cell culture plate (StemCell Technologies, Vancouver, BC, Canada) and cultured for 24 h in 150 μ l of 5% FBS/DMEM at 50,000 cells/well.

Conditioned media analysis. Conditioned media (CM) was collected from cells separated by FACS after 24 h and stored at -80° C. CM was pooled between the EX and CNT groups, respectively, and analyzed using a C-series Mouse Cytokine Assay C1 (RayBiotech, Norcross, GA) following the manufacturer's protocol. Assays were imaged using ChemiDoc XRS camera (BioRad, Hercules, CA) and analyzed using ImageJ (National Institutes of Health).

Gene expression analysis. The extensor digitorum longus (EDL) was obtained from previously euthanized mice and stored at -80° C. RNA was isolated using RNeasy Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocols. Total RNA was quantified using a NanoDrop 1000 spectrophotometer (ThermoScientific, Waltham, MA). RNA to cDNA reverse transcription was done using the High Capacity cDNA RT Kit (Life Technologies). Quantitative real-time polymerase chain reaction (qPCR) was done using Taqman ABI 7900 real-time PCR machine (Life Technologies) and analyzed using SDS software (Life Technologies). Changes in gene expression were calculated using the $2^{-\Delta\Delta CT}$ method (27) normalized to housekeeping genes. The housekeeping genes β -actin, SDHA, and MRPL14 were averaged and used for calculations due to stability pre- and postexercise. Due to variations in housekeeping genes between 15 min and 48 h postexercise time points, gene expression for the two time points was analyzed independently.

Statistical analysis. The data were checked for normal distribution by Shapiro-Wilk test. Data that were normally distributed (i.e., cell quantities in circulation and cell quantities in the bone marrow) were analyzed by parametric tests, while data that were not normally distributed (i.e., gene expression data and LSK⁺ quantity in the spleen) were analyzed by nonparametric tests. Cell quantities in

peripheral circulation were analyzed using a two-way ANOVA in GraphPad Prism 6 (GraphPad Software, La Jolla, CA) and cell quantities in the bone marrow were analyzed using *t*-tests in Microsoft Excel (Microsoft, Redmond, WA). Gene expression was analyzed using the Mann-Whitney-Wilcoxon test. Data are presented as means \pm SD with $P < 0.05$ considered significant.

RESULTS

Acute bout of exercise transiently mobilizes LSK cells into peripheral blood. Representative flow plots for LSK HSPCs are shown in Fig. 1A. The percentage of circulating HSPCs was significantly increased 15 min postexercise in the EX group vs. CNT (0.66 \pm 0.27% vs. 1.24 \pm 0.47%, $P < 0.001$) and no significant difference in EX vs. CNT mice at 60 min (Fig. 1B).

Acute exercise upregulates expression of factors conducive to HSPC homing and migration to spleen. Exercise has been shown to increase mobilization of HSPCs to skeletal muscle (34) and spleen (1). Thus we evaluated HSPC quantity in the spleen and gene expression of HSPC homing factors in skeletal muscle 15 min postexercise and 48 h postexercise. A significant increase in HSPCs was detected in the spleen 48 h postexercise (CNT 2.46 \pm 0.28% vs. EX 3.53 \pm 0.78%, $P = 0.01$) (Fig. 2A). The expression of SCF (35-fold, $P = 0.001$, Fig. 2B) CXCL12 (28-fold, $P = 0.001$, Fig. 2C) VEGFa (17-fold, $P = 0.001$, Fig. 2D), and ANG1 (31-fold, $P = 0.01$, Fig. 2E) were all significantly elevated in the skeletal muscle from EX vs. CNT mice. No significant differences were detected at 48 h postexercise for SCF (Fig. 2F), CXCL12 (Fig. 2G), VEGFa (Fig. 2H), and ANG1 (Fig. 2I).

Acute exercise stimulates HSPC proliferation with no effect on total quantity. Gating strategy for HSPC, LT-HSC, ST-HSC, and MPP is shown in representative flow plots in Fig. 3A. An acute bout of exercise did not significantly increase the percentage of total HSPCs (LSK; Fig. 3B), MPPs (Fig. 3C), ST-HSC (Fig. 3D), or LT-HSCs (Fig. 3E) in the bone marrow 48 h postexercise.

To evaluate the effects of an acute bout of exercise on HSPC proliferation, we evaluated the quantity of HSPCs, MPPs, ST-HSCs, and LT-HSCs expressing BrdU. An acute bout of exercise significantly increased the percentage of LSK BrdU⁺ (55.38 \pm 6.44% vs. 77.79 \pm 3.80%, $P < 0.001$, Fig. 4A), MPP BrdU⁺ (55.18 \pm 10.29% vs. 79.04 \pm 7.23%, $P < 0.001$, Fig. 4B), ST-HSC BrdU⁺ (40.37 \pm 7.23% vs. 65.13 \pm 10.21%, $P < 0.001$, Fig. 4C), and LT-HSC BrdU⁺ (40.64 \pm 5.68% vs. 62.9 \pm 10.03%, $P = 0.002$, Fig. 4D).

An acute bout of exercise stimulates proliferation of *lin*⁻ PDGFR α ⁺ cells in bone marrow and alters stromal cell secretome. Representative flow plots for PDGFR α ⁺ are shown in Fig. 5A following MACs sorting. An acute bout of exercise did not significantly affect the total number of PDGFR α ⁺ cells within the bone marrow (Fig. 5B, $P > 0.05$). An acute bout of exercise stimulated the proliferation in the MSC populations (Fig. 5C). The number of PDGFR α ⁺ BrdU positive cells was increased in the EX group, 68.43 \pm 4.43%, compared with CNT, 57.02 \pm 8.00% ($P = 0.01$).

To determine if an acute exercise bout altered paracrine factor secretion in bone marrow stromal cells, *Lin*⁻ CD45⁻

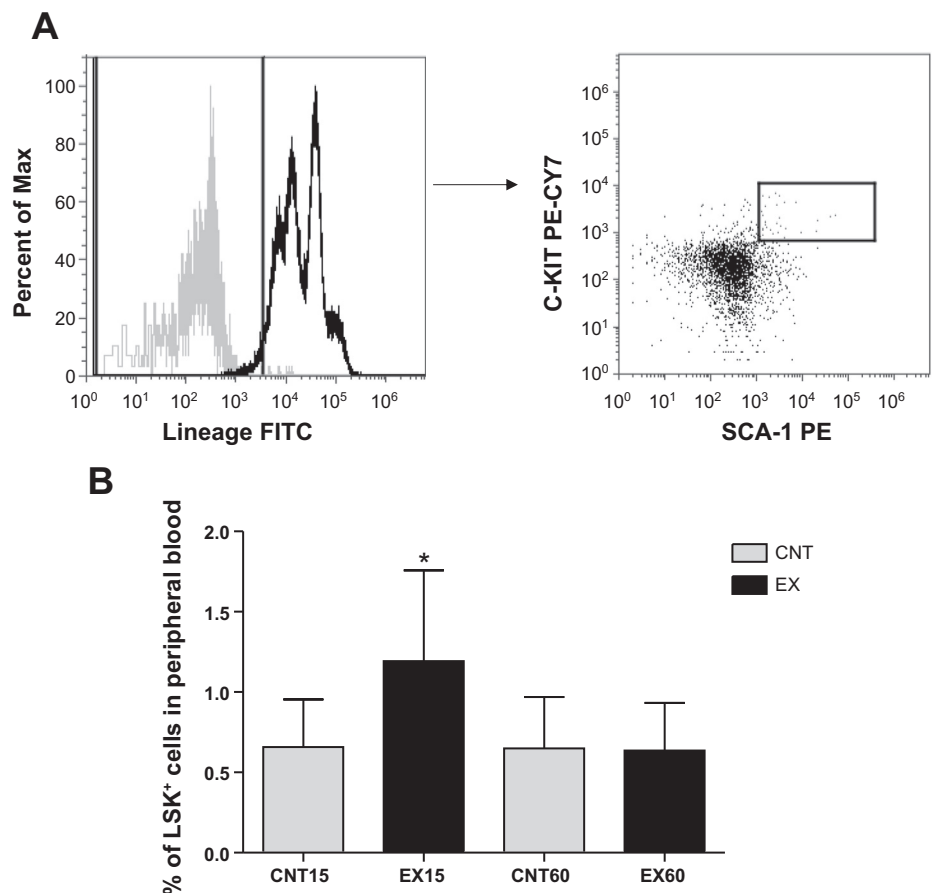


Fig. 1. Acute bout of exercise transiently mobilizes LSK cells into peripheral blood. **A**: representative flow plots of LSK cells in peripheral blood. **B**: percentage of LSK cells in peripheral circulation at 15 min and 60 min postexercise. Data are presented as means \pm SD from CNT15 ($n = 8$; 4 female and 4 male), EX15 ($n = 8$; 4 female and 4 male), CNT60 ($n = 10$; 5 female and 5 male), EX60 ($n = 10$; 5 female and 5 male). * $P < 0.01$.

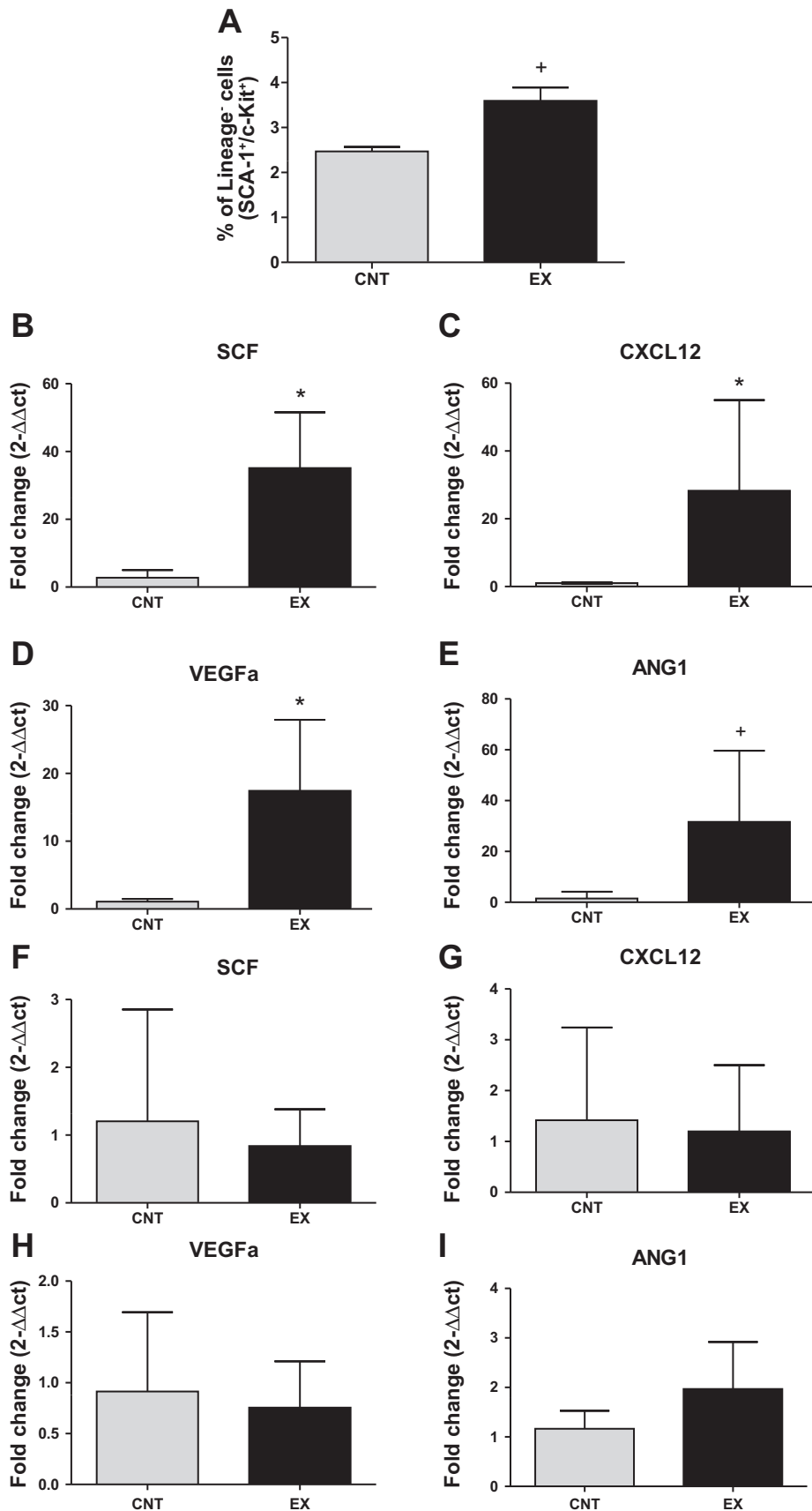


Fig. 2. Acute exercise promotes gene expression of factors conducive to hematopoietic stem and progenitor cells (HSPC) homing and migration to spleen. A: percentage of LSK cells in the spleen 48 h after exercise in exercise and control mice. Gene expression of SCF (B), CXCL12 (C), VEGFa (D), and ANG1 (E) 15 min postexercise. Gene expression of SCF (F), CXCL12 (G), VEGFa (H), and ANG1 (I) in EDL 48 h postexercise. Data are presented as means ± SD from CNT (n = 8; 4 female and 4 male) and EX (n = 8; 4 female and 4 male) in the spleen; and CNT (n = 10; 5 female and 5 male) and EX (n = 10; 5 female and 5 male) for gene expression in the EDL at 15 min and 48 h. *P < 0.001, +P = 0.01.

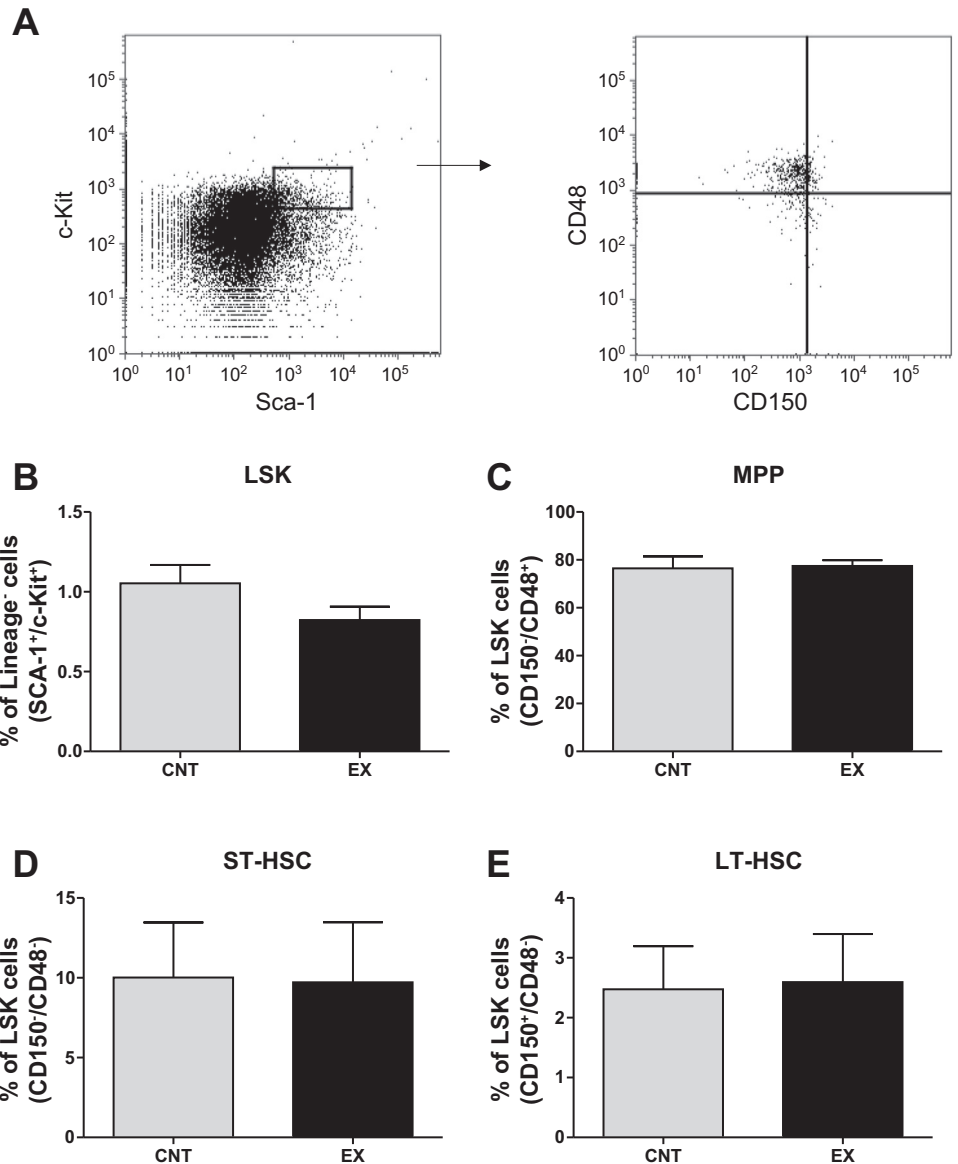


Fig. 3. An acute bout of exercise does not alter HSPC quantity within the bone marrow. *A*: representative flow plots of CD150⁺/CD48⁺ on Sca-1⁺/c-Kit⁺ hematopoietic stem and progenitor cells following MACs sorting of bone marrow cells 48 h postexercise. Percentage of cell SCA-1⁺ and c-Kit⁺ HSPCs in the bone marrow (*B*). Percentage of MPPs (*C*), ST-HSCs (*D*), and LT-HSCs (*E*) from Sca-1⁺/c-Kit⁺ cells. Data are presented as means \pm SD from CNT ($n = 10$; 5 female and 5 male) and EX ($n = 10$; 5 female and 5 male).

cells were isolated using a combined MACS/FACS isolation technique. Lin⁻ CD45⁻ MSCs were cultured for 24 h in serum-depleted media (5% FBS/DMEM) for evaluation of paracrine factor secretion in conditioned media (CM). The concentration of G-CSF (1.89-fold), SCF (1.63-fold), IL-2 (1.52-fold), IL-17 (1.56-fold), sTNFR1 (1.35-fold), IL-3 (1.26-fold), and thrombopoietin (THPO; 1.15-fold) were increased in all cells isolated from EX vs. CNT. IL-5 (0.73-fold) and INF- γ (0.80-fold) were decreased in cells isolated from EX vs. CNT (Fig. 5D). All other paracrine factors evaluated were not changed (Fig. 5D).

DISCUSSION

Developing a more complete understanding of the mechanisms governing HSPC mobilization following acute exercise is necessary to devise complementary therapies to current pharmacological interventions used in stem cell transplant. In pursuit of this aim, the present paper used a murine model to establish a time course for HSPC mobilization following ex-

ercise and identified, for the first time, exercise-induced paracrine factors released by MSCs in the HSC niche, including granulocyte-colony stimulating factor (G-CSF) and stem cell factor (SCF), and determined that gene expression for chemokines necessary for HSPC homing including CXCL12 and angiopoietin-1 (ANG1) were upregulated in skeletal muscle. Additionally, we observed that a single bout of exercise stimulated HSPC and MSC proliferation within the bone marrow. These observations support our hypothesis that exercise stimulates HSPC mobilization due to paracrine factors released within the bone marrow compartment by MSCs in the HSPC niche, and that chemoattractants produced in skeletal muscle draw HSPCs out of circulation.

Acute exercise has long been known to transiently increase HSPC content in peripheral blood. In the present study, HSPCs peaked 15 min after an acute exercise bout, and returned to levels similar to nonexercised mice by 1 h postexercise. This timeline is consistent with previous human studies showing peak HSPC quantity in circulation 15 min after an acute

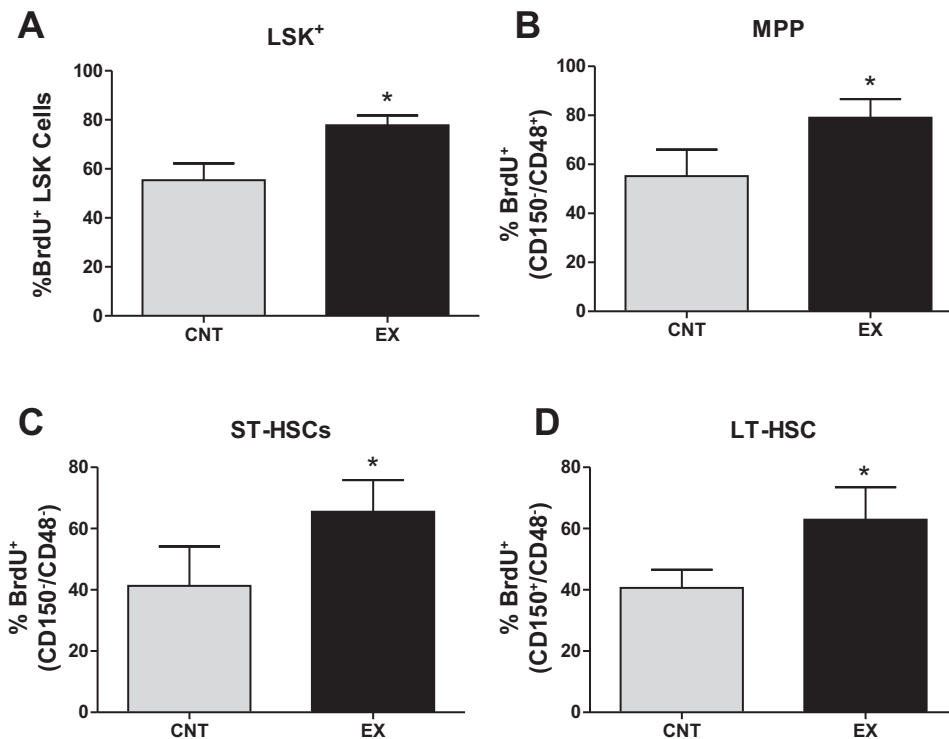


Fig. 4. An acute bout of exercise stimulates HSPC proliferation within the bone marrow. A: total percent of cells SCA-1⁺ and c-Kit⁺ hematopoietic stem and progenitor cells expressing BrdU 48 h postexercise. Total percentage of BrdU⁺ MPPs (B), ST-HSCs (C), LT-HSCs (D) from Sca-1⁺/c-Kit⁺ in the bone marrow. Data are presented as means \pm SD from CNT ($n = 10$; 5 female and 5 male) and EX ($n = 10$; 5 female and 5 male). * $P < 0.01$.

exercise bout and returning to baseline levels within 60 min of cessation of exercise (28, 29). A similar time course was established by Heal and Brightman (17), who utilized stair running as an acute bout of exercise and observed an increase in circulating progenitor cells after exercise that returned to baseline by 1 h postexercise. Taken together, these data highlight a parallel time course for HSPCs mobilization into peripheral blood between human and murine subjects when exposed to an intense acute bout of exercise, supporting the use of the murine model to elucidate underlying factors responsible for HSPC mobilization.

A major clinical limitation in the application of acute exercise as a stimulus to mobilize HSPCs for hematopoietic stem cell transplant is the short duration of increased HSPC content in circulation. Thus, developing a better understanding of the underlying mechanisms responsible for exercise-induced mobilization into and removal from peripheral blood could increase the efficacy of exercise as an adjuvant therapy to HSPC mobilization. Previous research has demonstrated that acute aerobic exercise bouts induce stress responses from skeletal muscle (31), and bone marrow-derived stem cells contribute to skeletal muscle repair following eccentric exercise (34), highlighting skeletal muscle as a potential target for HSPC migration. The expression of the chemokines CXCL12, ANG1, vascular endothelial growth factor (VEGF), as well as the cytokine stem cell factor (SCF), which is necessary for HSPC maintenance and proliferation (12), were all elevated following an acute exercise bout in skeletal muscle. These results are consistent with previous research that has demonstrated an upregulation of SCF and VEGF to promoting repair and revascularization of the skeletal muscle from stem cell populations (18). Injection of CXCL12, which interacts with CXCR4 on HSPCs, directly into skeletal muscle has been shown to increase skeletal muscle repair and regeneration as a

result of mechanical trauma, primarily due to the increased influx of CD34⁺ CXCR4⁺ progenitor cells (8). In another model of skeletal muscle damage, Bobadilla et al. (5) utilized cardiotoxin injection to induce skeletal muscle damage, which led to an upregulation of CXCL12 and enhanced skeletal muscle repair resulting from increased homing of stem cell populations. Within the bone marrow, ANG1 is necessary for HSPC retention and long-term maintenance (40). However, Hattori et al. (16) demonstrated that elevated levels of both VEGF and ANG1 stimulated hematopoiesis, vasculature remodeling, and capillary development. Given that these factors have been shown to facilitate HSPCs migration, retention, proliferation, and the contribution of bone marrow-derived stem cells to skeletal muscle repair, our data support our hypothesis that chemoattractants produced by skeletal muscle may be involved in removal of HSPCs from circulation to support skeletal muscle remodeling/repair following exercise.

The spleen is a secondary site of extramedullary HSPC residence. HSPCs migrate to the spleen during embryonic development and are maintained there in small quantities throughout life (42). Disruption of the bone marrow compartment, such as during chemotherapy treatment, increases the quantity of circulating progenitor cells that are likely entering the spleen or other extramedullary sites (36). Previous research has shown that as a result of an acute physiological challenge from infection, the quantity of HSPCs within the spleen increases (22). We also observed an increase of LSK⁺ HSPCs within the spleen 48 h postexercise, further suggesting that exercise is mobilizing HSPCs out of the bone marrow and into extramedullary sites, perhaps due to acute stress in the bone marrow compartment.

A decrease in HSPCs within the bone marrow may be expected with exercise-induced HSPC mobilization into circulation. However, in the present study, the content of HSPC

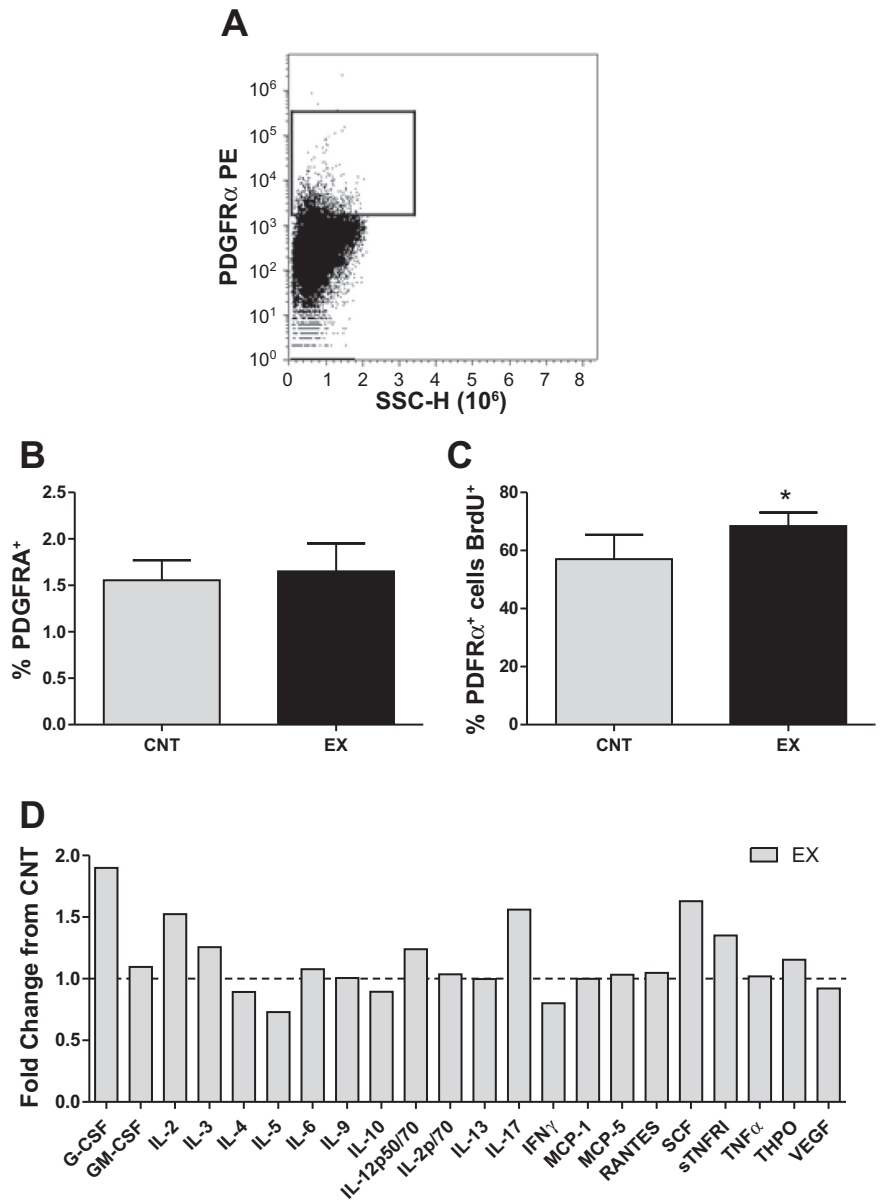


Fig. 5. An acute bout of exercise stimulates proliferation of Lin⁻ PDGFR α^+ cells in bone marrow and alters stromal cell secretome. A: representative flow plot of PDGFR α^+ cells follow MACS 48 h after exercise. Total percentage of PDGFR α^+ cells (B) within bone marrow and percentage of PDGFR α^+ cells expressing BrdU (C) 48 h postexercise. Analysis of conditioned media (CM) (D) collected from Lin⁻CD45⁻ cells after 24 h culture. Lin⁻CD45⁻ cells were obtained from mice bone marrow 15 min postexercise, as fold change from CNT. Data are presented as means \pm SD for B and C, and mean for D, from CNT ($n = 10$; 5 female and 5 male) and EX ($n = 10$; 5 female and 5 male). * $P < 0.01$. CM data samples were pooled between EX and CNT group.

subpopulations analyzed was not different between exercised and nonexercised mice. We did observe increased proliferation of all HSPCs populations within the bone marrow, including MPPs, ST-HSCs, and LT-HSCs as measured by BrdU incorporation, 48 h after exercise. These data suggest that acute exercise induced HSPC proliferation to replace the cells that had emigrated as a result of exercise. Chronic exercise training has been previously observed to alter the fate determination of MSCs (1) and increase the quantity of LSK⁺ population within the bone marrow (10). Thus our data suggest that increases in HSPC and MSC differentiation in response to an acute exercise bout are likely the initial steps towards long-term increases in content seen with chronic exercise training.

Previous exercise studies in humans have attempted to correlate changes in various cytokine concentrations in peripheral blood to alterations in HSPC content. These data have been mostly inconclusive, with no associations shown between HSPC and cytokine content (29). HSPCs reside within a

complex, but tightly regulated, niche within the bone marrow. MSCs are key cellular components of the HSPC niche that regulate HSPC cell fate decisions via a variety of surface antigens and secreted cytokines (23). Our data are the first to demonstrate that an acute bout of exercise increased Lin⁻PDGFR α^+ MSC proliferation within the bone marrow. Further, MSCs isolated from mice 15 min after exercise, the time point corresponding to peak HSPC mobilization, demonstrated increased secretion of G-CSF, SCF, and IL-3 compared with nonexercised mice. G-CSF, SCF, and IL-3 are thought to induce the secretion of matrix metalloproteinase-9 (MMP9) from cells in the bone marrow (35). MMP9 cleaves CXCL12, thereby freeing HSPCs and allowing for entrance into peripheral circulation (21, 26). It is likely these factors are being upregulated to stimulate mobilization of the HSPCs into the periphery. SCF and THPO are also involved with stimulating self-renewal and proliferation of HSPCs (39). We observed an increase of these factors from the CM of exercised mice. These

data suggest that an acute bout of exercise stimulated self-renewal/proliferation of HSPCs and it is likely caused by changes in the secretome of stromal cell populations within the bone marrow.

Sex may play a role in the response of the hematopoietic system following exercise. Sex has been implicated in the effectiveness of pharmacological stimulation of HSPC mobilization. Administration of G-CSF to men elicits a greater increase in HSPCs in peripheral circulation compared with women (3, 25). Conversely, Nakada and colleagues (33) demonstrated that an ovariectomy decreased HSPC proliferation but not castration. In addition, the HSPCs proliferation can be increased in mice through the administration of estradiol, a female sex hormone (33). In the present study, we did not observe any significant difference in the HSPC mobilization or proliferation (data not shown). Male and female mice were equally divided between the CNT and EX groups; however, the small sample size for each sex may limit the ability to identify differential responses to exercise. Future studies with larger sample sizes will be needed to delineate the response of the hematopoietic system following exercise.

In conclusion, our data demonstrate that an acute bout of exercise stimulates the transient release of HSPCs into peripheral circulation as well as stimulating proliferation of HSPCs and MSCs within the bone marrow. The transient nature of the increased HSPC content in peripheral blood is likely due to increased production of chemoattractants in extramedullary tissues, such as skeletal muscle and the spleen, that facilitate HSPC homing to those tissues to participate in repair. The acute alteration in HSPC content and proliferation in the bone marrow are likely mediated by MSC expansion, and alterations in the MSC secretome of the bone marrow. These data are the first to identify key paracrine factors produced by MSCs in the bone marrow following an acute exercise bout, and chemoattractants in the skeletal muscle involved in HSPC homing, thus providing mechanistic targets to enhance the efficacy of exercise as an adjuvant therapy for HSPC mobilization and collection for HSCT.

ACKNOWLEDGMENTS

We thank Dr. Barbara Pilas and Dr. Angela Kouris at the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana-Champaign for assistance with FACS sorting. We also thank A. Sadwilkar for assistance with mouse exercise and tissue collection.

GRANTS

Funding was provided by the University of Illinois at Urbana-Champaign Research Board and the APS STEP-UP program (O.O.).

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: R.E. and M.D.L. conception and design of research; R.E., G.N., O.O., and M.D.L. performed experiments; R.E., G.N., O.O., and M.D.L. analyzed data; R.E., G.N., and M.D.L. interpreted results of experiments; R.E. prepared figures; R.E. drafted manuscript; R.E., G.N., O.O., and M.D.L. approved final version of manuscript; G.N., O.O., and M.D.L. edited and revised manuscript.

REFERENCES

- Baker JM, De Lisio M, Parise G. Endurance exercise training promotes medullary hematopoiesis. *FASEB J* 25: 4348–4357, 2011.
- Bendall LJ, Bradstock KF. G-CSF: From granulopoietic stimulant to bone marrow stem cell mobilizing agent. *Cytokine Growth Factor Rev* 25: 355–367, 2014.
- Bertani G, Santoleri L, Martino M, Fedele R, Moscato T, Marengo P, Grillo G, Zucchetti E, Lotesoriere I, Lando G, Cesana C, Cairoli R, Rossini S. Identification of hematopoietic progenitor cell donor characteristics predicting successful mobilization: results of an Italian multicenter study. *Transfusion (Paris)* 54: 2028–2033, 2014.
- Beyer J, Schwella N, Zingsem J, Strohscheer I, Schwaner I, Oettle H, Serke S, Huhn D, Stieger W. Hematopoietic rescue after high-dose chemotherapy using autologous peripheral-blood progenitor cells or bone marrow: a randomized comparison. *J Clin Oncol* 13: 1328–1335, 1995.
- Bobadilla M, Sainz N, Abizanda G, Orbe J, Rodriguez JA, Páramo JA, Prósper F, Pérez-Ruiz A. The CXCR4/SDF1 axis improves muscle regeneration through MMP-10 activity. *Stem Cells Dev* 23: 1417–1427, 2014.
- Boxall SA, Jones E. Markers for characterization of bone marrow multipotential stromal cells. *Stem Cells Int* 2012: 975871, 2012.
- Broxmeyer HE, Orschell CM, Clapp DW, Hangoc G, Cooper S, Plett PA, Liles WC, Li X, Graham-Evans B, Campbell TB, Calandra G, Bridger G, Dale DC, Srour EF. Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. *J Exp Med* 201: 1307–1318, 2005.
- Brzoska E, Kowalewska M, Markowska-Zagrajek A, Kowalski K, Archacka K, Zimowska M, Grabowska I, Czerwińska AM, Czarnecka-Góra M, Stremińska W, Jańczyk-Ilach K, Ciemerych MA. Sdf-1 (CXCL12) improves skeletal muscle regeneration via the mobilisation of Cxcr4 and CD34 expressing cells. *Biol Cell* 104: 722–737, 2012.
- Challen GA, Boles N, Lin KKY, Goodell MA. Mouse hematopoietic stem cell identification and analysis. *Cytometry A* 75: 14–24, 2009.
- De Lisio M, Parise G. Characterization of the effects of exercise training on hematopoietic stem cell quantity and function. *J Appl Physiol (1985)* 113: 1576–1584, 2012.
- De Lisio M, Phan N, Boreham BR, Parise G. Exercise-induced protection of bone marrow cells following exposure to radiation. *Appl Physiol Nutr Metab* 36: 80–87, 2011.
- Ding L, Saunders TL, Enikolopov G, Morrison SJ. Endothelial and perivascular cells maintain haematopoietic stem cells. *Nature* 481: 457–462, 2012.
- Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8: 315–317, 2006.
- Ema H, Morita Y, Yamazaki S, Matsubara A, Seita J, Tadokoro Y, Kondo H, Takano H, Nakauchi H. Adult mouse hematopoietic stem cells: purification and single-cell assays. *Nat Protoc* 1: 2979–2987, 2006.
- Engelhardt M, Bertz H, Afting M, Waller CF, Finke J. High-versus standard-dose filgrastim (rhG-CSF) for mobilization of peripheral-blood progenitor cells from allogeneic donors and CD34(+) immunoselection. *J Clin Oncol* 17: 2160–2172, 1999.
- Hattori K, Dias S, Heissig B, Hackett NR, Lyden D, Tatenos M, Hicklin DJ, Zhu Z, Witte L, Crystal RG, Moore MA, Rafii S. Vascular endothelial growth factor and angiopoietin-1 stimulate postnatal hematopoiesis by recruitment of vasculogenic and hematopoietic stem cells. *J Exp Med* 193: 1005–1014, 2001.
- Heal JM, Brightman A. Exercise and circulating hematopoietic progenitor cells (CFU-GM) in humans. *Transfusion (Paris)* 27: 155–158, 1987.
- Heissig B, Rafii S, Akiyama H, Ohki Y, Sato Y, Rafael T, Zhu Z, Hicklin DJ, Okumura K, Ogawa H, Werb Z, Hattori K. Low-dose irradiation promotes tissue revascularization through VEGF release from mast cells and MMP-9-mediated progenitor cell mobilization. *J Exp Med* 202: 739–750, 2005.
- Hilbe W, Nussbaumer W, Bonatti H, Thaler J, Niederwieser D, Nachbaur D. Unusual adverse events following peripheral blood stem cell (PBSC) mobilisation using granulocyte colony stimulating factor (G-CSF) in healthy donors. *Bone Marrow Transplant* 26: 811–813, 2000.
- Huang GP, Pan ZJ, Jia BB, Zheng Q, Xie CG, Gu JH, McNiece IK, Wang JF. Ex vivo expansion and transplantation of hematopoietic stem/progenitor cells supported by mesenchymal stem cells from human umbilical cord blood. *Cell Transplant* 16: 579–585, 2007.
- Janowska-Wieczorek A, Marquez LA, Dobrowsky A, Ratajczak MZ, Cabuhat ML. Differential MMP and TIMP production by human marrow

- and peripheral blood CD34(+) cells in response to chemokines. *Exp Hematol* 28: 1274–1285, 2000.
22. Johns JL, Macnamara KC, Walker NJ, Winslow GM, Borjesson DL. Infection with *Anaplasma phagocytophilum* induces multilineage alterations in hematopoietic progenitor cells and peripheral blood cells. *Infect Immun* 77: 4070–4080, 2009.
 23. Kfoury Y, Scadden DT. Mesenchymal cell contributions to the stem cell niche. *Cell Stem Cell* 16: 239–253, 2015.
 24. Kroepfl JM, Pekovits K, Stelzer I, Fuchs R, Zelzer S, Hofmann P, Sedlmayr P, Dohr G, Wallner-Liebmann S, Domej W, Mueller W. Exercise increases the frequency of circulating hematopoietic progenitor cells, but reduces hematopoietic colony-forming capacity. *Stem Cells Dev* 21: 2915–2925, 2012.
 25. Lenk J, Bornhauser M, Kramer M, Hölig K, Poppe-Thiede K, Schmidt H, Wiesneth M, Schaefer-Eckart K, Schlenke P, Punzel M, Martin S, Kroschinsky F, Schmidt AH, Ehninger G, Thiede C. Sex and body mass index but not CXCL12 801 G/A polymorphism determine the efficacy of hematopoietic cell mobilization: a study in healthy volunteer donors. *Biol Blood Marrow Transplant* 19: 1517–1521, 2013.
 26. Liu F, Poursine-Laurent J, Link DC. Expression of the G-CSF receptor on hematopoietic progenitor cells is not required for their mobilization by G-CSF. *Blood* 95: 3025–3031, 2000.
 27. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. *Methods* 25: 402–408, 2001.
 28. Möbius-Winkler S, Hilberg T, Menzel K, Golla E, Burman A, Schuler G, Adams V. Time-dependent mobilization of circulating progenitor cells during strenuous exercise in healthy individuals. *J Appl Physiol (1985)* 107: 1943–1950, 2009.
 29. Morici G, Zangla D, Santoro A, Pelosi E, Petrucci E, Gioia M, Bonanno A, Profita M, Bellia V, Testa U, Bonsignore MR. Supramaximal exercise mobilizes hematopoietic progenitors and reticulocytes in athletes. *Am J Physiol Regul Integr Comp Physiol* 289: R1496–R1503, 2005.
 30. Morrison SJ, Uchida N, Weissman IL. The biology of hematopoietic stem cells. *Annu Rev Cell Dev Biol* 11: 35–71, 1995.
 31. Morton JP, Kayani AC, McArdle A, Drust B. The exercise-induced stress response of skeletal muscle, with specific emphasis on humans. *Sports Med Auckl NZ* 39: 643–662, 2009.
 32. Muguruma Y, Yahata T, Miyatake H, Sato T, Uno T, Itoh J, Kato S, Ito M, Hotta T, Ando K. Reconstitution of the functional human hematopoietic microenvironment derived from human mesenchymal stem cells in the murine bone marrow compartment. *Blood* 107: 1878–1887, 2006.
 33. Nakada D, Oguro H, Levi BP, Ryan N, Kitano A, Saitoh Y, Takeichi M, Wendt GR, Morrison SJ. Oestrogen increases haematopoietic stem-cell self-renewal in females and during pregnancy. *Nature* 505: 555–558, 2014.
 34. Palermo AT, Labarge MA, Doyonnas R, Pomerantz J, Blau HM. Bone marrow contribution to skeletal muscle: a physiological response to stress. *Dev Biol* 279: 336–344, 2005.
 35. Reddy RL. Mobilization and collection of peripheral blood progenitor cells for transplantation. *Transfus Apher Sci* 32: 63–72, 2005.
 36. Richman CM, Weiner RS, Yankee RA. Increase in circulating stem cells following chemotherapy in man. *Blood* 47: 1031–1039, 1976.
 37. Rutella S, Filippini P, Bertaina V, Li Pira G, Altomare L, Ceccarelli S, Brescia LP, Lucarelli B, Girolami E, Conflitti G, Cefalo MG, Bertaina A, Corsetti T, Moretta L, Locatelli F. Mobilization of healthy donors with plerixafor affects the cellular composition of T-cell receptor (TCR)- $\alpha\beta$ /CD19-depleted haploidentical stem cell grafts. *J Transl Med* 12: 240, 2014.
 38. Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells* 4: 7–25, 1978.
 39. Sharma S, Gurudutta GU, Satija NK, Pati S, Afrin F, Gupta P, Verma YK, Singh VK, Tripathi RP. Stem cell c-KIT and HOXB4 genes: critical roles and mechanisms in self-renewal, proliferation, and differentiation. *Stem Cells Dev* 15: 755–778, 2006.
 40. Suda T, Arai F, Shimmura S. Regulation of stem cells in the niche. *Cornea* 24: S12–S17, 2005.
 41. Tyndall A, Fassas A, Passweg J, Ruiz de Elvira C, Attal M, Brooks P, Black C, Durez P, Finke J, Forman S, Fouillard L, Furst D, Holmes J, Joske D, Jouet J, Kötter I, Locatelli F, Prentice H, Marmont AM, McSweeney P, Musso M, Peter HH, Snowden JA, Sullivan K, Gratwohl A. Autologous haematopoietic stem cell transplants for autoimmune disease—feasibility and transplant-related mortality. Autoimmune Disease and Lymphoma Working Parties of the European Group for Blood and Marrow Transplantation, the European League Against Rheumatism and the International Stem Cell Project for Autoimmune Disease. *Bone Marrow Transplant* 24: 729–734, 1999.
 42. Wolber FM, Leonard E, Michael S, Orschell-Traycoff CM, Yoder MC, Srouf EF. Roles of spleen and liver in development of the murine hematopoietic system. *Exp Hematol* 30: 1010–1019, 2002.
 43. Zhou BO, Yue R, Murphy MM, Peyer JG, Morrison SJ. Leptin-receptor-expressing mesenchymal stromal cells represent the main source of bone formed by adult bone marrow. *Cell Stem Cell* 15: 154–168, 2014.