

Resistance Exercise Increases Endothelial Progenitor Cells and Angiogenic Factors

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ABSTRACT

ROSS, M. D., A. L. WEKESA, J. P. PHELAN, and M. HARRISON. Resistance Exercise Increases Endothelial Progenitor Cells and Angiogenic Factors. *Med. Sci. Sports Exerc.*, Vol. 46, No. 1, pp. 16–23, 2014. **Introduction:** Bone marrow-derived endothelial progenitor cells (EPC) are involved in vascular growth and repair. They increase in the circulation after a single bout of aerobic exercise, potentially related to muscle ischemia. Muscular endurance resistance exercise (MERE) bouts also have the potential to induce muscle ischemia if appropriately structured. **Purpose:** The objective of this study is to determine the influence of a single bout of MERE on circulating EPC and related angiogenic factors. **Methods:** Thirteen trained men age 22.4 ± 0.5 yr (mean \pm SEM) performed a bout of MERE consisting of three sets of six exercises at participants' 15-repetition maximum without resting between repetitions or exercises. The MERE bout duration was 12.1 ± 0.6 min. Blood lactate and HR were 11.9 ± 0.9 mmol·L⁻¹ and 142 ± 5 bpm, respectively, at the end of MERE. Blood was sampled preexercise and at 10 min, 2 h, and 24 h postexercise. **Results:** Circulating EPC and serum concentrations of vascular endothelial growth factors (VEGF-A, VEGF-C, and VEGF-D), granulocyte colony stimulating factor, soluble Tie-2, soluble fms-like tyrosine kinase-1, and matrix metalloproteinases (MMP-1, MMP-2, MMP-3, MMP-9, and MMP-9) were higher ($P < 0.05$) in the postexercise period. Circulating EPC levels were unchanged at 10 min postexercise but higher at 2 h postexercise ($P < 0.05$). The concentration of most angiogenic factors and metalloproteinases were higher at 10 min postexercise (VEGF-A, +38%; VEGF-C, +40%; VEGF-D, +9%; soluble Tie-2, +15%; soluble fms-like tyrosine kinase-1, +24%; MMP-1, +62%; MMP-2, +3%; MMP-3, +54%; and MMP-9, +45%; all $P < 0.05$). Soluble E-selectin was lower ($P < 0.05$) at 2 and 24 h postexercise, with endothelial microparticles and thrombomodulin unchanged. **Conclusions:** Short intense bouts of MERE can trigger increases in circulating EPC and related angiogenic factors, potentially contributing to vascular adaptation and vasculoprotection. **Key Words:** VASCULAR ENDOTHELIAL GROWTH FACTOR, MATRIX METALLOPROTEINASE, MUSCULAR ENDURANCE, MUSCLE ISCHEMIA

Accumulating evidence indicates multiple roles for endothelial progenitor cells (EPC) in endothelial physiology, including neovascularization (17), endothelial repair (12), and restoration of endothelial function (39). Circulating EPC levels are reduced (30) and their functional ability impaired *in vitro* (15) in those with vascular-related diseases. The bone marrow is the principal reservoir for these mononuclear cells, from where they are mobilized into the circulation after chemical or mechanical injury to the

endothelium caused by tissue ischemia or trauma (8). Growth factors and cytokines such as vascular endothelial growth factor (VEGF), stromal cell-derived factor-1, and granulocyte colony stimulating factor (G-CSF) are known to have particularly potent mobilizing effects. Homing of EPC to sites of endothelial injury involves chemoattraction, tethering, and rolling, as occurs with activated leukocytes during an inflammatory response to an injured vessel (37). Circulating EPC subsets can contribute to growth and repair either by incorporating into damaged blood vessels (39) or by adhering to the site of vascular injury and secreting potent angiogenic growth factors (24). Circulating EPC levels are enumerated by flow cytometry on the basis of the expression of a stem/progenitor marker (CD34 or CD133) and an endothelial specific marker (VEGFR-2 or CD144).

Circulating EPC levels increase after intense bouts of aerobic exercise, including maximal exercise tests (1,3,21,25,28,36,40). These exercise-induced increases in EPC are of considerable interest to cardiovascular rehabilitation specialists and

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to exercise physiologists (29). Repeated increases in EPC may contribute to angiogenesis in skeletal muscle and to enhanced endothelial function. Although the exercise factors responsible are not fully understood, this acute effect on circulating EPC has been attributed to ischemia during exercise (1,27). An increase in VEGF has been documented in some study populations but not in others (1,3,21,25,40).

Although normally associated with muscle fiber hypertrophy, resistance exercise can also stimulate vascular adaptations. Resistance exercise training alters vascular structure and function, with increases in muscle capillarization (11), reactive hyperemia (19), and flow-mediated vasodilation (31). Acute bouts of resistance exercise increase angiogenic growth factor expression in muscle and in the circulation (10). Extracellular matrix (ECM) remodeling is an essential element in angiogenesis (5), and activation of the proteolytic matrix metalloproteinase (MMP) family of enzymes is critical for these angiadaptive processes. MMP expression is increased in muscle after leg endurance exercise (26), and circulating MMP increases acutely after resistance exercise (34). MMP enzymes appear to be involved in several angiogenic processes, including the liberation of VEGF and other angiogenic growth factors stored in the muscle ECM (32), the reactivation of VEGF angiogenic activity (13), and VEGF-mediated EPC mobilization from the bone marrow (14).

The stresses experienced by the vascular system in response to resistance exercise are likely to vary depending on the forces generated, number of repetitions completed, and recovery period allowed (19). Indeed, suitably structured resistance workouts that emphasize light to moderate resistance, high repetitions, and short recoveries as part of a muscular endurance workout may result in a greater ischemic stimulus in a wider range of muscle groups than that occurring during aerobic exercise. To our knowledge, no study has examined the effect of any resistance exercise protocol on EPC. The purpose of this study was to determine the influence of a single bout of endurance-type resistance exercise on circulating EPC in young trained men. A range of angiogenic factors, endothelial markers, and MMP were also measured. Angiogenic growth factors were measured as EPC mobilization factors. Endothelial markers were measured to indicate endothelial activation necessary for rolling and tethering of circulating EPC. MMP enzymes were measured not only as a potential indicator of ECM remodeling in exercised muscle but also because of their association with VEGF release and action. It was hypothesized that the resistance exercise bout would cause a significant increase in circulating EPC in the 24-h postexercise period attributable to increases in circulating mobilizing factors.

METHODS

Participants. Thirteen trained men (age, 22.4 ± 0.5 yr (mean \pm SEM); height, 178.2 ± 2.0 cm; weight, 82.4 ± 3.1 kg; percent body fat, $15.3\% \pm 1.4\%$) participated in a muscular endurance resistance exercise (MERE) protocol. To be eli-

gible for the study, participants had to be engaging in three exercise bouts weekly over the previous 3 yr, including resistance training. This was verified by a questionnaire. No exercise was undertaken during the 72 h before testing. The study was approved by the Waterford Institute of Technology Research Ethics Committee, and written informed consent was obtained from each volunteer.

Experimental protocol. The MERE bout consisted of three circuits of 15 repetitions of six exercises (leg press, seated chest press, leg curl, lat pulldown, knee extension, and tricep pushdown) performed at the 15-repetition maximum. All exercises were performed on machine resistance apparatus to eliminate technique issues. The 15-repetition maximum for each exercise was determined for each volunteer during a preliminary visit. Body fat was also determined during this preliminary visit by dual x-ray absorptiometry (Norland XR-46; Norland Corp., Fort Atkinson, WI). Volunteers were instructed to complete the exercises at a steady tempo but without pausing between repetitions to ensure a muscular fatiguing exercise stimulus. The exercises in the circuit were organized so that arm exercises always followed leg exercises, with no rest permitted between exercises. Approximately 1-min rest was necessary between circuits to facilitate blood lactate (BLa) sampling. BLa and HR were monitored during the exercise bout, BLa from a fingertip capillary blood sample (Lactate Pro; H/P/Cosmos, Nussdorf-Traunstein, Germany) and HR using a chest HR monitor (Polar, Finland). The MERE bouts were undertaken in the morning in the fasted state. Blood samples were taken pre-exercise and at 10 min, 2 h, and 24 h postexercise for determination of EPC and a range of angiogenic, proteolytic, and endothelial factors. Participants recorded their diet on the day before the MERE bout and repeated this after MERE in the lead-up to the 24-h postexercise blood sample to minimize the influence of diet on cellular and serum factors.

Blood processing. Peripheral blood samples were obtained from a prominent forearm vein by venepuncture using a 21-gauge needle with minimal tourniquet use. Participants were in a supine position. The first 3 mL was discarded to minimize the contamination of the samples with endothelial cells. Samples were collected into serum, ethylenediaminetetraacetic acid plasma, and sodium citrate plasma tubes. Serum tubes were allowed to stand at room temperature for 30 min followed by centrifugation for 15 min at 1500g. Sodium citrate tubes, for microparticle analysis, were processed using a double centrifugation protocol at room temperature, initially at 1500g for 15 min followed by 13,000g for 2 min. Serum and plasma were then divided into aliquots of approximately 350 μ L and frozen at -80°C until analysis.

Blood cell analysis. Circulating EPC counts were determined from ethylenediaminetetraacetic acid whole blood. Briefly, 200 μ L of blood was incubated with Fc receptor blocker (Beckman Coulter, Fullerton, CA) for 15 min in the dark. The sample was then incubated with 10- μ L CD34-PECy7 (Beckman Coulter), 12- μ L VEGF receptor-2

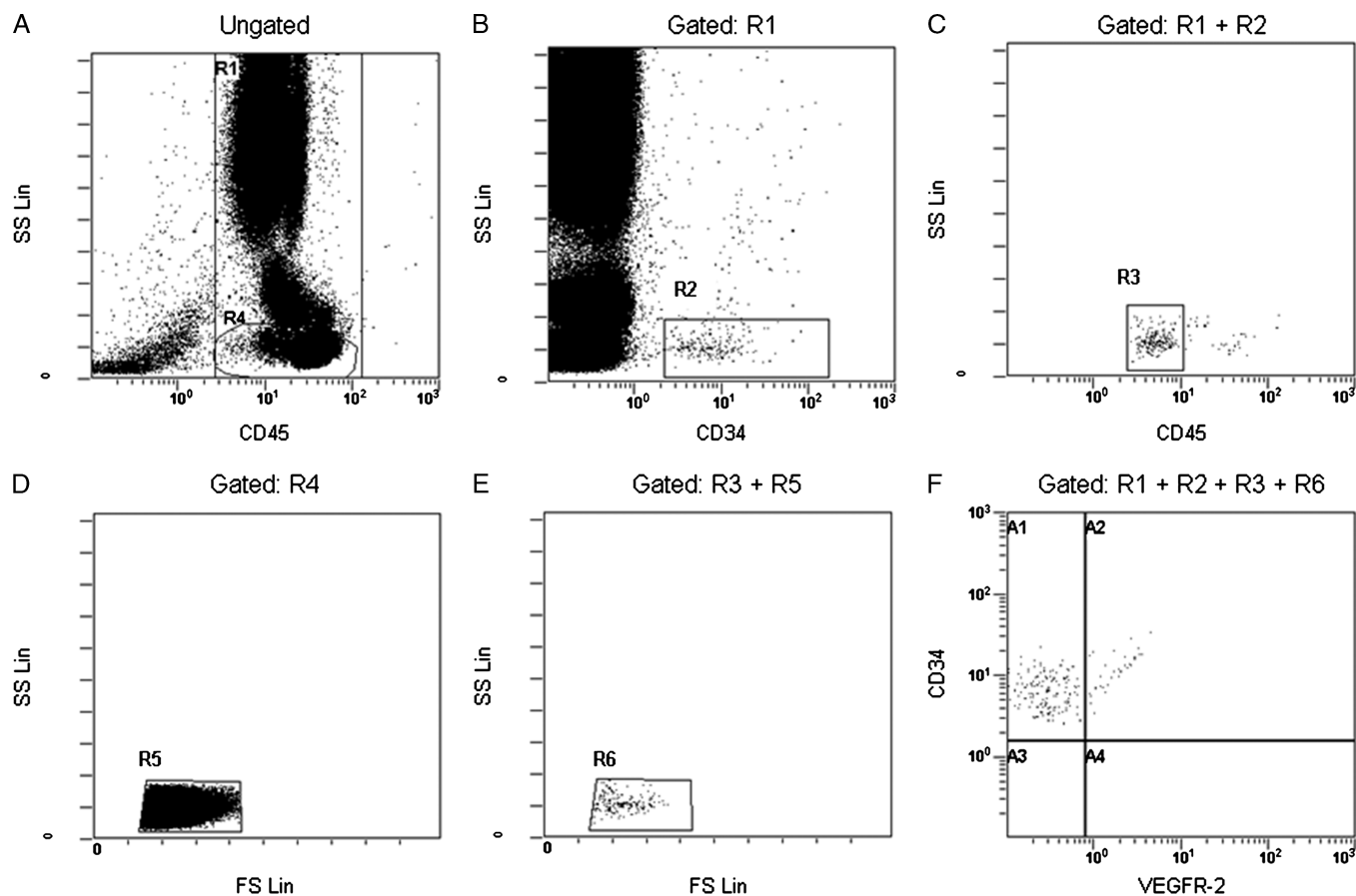


FIGURE 1—Flow cytometry quantification of EPC. A, CD45 vs SS to eliminate all red blood cells, platelets, and debris. B, CD34 vs SS to identify cluster of CD34 events. C, CD45 vs SS to distinguish CD34 events that are CD45^{dim} and CD45^{bright}. D, Forward scatter (FS) vs SS gated on R4 in plot A to identify lymphocyte size gate. E, FS vs SS to identify CD34⁺CD45^{dim} events with same FS/SS profile as lymphocytes (i.e., circulating progenitors). F, VEGFR-2 vs CD34 to identify endothelial progenitors.

(VEGFR-2)-PE (R&D Systems, Minneapolis, MN), and 10- μ L CD45-FITC (Beckman Coulter) for 30 min in the dark. Subsequently, 2 mL of Pharm LyseTM (BD Biosciences, Oxford, UK) was added to lyse erythrocytes before flow cytometric analysis. The sample was run through the flow cytometer (FC500, Beckman Coulter) for 30 min with a minimum of 500,000 CD45⁺ events collected.

Circulating progenitor cells and EPC were identified using the International Society for Hematotherapy and Graft protocol for progenitor cells (33), with the addition of the VEGFR-2 marker to identify progenitors of endothelial origin (Fig. 1). Thus, circulating progenitor cells were defined as CD34⁺CD45^{dim} and endothelial progenitors as CD34⁺VEGFR2⁺CD45^{dim}. The International Society for Hematotherapy and Graft protocol does not require isotype control samples to identify CD34⁺CD45^{dim} events because there is clear separation between positive and negative regions. Isotype control samples (IgG1-PE, R&D Systems) were used to distinguish VEGFR-2 positive and negative regions. Leukocyte counts were determined using a hematology analyzer (Ac-T diff2, Beckman Coulter) and checked daily with appropriate cell controls (4C-ES Cell Control, Beckman Coulter). Circulating progenitor and EPC events were converted to cells per milliliter using the flow cytometry CD45⁺

count in conjunction with the hematology analyzer-determined leukocyte count (Ac-T diff2, Beckman Coulter).

Endothelial microparticle analysis and immunoassays. Endothelial microparticles (EMP) were determined from washed sodium citrate plasma. Briefly, 500- μ L aliquots were washed twice in phosphate-buffered saline (PBS)-citrate at 19,000g for 30 min, after which, the pellet was resuspended in PBS-citrate and vortexed vigorously. Because of their low numbers in circulation, EMP was enumerated using a monochrome multimarker approach (9) that uses three endothelial-specific antibodies. Antibodies were diluted in PBS and washed at 19,000g for 5 min to remove aggregates. The concentrations of CD144-PE, CD146-PE, and CD105-PE (BD Biosciences) in this antibody dilution were 0.53, 0.35, and 1.16 μ g \cdot mL⁻¹, respectively, with 10 μ L of the dilution added to each sample. A microparticle size gate <1.0 μ m but above the noise of the instrument was established on forward versus side scatter (SS) using sizing beads (Megamix; Biocytex, France). Events within this gate and with phycoerythrin fluorescence greater than that of negative control samples were regarded as EMP. Negative control samples were prepared by staining with a concentration-matched isotype antibody (IgG1-PE, BD Biosciences). Events were converted

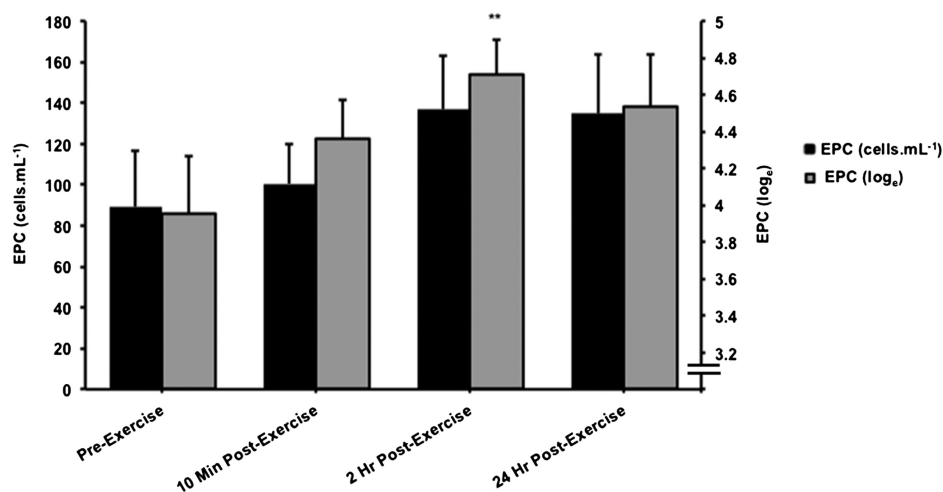


FIGURE 2—Influence of an acute bout of MERE on circulating EPC ($N = 13$). Values are mean \pm SEM. Statistical analysis performed on natural log-transformed data, which are presented in conjunction with untransformed data. ** $P < 0.01$ compared with preexercise.

to events per microliter using flow count beads (Spherotech, Lake Forest, IL). Concentrations of angiogenic factors (VEGF-A, VEGF-C, VEGF-D, soluble Tie-2 (sTie-2), soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PIGF), and basic fibroblast growth factor (bFGF)), MMP (MMP-1, MMP-2, MMP-3, MMP-9, and MMP-10), and endothelial markers (sE-selectin, sP-selectin, and thrombomodulin) were determined at each time point from serum samples using multiplex immunoassay technology with electrochemiluminescence detection (Mesoscale Discovery, Gaithersburg, MD). The coefficient of variation for all immunoassays was between 2.3% and 5.7%.

Data analysis. The significance of changes over the four time points was determined using a one-way repeated-measures ANOVA, with the Greenhouse–Geisser correction applied for violations of sphericity. Significant F -ratios were followed up by the least significant difference *post hoc* procedure. Data that were not normally distributed were

natural log-transformed before statistical analysis. Associations between variables were determined using Pearson correlations. Significance was set at $P < 0.05$. Values are presented as mean \pm SEM.

RESULTS

The MERE bout was 12.1 ± 0.6 min in duration. The BLA (1.5 ± 0.2 vs 11.9 ± 0.9 mmol·L⁻¹) and HR (60 ± 3 vs 142 ± 5 bpm) increased significantly ($P < 0.05$) from preexercise levels as a result of the MERE bout.

EPC counts increased postexercise (Fig. 2), with EPC (log_e) highest at 2 h postexercise ($P < 0.05$). Circulating EPC (log_e) were also elevated at 24 h postexercise, although these 24-h values were not significantly different to preexercise ($P = 0.13$). Circulating leukocytes were higher ($P < 0.05$) at 10 min and 2 h postexercise but had reverted back to preexercise values by 24 h (Fig. 3). Circulating

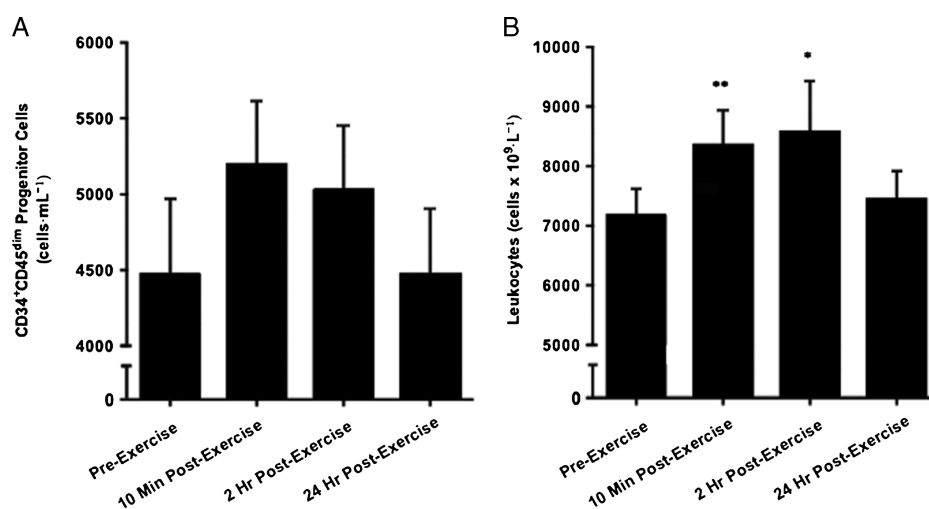


FIGURE 3—Influence of an acute bout of MERE on circulating (A) progenitor cells and (B) leukocytes ($N = 13$). Values are mean \pm SEM. * $P < 0.05$, ** $P < 0.01$ compared with preexercise.

TABLE 1. Influence of an acute bout of MERE on angiogenic factors, MMP, and markers of endothelial activation ($N = 13$).

	Preexercise	10 min Postexercise	2 h Postexercise	24 h Postexercise
VEGF-A (pg·mL ⁻¹)	289 ± 70	398 ± 87*	316 ± 79	291 ± 78
VEGF-C (pg·mL ⁻¹)	215 ± 22	301 ± 30*	197 ± 15	190 ± 20
VEGF-D (pg·mL ⁻¹)	290 ± 37	317 ± 38**	315 ± 40**	294 ± 38
G-CSF (pg·mL ⁻¹)	2.7 ± 0.3	2.6 ± 0.3	3.5 ± 0.4**	2.3 ± 0.3*
bFGF (pg·mL ⁻¹)	0.87 ± 0.23	0.69 ± 0.10	0.68 ± 0.14	0.72 ± 0.15
PIGF (pg·mL ⁻¹)	7.5 ± 0.4	7.5 ± 0.4	6.5 ± 0.3**	7.9 ± 0.3
sTie-2 (pg·mL ⁻¹)	2497 ± 298	2866 ± 369**	2630 ± 330	2510 ± 288
sFlt-1 (pg·mL ⁻¹)	58 ± 4	72 ± 7*	76 ± 8**	53 ± 3
MMP-1 (pg·mL ⁻¹)	684 ± 109	1110 ± 246*	704 ± 146	586 ± 75
MMP-2 (pg·mL ⁻¹)	80,350 ± 2338	82,632 ± 2034**	83,360 ± 2270*	80,882 ± 2450
MMP-3 (pg·mL ⁻¹)	1155 ± 148	1777 ± 241**	1438 ± 218**	1037 ± 188
MMP-9 (pg·mL ⁻¹)	6293 ± 646	9140 ± 961**	9066 ± 867**	6539 ± 704
MMP-10 ^a (pg·mL ⁻¹)	1393 ± 117	1412 ± 126	1493 ± 131*	1488 ± 132
EMP (counts·μL ⁻¹)	2.3 ± 0.6	2.5 ± 0.4	1.9 ± 0.4	2.3 ± 0.6
sE-selectin (ng·mL ⁻¹)	22.6 ± 2.0	22.1 ± 2.1	21.8 ± 2.1*	19.9 ± 1.7**
Thrombomodulin (ng·mL ⁻¹)	1.84 ± 0.10	1.98 ± 0.12	1.88 ± 0.10	1.82 ± 0.10
sP-selectin (ng·mL ⁻¹)	58.2 ± 4.2	70.9 ± 9.0*	58.8 ± 4.9	53.4 ± 5.0

Values shown are mean ± SEM.

* $P < 0.05$ denotes significantly different from preexercise values.

** $P < 0.01$ denotes significantly different from preexercise values.

^aData natural log transformed before statistical analysis.

progenitor cells were not significantly influenced by exercise ($F = 2.58$, $P = 0.07$ on log_e data), but the trend across time was similar to that of leukocytes (Fig. 3).

Several growth factors (proangiogenic) and soluble growth factor receptors (antiangiogenic) increased after MERE (Table 1). VEGF-A, VEGF-C, VEGF-D, Tie-2, and sFlt-1 were higher ($P < 0.05$) at 10 min postexercise, with VEGF-D and sFlt-1 still higher ($P < 0.05$) at 2 h postexercise. G-CSF was elevated at 2 h postexercise only. PIGF was lower at 2 h postexercise. All had reverted back to preexercise levels at the 24-h time point. bFGF did not change in the post-exercise period.

Several MMP were also elevated after MERE (Table 1). MMP-1, MMP-2, MMP-3, and MMP-9 were higher ($P < 0.05$) at 10 min postexercise, with MMP-2, MMP-3, and MMP-9 still higher ($P < 0.05$) at 2 h postexercise. MMP-10 (log_e) was higher ($P < 0.05$) at 2 h postexercise only. All had reverted back to preexercise levels at the 24-h time point.

Markers of endothelial activation were also measured (Table 1). Soluble E-selectin decreased in the postexercise period, with values significantly lower ($P < 0.05$) at 2 and 24 h postexercise. Soluble P-selectin was higher at 10 min postexercise ($P < 0.05$). EMP and thrombomodulin did not change in the postexercise period.

The preexercise to 2-h postexercise (peak value) change in EPC was not associated with the change in any angiogenic factor or MMP. The preexercise to 10-min post-exercise changes in VEGF-A and VEGF-C were correlated ($r = 0.80$, $P < 0.05$). There were associations between the preexercise to 10-min postexercise changes in MMP-1 and VEGF-A ($r = 0.61$, $P < 0.05$), MMP-1 and VEGF-C ($r = 0.80$, $P < 0.05$), MMP-1 and sFlt-1 ($r = 0.69$, $P < 0.05$), and MMP-9 and VEGF-C ($r = 0.62$, $P < 0.05$). There were also preexercise to 10-min postexercise associations between the changes in MMP-3 and MMP-9 ($r = 0.66$, $P < 0.05$) and preexercise to 2-h postexercise associations between the

changes in MMP-2 and MMP-3 ($r = 0.78$, $P < 0.05$) and MMP-3 and MMP-10 ($r = 0.81$, $P < 0.05$).

DISCUSSION

This study has shown that a bout of MERE can increase circulating EPC in trained young men. It can also activate angiogenic pathways and the MMP system, potentially relevant to EPC mobilization and vascular growth. Light to moderate weights were used with little recovery between repetitions or exercises to maximize the endurance stimulus, triggering high concentrations of BLA and an increase in VEGF. These elements of the resistance exercise bout may be relevant to the EPC response.

To our knowledge, this is the first study to demonstrate an increase in EPC from a bout of resistance exercise. Circulating EPC levels were not significantly elevated at 10 min postexercise but was increased at the 2-h time point. Results at 24 h postexercise are equivocal. Although mean EPC counts were similar at 2 and 24 h postexercise, results did not reach statistical significance, possibly because of greater interindividual variation after the 2 h time point. Circulating leukocytes were higher at 10 min and 2 h postexercise but had reverted back to preexercise levels by 24 h postexercise. Circulating progenitor cells (CD34⁺CD45^{dim}) followed a similar trend, although changes did not reach statistical significance. Because the changes in EPC followed a different trend, the mechanisms are likely to be different. Whereas the postexercise increase in leukocytes is generally attributed to the demargination of previously released pools, the increase in EPC more likely reflects increased mobilization from the bone marrow and differentiation along an endothelial lineage.

Not all studies of aerobic exercise examine the time course for the changes in EPC. Single time point studies generally demonstrate an increase in circulating EPC in the

immediate postexercise period (3,25,36). Other studies report an increase immediately after exercise with counts back to baseline at 6 h (21), an increase immediately after exercise with values back to baseline at 12 h but rebounding upward again at 24 h (35), and also a delayed response peaking at 24 h postexercise (1,28). In the present study involving resistance exercise, the increase in EPC was delayed, evident at 2 h postexercise but potentially longer. Future work should address limitations in the present study by tracking changes in EPC back to preexercise values and with a sample size sufficient to detect significant differences at later time points when divergent responses become evident.

Several proangiogenic growth factors were elevated postexercise: VEGF-A and VEGF-C at 10 min only, G-CSF at 2 h only, and VEGF-D at the 10-min and 2-h time points. PIGF was lower at 2 h postexercise. Both VEGF-A and PIGF (a member of the VEGF family) can not only mobilize EPC from the bone marrow (14) but also have independent effects on neovascularization (22). VEGF-C and VEGF-D are more usually associated with lymphangiogenesis, with evidence that VEGF-C can stimulate bone marrow stromal cells to differentiate into lymphatic endothelial cells (7). Soluble Flt-1 was elevated at 10 min and 2 h postexercise and sTie-2 at 10 min only. These soluble growth factor receptors are considered to have antiangiogenic properties, binding with proangiogenic growth factors and therefore reducing their bioavailability for cell surface receptors. One possibility is that the net balance of pro- and antiangiogenic factors regulates these angiogenic processes. However, other functional roles may exist particularly for sFlt-1, whose release from endothelial cells has been proposed as ensuring a more productive and regulated vessel branching (18).

The importance of an ischemic stimulus for increases in EPC is emphasized in some articles (1,27), although not ruling out the possibility of a contribution from other exercise factors. Whereas increases in circulating EPC have been documented in conjunction with (1,28,36) and in the absence of (1,3,21,25,40) increases in VEGF, to our knowledge, no study has reported an increase in VEGF in the absence of a change in EPC. The trained status of our volunteers may have increased the VEGF response in this study (32). In some studies involving clinical groups, increases in circulating EPC and VEGF were only evident in coronary artery and peripheral artery disease groups with ischemia and absent in similar patient groups without ischemia (1,27). There is also evidence in young healthy individuals of the importance of anaerobic metabolism and muscle ischemia for increases in VEGF. In one exercise study, VEGF increased after four anaerobic sprints but not after 1 h of moderate-intensity exercise (38). In another nonexercise study, 15 min of limb occlusion triggered an increase in VEGF and EPC (28). In the present study, an increase in VEGF-A, VEGF-C, and VEGF-D was evident at 10 min postexercise, which preceded the increase in EPC. An early rise in circulating VEGF followed by a later rise in EPC after exercise has been observed previously (1,23,28). Indeed, it

took 24 h for a rise in circulating EPC to be observed after initial VEGF injection in mice (2). A time delay may therefore exist between the stimulated release of VEGF into the bloodstream and the liberation of EPC from the bone marrow. The MERE bout was structured to ensure considerable local muscular fatigue, and the high BLA values suggest that it was successful in this regard. Indeed, it is possible that such exercise protocols may be more effective than aerobic exercise in triggering muscle ischemia and increases in VEGF.

Resistance exercise activated the MMP family of enzymes. MMP-2, MMP-3, and MMP-9 were all elevated at 10 min and 2 h postexercise. MMP-1 was elevated at 10 min only, with MMP-10 elevated at the 2-h time point only. The increases in MMP-1, MMP-3, and MMP-9 were of considerable magnitude; the increases in MMP-2 and MMP-10 were small, although still significant. Some of these pre- to postexercise changes were highly correlated, indicating similar intraindividual MMP family activation. Little comparative data exist for resistance exercise and MMP. One previous study (34) has demonstrated an increase in plasma MMP-1 and MMP-3, with little effect on MMP-2 and MMP-9. The absence of muscle-specific assessments represents a limitation of this study, however. In the absence of muscle mRNA, protein content, interstitial dialysate, or arteriovenous difference assessments, the extent to which postexercise MMP increases derived from the muscle ECM is uncertain. Peritendinous tissue (20) and circulating leukocytes (4) may be additional sources of plasma MMP after acute exercise. Regardless of the MMP source, the associations of MMP and VEGF are intriguing and deserve further attention because various MMP may reactivate VEGF activity (13) and VEGF-A attracts MMP-9 delivering neutrophils to ischemic tissue (6). In addition, MMP-9 has been identified as having novel roles in VEGF-mediated EPC mobilization and migration and in EPC colony and tube formation (16).

These changes in circulating EPC, angiogenic markers, and MMP appear to form part of a coordinated response to acute exercise. MMP performs multiple roles in this response. Different MMP enzymes are responsible for degradation of the endothelial cell basal membrane and specific components of the ECM to create a pathway for capillary sprouts during angiogenesis (5). These actions of MMP also trigger the release of growth factors, including those stored in the ECM in inactive form. Early postexercise increases in VEGF and other angiogenic factors, as observed in the current study, likely resulted from these ECM stores, preceding any increase in transcription and translation (32). The correlations observed in this study between the postexercise changes in MMP and angiogenic factors, although not demonstrating causality, are consistent with this concept. VEGF-A and G-CSF, which both increased considerably postexercise in this study, are potent triggers for EPC mobilization, VEGF-A by triggering the release of MMP-9 in bone marrow and G-CSF by triggering the release of

elastase from neutrophils (37). The increase in circulating MMP-9 may supplement the action of VEGF on bone marrow stromal cells. There was no correlation, however, between the pre- to postexercise change in EPC and any angiogenic factor, making it difficult to attach greater importance to any single mobilizing factor. The time course of these changes is also interesting, with early postexercise increases in MMP and angiogenic growth factors triggering a delayed increase in EPC. After EPC mobilization, the next critical steps involve cell homing to sites of injury and growth, followed by rolling, binding, and tethering to the vascular endothelium, mediated by integrins and selectins including E-selectin. VEGF, among other growth factors, and cytokines can act as chemoattractants for EPC homing to sites of need (37). There is little evidence, however, in the current study for an increase in endothelial activation. Soluble E-selectin and EMP did not increase postexercise. Although soluble P-selectin was increased, we cannot say with any certainty that the endothelium was the source of this molecule because it is also expressed on platelets.

The results of this study have shown that a short bout of resistance exercise structured to maximize muscle fatigue is an effective means of increasing circulating EPC. Resistance exercise can also activate the MMP system and increase angiogenic factors. Increases in circulating EPC have only been documented previously after aerobic exercise. The stimulus for triggering each event in the cascade is not

clear. The “hybrid” type exercise bout is likely to have involved multiple stimuli, including muscle overload, muscle ischemia and acidosis, mechanical deformation of the ECM, and increased vascular shear stress and cyclic strain. It is unclear if resistance protocols involving heavier lifting with longer recoveries will trigger the same responses. However, regardless of mechanisms, the findings highlight the potential value of resistance exercise for vascular adaptation. The findings also highlight the responses that can accrue from bouts of appropriately structured exercise that are of shorter duration than that recommended in the literature. Future studies should consider the effect of exercise training using this mode of exercise on circulating EPC and related pathways.

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M. D. Ross and M. Harrison conceived the study. M. D. Ross, A. L. Wekesa, and J. P. Phelan were involved in data collection and blood sample analysis. M. D. Ross and M. Harrison performed the statistical analysis. M. Harrison and M. D. Ross wrote the manuscript, with A. L. Wekesa and J. P. Phelan contributing as reviewers and revisers. All authors approved the final manuscript.

None of the authors has declared a conflict of interest.

None of the work presented in this article was undertaken while Dr. Mark Ross was at Edinburgh Napier University.

The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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