Monocyte Recruitment after High-Intensity and High-Volume Resistance Exercise

ADAM J. WELLS1, JAY R. HOFFMAN2, ADAM R. JAJTNER2, ALYSSA N. VARANOSKE2, DAVID D. CHURCH2, ADAM M. GONZALEZ3, JEREMY R. TOWNSEND2, CARLEIGH H. BOONE2, KAYLA M. BAKER2, KYLE S. BEYER2, GERALD T. MANGINE4, LEONARDO P. OLIVEIRA2, DAVID H. FUKUDA2, and JEFFREY R. STOUT2

1School of Health and Kinesiology, Georgia Southern University, Statesboro, GA; 2Institute of Exercise Physiology and Wellness; University of Central Florida, Orlando, FL; 3Department of Health Professions, Hofstra University, Hempstead, NY; and 4Department of Exercise Science and Sport Management, Kennesaw State University, Kennesaw, GA

ABSTRACT

WELLS, A. J., J. R. HOFFMAN, A. R. JAJTNER, A. N. VARANOSKE, D. D. CHURCH, A. M. GONZALEZ, J. R. TOWNSEND, C. H. BOONE, K. M. BAKER, K. S. BEYER, G. T. MANGINE, L. P. OLIVEIRA, D. H. FUKUDA, and J. R. STOUT. Monocyte Recruitment after High-Intensity and High-Volume Resistance Exercise. Med. Sci. Sports Exerc., Vol. 48, No. 6, pp. 1169–1178, 2016. The innate immune response is generally considered to have an important role in tissue remodeling after resistance exercise. Purpose: The purpose of this study was to compare changes in markers of monocyte recruitment after an acute bout of high-intensity (HVY) versus high-volume (VOL) lower-body resistance exercise. Methods: Ten resistance-trained men (24.7 ± 3.4 yr, 90.1 ± 11.3 kg, 176.0 ± 4.9 cm) performed each protocol in a randomized, counterbalanced order. Blood samples were collected at baseline, immediately (IP), 30 min (30P), 1 h (1H), 2 h (2H), and 5 h (5H) postexercise. Plasma concentrations of monocyte chemoattractant protein 1 (MCP-1), tumor necrosis factor alpha (TNF-α), myoglobin, and cortisol were measured via assay. Tumor necrosis factor receptor 1 (TNFr1), macrophage-1 antigen (cluster of differentiation 11b [CD11b]), and C-C chemokine receptor 2 (CCR2) expression levels were measured using flow cytometry. TNFr1 and CD11b were assessed on CD14++CD16<sup>+</sup> monocytes, whereas CCR2 was assessed on CD14<sup>+</sup> monocytes. Results: Plasma myoglobin concentrations were significantly greater after HVY compared with VOL (P < 0.001). Changes in plasma TNF-α, MCP-1, and expression levels of CCR2 and CD11b were similar between HVY and VOL. When collapsed across groups, TNF-α was significantly increased at IP, 30P, 1H, and 2H (P < 0.05), whereas MCP-1 was significantly elevated at all postexercise time points (P values < 0.05). CCR2 expression on CD14<sup>+</sup> monocytes was significantly lower at IP, 1H, 2H, and 5H (P values < 0.05). CD11b expression on CD14<sup>+</sup>CD16<sup>−</sup> monocytes was significantly greater at IP (P < 0.014) and 1H (P = 0.009). TNFr1 expression did not differ from baseline at any time point. Plasma cortisol concentrations did not seem to be related to receptor expression. Conclusions: Results indicate that both HVY and VOL protocols stimulate a robust proinflammatory response. However, no differences were noted between resistance exercise training paradigms. Key Words: INFLAMMATION, MONOCYTE CHEMOATTRACTANT PROTEIN 1 (MCP-1), TUMOR NECROSIS FACTOR ALPHA (TNF-α), C-C CHEMOKINE RECEPTOR 2 (CCR2), MACROPHAGE-1 ANTIGEN (CD11b/MAC-1)

Skeletal muscle demonstrates a profound capacity to adapt to an array of physiological demands. Episodic bouts of muscle contraction are potent stimuli in this regard, which under optimal conditions lead to the remodeling and functional adaptation of skeletal muscle in response to mechanical stress (11). This stimulus is primarily modulated through changes in acute program variables such as exercise intensity, volume, and rest intervals, which evoke distinct mechanical and biochemical responses that subsequently result in differing phenotypical characteristics. Recent evidence suggests that exercise-induced myotrauma may not be necessary for muscle remodeling to occur (12). Nevertheless, acute myotrauma seems to play a role in the activation and proliferation of myogenic precursor cells (25), which has recently been associated with training induced muscle growth (2).

Previous research suggests that myogenic precursor cells depend on the support of stromal cells such as macrophages to develop their myogenic properties (8). Inflammatory macrophages are derived from classical monocytes, which are recruited to the site of muscle damage from the circulation. Upon entry into the tissue, cytokines and chemokines produced by local innate immune cells drive monocyte differentiation toward the proinflammatory (M1) macrophage phenotype. The abrogation of receptors involved in classical
monocyte trafficking, such as C-C chemokine receptor 2 (CCR2) and/or endothelial adhesion receptor cluster of differentiation 11b (CD11b), have been shown to result in an attenuated macrophage accumulation and the subsequent prevention of muscle regeneration (1). Consequently, the successful infiltration of monocytes into damaged tissue seems to be paramount for optimal tissue remodeling.

Classical monocytes are reported to possess gene enrichment in areas that make them particularly receptive to environmental stimuli. These stimuli include bacterial components, toxins, drugs, hypoxia, nutrient levels, and hormones (42). Resistance exercise alone is a potent stimulus for acute elevations in the concentrations of circulating hormones. Perturbations to the systemic hormone profile may subsequently dictate the timing and magnitude of the inflammatory response, which in turn may lead to altered tissue adaptation. Glucocorticoids in particular have been implicated with innate response, which in turn may lead to altered tissue adaptation. Glucocorticoids in particular have been implicated with innate immune regulation, potentiating an upregulation in the expression of chemotactic receptor CCR2 on human monocytes in vitro (28). High-volume resistance exercise protocols may stimulate a differential pattern of receptor expression because these protocols are often reported to result in greater circulating cortisol concentrations compared with low-volume resistance exercise. Nevertheless, a comparison of monocyte cell surface protein expression after different resistance training protocols, to our knowledge, has not yet been conducted.

The presence of several other ligands within the circulation may also regulate the expression of specific cell surface receptors. For example, tumor necrosis factor alpha (TNF-α) (36) and monocyte chemoattractant protein 1 (MCP-1) (37) have been shown to modulate CD11b receptor expression on monocytes. However, the effect of different resistance exercise protocols on circulating MCP-1 and TNF-α levels is not known. It remains unclear whether the innate proinflammatory immune response varies with different resistance training protocols and how it may impact recovery and the muscle remodeling process. Therefore, the purpose of this investigation was to examine temporal changes in the expression of chemotactic and adhesion receptors on classical monocytes after an acute bout of high-volume, moderate-intensity (VOL) versus high-intensity, low-volume (HVY) lower-body resistance exercise in resistance-trained men. In particular, the expression levels of tumor necrosis factor receptor 1 (TNFRI), C-C chemokine 2 (CCR2), and CD11b were assessed. Changes in receptor expression were assessed in conjunction with changes to plasma concentrations of TNF-α, MCP-1, and cortisol.

METHODS

Participants. Ten resistance-trained men (24.7 ± 3.4 yr, 90.1 ± 11.3 kg, 176.0 ± 4.9 cm, 14.1% ± 6.1% body fat) were recruited to participate in this randomized crossover design research study. Using the procedures described by Gravettier and Wallhuin (15) for estimating sample sizes for repeated-measures designs, a sample size of n = 10 produced a statistical power (1 − β) of 0.8, based on changes in CD11b receptor expression reported by Jajtner et al. (21). Strict recruitment criteria were implemented to increase homogeneity of the sample. Inclusion criteria required participants to be between the ages of 18 and 35 yr, with a minimum of 1 yr of resistance training experience, and to have the ability to squat a weight equivalent to their body mass (confirmed during one-repetition maximum [1RM] testing). Participants had 6.7 ± 4.6 yr of resistance training experience with an average maximum barbell back squat of 172.7 ± 25.2 kg. All participants were free of any physical limitations that may have affected performance. In addition, all participants were free of any prescription or over-the-counter medications, performance-enhancing drugs, and/or ergogenic aids, including the use of creatine, beta-alanine, or any herbal/vitamin supplement, as determined by a health and activity questionnaire. After an explanation of all procedures, risks, and benefits, each participant provided his written informed consent before participation in this study. The research protocol and the informed consent document were approved by the New England Institutional Review Board before participant enrollment.

Maximal strength testing. Before experimental trials, participants reported to the Human Performance Laboratory (HPL) to establish the maximal strength (1RM) on all lifts involved in the exercise protocol. Participants performed a standardized warm-up consisting of 5 min on a cycle ergometer against a light resistance, 10 body weight squats, 10 body weight walking lunges, 10 dynamic walking hamstring stretches, and 10 dynamic walking quadriceps stretches. After the warm-up, 1RM testing for the barbell back squat and leg press exercises were performed. Briefly, each participant performed two warm-up sets using a resistance of approximately 40%–60% and 60%–80% of his perceived maximum, respectively. For each exercise, three to four subsequent trials were performed to determine the 1RM. A 3- to 5-min rest period was provided between each trial. Maximum strength testing for the back squat and leg press was administered by the same certified strength and conditioning specialist to ensure that each participant reached the parallel position for each repetition of the squat and that the exercise technique was consistent between sessions. For the leg press, hamstring curl, and calf raise, 1RM was predicted using the Brzycki equation (6). Trials were discarded if the range of motion criteria for each exercise were not met, the repetitions performed for predicted 1RM were greater than 10, or the proper technique was not used, as determined by the certified strength and conditioning specialist.

Experimental trials. On the morning of each trial, participants reported to the HPL after a 10-h overnight fast and having refrained from all forms of moderate to vigorous exercise for the previous 72 h. Experimental trials were performed in a randomized counterbalanced order, and each experimental trial was separated by a minimum of 1 wk to ensure adequate recovery. During each experimental trial, participants performed the standardized warm-up routine as
described previously, followed by a lower-body resistance exercise protocol. The VOL protocol required participants to perform 10–12 repetitions with a load equating to 70% of their 1RM and a 1-min rest period between each set and exercise. The HVY protocol required participants to perform three to five repetitions with a load equating to 90% of their 1RM and a 3-min rest period between each set and exercise. Both protocols included six sets of barbell back squats and four sets of bilateral leg press, bilateral hamstring curls, bilateral leg extensions, and seated calf raises. Pilot testing revealed that exercise time for the HVY protocol was approximately 20 min greater when compared with the VOL protocol. Consequently, a 20-min time delay was incorporated between the baseline (BL) blood draws and the onset of the exercise during the VOL protocol. This was conducted to ensure that each participant completed each protocol at approximately the same time of day to control for diurnal variations. During each protocol, participants were verbally encouraged to complete all repetitions for each set. If the participant was unable to complete the desired number of repetitions, spotters provided assistance until the participant completed the remaining repetitions. Subsequently, the load for the next set was adjusted so that participants were able to perform the specific number of repetitions for each set. After each resistance exercise protocol, participants remained in the laboratory for all postexercise assessments. Blood samples were collected at six time points during each experimental condition: BL, immediately postexercise (IP), 30 min postexercise (30P), 1 h postexercise (1H), 2 h postexercise (2H), and 5 h postexercise (5H). To control for diet, participants were provided a standardized low protein, low carbohydrate breakfast (7 g protein, 3 g carbohydrate, and 13 g fat) after BL assessments. Immediately after IP blood sampling, participants were also provided a flavored drink (355 mL; 0 g protein, 2.5 g carbohydrates, 0 g fat). Participants were permitted to drink water ad libitum during the experimental trials and postexercise period, and water consumption was monitored.

**Dietary logs.** Participants were instructed to maintain their normal dietary intake leading up to experimental trials. Participants were then instructed to record as accurately as possible everything they consumed 24 h before the first experimental trial. For the next experimental trial, participants were required to duplicate the content, quantity, and timing of their daily diet 24 h preexercise. Participants were instructed not to eat or drink (except water) within 10 h of reporting to the HPL for experimental trials.

**Blood measurements.** During each experimental trial, blood samples were collected using a Teflon cannula placed in a superficial forearm vein using a three-way stopcock with a male Luer lock adapter and plastic syringe. The cannula was maintained patent using a nonheparinized isotonic saline solution (Becton Dickinson, Franklin Lakes, NJ). BL blood samples were collected after a 15-min equilibration period. IP blood samples were collected within 1 min of exercise cessation. Participants were instructed to lie in a supine position for 15 min before 30P, 1H, 2H, and 5H blood draws.

All blood samples placed in three 6-mL Vacutainer® tubes. Blood samples were drawn into plain, sodium heparin, or K2-EDTA-treated tubes. A small aliquot of whole blood was removed and used for determination of hematocrit and hemoglobin concentrations. The blood in the plain tube was allowed to clot at room temperature for 30 min and subsequently centrifuged at 3000g for 15 min, along with the remaining whole blood from the other tubes. The resulting serum and plasma was placed into separate microcentrifuge tubes and frozen at −80°C for later analysis.

**Biochemical analysis.** Blood lactate concentrations were analyzed from plasma using an automated analyzer (Analogix Instruments USA, Lunenburg, MA). Hematocrit concentrations were analyzed from whole blood using a microcentrifugation technique (CritSpin, Westwood, MA) and microcapillary technique. Hemoglobin concentrations were analyzed from whole blood using an automated analyzer (HemoCue, Cypress, CA). Plasma volume shifts were calculated using the formula established by Dill and Costill (10). To eliminate interassay variance, all samples were assayed in duplicate by a single technician. Coefficients of variation for each assay were 1.4% for blood lactate, 0.4% for hematocrit, and 0.6% for hemoglobin.

Plasma concentrations of myoglobin and cortisol (Calbiotech, Spring Valley, CA) were assessed via enzyme-linked immunosorbent assays and a spectrophotometer (BioTek Eon, Winoski, VT) using commercially available kits. To eliminate interassay variance, all samples for each assay were thawed once and analyzed in duplicate in the same assay run by a single technician. Coefficients of variation for each assay were 4.1% for myoglobin and 5.3% for cortisol.

Plasma samples were assayed for concentrations of MCP-1 and TNF-α, using a multiplex cytokine assay (Milliplex, catalog no. HCYTMAG-60K; Millipore, Billerica, MA) on a MAGPIX instrument (Luminex, Austin, TX), according to the manufacturer’s instructions. All samples were run in duplicate with a mean intra-assay variance of 8.77% for MCP-1 and 7.00% for TNF-α.

**Cell staining.** Cell staining was performed as described previously (39). The analysis of target receptor expression on monocytes was completed at BL, IP, 1H, 2H, and 5H time points. K2-EDTA-treated peripheral whole blood was used to identify monocytes and to quantify target receptor expression by direct immunofluorescence and flow cytometry (BD Biosciences, San Jose, CA). Erythrocytes were first lysed from 350 μL of K2-EDTA-treated whole blood with BD Pharm Lyse solution (BD Biosciences) within 30 min of collection. Samples were then washed in staining buffer containing 1× phosphate-buffered saline and fetal bovine serum (BD Pharmingen Stain Buffer, BD Biosciences) followed by centrifugation and aspiration for a total of three washes. Leukocytes were then resuspended in 100 μL BD Pharmingen stain buffer (BD Biosciences). Direct staining methods were used to label CD14 and CD16 (monocyte identifiers), CCR2 (monocyte chemotaxis), CD120a (receptor for TNF-α), and CD11b (monocyte adhesion). Because of
the number of receptors of interest, two separate cell preparations were performed.

For cell preparation 1, Alexa Fluor® 488–conjugated anti-CD120a (TNFr1, FAB225G, IgG; R&D Systems, Minneapolis, MN), allophycocyanin (APC)-conjugated anti-CD11b (550019, IgG1; BD Pharmingen™), PerCP Cy5.5–conjugated anti-CD14 (562692, IgG2b; BD Pharmingen™), and PE-conjugated anti-CD16 (561313, IgG1; BD Pharmingen™) were used in the receptor labeling process. Surface staining for preparation 1 was performed by adding 5 μL of directly conjugated Alexa Fluor® 488 anti-CD120a, 20 μL of directly conjugated APC anti-CD11b, 5 μL of directly conjugated PerCP Cy5.5 anti-CD14, and 5 μL of directly conjugated PE anti-CD16 to the cell suspension, followed by incubation in the dark for 30 min at room temperature. Cells were then resuspended in 1.0 mL of stain buffer for immediate flow cytometry analysis.

For cell preparation 2, PerCP Cy5.5–conjugated anti-CD14 (562692, IgG2b; BD Pharmingen) and APC-conjugated anti-CCR2 (FAB151A, IgG2b; R&D Systems) were used in the receptor labeling process. Surface staining for preparation 2 was performed by adding 5 μL of directly conjugated Alexa Fluor® 488 anti-CD120a and 10 μL of directly conjugated APC anti-CCR2 to the cell suspension, followed by incubation in the dark for 30 min at room temperature. Cells were then washed in staining buffer followed by centrifugation and aspiration. Cells were subsequently resuspended in 250 μL of fixation and permeabilization solution (BD cytofix/cytoperm™, BD Biosciences) and set to incubate for 20 min in the dark at 4°C. After incubation, cells were washed in 1 mL of Perm Wash Buffer (BD Perm/Wash™, BD Biosciences), followed by centrifugation and aspiration. Cells were resuspended in 50 μL of Perm Wash Buffer, followed by a second incubation in the dark for 30 min at 4°C. Cells were then washed in 1.0 mL of Perm Wash Buffer and resuspended in 1.0 mL of stain buffer for flow cytometry analysis after centrifugation and aspiration.

Flow cytometry. The flow cytometric analysis of stained cells was performed on a BD Accuri C6 flow cytometer (BD Biosciences), equipped with BD Accuri analysis software (BD Biosciences). Forward and side scatter, along with four fluorescent channels of data, were collected using two lasers, providing excitation at 488 and 640 nm. A minimum of 10,000 events, defined as CD14+ monocytes, were obtained with each sample. Compensation for fluorescence spillover was achieved through a single staining of anti-mouse Ig, κ/negative control compensation particles (BD CompBeads, BD Biosciences). Unstained leukocytes from human peripheral blood taken at BL were used as a negative control for CD14, CD16, CD120a, CD11b, and CCR2 expression. Viable cells were obtained using forward-scatter height (FSC-H) × forward-scatter area (FSC-A) gating to eliminate debris, necrotic cells, and artifactual. The mean fluorescence intensity, which represents the mean density of each receptor per cell, was quantified by overlaying the histogram plots of target receptors to the control samples.

**Gating procedure.** Cell preparation 1: Classical monocytes were determined based on CD14 and CD16 expression as previously described (43) and is depicted in Figure 1. The analysis of TNFr1 and CD11b receptor expression was completed on CD14++CD16− cells using one-dimensional histograms relative to negative control.

Cell preparation 2: Monocytes were determined via a one-dimensional histogram analysis of CD14+ cells relative to negative control. CCR2 is reported to be expressed almost solely on CD14++CD16− monocytes (42). Therefore, CD16 was not used for cellular differentiation. Accordingly, CCR2 expression was assessed on CD14+ monocytes via one-dimensional histogram analysis relative to negative control.

**Statistical analysis.** Before statistical procedures, all data were assessed for sphericity. If the assumption of sphericity was violated, a Greenhouse–Geisser correction was applied. Biochemical and receptor expression changes were analyzed using a two factor (time × trial) repeated-measures ANOVA. In case of a significant F-ratio, a follow-up one-way repeated-measures ANOVA was used to determine time effects for each treatment, and a least significant difference (LSD) post hoc tests were used for pairwise comparisons across time. Dependent t-tests were used for pairwise comparisons between trials at each time point. Comparisons between trials were further analyzed using Cohen’s d. Interpretations of Cohen’s d were evaluated in accordance with Thalheimer and Rosenthal (38) at the following levels: negligible effect (≥0.15 and <0.15), small effect (≥0.15 and <0.40), medium/moderate effect (≥0.40 and <0.75), large effect (≥0.75 and <1.10), very large effect (≥1.10 and <1.45), and huge effect (≥1.45). Time effects were further analyzed using partial eta squared (ηp2). Interpretations of ηp2 were evaluated in accordance with Cohen’s criteria.
TABLE 1. Average percentage of 1RM completed.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Exercise</th>
<th>SET 1 (%)</th>
<th>SET 2 (%)</th>
<th>SET 3 (%)</th>
<th>SET 4 (%)</th>
<th>SET 5 (%)</th>
<th>SET 6 (%)</th>
<th>Mean (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HVY</td>
<td>Squat</td>
<td>90</td>
<td>90</td>
<td>89</td>
<td>88</td>
<td>86</td>
<td>84</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>Leg press</td>
<td>90</td>
<td>81</td>
<td>79</td>
<td>79</td>
<td>—</td>
<td>—</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>Hamstring curl</td>
<td>90</td>
<td>88</td>
<td>85</td>
<td>84</td>
<td>—</td>
<td>—</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>Leg extension</td>
<td>90</td>
<td>86</td>
<td>85</td>
<td>84</td>
<td>—</td>
<td>—</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>Calf raise</td>
<td>90</td>
<td>95</td>
<td>97</td>
<td>99</td>
<td>—</td>
<td>—</td>
<td>98</td>
</tr>
<tr>
<td>VOL</td>
<td>Squat</td>
<td>70</td>
<td>69</td>
<td>67</td>
<td>62</td>
<td>57</td>
<td>54</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Leg press</td>
<td>70</td>
<td>61</td>
<td>53</td>
<td>52</td>
<td>—</td>
<td>—</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>Hamstring curl</td>
<td>70</td>
<td>63</td>
<td>59</td>
<td>49</td>
<td>—</td>
<td>—</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Leg press</td>
<td>70</td>
<td>62</td>
<td>59</td>
<td>56</td>
<td>—</td>
<td>—</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Calf raise</td>
<td>70</td>
<td>72</td>
<td>72</td>
<td>69</td>
<td>—</td>
<td>—</td>
<td>71</td>
</tr>
</tbody>
</table>

Cohen (9) at the following levels: small effect (0.01–0.058), medium effect (0.059–0.137), and large effect (>0.138). The net area under the curve (AUC) was also calculated for biochemical measures using a standard trapezoidal technique and was assessed using paired-samples t-tests. Pearson product–moment correlations were used to examine the association between markers of muscle damage, circulating hormones, cytokines, and cellular receptor expression on classical monocytes. Significance was accepted at an alpha level of 0.05. Data were analyzed using IBM SPSS Statistics for Windows (version 21.0; IBM Corp., Armonk, NY). All data are presented as mean ± SD.

RESULTS

Resistance exercise protocol. Training volume (sets × load × reps) was significantly greater for VOL (53359.0 ± 14178.1 kg) compared with HVY (37633.5 ± 6253.1 kg) (+42%, d = 1.53, P = 0.002). The average percentage values of 1RM completed for each exercise are presented in Table 1.

Changes in plasma lactate concentrations are depicted in Table 2. A significant interaction between trials was observed for plasma lactate concentrations (F = 41.7, P ≤ 0.001, \( \eta_p^2 = 0.82 \)). Plasma lactate was significantly greater during VOL at IP (d = 2.82, P ≤ 0.001), 30P (d = 2.10, P ≤ 0.001), and 1H (d = 1.77, P ≤ 0.001) compared with HVY. AUC was significantly greater for VOL compared with HVY (d = 1.28, P = 0.009).

Biochemical analysis. Changes in plasma myoglobin concentrations are presented in Table 2. The time × group repeated-measures ANOVA indicated a significant time effect for plasma myoglobin (F = 30.2, P ≤ 0.001, \( \eta_p^2 = 0.77 \)). A significant interaction between trials was also observed (F = 8.3, P = 0.003, \( \eta_p^2 = 0.48 \)). Compared with BL, plasma myoglobin was significantly elevated at all postexercise time points during both HVY (P values = 0.001) and VOL (P values < 0.001). Myoglobin concentrations at IP (d = 1.11, P = 0.022) and 30P (d = 1.28, P = 0.009) were significantly greater during HVY compared with VOL. In addition, myoglobin concentrations tended to be higher during HVY at 1H compared with VOL (d = 0.74, P = 0.054). AUC analysis revealed no significant differences between trials (d = 0.60, P = 0.111).

Correlations between myoglobin, inflammatory markers, and monocyte receptor expression are presented in Table 3. With both trials combined, plasma myoglobin was significantly correlated with CCR2 expression at 2H and 5H postexercise (r = −0.507, P = 0.023 and r = −0.454, P = 0.044, respectively).

Changes in plasma TNF-\( \alpha \) and MCP-1 concentrations are presented in Table 3. The time × group repeated-measures ANOVA indicated a significant time effect for both TNF-\( \alpha \) (F = 12.7, \( \eta_p^2 = 0.59 \), P ≤ 0.001) and MCP-1 (F = 6.4, P < 0.001, \( \eta_p^2 = 0.42 \)); however, no significant main effect for group or time–group interactions (P > 0.05) were noted. With both trials combined, plasma TNF-\( \alpha \) was significantly elevated at IP, 30P, 1H, and 2H (P values ≤ 0.002). Plasma MCP-1 was significantly elevated above BL at all postexercise time points (P = 0.002–0.033). AUC analysis revealed no significant differences between trials for TNF-\( \alpha \) (d = 0.31, P = 0.263), or MCP-1 (d = 0.20, P = 0.592).

Changes in plasma cortisol concentrations are presented in Table 2. The time × group repeated-measures ANOVA indicated a significant time effect for cortisol (F = 24.0, P ≤ 0.001, \( \eta_p^2 = 0.73 \)). A significant interaction between trials was also observed (F = 10.7, P ≤ 0.001, \( \eta_p^2 = 0.54 \)). During HVY, cortisol concentrations were significantly lower than BL at 2H and 5H (P = 0.028 and P ≤ 0.001, respectively). During VOL, significant elevations in cortisol concentrations were noted at IP, 30P, and 1H (P ≤ 0.004), whereas a

TABLE 2. Biochemical response to resistance exercise.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Trial</th>
<th>BL</th>
<th>IP</th>
<th>30P</th>
<th>1H</th>
<th>2H</th>
<th>5H</th>
</tr>
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<tbody>
<tr>
<td>Lactate (mmol)</td>
<td>HVY</td>
<td>1.2</td>
<td>6.1</td>
<td>3.4</td>
<td>2.3</td>
<td>1.8</td>
<td>2.4</td>
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<tr>
<td></td>
<td>VOL</td>
<td>1.4</td>
<td>12.6</td>
<td>7.0</td>
<td>9.0</td>
<td>8.0</td>
<td>2.7</td>
</tr>
<tr>
<td>Myoglobin (ngmL(^{-1}))</td>
<td>HVY</td>
<td>29.3</td>
<td>164.3</td>
<td>201.6</td>
<td>199.9</td>
<td>159.7</td>
<td>112.9</td>
</tr>
<tr>
<td></td>
<td>VOL</td>
<td>35.0</td>
<td>91.9</td>
<td>114.9</td>
<td>141.9</td>
<td>141.1</td>
<td>92.9</td>
</tr>
<tr>
<td>Cortisol (nmolL(^{-1}))</td>
<td>HVY</td>
<td>562.3</td>
<td>682.5</td>
<td>548.2</td>
<td>522.3</td>
<td>396.2</td>
<td>281.5</td>
</tr>
<tr>
<td></td>
<td>VOL</td>
<td>550.5</td>
<td>1074.3</td>
<td>622.8</td>
<td>622.8</td>
<td>288.0</td>
<td>221.9</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.
*Significant difference between trials.
†Significant increase relative to BL (P ≤ 0.05).
‡Significant decrease relative to BL (P ≤ 0.05).
significant decrease was noted at 5H relative to BL ($P \leq 0.001$). Cortisol concentrations at were significantly greater during VOL compared with HVY at IP ($d = 1.08, P = 0.012$), 30P ($d = 2.12, P \leq 0.001$), 1H ($d = 2.00, P = 0.003$), and 2H ($d = 1.19, P = 0.018$). AUC was significantly greater for VOL compared with HVY ($d = 1.51, P = 0.003$).

Supplemental effect sizes and their associated $P$ values for biochemical analyses are presented in Table 4.

**Plasma volume shifts.** Relative to BL, plasma volume shifts were significantly different between trials at IP ($P = 0.16$). The difference between trials was not significant for any other time point. During VOL, plasma volume decreased at IP, $-8.0\% \pm 7.7\%$; increased at 30P, $2.1\% \pm 9.4\%$; increased at 1H, $7.2\% \pm 14.0\%$; increased at 2H, $3.7\% \pm 5.0\%$; and decreased at 5H, $-1.6\% \pm 5.5\%$. During HVY, plasma volume decreased at IP, $-1.6\% \pm 3.1\%$; increased at 30P, $3.3\% \pm 3.6\%$; increased at 1H, $4.0\% \pm 3.0\%$; increased at 2H, $7.2\% \pm 7.3\%$; and decreased at 5H, $-2.6\% \pm 4.0\%$. Blood variables were not corrected for plasma volume shifts due to the importance of molar exposure at the tissue receptor level.

No significant differences were noted for water consumption between each exercise protocol ($P = 0.337$).

**Receptor expression.** Changes in CD11b, CCR2, and TNFr1 expression levels are presented in Figure 2. A significant time effect was observed for CCR2 ($F = 4.6, P = 0.005, \eta_p^2 = 0.37$); however, no significant main effect for group or time–group interaction ($P > 0.05$) was noted. With both trials combined, CCR2 expression was significantly lower at IP ($d = 1.32, P = 0.033$), 1H ($d = 1.37, P = 0.020$), 2H ($d = 1.32, P = 0.040$), and 5H ($d = 1.43, P = 0.024$) relative to BL.

A significant time effect was observed for CD11b ($F = 7.1, P < 0.001, \eta_p^2 = 0.44$); however, no significant main effect for group or time–group interaction ($P > 0.05$) was noted. With both trials combined, CD11b was significantly elevated at IP ($d = 0.86, P = 0.014$) and 1H ($d = 1.04, P = 0.009$). A trend toward a decrease in CD11b receptor expression relative to BL was observed at 5H ($d = 0.59, P = 0.095$). CD11b receptor expression was positively correlated with circulating MCP-1 at IP ($r = 0.576, P = 0.008$) and 1H.

| TABLE 3. Correlations between myoglobin, MCP-1, TNF-α, TNFr1, CCR2, and CD11b. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | IP              | 30P             | 1H              | 2H              | 5H              |
| Myoglobin       | $r$             | $P$             | $r$             | $P$             | $r$             | $P$             |
| MCP-1           | -0.037          | 0.878           | -0.067          | 0.078           | 0.098           | 0.680           | 0.214           | 0.365           | 0.340           | 0.142           |
| TNF-α           | -0.082          | 0.797           | -0.138          | 0.560           | -0.005          | 0.984           | 0.002           | 0.993           | 0.117           | 0.624           |
| TNF-ρ1          | -0.033          | 0.889           | —               | —               | 0.151           | 0.526           | -0.014          | 0.954           | 0.207           | 0.380           |
| CCR2            | -0.320          | 0.169           | —               | —               | -0.084          | 0.726           | -0.507          | 0.023           | -0.454          | 0.044           |
| CD11b           | 0.056           | 0.816           | —               | —               | -0.009          | 0.971           | -0.271          | 0.247           | -0.111          | 0.643           |

$r$, Pearson correlation coefficient.
**DISCUSSION**

The findings of this study indicate that changes in CCR2 and CD11b expression levels on classical monocytes are similar after acute bouts of high-intensity and high-volume resistance exercise. When collapsed across groups, CCR2 expression was significantly lower at IP, 1H, 2H, and 5H postexercise, whereas CD11b expression was significantly elevated at IP and 1H postexercise. Plasma concentrations of myoglobin were significantly higher after HVY compared with VOL; however, both protocols resulted in significant elevations above BL at all postexercise time points. Changes in plasma TNF-α and MCP-1 concentrations were similar between HVY and VOL. As expected, significant elevations in plasma cortisol concentrations were observed after VOL only, although this did not seem to be related to changes in receptor expression. TNFR1 receptor expression did not seem to be affected by resistance exercise.

TNF-α plays several important roles in inflammation, including inducing the secretion of MCP-1 (27) and the upregulation of CD11b expression (36). Our results indicate that plasma TNF-α is rapidly increased after resistance exercise. Relative to BL, plasma TNF-α was significantly increased at IP, 30P, 1H, and 2H postexercise, and this was similar between HVY and VOL, suggesting that resistance exercise, irrespective of HVY or VOL, results in a robust postexercise inflammatory response. These findings are consistent with the findings of Townsend et al. (39), who reported significant increases in plasma TNF-α immediately and 30 min postexercise after an acute bout of high-volume resistance exercise in well-trained males. In contrast to our findings, several studies report no changes in plasma TNF-α after high-volume resistance exercise (5,34). However, these studies employed untrained men, suggesting that the cytokine response may differ between trained and untrained individuals. Previous reports suggest that the cytokine response may be delayed in untrained individuals, which may be related to the degree of tissue damage (29).

TNFR1 is reported to be the key mediator of TNF-α signaling (16). TNFR1 expression did not change significantly in response to HVY or VOL resistance exercise. We have previously reported a significant increase in TNFR1 receptor expression on monocytes after acute bouts of high-volume resistance exercise in well-trained males (39). However, in contrast to the present study, this observation was made on CD14⁺ monocytes, without further differentiation based on the expression of CD16 antigen. Recent research suggests that intermediate monocytes express TNFR1 to a significantly greater extent compared with classical and nonclassical monocytes (18). It is therefore possible that the change in TNFR1 expression previously reported by our laboratory (39) occurred in intermediate CD14⁺CD16⁻ monocytes rather than in CD14⁺CD16⁻ classical monocytes. Nevertheless, examinations of temporal changes in the expression of TNFR1 on monocytes after resistance exercise are lacking.

Our results indicate that circulating MCP-1 is also rapidly increased after damaging resistance exercise. Under inflammatory conditions, MCP-1 is the primary cytokine involved in mediating classical monocyte chemotaxis (32). Relative to BL, MCP-1 was significantly elevated at all postexercise time points, and this response was similar between HVY and VOL. This is consistent with the reports indicating that circulating concentrations of MCP-1 parallel early inflammation (33) and suggests that both HVY and VOL protocols likely resulted in a significant recruitment of monocytes.

To our knowledge, only one other study has examined changes in plasma MCP-1 in conjunction with an acute bout of resistance exercise (20). In contrast to our findings, Ihalainen et al. (20) reported a significant decrease in plasma MCP-1 concentrations, after an acute bout of high-volume leg press exercise [5 × 10 (80% 1RM)] in healthy untrained males. Data regarding the effect of training status on the plasma MCP-1 response are not well understood. However, differences between findings could be related to the magnitude of the exercise stimulus. We used an exercise protocol consisting of five exercises designed to target the entire lower extremity. By contrast, Ihalainen et al. used only the

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**TABLE 4. Effect sizes and \( P \) values for biochemical analyses.**

<table>
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<tr>
<th></th>
<th>IP</th>
<th>30P</th>
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<th>2H</th>
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<tr>
<td></td>
<td>( P )</td>
<td>ES (d)</td>
<td>( P )</td>
<td>ES (d)</td>
<td>( P )</td>
</tr>
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<tr>
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</tr>
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</table>

ES (d), effect size (Cohen’s \( d \); HVY = high-intensity exercise protocol; VOL = high-volume exercise protocol; COM, HVY and VOL trials combined.
† Significant increase relative to BL.
‡ Significant decrease relative to BL.

\( r = 0.706, P = 0.001 \), and with circulating TNF-α at 1H \( (r = 0.666, P = 0.001) \).

No significant time effect, main effect for group, or time–group interaction was observed for TNFR1 \( (P > 0.05) \).
leg press exercise. Given the large disparity in total work between studies, it is possible that a minimal exercise volume may be required to stimulate MCP-1 and that this was not attained with the leg press alone. Nevertheless, Ihalainen et al. (20) only examined plasma MCP-1 concentrations up to 30 min postexercise. Therefore, a delayed increase in plasma MCP-1 cannot be discounted.

MCP-1 exerts its chemotactic function via exclusive binding to the G protein–coupled receptor CCR2 (31). Our results indicate that CCR2 expression is significantly down-regulated at all postexercise time points after both high-intensity and high-volume resistance exercises. The observed findings may be the result of agonist-mediated receptor internalization, a well-characterized mechanism contributing to the tight control of inflammation (3,4,23). However, to the best of our knowledge, no other study has evaluated changes in CCR2 expression after an acute bout of resistance exercise.

Changes in CCR2 expression have been reported after examinations of MCP-1/CCR2-mediated monocyte chemotaxis in vitro (13,17). Handel et al. (17) observed a dose-dependent decrease in CCR2 expression after incubation of murine monocytes with MCP-1. A substantial downregulation of CCR2 receptors was apparent within 30 min, whereas 1 h of incubation was sufficient to induce a 60% downregulation of CCR2 receptors. Upon removal of the MCP-1 ligand, CCR2 expression began to recover. Nevertheless, maximal receptor expression was not regained for an additional 4 h (17). Consistent with this, low concentrations of MCP-1 have been shown to be sufficient to induce a substantial downregulation of CCR2. Further, this effect was achieved within 5–10 min of MCP-1 exposure (13).

Together, these results suggest that MCP-1 may induce the internalization of CCR2 receptors, possibly as part of a mechanism designed to modulate the magnitude of the monocyte response.

A recent investigation by Volpe et al. (41) indicates that the internalization of CCR2 does not reduce the responsiveness of monocytes to MCP-1. These investigators suggest that after interaction with MCP-1, internalized receptors rapidly recycle back to the cell surface to maintain the responsiveness of the cell toward the chemokine. Physiologically, this action provides for continuous signaling from receptors at the leading edge of the monocyte, which is required for directional migration. This mechanism would allow monocytes to proceed along an increasing chemokine gradient without becoming desensitized. Nevertheless, under conditions of continuous stimulation, receptor cycling may still lead to a decrease in CCR2 receptor expression. However, given the need to maintain cell polarity, it is likely that this response would be limited to a particular threshold rather than being dose dependent. This is consistent with the plateau observed in the present study and may also explain why we did not find a significant inverse relationship between plasma MCP-1 concentrations and CCR2 expression. Significant inverse correlations were observed between plasma myoglobin concentrations and CCR2 expression at 2H and 5H postexercise.

However, it remains unclear whether myoglobin is able to modulate CCR2 expression.

Cortisol has been shown to modulate the expression of CCR2 on human monocytes in vitro (28,30). Despite a significant increase in plasma cortisol after the high-volume protocol, changes in the expression of CCR2 were not different between trials. Consequently, our results do not support the in vitro observations of others. It is worth mentioning, however, that at least 4–6 h of incubation were required before an upregulation of CCR2 was observed in vitro (28,30). Further, 60 min of cycling at 70% $V_{O2peak}$ was unable to stimulate an upregulation of CCR2 on monocytes in vivo, even after 24 h of additional incubation time in vitro (28). In the present study, plasma cortisol concentrations did not correlate with any outcome measure to a physiologically meaningful degree.

CD11b mediates monocyte intravascular crawling, which is the direct prerequisite to transendothelial migration (24,36). Our results indicate a moderate but significant upregulation in CD11b at IP and 1H postexercise. Further, this change was similar between high-intensity and high-volume trials. The upregulation of CD11b indicates a greater potential for monocyte adhesion and is consistent with the temporal appearance of monocytes at sites of tissue damage reported by McLennan (26). We have previously reported similar increases in CD11b on CD14+ monocytes after an acute bout of high-volume resistance exercise in well-trained males (21).

Our reports seem to be consistent with the findings of others (19). Hong et al. (19) observed a significant increase in the expression of CD11b on CD14+CD16– monocytes immediately and 10 min postexercise, after a 20-min bout of treadmill exercise at 65%–70% $V_{O2peak}$. In another study, a significant upregulation in CD11b expression was observed after maximal treadmill exercise and marathon running, albeit on granulocytes (22). Interestingly, a bout of moderate treadmill exercise was not sufficient to cause an upregulation in CD11b (22). This could indicate that exercise must be of a sufficient volume and/or intensity for CD11b expression to be increased.

The observed increase in CD11b expression correlated strongly with plasma concentrations of MCP-1 at IP ($r = 0.576$) and 1H ($r = 0.706$), and with plasma concentrations of TNF-α at 1H ($r = 0.666$). Previous research indicates that for a given receptor, the signals required for rapid adhesion to vascular integrin ligands are different from those required for chemotaxis, illustrating that two distinct G protein–linked receptor-depente events are required for leukocyte extravasation from the blood (7). This suggests that the TNF-α–mediated upregulation of CD11b is more likely because MCP-1 mediates chemotaxis. Nevertheless, we are unable to delineate the contributions of MCP-1 and/or TNF-α in the regulation of CD11b expression.

Resistance exercise is associated with an acute monocytosis that peaks immediately postexercise (14). One of the limitations of this study is that we did not assess monocyte cell counts. It is possible that an increased number of
circuitulating CD14++/CD16− classical monocytes may result in a more pronounced inflammatory response postresistance exercise. Nevertheless, although a transient increase in monocyte count is commonly observed after exercise, it has been suggested that this response is dominated by intermediate (CD14++/CD16−) and nonclassical (CD14+/CD16++) monocytes (40). Further, it has been reported that this response is mainly driven by hemodynamic changes as opposed to increased bone marrow egress of monocytes (35). Consequently, there is limited evidence to suggest that classical monocyte counts increase after resistance exercise. Future studies may benefit from analyzing changes in receptor expression in conjunction with changes in cell counts for monocyte subtypes.

In conclusion, the present study investigated the acute proinflammatory response after two typical lower-body resistance exercise protocols in experienced resistance-trained men. Markers of muscle damage were elevated to a significantly greater extent in HVY, whereas plasma cortisol concentrations were significantly greater after VOL. Nevertheless, changes in plasma concentrations of TNF-α and MCP-1 were similar between HVY and VOL, as was the temporal response of TNFR1, CCR2, and CD11b receptors on classical monocytes. Consequently, our results do not support a role for cortisol in the modulation of these receptors in vivo, whereas the degree of muscle damage does not seem to influence plasma concentrations of TNF-α or MCP-1. It is therefore likely that both HVY and VOL protocols constitute an exercise stimulus that is sufficient enough to promote a robust proinflammatory response, which is similar in timing and magnitude.

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