Leukocyte IGF-1 Receptor Expression during Muscle Recovery

MAREN S. FRAGALA, ADAM R. JAJTNER, JEREMY R. TOWNSEND, ADAM M. GONZALEZ, ADAM J. WELLS, LEONARDO P. OLIVEIRA, JAY R. HOFFMAN, JEFFREY R. STOUT, and DAVID H. FUKUDA

Department of Educational and Human Sciences, Institute of Exercise Physiology and Wellness, Sport and Exercise Science, University of Central Florida, Orlando, FL

ABSTRACT

FRAGALA, M. S., A. R. JAJTNER, J. R. TOWNSEND, A. M. GONZALEZ, A. J. WELLS, L. P. OLIVEIRA, J. R. HOFFMAN, J. R. STOUT, and D. H. FUKUDA. Leukocyte IGF-1 Receptor Expression during Muscle Recovery. Med. Sci. Sports Exerc., Vol. 47, No. 1, pp. 92–99, 2015. Introduction: The insulin-like growth factor 1 (IGF-1) system plays a central role in anabolic cellular processes. Recently, a regulatory role of IGF-1 in the immune response for muscle repair has been suggested, but how it modulates the inflammatory process is largely unknown. We evaluated changes in leukocyte expression of IGF-1 receptors (IGF-1R) during recovery from resistance exercise to determine whether changes in the potential for IGF-1 interactions with leukocytes may mediate the role of IGF-1 in muscle repair. Methods: Twenty resistance-trained men (18–35 yr) performed resistance exercise followed by cold water immersion (CWI) or control treatment (CON) on three consecutive days. Blood was sampled at baseline (PRE), immediately (IP), 30 min (30P), 24 h (24H), and 48 h after (48H) exercise. Circulating IGF-1 was assayed, and IGF-1 receptor expression (CD221) on gated circulating leukocytes (monocytes, granulocytes, and lymphocytes) was measured by flow cytometry. Time and treatment effects were analyzed with ANCOVA. Results: Circulating IGF-1 significantly increased from PRE to IP as a result of resistance exercise, but no differences between CON and CWI were observed. Mean fluorescence intensity of CD221 on monocytes and granulocytes and percent of CD221+ granulocytes significantly increased at 30P (P < 0.000) and returned to preexercise levels by 24H. No treatment effects on monocytes or granulocytes were observed. On lymphocytes, mean fluorescence intensity of CD221+ significantly increased from PRE to 30P in CWI. Conclusions: Changes in IGF-1 and its receptor on monocytes and granulocytes seem to be part of the mechanism that facilitates recovery from resistance exercise during earlier stages of muscle recovery. In addition, CWI seems to alter IGF-mediated responses on slower-acting lymphocytes, suggesting that its effects may be seen in later stages of muscle repair. Key Words: MUSCLE REPAIR, IMMUNE FUNCTION, COLD WATER IMMERSION, CRYOTHERAPY, RESISTANCE EXERCISE, INFLAMMATION

The insulin-like growth factor 1 (IGF-1) system contains a family of proteins essential to mediating anabolic and metabolic cellular processes (29). Among its ubiquitous functions, IGF-1 is an anabolic growth factor important for initiating the sequence of events involved in muscle repair and remodeling (11,44). The binding of IGF-1 to its cellular receptor (IGF-1R) triggers signaling via the Akt/mTOR pathway for muscle hypertrophy to proceed (16). IGF-1 exerts its hypertrophic function by both increasing protein synthesis and decreasing protein degradation (1). Whereas IGF-1 is known to play a central role in the mechanisms underlying muscle hypertrophy, its role in immune communication is largely unknown. Interestingly, previous research has demonstrated a regulatory role of IGF-1 in the immune response during muscle repair (11, 23,44) where both IGF-1 messenger RNA and protein expression are markedly up-regulated in injured muscles (23) and the up-regulation of IGF-1 in skeletal muscle seems to be protective against muscle damage (11) and beneficial for regeneration (44).

Among the several cell types that contain IGF-1 receptors (IGF-1R) to elicit cellular growth are immune cells (38). IGF-1R are prevalent on the surface of human peripheral blood mononuclear cells identified by cell differentiation marker 221 (CD221). Their presence signifies IGF-1’s communication with the immune system in muscle repair processes (38). Earlier work has shown that the cell surface receptors for IGF-1 are required for regulating cellular immune responses including proliferation and differentiation (22). In addition, IGF-1R on immune cells are known to play a role in inflammation through regulating macrophage infiltration and accumulation (23). The macrophages that infiltrate damaged skeletal muscles are known to express high concentrations of IGF-1 and play a role in muscle regeneration (23).

The mechanical stress of physical exercise causes physical disruption to the muscle’s myofilaments and connective tissue, which initiates an inflammatory cascade (10,40). Consequently, treatments to facilitate recovery have traditionally
focused on attenuating inflammation. One common treatment used by athletes to alleviate muscle soreness is cryotherapy via cold water immersion (CWI). CWI is believed to modulate tissue damage by attenuating the inflammatory response (33). Its anti-inflammatory actions are primarily attributed to reflexive vasoconstriction, which reduces local blood flow (14). In addition, CWI has been shown to attenuate pro-inflammatory cytokine signaling (31) and leukocytosis (30). Although previous studies investigating the role of IGF-1–related signaling in immune responses have yielded important mechanistic insights, it is not presently known how these mechanisms apply to exercise-induced muscle recovery or how recovery treatments, such as CWI, may influence these processes. Thus, the purpose of the present investigation was to evaluate changes in leukocyte expression of IGF-1R during recovery from resistance exercise with and without CWI treatment to determine whether changes in the potential for IGF-1 interactions with specific immune cells may mediate the role of IGF-1 in muscle repair. The resistance exercise protocol was expected to result in at least two- to threefold increases in circulating creatine kinase at 24 h after exercise, in line with previous acute resistance exercise protocols (21,43). Similarly, perceived muscle soreness was expected to significantly increase over preexercise ratings at 24 h after exercise, similar to previous heavy acute resistance exercise protocols in resistance-trained volunteers (21,36).

**METHODS**

**Participants.** Twenty resistance-trained men volunteered to participate in this study. Recruitment criteria required that participants be age 18–35 yr, have at least 1 yr of resistance training experience (including the barbell back squat), refrain from using nutritional supplements (i.e., creatine, amino acids, Beta-hydroxy-beta-methylbutyrate [HMB], herbal supplements) or medication (i.e., prescribed or over-the-counter medications such as cold medications or nonsteroidal anti-inflammatory drugs [NSAID]) during the study, and agree to not engage in any additional recovery strategies (i.e., saunas, stretching, foam rollers, massage, and supplementation) outside the study procedures. Before enrollment in the study, all volunteers gave a written informed consent after being informed of all study procedures, risk, and benefits. The study protocol was approved by the university’s institutional review board for the protection of human participants. Volunteers were randomized into one of two treatment groups: control (CON) (n = 10, 23.8 ± 3.0 yr, 178 ± 6 cm, 85.7 ± 5.4 kg) or CWI (n = 10, 22.5 ± 3.0 yr, 171 ± 7 cm, 77.1 ± 7.7 kg). Participants had an average of 5.7 ± 3.4 yr (CON) and 7.6 ± 4.2 yr (CWI) of resistance training experience using multiple-set periodized programs including heavy-load phases. Participants had squat strength (one-repetition maximum [1RM]) of 148.0 ± 30.9 kg (CON) and 148.6 ± 31.9 kg (CWI), which corresponded to relative squat strength to body mass of 1.73 ± 0.35 (CON) and 1.94 ± 0.43 (CWI).

**Protocol.** Using a randomized controlled design, all participants performed a high-volume resistance exercise protocol on day 1, which consisted of four sets of up to 10 repetitions of the barbell back squat at 80% of their previously determined 1RM and barbell dead lift and split squat at 70% of their previously determined 1RM, with 90-s rest intervals allowed between each set and exercise. On days 2 (24 h after) and 3 (48 h after), participants again performed resistance exercise consisting of four sets of up to 10 repetitions of the barbell back squat exercise only at 80% of 1RM with 90-s rest intervals. Immediately after exercise on days 1 and 2, assigned treatment was administered as described in the following sections. All testing was performed in the morning after a 10-h overnight fast (except for water). Each participant was scheduled for testing at the same time on days 1, 2, and 3 (between 07:00 and 10:00 a.m.).

**CWI.** Participants randomized to the CWI treatment group were immersed in a cold water ice bath in a metal tub (58.4 × 121.9 cm) for 10 min after exercise on days 1 and 2. The water temperature was maintained at 10°C to 12°C (31), and water depth was set to 22.9 cm, which fully immersed the lower body while seated and reached the approximate height of the umbilicus. After the bath, participants remained in the laboratory for 20 additional minutes until collection of data at 30 min after exercise on day 1.

**Control.** Participants randomized to the control group completed the same resistance exercise protocol at the same relative intensity as those assigned to the CWI treatment group. However, after exercise on days 1 and 2, those assigned to the control group received no treatments after exercise and remained in the laboratory until collection of data at 30 min after exercise on day 1.

**Biological sampling.** Blood samples were collected from a superficial forearm vein at the following time points: before exercise (PRE), immediately after exercise (IP), 30 min after exercise (30P), 24 h after exercise (24H), and 48 h after exercise (48H). During the first exercise session on day 1, the blood samples collected at PRE, IP, and 30P were obtained using a 20-gauge Teflon cannula with a three-way stopcock and male luer lock adapter. A 15-min equilibration period after cannula insertion was allowed with the participant lying supine before collection of the PRE blood sample. The participant returned to the supine position for the IP and 30P blood draws to reduce the influence of fluid volume shifts. The cannula was kept patent between blood draws using an isotonic saline solution. The cannula was removed after the 30P blood sample. Participants returned to the laboratory for blood sample collection on days 2 (24H) and 3 (48H). Blood samples at 24H and 48H were collected before exercise using a 20-gauge disposable needle equipped with a Vacutainer® tube holder (Becton Dickinson, Franklin Lakes, NJ), with the participant in a supine position for at least 15 min before collection. All blood samples were collected into prechilled plasma collection Vacutainer® tubes containing K$_2$EDTA. A volume of 350 μL of K$_2$EDTA-treated fresh whole blood was reserved for isolation of peripheral blood mononucleated...
cells (PBMC). Remaining blood was centrifuged at 3000g for 15 min. Resulting plasma was aliquoted into separate 1.6-mL microcentrifuge tubes and frozen at −80°C for subsequent analysis.

Biochemical analysis. Plasma IGF-1 was assayed by commercially available Quantikine® enzyme-linked immunosorbent assay (Catalog No: DG100; R&D Systems, Minneapolis, MN) according to manufacturer specifications. Before the assay, 20 μL of plasma samples were pretreated with the acidic dissociation solution to release IGF-1 from binding proteins and reconstituted with a protein buffer. Reconstituted samples of 50 μL were added to each well for the assay. All samples were run in duplicate and thawed only once before an analysis. The sensitivity of the assay was 0.026 ng mL−1, and intraassay precision was 4.6%.

Cell staining. Peripheral whole blood treated with K2EDTA was used to identify leukocytes and quantify the IGF-1 receptor expression by direct immunofluorescence and flow cytometry. Erythrocytes were lysed from 350 μL of K2EDTA-treated whole blood with BD Pharm Lyse solution (BD Biosciences, Franklin Lakes, NJ) within 30 min of collection. Remaining leukocytes were then resuspended in 100 μL BD Pharmingen stain buffer (BD Biosciences) containing phosphate buffered saline (PBS), fetal bovine serum, metabolic inhibitor, and sodium azide (NaN3) to block Fc receptors. Direct staining methods were used to label CD221 with PE-conjugated anti-CD221 (catalog #555999, Clone 1H7; BD Pharmingen). Staining was performed by adding 10 μL of directly conjugated PE-anti-CD221 to the cell suspension and incubating in the dark for 30 min at 20°C. Cells were resuspended in 1.0 mL of stain buffer for flow cytometry analysis.

Flow cytometry. Flow cytometry analysis of stained cells was performed on a BD C6 Accuri flow cytometer (BD Biosciences, San Jose, CA) equipped with BD Accuri analysis software (BD Biosciences). Cell scatter and fluorescence were collected using two lasers providing excitation at 488 and 640 nm. Leukocyte populations (monocytes, granulocytes, and lymphocytes) were gated (Fig. 1) using forward scatter versus side scatter, as previously described (13). Gating was set to exclude dead cell debris. A minimum of 10,000 cells per gate were obtained with each sample. Analysis of CD221+ cell subpopulations was completed by quadrant analyses. Mean fluorescence intensity (MFI) of CD221+ on cell populations was recorded, representing the mean density of IGF-1R per cell. To control for fluorescence spillover, correction subtraction values were applied when necessary as per manufacturer instructions (BD Biosciences). Data are reported as the percent of CD221+ positive cells and MFI of CD221 on positively stained cells.

Statistical analysis. Effects of exercise and treatment were analyzed using two-way (group–time) repeated-measures ANCOVA. All analyses used the PRE measure as the covariate. In the event of a significant F score, post hoc one-way repeated-measures ANOVA and Tukey comparisons were run. Before analysis, all data were assessed for homogeneity of variance and sphericity. If the assumption of sphericity was violated, a Greenhouse–Geisser correction was applied. An alpha level of $P \leq 0.05$ was considered statistically significant for all comparisons. $P \leq 0.10$ was interpreted as a meaningful trend in the analysis to reduce the chance of making a type 2 error and missing an important change because of the heterogeneity of immune response in humans. Thus, post hoc analyses were run when main effects were $P \leq 0.10$. All data are reported as mean ± SE unless otherwise noted.

RESULTS

Participants assigned to the CWI and CON groups were similar in physical characteristics including years of resistance training experience ($7.1 \pm 3.8$ yr) and squat 1RM ($145.6 \pm 30.3$ kg). Subject characteristics and performance data have been previously described in a related study (15). Briefly, the exercise protocol resulted in significant increases in circulating creatine kinase ($154.7 \pm 139.8$ U·L−1 at PRE to $468.9 \pm 358.7$ U·L−1 at 24H in CON and $103.4 \pm 62.2$ U·L−1 at PRE to $510.9 \pm 385.2$ U·L−1 at 24H in CWI), with no significant difference seen between the groups.

Circulating IGF-1 (Fig. 2) increased from PRE ($72.0 \pm 14.8$ ng·mL−1) to IP ($75.5 \pm 13.9$ ng·mL−1) ($P = 0.045$) and returned to preexercise levels at 30P ($72.8 \pm 13.3$ ng·mL−1). At 24H ($74.4 \pm 12.5$ ng·mL−1) and 48H ($75.2 \pm 15.1$ ng·mL−1), circulating IGF-1 tended to increase above PRE levels, albeit not significantly ($P = 0.129$ and 0.083, respectively). No differences between treatment groups were observed for circulating IGF-1.

Granulocytes. MFI of CD221 (relative expression of IGF-1R) on granulocytes increased at 30P ($139.8$ U·L−1) and 48H ($135.3$ U·L−1) from PRE ($55.6$ U·L−1) ($P \leq 0.000$) and returned to PRE levels at 24H ($49.38 \pm 93.24$) (Fig. 3a). No differences
between treatment groups were observed for MFI of CD221 on granulocytes. When collapsed across groups, percent of granulocytes expressing IGF-1R (Fig. 3b) significantly decreased from PRE (31.6% ± 10.7%) to IP (27.2% ± 10.0%) (P = 0.004) and increased at 30P (41.7% ± 12.8%) from PRE (P < 0.000) and IP (P = 0.019). At 24H (33.0% ± 10.4%) and 48H (32.7% ± 11.4%), percent of granulocytes expressing IGF-1R decreased from 30P (P = 0.006, P < 0.000) and returned to preexercise levels. No treatment–time interaction (P = 0.284) was observed in percent of granulocytes expressing IGF-1R, although at 48H postexercise CWI (30.9% ± 12.0%), it seemed to reduce percent of granulocytes expressing IGF-1 compared with CON (34.6% ± 11.0%) at 48H.

**Monocytes.** MFI of CD221 (relative expression of IGF-1R) on monocytes showed a significant time effect (P = 0.002) where MFI on monocytes significantly increased at 30P (5846.6 ± 1346.2) from PRE (4514.1 ± 760.1) (P < 0.000) and IP (4753.6 ± 1039.7) (P = 0.001) levels (Fig. 4a). By 24H (4983.1 ± 1223.8) (P = 0.015) and 48H (4847.2 ± 950.3) (P = 0.001), MFI had decreased from 30P and returned to preexercise levels. No treatment–time interaction was observed in MFI of CD221 on monocytes.

**Lymphocytes.** MFI of CD221 (relative expression of IGF-1R) on lymphocytes showed a significant time effect (P = 0.012) and trended toward a treatment–time interaction (P = 0.071) (Fig. 5a). Both groups tended to increase MFI from PRE (1621.4 ± 165.3) to IP (1706.8 ± 206.9) (P = 0.087). At 30P, MFI increased only in the CWI group (1868.9 ± 333.7) (P = 0.004) and remained above PRE at 24H (1751.5 ± 52.6) (P = 0.041). In the CON group, MFI did not significantly increase above PRE at 30P (1694.4 ± 174.4). Percent of lymphocytes expressing IGF-1R (Fig. 5b) increased from PRE (42.6% ± 10.7%) to IP (48.7% ± 10.9%) (P = 0.003) and decreased at 30P (35.8% ± 11.1%) (P < 0.000) in both groups. Percent of lymphocytes expressing IGF-1R (43.4% ± 11.0%) returned to preexercise levels by 24H. No significant treatment–time interaction was observed in percent of lymphocytes expressing IGF-1R.

**DISCUSSION**

The expression of IGF-1R on target cells provides important insights into the physiological role of IGF-1. This investigation sought to examine its relative expression on leukocyte populations in response to resistance exercise and CWI therapy. Whereas the endocrine–immune interactions of other hormones and corresponding receptors have been previously reported (12,13), the potential of IGF-1 immune interactions in humans, to our knowledge, has not been reported and may be the key to explaining the endocrine–immune

---

**FIGURE 2**—Circulating IGF-1 before and after resistance exercise. Data are covaried by a PRE value of 72.02 ng mL⁻¹. *Indicates P < 0.05 for time point from PRE (CWI, solid line; CON, dashed line).

**FIGURE 3**—A, MFI of CD221+ (IGF-1R) on circulating granulocytes before and after resistance exercise. Data are covaried by a PRE value of 4724.61. B, Percent CD221+ (IGF-1R) granulocytes before and after resistance exercise. Data are covaried by a PRE value of 31.58%. *Indicates P < 0.05 from PRE (CWI, solid line; CON, dashed line).
interactions in muscle recovery. Because IGF-1 has been previously shown to be protective against muscle damage (11) and beneficial for regeneration (44), we hypothesized that the stress of the resistance exercise would result in altered cellular expression of IGF-1R that may be attenuated by CWI treatment after exercise. The main finding of this investigation was that resistance exercise seems to increase IGF-1R expression on monocytes and granulocytes, supporting the evidence for IGF-1’s role in communicating with immune cells in muscle tissue repair. In addition, CWI therapy after resistance exercise did not seem to alter IGF-1R expression on monocytes and granulocytes. However, CWI therapy after resistance exercise seemed to alter IGF-mediated responses on lymphocytes, the immune cells that are important for the latter stages of tissue repair and immunosuppression observed after injury (35).

Circulating IGF-1 somewhat increased in response to the acute resistance exercise protocol on day 1. Similar to our findings, previous research has also reported that resistance exercise results in marginal increases in circulating IGF-1 levels in men (28). Interestingly, some research has also shown that the effects of resistance exercise on IGF-1 seem to be in the partitioning of IGF-1 among its binding proteins (28). This work has suggested that the bioavailability of IGF-1 in response to resistance exercise may be more attributable to changes in its circulating binding proteins than the free or total IGF-1 in circulation (28). Regardless of the mechanism for increased circulating IGF-1 in response to exercise, the observed increase in circulating IGF-1 at IP suggests that it may have a role in subsequent anabolic muscle regeneration processes. IGF-1 is known to act as a stimulator of both the skeletal muscle regeneration and hypertrophy (26) via the activation of satellite cells (27). However, in order for IGF-1 to stimulate myofiber hypertrophy, mechanical loading seems necessary (5). The inflammatory process triggered by muscle-damaging exercise may help explain this connection. Muscle-damaging exercise triggers the release of chemotactic factors to recruit leukocytes (neutrophils and macrophages),

![FIGURE 4](http://www.acsm-msse.org)

**FIGURE 4**—A, MFI of CD221+ (IGF-1R) on circulating monocytes before and after resistance exercise. Data are covaried by a PRE monocyte MFI value of 4514.42. B, Percent of CD221+ (IGF-1R) monocytes before and after resistance exercise. Data are covaried by a PRE value of 6.06%. *Indicates $P < 0.05$ from PRE (CWI, solid line; CON, dashed line).

![FIGURE 5](http://www.acsm-msse.org)

**FIGURE 5**—A, MFI of CD221+ (IGF-1R) on circulating lymphocytes before and after resistance exercise. Data are covaried by a PRE value of 1621.36. B, Percent of CD221+ (IGF-1R) lymphocytes before and after resistance exercise. Data are covaried by a PRE value of 42.63%. *Indicates $P < 0.05$ from PRE. #Indicates $P < 0.05$ from PRE in the CWI group (CWI, solid line; CON, dashed line).
which both remove the damaged tissue and activate satellite cells to repair the tissue (10,40). Most leukocyte sub-populations express IGF-1R (9), and previous research has shown that IGF-1 mediates muscle repair and regeneration by attenuating chronic inflammatory response (26). Thus, taken together, in addition to its role in muscle hypertrophy, IGF-1 may be an important mediator of the inflammatory cascade to repair muscle tissue in response to exercise. However, although CWI therapy was expected to attenuate the inflammatory response after resistance exercise, it did not seem to alter the circulating IGF-1 response in the present study. Thus, in the present study, CWI treatment did not seem to alter the acute endocrine response to resistance exercise.

Granulocytes. MFI of CD221 (relative expression of IGF-1R) on granulocytes increased at 30P and returned to preexercise levels by 24H. At the same time, the percent of granulocytes expressing IGF-1R similarly increased at 30P and returned to preexercise levels by 24H. Both the observed increased expression of the IGF-1 receptor and increased number of cells expressing the IGF-1 receptor after exercise increase the potential for IGF-1–mediated responses in granulocytes after exercise. Although the mechanisms for the interaction of IGF-1 with granulocytes require further investigation previous research has shown that IGF-1 may be important in promoting granulocyte activity in several ways. IGF-1 has been shown to increase the longevity of granulocytes by inhibiting their spontaneous apoptosis (18). In addition, IGF-1 has been reported to enhance other granulocyte functions such as phagocytosis, degranulation, and oxidative burst (6). Moreover, previous work has shown that IGF-1 enhances interleukin 8 (IL-8) cytokine secretion by peripheral blood mononuclear cells (18). Because granulocytes produce large amounts of IL-8 (8), it may be that the role of increased expression of IGF-1R is to help mediate the secretion of IL-8, an important cytokine for neutrophil accumulation in muscle repair (3). Thus, although speculative, our observed increase in IGF-1R on granulocytes after resistance exercise may be important for promoting granulocyte longevity and activity mediated by IGF-1.

CWI treatment did not seem to affect the MFI of CD221 on granulocytes or the percent of granulocytes expressing IGF-1R compared with control after exercise. We had expected to see an attenuation in IGF-1R expression in the cold water treatment group because granulocytes (mainly composed of neutrophils) are still being recruited to the damaged tissue if the damage is sustained at 24H and 48H (24) and cold may modulate damage by reducing the inflammatory response intensity and preserving morphology (33). Nevertheless, the observed reduction in the percent of granulocytes expressing IGF-1R and percent of CD221+ granulocytes at 24 and 48 h from 30P in both groups could mean that subsequent inflammatory actions of IGF-1 occurred within the first 24 h during recovery. Considering that this is the first study to attempt to explore the time sequence, future studies incorporating more time points during the first day of the recovery process may provide further insights.

Monocytes. MFI of CD221 (relative expression of IGF-1R) on monocytes increased at 30P and returned to preexercise levels by 24H. Increased expression of the IGF-1R on monocytes increases the availability for IGF-1's interaction with its receptor to mediate a response. Thus, our data would support that at 30P, there is an increased potential for IGF-1–mediated responses in circulating monocytes. Monocytes are immune cells in circulation fated to become macrophages once they enter the damaged tissue (34). Once they infiltrate the damaged tissue, macrophages play an important role in phagocytosis (25). Previous research has shown that IGF-1 can enhance phagocytosis and the oxidative burst of monocytes (4). Thus, it may be that the purpose of increased expression of IGF-1R on monocytes at 30P is to “prime” these future macrophages in their phagocytic roles once they enter the tissue.

Interestingly, at the same time that the relative expression of IGF-1R was increasing, the percent of monocytes expressing the IGF-1R decreased at IP and 30P. While the percent of CD221+ positive monocytes (approximately 5%) is relatively low compared with that of granulocytes (approximately 35%) and lymphocytes (approximately 40%) in the present study, it seems that the decrease in the percent of CD221+ (expressing the IGF-1R) monocytes may be due to the interaction of IGF-1 with its receptor on monocytes. If IGF-1 is interacting with its receptor, the percentage of monocytes expressing IGF-1R would potentially decline. This may explain the decline in CD221+ monocytes. In addition, the decrease in CD221+ monocytes from circulation may be due to their movement into the tissue in response to the exercise to elicit their local effects. When muscle damage occurs, injured skeletal muscle recruits monocytes for phagocytosis and for stimulation of myogenesis and muscle fiber growth (2). Previous research has shown that intense exercise lasting for only 1 min can significantly increase the monocytes in circulation (37) partly because of the exercise-induced elevations in catecholamines (13,37). However, monocye/macrophage infiltration does not seem to be evident until 90 min to 24 h after tissue damage (13). In addition, although CWI treatment was hypothesized to affect circulatory responses and thus immune cells in circulation, no differences in IGF-1R expression on monocytes were observed in those receiving CWI treatment after exercise compared with those receiving no treatment. Thus, CWI effects for recovery likely act on pathways other than IGF-1–mediated monocyte responses. Because this is the first study, to our knowledge, to evaluate IGF-1R on monocytes in response to acute exercise, further studies are needed to elaborate on our results to determine the specific monocytes that are responsible for this effect.

Lymphocytes. MFI of CD221 on lymphocytes increased at IP. At the same time, the percent of lymphocytes expressing IGF-1 increased at IP. Increased relative expression of IGF-1R on lymphocytes and percent of lymphocytes expressing IGF-1 at IP would increase the opportunity for IGF-1–mediated responses in these immune cells. During the muscle repair processes, lymphocytes are typically not
recruited into the muscle until several days after exercise (10,40). However, IGF-1 has been shown to influence the development and function of T and B lymphocytes in earlier stages of lymphocyte involvement (17,20). Considering that IGF-1 has been shown to enhance lymphopoiesis, cell proliferation, and cell survival (9,19), IGF-1–mediated responses on lymphocytes may be occurring before the lymphocytes infiltrate the damaged tissue. In support of the delayed response in tissue repair, previous research has shown that IGF-1 is involved in the initiation of T lymphocyte activation (7). In addition, previous studies have shown that IGF-1 both inhibits apoptosis of activated T lymphocytes (42) and acts as a chemotactic factor for T lymphocytes (39). Thus, it is likely that the early increases in IGF-1R on lymphocytes observed in the present study are indicative of acute IGF-1–mediated interactions with lymphocytes that are “informing” the cells early of their subsequent responsibilities in the regeneration cascade.

Interestingly, CWI treatment immediately after acute resistance exercise seemed to increase the MFI of CD221 on lymphocytes at 30 min and 24H whereas those receiving no treatment had levels return to preexercise levels by 30P. The physiological significance of this difference is not known but likely indicates differences in IGF-1–mediated actions in lymphocytes after CWI treatment. Because CWI is known to reduce local blood flow (14), attenuate pro-inflammatory cytokine signaling (32,41), and decrease leukocytosis (30), CWI treatment may have altered the population of lymphocytes in circulation after exercise. However, both activated and inactivated lymphocytes have been shown to contain functional IGF-1R (39). Although the subsequent effects on muscle regeneration remain to be elucidated, differences in IGF-1R expression may mediate lymphocyte action through chemotaxis and lymphocyte growth (39). In addition, because lymphocytes are not required for the initiation of repair but are essential for a normal outcome of tissue repair (35), the cold water treatment may alter the repair outcome. Although it is unclear whether the alteration is ultimately beneficial or detrimental particularly because our study only provides limited snapshots in time during the recovery process, enhanced lymphocyte action may potentially facilitate repair outcomes.

Although this investigation is the first, to our knowledge, to report on the IGF-1R expression on immune cells to an acute resistance exercise stress, it is important to interpret the results within the specific context of this study and to consider the potential limitations. First, this investigation sought to explore the potential mechanisms of IGF-1’s interaction with immune cells. Although a novel and relevant question, it is important to keep in mind that several other hormones and cytokines contribute to the entire scope of how immune cells respond to tissue damage. In addition, it is important to acknowledge that IGF-1 functions as a system containing various binding proteins, which determine its bioavailability. Thus, results are intended to contribute evidence to the larger paradigm of endocrine–immune interactions. Second, as the first study of its kind, we attempted to somewhat globally identify immune cell populations as granulocytes, monocytes, and lymphocytes. However, it is important to consider that within each leukocyte subpopulation, there are various cells with specialized functions. Although our data may drive hypotheses for future work, further investigation is needed to more closely examine targeted immune cell populations. Finally, interpreting immune data in human volunteers is complex because of the heterogeneity of data. Thus, we opted to interpret the present data using covariate analysis with a more lenient P value of 0.10 to reduce the likelihood of missing a potentially relevant change. Because optimal statistic tools to evaluate heterogeneous data are a topic of debate, we opted to report all P values to allow different interpretations of the data.

In conclusion, changes in IGF-1 and its receptor expression on leukocyte populations seem to be part of the mechanism that helps facilitate recovery from resistance exercise. Resistance exercise seems to increase IGF-1R expression on monocytes and granulocytes, suggesting that IGF-1 likely mediates their role in the earlier stages of muscle recovery. Thus, in addition to its anabolic role, IGF-1 seems to communicate with the immune system via cellular receptors to facilitate recovery. In addition, CWI treatment after resistance exercise seems to increase the potential for IGF-1–mediated responses on lymphocytes. Whether this increase is ultimately favorable or detrimental to muscle recovery outcomes remains unclear. However, the observed increase in potential for IGF-1–mediated responses on lymphocytes may contribute to the mechanisms underlying the previously reported benefits of CWI treatment for recovery. Because this study aimed to explore for the first time the in vivo potential for IGF-1 interaction with leukocytes during recovery from resistance exercise, further work is needed to elucidate the outcomes of these effects.

This research was supported by the Young Investigator Grant awarded by the National Strength and Conditioning Association. The authors have no conflicts of interest to report. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

REFERENCES

4. Balteskard L, Unneberg K, Halvorsen D, Hansen JB, Revhaug A. Effects of insulin-like growth factor I on neutrophil and monocyte...


