

Systemic inflammatory responses to maximal versus submaximal lengthening contractions of the elbow flexors.

Muscle damage and systemic inflammation

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ABSTRACT

We compared changes in markers of muscle damage and systemic inflammation after submaximal and maximal lengthening muscle contractions of the elbow flexors. Using a cross-over design, 10 healthy young men not involved in resistance training completed a submaximal trial (10 sets of 60 lengthening contractions at 10% maximum isometric strength, 1 min rest between sets), followed by a maximal trial (10 sets of three lengthening contractions at 100% maximum isometric strength, 3 min rest between sets). Lengthening contractions were performed on an isokinetic dynamometer. Opposite arms were used for the submaximal and maximal trials, and the trials were separated by a minimum of two weeks. Blood was sampled before, immediately after, 1 h, 3 h, and 1-4 d after each trial. Total leukocyte and neutrophil numbers, and the serum concentration of soluble tumor necrosis factor- α receptor 1 were elevated after both trials ($P < 0.01$), but there were no differences between the trials. Serum IL-6 concentration was elevated 3 h after the submaximal contractions ($P < 0.01$). The concentrations of serum tumor necrosis factor- α , IL-1 receptor antagonist, IL-10, granulocyte-colony stimulating factor and plasma C-reactive protein remained unchanged following both trials. Maximum isometric strength and range of motion decreased significantly ($P < 0.001$) after both trials, and were lower from 1-4 days after the maximal contractions compared to the submaximal contractions. Plasma myoglobin concentration and creatine kinase activity, muscle soreness and upper arm circumference all increased after both trials ($P < 0.01$), but were not significantly different between the trials. Therefore, there were no differences in markers of systemic

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inflammation, despite evidence of greater muscle damage following maximal versus submaximal lengthening contractions of the elbow flexors. (Exerc. Immunol. Rev. 12, 2006: 72-85)

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INTRODUCTION

Lengthening contractions (eccentric exercise) cause damage in skeletal muscle. This damage is demonstrated by histological evidence of transient alterations in the content and position of myofilaments within skeletal muscle (1, 7, 35, 36). Other markers of muscle damage that have been used include loss of muscular strength, reduced range of motion, muscle soreness and swelling, and elevated blood concentrations of muscle proteins (e.g., creatine kinase, myoglobin) (14, 16-19, 21, 31). Among these latter markers of muscle damage, muscular strength and range of motion are deemed to be the most valid and reliable (34). The extent of muscle damage after lengthening contractions appears to relate to mechanical loading of muscle during exercise. Compared with heavy loading (100% maximum voluntary force), light loading ($\leq 50\%$ maximum voluntary force) causes a smaller loss of muscular strength (18, 19).

Lengthening muscle contractions elicit an inflammatory response. Within skeletal muscle, pro-inflammatory cytokines are produced, and phagocytic cells invade damaged muscle tissue (for review see ref. (22)). Within the systemic circulation, the complement cascade is activated, leukocytes are mobilized, and cytokine concentrations increase (22). These inflammatory responses share some similarities with the responses to trauma injury (8). Many of the original studies that examined the relationship between contraction-induced muscle injury and inflammation were performed using animals. However, electrically-stimulated muscle contractions likely produce different contractile responses than do voluntary contractions. Consequently, the results of these animal studies cannot necessarily be applied to humans (15).

The inflammatory response to contraction-induced injury may be proportional to the severity of muscle damage, which in turn is dependent on mechanical loading of muscle during exercise. This relationship is important because the magnitude of the inflammatory response regulates adaptation to muscle injury (32). Few studies have directly examined the relationship between inflammation and the degree of muscle damage after lengthening contractions. There is indirect evidence from studies demonstrating that adaptation to repeated bouts of lengthening contractions is characterized by less muscle damage (as indicated by plasma creatine kinase activity), lower blood neutrophil counts, and reduced expression of cell surface adhesion molecules (27, 29). Furthermore, muscle damage after lengthening contractions (as indicated by loss of muscular strength) correlates with blood leukocyte counts and serum C-reactive protein concentration (21). More direct evidence of a relationship between inflammation and the degree of muscle damage comes from studies that modulated the degree of muscle damage by imposing different mechanical loads. For example, in a study of runners, running downhill caused greater muscle damage (as indicated by plasma creatine

	PRE	POST	1 h	3 h	1 d	2 d	3 d	4d
Total leukocytes (cells × 10 ⁹ /l)								
Submaximal	6.4 (1.6)	6.9 (1.7)	7.2 (2.6)	8.1 (2.7) *	6.8 (1.6)	6.8 (1.6)	7.4 (1.8)	5.9 (2.0)
Maximal	6.6 (1.3)	7.4 (2.3)	7.4 (1.9)	8.3 (2.0) *	7.0 (1.8)	6.6 (1.0)	7.0 (1.5)	6.8 (2.1)
Neutrophils (cells × 10 ⁹ /l)								
Submaximal	3.7 (1.3)	4.0 (1.4)	4.7 (2.6)	5.2 (2.7) *	4.2 (1.4)	4.2 (1.2)	4.6 (1.7)	3.3 (1.5)
Maximal	3.8 (1.1)	4.6 (2.1)	4.6 (1.9)	5.1 (1.8) *	4.2 (1.5)	4.0 (1.2)	4.2 (1.1)	4.1 (1.7)
Lymphocytes (cells × 10 ⁹ /l)								
Submaximal	1.9 (0.6)	2.0 (0.5)	2.1 (0.9)	2.0 (0.5)	1.9 (0.5)	1.9 (0.6)	2.0 (0.5)	1.8 (0.7)
Maximal	1.9 (0.4)	1.9 (0.4)	2.0 (0.4)	2.3 (0.4)	1.9 (0.5)	1.9 (0.5)	1.0 (0.5)	1.9 (0.5)
Monocytes (cells × 10 ⁹ /l)								
Submaximal	0.5 (0.2)	0.5 (0.3)	0.6 (0.5)	0.6 (0.3)	0.5 (0.3)	0.5 (0.3)	0.5 (0.3)	0.5 (0.3)
Maximal	0.5 (0.2)	0.6 (0.2)	0.6 (0.2)	0.7 (0.2)	0.6 (0.2)	0.5 (0.1)	0.6 (0.2)	0.6 (0.3)

Data are means (SD). * $P < 0.05$ versus pre-exercise values.

Table 1. Leukocyte counts before (PRE), immediately after (POST), 1 h, 3 h, and 1-4 days after submaximal and maximal eccentric exercise.

kinase activity), mobilization of neutrophils and lymphocytes, and activation of T-lymphocytes than did level running (30). In contrast, a more recent study found that although muscle damage was greater following downhill running at -8° versus -4° , the two exercise protocols did not differ in their effects on markers of inflammation, such as blood leukocyte counts, cytokine concentrations, and immunohistochemical staining for leukocytes and cytokines in skeletal muscle and epimysium (16). Therefore, evidence for a relationship between inflammation and the muscle degree of damage following exercise is equivocal.

Interleukin (IL)-6 plays an important role in mediating inflammatory responses to exercise. IL-6 is believed to exert anti-inflammatory effects during exercise by inhibiting the production of the pro-inflammatory cytokine tumor necrosis factor (TNF)- α , and stimulating the synthesis of other anti-inflammatory cytokines such as IL-1 receptor antagonist (IL-1ra), IL-10 and soluble TNF- α receptor 1 (sTNF- α R1) (25). IL-6 is produced within, and released from skeletal muscle during exercise in response to glycogen depletion, calcium signaling, changes in blood glucose availability, and to a lesser extent sympathetic activation (9, 11, 13). During concentric exercise, IL-6 release from contracting muscle is dependent on exercise intensity (9). Several studies have compared changes in IL-6 gene expression and plasma IL-6 concentration after shortening versus lengthening muscle contractions (2, 12, 24). However, data from Malm et al. (16) relating to the relationship between the extent of muscle damage and changes in serum IL-6 concentration after downhill running were inconclusive.

The aim of our study was to further investigate the relationship between inflammation and muscle damage after lengthening muscle contractions. We modulated the degree of muscle damage using two different protocols of lengthening muscle contractions using the elbow flexors (18, 19), and compared the inflammatory responses to each protocol. We hypothesized that if the magnitude of the inflammatory response is related to the degree of muscle damage, changes in markers of systemic inflammation such as systemic leukocyte numbers, cytokines, C-reactive protein and calprotectin (marker of neutrophil activation) would be greater after maximal than after submaximal lengthening contractions.

METHODS

Participants

Ten healthy young men volunteered to take part in this study. The mean \pm SD age, body mass and height of the participants were 22.9 ± 4.7 yrs, 76.2 ± 11.8 kg and 1.80 ± 0.08 m, respectively. None of the participants took part in regular resistance training exercise, and all participants were told to avoid taking any anti-inflammatory or pain-killer medication during the study. All subjects completed a medical questionnaire and gave written informed consent prior to the study. The experimental procedure was approved by the Human Research Ethics Committee at Edith Cowan University.

Study design

The study involved a cross-over design. All participants completed the submaximal lengthening contractions before the maximal lengthening contractions. The two trials were separated by a minimum period of two weeks. The use of the dominant versus non-dominant arm for the first trial was randomized and

counterbalanced among the 10 participants. After the submaximal exercise had been completed, the contralateral arm was used for the maximal exercise. We used this design for two reasons: (a) to avoid the prolonged adaptation that results from a single bout of lengthening contractions using one arm, and (b) to reduce the well known variability in markers of muscle damage between individuals. Furthermore, isolated muscle contractions have a low metabolic cost compared to other types of exercise such as downhill running (21). One week prior to any of the actual exercise trials, all participants took part in a familiarization session, during which they were

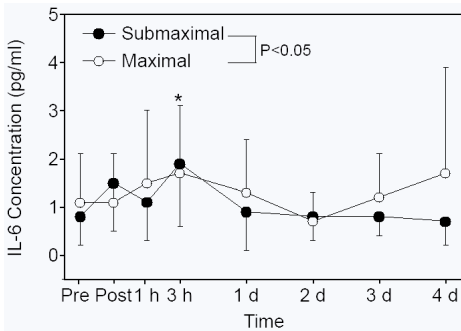


Figure 1: Serum interleukin (IL)-6 concentration before (PRE), immediately after (POST), 1 h, 3 h, and 1 – 4 days after exercise. * $P < 0.05$ significantly different versus pre-exercise values for submaximal exercise. Data are means (SD)

introduced to the testing procedures. Height, body mass, maximal isometric strength, range of motion of the elbow joint, upper arm circumference, and muscle soreness and pain pressure threshold were measured. To establish reliability, the values recorded for maximal isometric strength, range of motion of the elbow joint, upper arm circumference, and muscle soreness and pain pressure threshold were compared with the values taken immediately before the actual exercise.

Exercise protocols

Two exercise protocols were conducted on a preacher curl bench placed alongside an isokinetic dynamometer (Cybex 6000, Lumex Inc., Ronkonkoma, NY, USA).

	PRE	POST	1 h	3 h	1 d	2 d	3 d	4d
IL-1ra (pg/ml)								
Submaximal	238 (88)	266 (80)	262 (170)	256 (130)	235 (81)	240 (105)	234 (72)	207 (61)
Maximal	259 (78)	311 (107)	257 (106)	275 (68)	246 (83)	306 (159)	239 (77)	267 (100)
IL-10 (pg/ml)								
Submaximal	1.7 (1.3)	2.1 (1.9)	2.1 (1.4)	2.0 (1.8)	1.7 (1.3)	1.7 (1.5)	1.7 (1.4)	1.7 (1.5)
Maximal	1.5 (1.4)	1.6 (1.8)	1.7 (1.6)	2.0 (1.4)	2.4 (1.7)	2.3 (1.6)	1.3 (1.0)	2.0 (1.4)
TNF- α (pg/ml)								
Submaximal	1.1 (0.4)	1.3 (0.7)	1.0 (0.4)	1.0 (0.4)	0.9 (0.5)	1.0 (0.3)	0.9 (0.3)	1.3 (0.5)
Maximal	1.5 (1.6)	0.9 (0.3)	1.0 (0.3)	1.0 (0.4)	1.2 (0.4)	1.0 (0.3)	1.1 (0.6)	1.2 (0.7)
G-CSF (pg/ml)								
Submaximal	2.0 (1.5)	2.2 (1.1)	2.5 (1.4)	2.2 (1.0)	2.3 (0.9)	1.7 (0.9)	2.2 (1.0)	1.6 (0.7)
Maximal	2.7 (1.9)	2.4 (1.8)	2.7 (1.7)	3.0 (1.2)	3.3 (1.6)	2.6 (1.3)	2.4 (1.6)	2.8 (2.2)

Data are means (SD).

Table 2. Serum concentrations of interleukin-1 receptor antagonist (IL-1ra), IL-10, tumor necrosis factor (TNF)- α and granulocyte-colony stimulating factor (G-CSF) before (PRE), immediately after (POST), 1 h, 3 h, and 1-4 days after submaximal and maximal eccentric exercise.

The protocol for the submaximal contractions involved 10 sets of 60 lengthening contractions of the elbow flexors of one arm at 10 % of maximal isometric strength. Participants watched a visual representation of their strength output on a computer screen, and were told to match their strength against a line on the screen that corresponded to 10 % of their maximal isometric strength. The protocol for the maximal contractions involved 10 sets of three lengthening contractions of the contralateral arm, in which subjects were asked to maximally resist against the lengthening motion of the dynamometer. The muscle actions in both trials involved extending the elbow joint from a starting angle of 120° down to a fully extended angle of 180°. The angular velocity for both protocols was 30°/s. The rest intervals between sets for the submaximal and maximal trials were 1 min and 3 min, respectively. Two seconds of passive recovery were allowed between each contraction as the mechanical arm of the dynamometer returned the participants' arm to 120°. The total muscle activation time for the submaximal contractions was 20 min (2 s x 60 contractions x 10 sets), whereas the total muscle activation time for the maximal contractions was 2 min (2 s x 3 contractions x 10 sets). The total work completed during the submaximal and maximal contractions was 6791 \pm 187 J and 1288 \pm 36 J, respectively.

Markers of muscle damage

The following variables were measured before exercise, immediately after, 3 h, 1 d, 2 d, 3 d and 4 d after exercise: maximal isokinetic torque at 30, 180 and 300°/s, isometric torque at 90°, range of motion, upper arm circumference (swelling), muscle soreness on palpation and pain pressure threshold. The test-retest reliability of these measures was established by an intraclass correlation coefficient (R) comparing the values from familiarization session and pre-exercise measurements. R-value for maximal isometric strength, range of motion, upper arm circumference, muscle soreness and pain pressure threshold was 0.94, 0.90, 0.89, 0.95, and 0.88, respectively.

Maximal isometric strength was used, as opposed to maximal eccentric strength because testing of eccentric strength itself causes some muscle damage. The changes in maximal isokinetic strength at all three angular velocities (30, 180 and 300°/s) were very similar to the change in isometric torque at 90°. Therefore,

we chose to represent changes in muscular strength only by reporting data for isometric torque at 90°. Range of motion was calculated as the difference between flexed and extended elbow joint angles. Upper arm circumference was measured at a point 11 cm beyond the elbow joint. To assess muscle soreness, the elbow flexors were palpated at the mid-belly of biceps brachii. Participants were asked to rate the soreness on a visual analog scale that had a 100-mm line with "no pain" on one end and "extremely painful" on the other. Pain pressure threshold was assessed using a pressure algometer (Type II, Somedic Production AB, Sollentuna, Sweden). For this assessment, the probe head (1 cm²) of the algometer was placed perpendicular to the mid-belly of biceps brachii, and force was gradually applied at a rate of 50 kPa per second until the participant reported the first feeling of noticeable pain. The value (in kPa) corresponding to the amount of force applied was then recorded. All measurements of strength, range of motion, upper arm circumference, muscle soreness and pain pressure threshold were performed twice. The data presented in the results represent the average of the two numbers.

Blood sampling

Venous blood samples were drawn from a forearm vein before exercise, immediately after, 1 h, 3 h, 1 d, 2 d, 3 d and 4 d after exercise. Due to demands on the participants' time on the day of exercise, it was not possible to collect blood samples between 3 h and 1 d after exercise. Blood was collected into sterile vacutainers containing K₂-EDTA (Becton Dickinson, Franklin Lakes, NJ, USA) or serum separation tubes. Before the K₂-EDTA tube was centrifuged, 1 ml blood was removed to obtain a complete blood cell count. The K₂-EDTA tube was then centrifuged for 10 min at 1000 × g at 4°C to obtain plasma. The serum separation tube was left at room temperature for the blood to clot, and then centrifuged for 10 min at 1000 × g at 4°C. The plasma and serum samples were stored in 0.7-ml aliquots at -80°C until the day of analysis.

Blood analysis

EDTA-treated whole blood was analysed to obtain a complete blood cell count using a Beckman Coulter-Counter, Gen-S (France SA, Villepinte, France). Plasma samples were analyzed for myoglobin, calprotectin and C-reactive protein concentrations, in addition to CK activity. Myoglobin was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (BioCheck, Foster City, CA, USA). Calprotectin was also measured by ELISA (HyCult Biotechnology, Uden, The Netherlands). CK was measured using an automated analyser (Model 7450 or Model 7170, Hitachi, Japan). Plasma C-reactive protein concentration was measured using an immunoturbidimetric assay (Kamaya Biomedical Company, Seattle, WA, USA) on an automated analyzer (Cobas Mira, Roche Diagnostics, Indianapolis, IN, USA). Serum samples were analyzed by ELISA for IL-1ra, IL-6, TNF-α (Quantikine® High Sensitivity ELISA, R&D Systems, Minneapolis, MN, USA), sTNF-αR1, IL-10 (Opt EIA, Becton Dickinson, San Diego, CA, USA) and granulocyte-colony stimulating factor (G-CSF) (Immuno Biological Laboratories, Gunma, Japan). ELISA measurements were performed using a microplate reader (VERSAmix, Molecular Devices, Sunnyvale, CA, USA). The coefficient of variation for duplicate measurements using ELISA was <6%. Leukocyte counts were

adjusted for percentage changes in blood volume, whereas plasma and serum variables were adjusted according to percentage changes in plasma volume, as calculated from hemoglobin and hematocrit (5).

Statistical analysis

Data are presented means \pm SD. All data were checked for normal distribution, and when necessary the data were log transformed to obtain a normal distribution before further statistical analysis. The data requiring log transformation included leukocyte and neutrophil counts, IL-1ra, IL-6, IL-10, C-reactive protein and CK. The data for upper arm circumference and muscle soreness could not be log transformed to follow a normal distribution. Therefore, these data were analyzed non-parametrically using Friedman's repeated measures analysis of variance and sign ranked t-tests. The data for strength, range of motion and

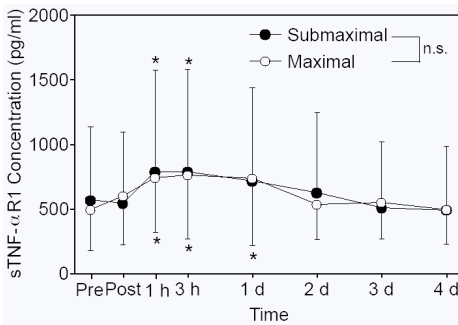


Figure 2: Serum soluble tumor necrosis factor (TNF)- α receptor 1 (sTNF- α R1) concentration before (PRE), immediately after (POST), 3 h, and 1 – 4 days after exercise. * $P < 0.05$ significantly different versus pre-exercise values for the maximal trial. Data are means (SD).

pain pressure threshold were analyzed using a 2 (trials) \times 7 (time points) repeated measures analysis of variance. The blood data were analyzed using a 2 (trials) \times 8 (time points) repeated measures analysis of variance. Statistical significance was set at $P < 0.05$ for the main effects of time and time \times trial interactions. Student's paired t-tests were used to compare differences between the trials and individual time points. The false discovery rate procedure was used for these multiple comparisons (4). Statistical analysis was performed using SigmaStat 3.1 software (System, Point Richmond, CA, USA).

RESULTS

Leukocyte numbers

The number of total leukocytes and neutrophils increased after both trials (time effect $P = 0.0005$), reaching a peak at 3 h after exercise (Table 1). There were no differences between the trials. There were no significant changes in either lymphocyte (time effect $P = 0.23$) or monocyte numbers (time effect $P = 0.17$) after either trial.

Serum cytokine concentrations

Serum IL-6 concentration increased after exercise (Figure 1). The pattern of changes in serum IL-6 was different between the trials (interaction effect $P = 0.044$); serum IL-6 was elevated 3 h after the submaximal contractions, but not after the maximal contractions. The serum concentration of sTNF- α R1 also increased significantly after exercise (time effect $P = 0.0001$), but there were no

differences between the trials (Figure 2). Serum IL-1ra concentration tended to increase after exercise ($P = 0.068$) (Table 2), while the serum concentrations of IL-10 (time effect $P = 0.36$), TNF- α (time effect $P = 0.48$) and G-CSF (time effect $P = 0.36$) remained unchanged after exercise (Table 2).

Plasma CK activity, myoglobin calprotectin and C-reactive protein concentrations

Plasma myoglobin concentration and CK activity increased after exercise (time effect $P = 0.001$) (Table 3), but there were no differences between the trials. There were no significant changes in plasma calprotectin or C-reactive protein concentrations after either trial (Table 3).

	PRE	POST	1 h	3 h	1 d	2 d	3 d	4 d
Myoglobin (ng/ml)								
Submaximal	27 (10)	28 (13)	55 (26) *	55 (31) *	49 (52)	54 (68)	28 (13)	43 (51)
Maximal	37 (21)	45 (31)	80 (50) *	60 (31) *	43 (21)	41 (27)	57 (21) *	65 (45) *
CK (IU/l)								
Submaximal	248 (165)	269 (174)	258 (168)	276 (164)	337 (220)	364 (419)	492 (818)	836 (1546) *
Maximal	342 (310)	352 (326)	355 (334)	356 (295)	389 (286)	420 (295)	469 (248) *	770 (589) *
CRP (mg/l)								
Submaximal	0.2 (0.3)	-	-	-	0.2 (0.1)	0.2 (0.2)	0.5 (1.0)	0.5 (1.3)
Maximal	0.5 (0.7)	-	-	-	0.7 (0.8)	0.7 (0.9)	0.5 (0.5)	0.5 (0.8)
Calprotectin (ng/l)								
Submaximal	3.9 (2.7)	4.9 (2.7)	3.8 (1.8)	4.4 (3.1)	4.7 (3.2)	4.2 (2.2)	2.7 (1.5)	3.3 (2.0)
Maximal	2.6 (1.9)	2.8 (1.8)	2.9 (1.8)	2.6 (2.6)	4.0 (2.6)	3.5 (2.5)	2.8 (2.2)	2.3 (1.5)

Data are means (SD). * $P < 0.05$ versus pre-exercise values.

Table 3. Plasma myoglobin concentration, plasma creatine kinase (CK) activity, C-reactive protein (CRP) and calprotectin concentrations before (PRE), immediately after (POST), 1 h, 3 h, and 1-4 days after submaximal and maximal eccentric exercise.

	PRE	POST	3 h	1 d	2 d	3 d	4 d
Range of motion (degrees)							
Submaximal	133 (5)	118 (9) *	124 (10) *	126 (5) *	129 (5)	130 (6)	131 (5)
Maximal	138 (4)	126 (5) *	130 (6) *	128 (8) *	127 (9) *	129 (11) *	128 (16) *
Upper arm circumference (mm)							
Submaximal	279 (32)	287 (32) *	282 (31)	285 (32)	287 (33) *	286 (32)	286 (32)
Maximal	285 (32)	289 (31)	288 (33)	291 (32) *	292 (32) *	291 (31) *	291 (31)
Muscle soreness (mm)							
Submaximal	0 (0)	0 (0)	9 (16)	15 (8) *	15 (7) *	7 (9)	3 (4)
Maximal	0 (0)	0 (0)	0 (0)	14 (11)	21 (17) *	25 (21) *	18 (19) *
Pain pressure threshold (kPa)							
Submaximal	394 (81)	410 (132)	392 (112)	238 (91) *	248 (110) *	292 (93) *	362 (126)
Maximal	339 (94)	367 (82)	376 (80)	242 (87) *	232 (82) *	278 (138)	316 (146)

Data are means (SD). * $P < 0.05$ versus pre-exercise values. N.B. Participants rated their muscle soreness on a 100-mm visual analog scale ranging from "no pain" to "extremely painful".

Table 4. Range of motion, upper arm circumference (swelling), muscle soreness assessed by palpation of the biceps and pain pressure threshold before (PRE), immediately after (POST), 3 h, and 1-4 days after submaximal and maximal eccentric exercise.

Strength and range of motion

Maximal isometric strength decreased after exercise, and the pattern of changes was different between the trials (interaction effect $P < 0.0001$) (Figure 3). Strength decreased by 30–40% immediately after both trials. No further strength decrement was evident 3 h after the submaximal exercise, whereas strength continued to decrease at this time after the maximal exercise. At 1 d after exercise, strength remained below pre-exercise values for both trials; however, strength was lower at this time following the maximal exercise when compared to the submaximal exercise. Whereas strength had almost returned to the baseline 2 d after the submaximal exercise, strength remained ~30% below pre-exercise values at 4 d after the maximal exercise. Range of motion at the elbow joint decreased by ~10% immediately after exercise, and the pattern of changes was different between the trials (interaction effect $P = 0.002$) (Table 4). Whereas range of motion had returned towards normal 2 d after the submaximal exercise, it remained lower than normal at 4 d after the maximal exercise.

Upper arm circumference, soreness and pain pressure threshold

As an indicator of swelling, upper arm circumference increased after exercise (time effect $P < 0.0001$) (Table 4), but there were no differences between the two trials. When biceps brachii was palpated, the participants reported greater muscle soreness after exercise (time effect $P < 0.0001$) (Table 4). Participants were also more sensitive to pain when force was applied against their biceps brachii at 1, 2 and 3 d after both trials (Table 4). Neither muscle soreness nor pain pressure threshold was different between the trials.

DISCUSSION

The aim of this study was to compare systemic inflammatory responses to submaximal versus maximal lengthening contractions. Our data indicate that there were no significant differences in systemic markers of inflammation, despite evidence of greater muscle damage (as indicated by impaired muscular strength) following the maximal versus submaximal contractions. The magnitude of systemic inflammation was relatively small compared to lengthening contractions involving multiple and/or larger muscle groups. This factor may account for the lack of differences between submaximal and maximal contractions. It is also possible the maximal contractions caused greater local inflammation within skeletal muscle that were not reflected by the systemic changes.

Elevated blood neutrophil counts are a consistent finding after lengthening contractions of the elbow flexors (17, 27–29). Neutrophils are most likely mobilized from endothelial surfaces into the circulation in response to tissue injury such as exercise-induced muscle damage. Once in the circulation, neutrophils travel to the site of injury, and then bind and break down damaged tissue fragments. In our study, neutrophil numbers were highest 3 h after exercise. Unfortunately, due to constraints on the time availability of the participants we could not obtain blood samples to assess neutrophil numbers in the period between 3 h and 1 d after exercise. Others have reported that neutrophil numbers peak up to 12 h after lengthening contractions of the elbow flexors (27–29). In contrast

to our findings, some studies have also found a secondary increase in blood neutrophils 2 d after exercise (17, 29). We used untrained participants and a similar protocol to these studies for the maximal lengthening contractions. Therefore, it is difficult to explain why this secondary increase did not occur in our study.

We hypothesized that blood neutrophil or total leukocyte numbers would be higher after the maximal contractions. This hypothesis was based on data from several studies indicating that (a) a reduction in muscle damage (plasma CK activity) was accompanied by lower blood neutrophil counts (27, 29, 30), and (b) muscle damage (decline in muscular strength) correlated with blood leukocyte counts (21). However, Malm et al. (16) also reported no significant differences in blood leukocyte numbers after downhill running at -8° versus -4° . Taken together, these findings suggest that the leukocytosis following lengthening contractions is not always proportional to the degree of muscle damage.

Compared with studies of endurance exercise, relatively few studies have investigated changes in systemic cytokine concentrations after lengthening contractions using the elbow flexors. Two studies have reported that plasma IL-6 concentration increased after three sets of 10 maximal lengthening contractions of the elbow flexors (3, 26). We previously found no significant change in plasma IL-6 concentration following six sets of five lengthening contractions of the elbow flexors at 40% maximum voluntary force, despite evidence of muscle damage (10). The changes in serum IL-6 concentration that we observed in the present study do not appear to be related to muscle damage. In their comparison of downhill running at different gradients, Malm et al. (16) found no significant change in serum IL-6 concentration. However, this result may have been due to the low sensitivity of the assay used to measure IL-6. The small increase in serum IL-6 concentration after the submaximal contractions could represent the release of IL-6 from muscle in response to muscle glycogen depletion (13). The greater amount of work completed during the submaximal versus the maximal

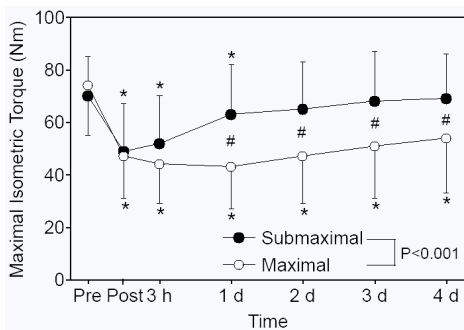


Figure 3: Maximal isometric strength before (PRE), immediately after (POST), 3 h, and 1–4 days after exercise. * $P < 0.05$ significantly different versus pre-exercise values for both trials. # $P < 0.05$ significant difference between submaximal and maximal eccentric exercise. Data are means (SD)

contractions (6791 ± 187 J versus 1288 ± 36 J, respectively) likely caused greater muscle glycogen depletion. Others have reported that the systemic concentration of IL-6 is significantly elevated at 6 h (14, 21), 12 h, 1 d and 3 d (31) after lengthening contractions using larger muscle groups. If we had been able to take blood samples between 3 h and 1 d after exercise in the present study, it is possible that we may have seen similar responses. The use of larger muscle groups may also partially explain these different findings. Other factors such as muscle glycogen depletion, calcium sig-

naling and blood glucose availability also influence changes in plasma IL-6 concentration following exercise (9, 11, 13). But it is unlikely that these factors can explain delayed changes in plasma IL-6 concentration in the hours and days following lengthening muscle contractions.

The delayed increase in the serum concentration of sTNF- α R1 after the maximal lengthening contractions was similar to that seen after marathon running (20) and eccentric cycling (33). sTNF- α R1 may be produced as an anti-inflammatory response (25). Our data suggest that the production of sTNF- α R1 is not dependent on the extent of muscle damage. There were no significant changes in the serum concentrations of IL-1ra, G-CSF, TNF- α or IL-10 after exercise in the present study. These findings contrast with our own study described previously (10), and findings from other groups who have also examined lengthening muscle contractions (14, 21, 31). Because we used maximal lengthening contractions and untrained subjects, it is difficult to explain these differences.

The plasma concentration of calprotectin remained unchanged after exercise. This result contrasts with the findings of another recent study which reported that plasma calprotectin concentration increases after aerobic exercise (6). Calprotectin is a marker of neutrophil activation. The factors affecting the release of calprotectin from neutrophils during exercise are unknown, but our data suggest that muscle damage is not a strong stimulus for its release. Other neutrophil functions, such as oxidative burst activity, are stimulated by growth hormone and cytokines such as IL-6 and IL-8 (23). Therefore, growth hormone and cytokines may also stimulate the release of calprotectin after strenuous endurance exercise, independently of muscle damage. We and others have also found little or no change in plasma myeloperoxidase concentration (another marker of neutrophil activation) following maximal lengthening contractions of the elbow flexors (3, 10). Taken together, these data suggest that damage resulting from lengthening contractions of small muscle groups does not activate neutrophils, at least in the systemic circulation.

Debate exists regarding the best method for assessing muscle damage. Warren et al. (34) contend that changes in muscular strength and range of motion are valid and reliable indicators of the extent of muscle damage. Our data indicated that from 1-4 d after exercise, muscular strength was significantly lower after the maximal versus submaximal contractions. We interpret this difference in muscular strength as evidence of greater muscle damage following the maximal contractions. The finding that strength decreased to a similar extent immediately after both submaximal and maximal lengthening contractions may reflect a greater contribution of muscle fatigue during the submaximal contractions (19). This idea is supported by the greater total amount of work completed in the submaximal than in the maximal trial.

The alterations in plasma myoglobin concentration and CK activity in our study were smaller than those reported in other studies involving lengthening contractions of the elbow flexors (17-19, 28, 29). We can only speculate about the reasons for this. One possibility is that although the participants in our study were not regularly involved in resistance-type exercise, they could have been more physically active in general than participants in other studies that reported larger changes in myoglobin and CK after lengthening contractions. Participation in general physical activity may prevent large changes in myoglobin and CK follow-

ing lengthening muscle contractions. The small changes in myoglobin and CK in our study may raise the question of whether (a) muscle damage did in fact occur and (b) there were any differences in muscle damage between the submaximal and maximal contractions. However, the minor changes in myoglobin and CK in our study should not be over-emphasized, because changes in these proteins do not always correlate with, or follow the pattern of changes in muscle function following lengthening contractions (34).

In conclusion, the present data indicate that although maximal lengthening contractions of the elbow flexors may result in greater muscle damage, this is not accompanied by a greater systemic inflammatory response. The magnitude of the systemic inflammatory response may differ when comparing lengthening contractions of small muscle groups such as the elbow flexors with larger muscle groups. Furthermore, although we could not detect differences in systemic inflammation, differences could exist locally within skeletal muscle.

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