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RESEARCH ARTICLE

Muscle damage and muscle remodeling: no pain, no gain?

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SUMMARY

Skeletal muscle is a dynamic tissue that responds adaptively to both the nature and intensity of muscle use. This phenotypic plasticity ensures that muscle structure is linked to patterns of muscle use throughout the lifetime of an animal. The cascade of events that result in muscle restructuring – for example, in response to resistance exercise training – is often thought to be initiated by muscle damage. We designed this study to test the hypothesis that symptomatic (i.e. detectable) damage is a necessary precursor for muscle remodeling. Subjects were divided into two experimental populations: pre-trained (PT) and naive (NA). Demonstrable muscle damage was avoided in the PT group by a three-week gradual 'ramp-up' protocol. By contrast, the NA group was subjected to an initial damaging bout of exercise. Both groups participated in an eight-week high-force eccentric-cycle ergometry program (20 min, three times per week) designed to equate the total work done during training between the groups. The NA group experienced signs of damage, absent in the PT group, as indicated by greater than five times higher levels of plasma creatine kinase (CK) and self-reporting of initial perceived soreness and exertion, yet muscle size and strength gains were not different for the two groups. RT-PCR analysis revealed similar increases in levels of the growth factor IGF-1Ea mRNA in both groups. Likewise, the significant (P<0.01) increases in mean cross-sectional area (and total muscle volume) were equal in both groups. Finally, strength increases were identical for both groups (PT=25% and NA=26% improvement). The results of this study suggest that muscle rebuilding – for example, hypertrophy – can be initiated independent of any discernible damage to the muscle.

Key words: eccentric exercise, hypertrophy, creatine kinase, IGF.

INTRODUCTION

Skeletal muscle retains a large degree of phenotypic plasticity throughout the lifetime of an individual, allowing muscle to respond adaptively to changes in both the nature and intensity of muscle use. Hence, repeated bouts of resistance exercise produce compensatory growth (hypertrophy) of skeletal muscle, even late in life (Drummond et al., 2008), characterized by an increase in the cross-sectional area of individual muscle fibers as well as the volume of whole muscle. Because force production is a function of the cross-sectional area of a muscle, the consequence of increased muscle mass is greater force production (strength). Although muscle growth in response to resistance exercise has long been recognized, it is only now that the details of the mechanisms underlying this response are becoming clear.

A key element in muscle growth is the regulation of skeletal muscle protein synthesis, which involves several intracellular signaling pathways (Nadar et al., 2002; Rennie et al., 2004). In particular, the anabolic mediator and myogenic growth factor insulin-like growth factor 1 (IGF-1) is known to be upregulated during muscle hypertrophy. IGF-1 stimulation alone has been shown to be sufficient for induction of skeletal muscle hypertrophy (Coleman et al., 1995; Musaro et al., 2001); moreover, a muscle-specific isoform of IGF-1 – IGF-1Ea – has been described as contributing to muscle regeneration (Chakravathy et al., 2000; Rotwein et al., 1986; Yang et al., 1996).

This study was designed to investigate whether muscle hypertrophy is possible in the absence of the symptoms of damage. This issue becomes most crucial for those individuals who are exercise limited or exercise intolerant owing to existing cardio-pulmonary pathologies. Can interventions be designed for these individuals that do not involve damage sufficient to initiate a repair response (and hence a potentially harmful inflammatory response) as a precursor to muscle hypertrophy?

Since Goldberg and colleagues (Goldberg et al., 1975) first proposed that the development of muscle force is the crucial event in initiating muscle growth in mammals, efforts have been made to identify the 'optimal' exercise regime to maximize muscle force production and the resultant hypertrophy and improved performance. Because the greatest magnitude of muscle force production occurs during lengthening (eccentric) contractions, high-force eccentric exercise might be the most powerful stimulus to induce hypertrophy (Hortobagyi, 2003; LaStayo et al., 2000; LaStayo et al., 2003). Furthermore, this kind of muscle use often results in an initial damaging bout of exercise, which is often thought to be a pre-requisite for the initiation of muscle hypertrophy (Evans and Cannon, 1991; Folland et al., 2002; Goldspink, 2003; Hawke and Garry, 2001; Smith et al., 1999). Indeed, high-force lengthening (eccentric) forces often result in muscle ultrastructural damage (Ebbeling and Clarkson, 1989; Newham, 1988) – however, these need not cause muscle damage (Crameri et al., 2007). In this study,

eccentric exercise was used both to induce damage to the muscle as well as to function as the intervention to provoke muscle growth.

MATERIALS AND METHODS

Participants

All participants consented to 11 weeks of lower-extremity eccentric resistance exercise training (although half would be assigned to an eight-week exercise regime), with the possibility of considerable muscle soreness, and all agreed to refrain from any other exercise. The institutional review board at Northern Arizona University approved the study protocol. Fourteen healthy university students, eight males and six females, were divided into two groups with a composition that equalized age, sex, height, body mass and strength of quadriceps. Participants were excluded from the study if they had engaged in any regular lower-extremity resistance exercise program within the previous year. Quadriceps strength was determined by means of a maximum voluntary isometric contraction (MVIC) of the knee extensors. Thus, the two mixed-gender groups were nearly identical in mean height, mass and quadriceps strength. This allowed us to equalize workloads between the groups as well. Once the two groups were formed, the final assignment of these groups as (1) pre-trained (PT) and (2) naive (NA) occurred by a stochastic process.

Eccentric resistance exercise

All training was performed on a recumbent, high-force, eccentric, leg cycle ergometer (Eccentron; BTE Technologies, Inc., Hanover, MD, USA) (Fig. 1). The ergometer is powered by a three-horsepower motor that drives the pedals in a 'backwards' direction (towards the participant). The participants attempt to resist this motion by pushing on the pedals as they move towards them. Because the magnitude of the force produced by the machine exceeds that of the participant, the pedals continue to move towards the participant at a constant velocity, resulting in lengthening contractions of the knee and hip extensors, including the quadriceps muscles. The resistance applied by the participant (i.e. load on the motor) is displayed along with a pre-set target-level resistance on a monitor facing the participant.

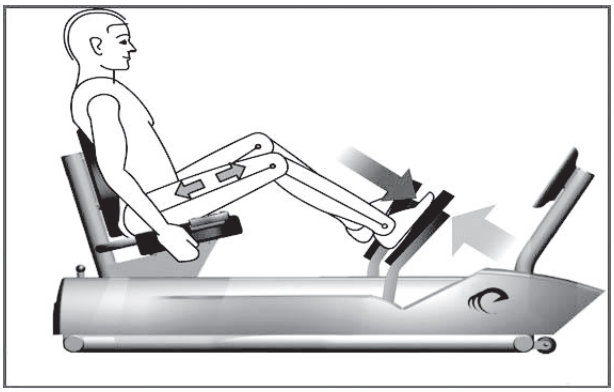

Fig. 1. All resistance exercises were performed on an Eccentron ergometer. The ergometer is powered by a three-horsepower motor that drives the pedals in a backwards (towards the participant, largest arrow) direction while the participant attempts to resist this motion by pushing on the pedals as they move closer (smaller arrows). The result is a lengthening of the quadriceps muscles. The resistance applied by the participants is monitored on a screen facing them, and they try to match a pre-set target-level resistance denoted on the screen. Workout duration, pedal speed, stroke length and 'target' force resistance were all controlled for each training session.

Experimental design

The intent of the experimental design was that the NA subjects would experience demonstrable muscle damage, whereas the PT subjects, by using a slow gradual acclimation to the exercise, would experience no symptoms of structural muscle damage. The initial bouts of exercise thus varied greatly between the two groups: the PT group experiencing three additional weeks of gradual 'ramp-up'. By introducing lengthening contractions progressively, our experience is that individuals can produce high-force chronic eccentric forces with no symptoms of muscle injury or damage. This gradual ramp-up protocol has been used successfully in our laboratory with both young (LaStayo et al., 2000) and frail elderly (LaStayo et al., 2003) participants, to increase force and exercise duration gradually without muscle soreness or detectable signs of muscle damage. Each participant was assigned a unique identifier on the ergometer that allowed us to (1) standardize the progression of each workout session and (2) record and save the data from each exercise bout for each subject.

The PT participants all used a set routine that programmed a gradual increase in duration and resistance intensity over three weeks, increasing the total integrated force generated by the participant slightly above his/her previous workout. The routine began with a workout for 5 min at the lowest intensity level on day one and increased to a duration of 20 min at progressively higher levels, by the end of week three. Perceived exertion was assessed after every training session using the Borg rating of perceived exertion (RPE) scale. This scale starts with a level '6' corresponding to the least effort 'very light' and tops at '20 – maximum exertion'. For the remaining 8 weeks, the participants continued to exercise for 20 min at a perceived exertion level of 'somewhat hard' ('13' on the Borg scale) three times a week.

By contrast, the NA participants were introduced to the experiment in week four; they began and continued to exercise at a 'somewhat hard' exertion level for 20 min three times a week for eight weeks (identical to the PT group following the pre-training). Exercise intensity was the same in both groups during this period. The introduction of high-force lengthening contractions in the NA group was expected to result in both muscle discomfort (initial soreness) and muscle damage. The total amount of (negative) muscle work, integrated over the entire training period (8 or 11 weeks), was designed to be identical for both groups by the end of the study. We did this by calculating a 'total work' integral daily for each subject and normalizing for the groups over the entire exercise period, which included the ramp-up of the PT group. In other words, workloads were adjusted so that participants in both groups did the same cumulative training over the course of the study.

In addition to initial soreness and CK, a key marker of muscle damage, measurements were made in both groups of quadriceps muscle size (i.e. volume), isometric strength and levels of the mRNA encoding the mechano growth factor IGF-1Ea. The volume of the quadriceps was determined by quantitative MRI before and after the study for all participants. In addition, muscle strength was determined before and after training by quantifying MVIC. Perceived muscle soreness and rating of perceived exertion were recorded immediately after all workouts for all subjects. Creatine kinase (CK) was measured pre- and post-training, as well as weekly throughout the study; it served as the primary measure of muscle damage. Finally, biopsies of the vastus lateralis were taken pre-training and at the eighth week of training to measure levels IGF-

1Ea mRNA levels, as this should coincide with the largest upregulation of the growth factors (Bickel et al., 2005; Goldspink, 2005). IGF-1Ea mRNA levels were measured by means of RT-PCR.

Measurement of quadriceps muscle volume

The volume of the thigh was measured using nuclear magnetic resonance imaging (MRI). The image was taken with the participants lying in a supine position with their legs extended and relaxed. All scans were performed on a 1.0-Tesla whole-body MR imager (Signa 9.0; General Electric Medical Systems, Milwaukee, WI, USA). To identify the region of interest (ROI), a coronal gradient echo scout scan was used to pinpoint the superior and inferior boundaries of the scans (the femoral head and the tibiofemoral joint line). Once this ROI was established, axial T1 weighted images were acquired in standard body coil using a fast spin echo sequence with TR/TE=700/20; 8 mm slice thickness, 23 mm inter-slice distance and a 256×192 matrix. The axial MRI images were then digitized (DICOM viewer, GE Medical Systems) and saved to an optical disk for later analysis.

After electronic data transfer of images, cross-sectional area (CSA) measurements and calculations were performed by use of custom image analysis software (MatLab, Mathworks, Natick, MA, USA) on a desktop personal computer. As a minimum of 12 slices across various volume measurement approaches is suggested to maximize validity (Nordez et al., 2009), we used at least 13 (depending on muscle length) cross-sectional images each for the right and left leg. On each of the cross-sectional images, outlines of the quadriceps (rectus femoris, vastus intermedius, vastus lateralis, vastus medialis) were quantitatively identified and sectioned, thus isolating the muscle and allowing overall CSA to be computed automatically. The outcome variable (muscle volume) was then determined by summing the volumes from each slice (area × slice thickness) to give total volume, as described by previous researchers (Dibble et al., 2006; Gerber, 2006; Tracy et al., 2003). We have previously investigated the reliability and validity of these measures by asking an investigator, blinded to time-point of the scan and slice location, to perform measurements of individual participants before and after training. To establish the intra-investigator reliability of CSA measurement, the same investigator performed two separate measurements of quadriceps CSA of 18 different images of participants. The repeat measurements were separated by measurement of other images or rest periods. The median interclass correlation coefficient (ICC), across the 18 images, was 0.99 (range 0.89–0.99). The validity of the volume measurement was determined by analysis of images obtained from a cadaveric thigh phantom that approximated the size of the quadriceps femoris muscle group. The volume of the phantom, measured by water displacement five hours after MRI scanning, was 100.7% of the MRI-determined value. There was a 0.012% difference between repeat volume displacement measurements of the phantom by the same investigator.

Isometric strength

Maximum voluntary isometric contraction (MVIC) strength was assessed unilaterally during knee extension for all participants. This measurement was taken at 45 deg. of knee flexion and was measured three times, before, immediately after and 2 weeks following the 11-week training program. The location of the dynamometer was located 3.5 cm proximal to the medial malleolus for each subject, and this position was recorded and used in all subsequent measures. This measurement was made with a dynamometer (Microfet, Hoggan Health Industries, Murray, UT, USA), which was secured to an immovable frame. Participants were instructed to push as hard as possible and were encouraged verbally throughout each trial. The force output was recorded and measured as Newtons of isometric force. The average of three repetitions (2 min rest between repetitions) was recorded.

Perceived muscle soreness and exertion

Muscle soreness was assessed before each training session on a 15 cm visual analog scale anchored at one end of the scale (0 cm) by the descriptor ‘no soreness’ and at the other end (15 cm) by ‘worst possible soreness’. The numerical muscle soreness score was determined by measuring (in centimetres) where the participant marked with pen their leg soreness along this scale.

Creatine kinase levels

Blood samples were taken before training as well as weekly from all participants by trained phlebotomists at the Medical Center on the Northern Arizona University Campus. 5 ml of blood was collected by venipuncture in plastic serum separation tubes (Fisher Scientific, Houston, TX, USA). The blood was centrifuged and the serum was collected and stored in a –80°C freezer for analysis. Quantitative determination of total creatine kinase (CK) activity was made using a creatine kinase reagent kit (Teco Diagnostics, UV-Kinetic Method, Anaheim CA, USA). The procedure was run and analyzed in a standard UV spectrophotometer at 340 nm. Abnormal and normal human serum controls were run every 10 samples to assure accurate readings.

Biopsy technique

Muscle biopsy samples were taken from the right vastus lateralis, at mid-thigh level, using a percutaneous biopsy technique. The biopsy was taken using an automated biopsy device (#MC1410, Bard) with a 14-gauge needle. Biopsies were taken from each participant both pre-training and at mid-exercise, week 8 (NA) and week 11 (PT), one day after their most recent exercise bout. Biopsy samples (~10 mg each) were quick frozen with liquid nitrogen and then stored at –80°C until analyzed.

RT-PCR

IGF-1Ea mRNA levels from all 28 biopsy samples were compared by relative quantification of mRNA levels using RT-PCR. The average mass of each sample was 18 mg (range 4–32 mg). Each entire sample was taken from storage at –80°C, placed in 500 µl of TRI Reagent (MRC, Molecular Research Corporation, Cincinnati, OH, USA) and shredded in a polytron-homogenizer for 10 s. RNA was prepared according to the Molecular Research Corporation protocol and then dissolved in 60 µl formazol (stabilized formamide, MRC) to discourage RNA degradation. RNA concentrations and purities were measured spectrophotometrically. First-strand cDNA synthesis used Superscript III reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA, USA) according to that manufacturer’s protocol, with 1.6 µg RNA, and 125 pmol l^{-1} of T14VN oligo-dT first-strand primer (Lekanne Deprez et al., 2002), in a 20 µl cDNA synthesis reaction per sample. Following synthesis, each cDNA sample was purified using a QIAQuick PCR-purification spin column (Qiagen, Valencia, CA, USA) and then stored before PCR at 4°C in 1 mmol l^{-1} Tris pH 8.0, 0.1 mmol l^{-1} EDTA. Quantitative PCR was performed with a Roche LightCycler using primers designed using Primer3 (Rozen and Skaletsky, 2000) and using a normalization strategy and methods with SYBR Green I dye that were similar to those used previously (Cooksey et al., 2004). Forward and then reverse primers used for each of the following amplicons were: IGF-1Ea, 219-bp: 5′-TGGAGACAGGGGCT-

Table 1. Participant demographics pre-training

	Age (years)	Height (cm)	Mass (kg)	Quadriceps strength (N)
Pre-trained group (PT)	20.3±4	172±13	68.2±7.3	105±65
Naive group (NA)	19.7±3	170±10	70.4±9.5	108±81

Mean age, height, mass and quadriceps strength of the PT and NA groups (N=14, ±s.e.m.) before the 12-week resistance training period.

TTTATTTC-3′, 5′-TTCAAATGTACTTCCTTCTGGGTCT-3′; cyclophilin A (peptidylprolyl isomerase A), 222-bp: 5′-GCATACGGGTCCTGGCATC-3′, 5′-TCGAGTTGTCCACAGTCAGCA-3′; RPL13a (ribosomal protein L13a), 147-bp: 5′-GCAAGCGGATGAACACCAAC-3′, 5′-TGCCGTCAAACACCTTGAGAC-3′. Approximately 25 ng cDNA was used in each 10 µl PCR reaction. The same mix per cDNA sample (containing all reagents except PCR primers) was used for both the IGF-1Ea PCR run and the PCR runs of the two normalizer transcripts. Standard curves (log cDNA *vs* crossing-point cycle number) were constructed by the LightCycler software for each amplicon, within each PCR run, using a nine-point titration (6–36 ng) of compiled cDNA from of all 28 cDNA samples. Two multi-run PCR experiments were performed, each containing either one or two IGF-1Ea runs plus one run of each of both normalizers. The raw IGF-1Ea mRNA value per PCR experiment was divided by the average of the raw values of the two normalizers per experiment, to arrive at each of the normalized ICF-1Ea values of the 28 cDNAs. Finally, the normalized IGF-1Ea mRNA values of the two experiments were averaged, for each cDNA sample and the four categories of seven samples each were compared. Note that we took the pre/post ratio of normalized IGF-1Ea mRNA level per subject and then averaged that ratio for each regimen category.

Statistical analysis

Statistical analyses were performed using the Wilcoxon matched pairs test, and two-sample *t*-tests at a preset level of significance of α=0.05. The paired variables that were compared using the Wilcoxon test were the muscle volumes pre-training and post-training, and isometric strength values pre-training and post-training. Paired two-sample *t*-tests were used to compare weekly differences between the NA and PT groups including: mean CK values, muscle volume, perceived soreness, perceived exertion, muscle strength and total work, and they were used to compare mean group and per-subject pre- or post-normalized IGF-1Ea mRNA ratio levels in the two biopsy samples.

RESULTS

Both the pre-trained (PT) group and the naive (NA) group comprised three women and four men. The mean age, body mass, height and quadriceps volume were statistically the same for both groups before training (Table 1).

Increasing work totals were observed throughout the study for both the PT and NA group; the mean weekly work totals more than doubled for both groups by the end of the study. Overall, the 'work equivalent' [the training effort as described previously (Dibble et al., 2006; Gerber et al., 2006)], over the entire 8- or 11-week training session was the same (3.2×10^3 kJ) for both groups. These work totals included the three-week ramp-up session (weeks 1–3) for the PT group. This was accomplished through slightly higher, but not significantly higher (P>0.05), values for weekly work averages for the NA group from week 4 through to week 11 (Fig. 2).

The two groups experienced significantly different levels of muscle damage, as assessed through determining the levels of plasma CK. The PT group had a mean CK level that remained below 107 U l^{-1} [≤150 U l^{-1} (=normal)], indicating no demonstrable muscle damage throughout the training period. By contrast, the NA group recorded mean CK values well above normal, peaking on week 5 at 580±160 U l^{-1}. Plasma CK remained significantly elevated in weeks 4–7 (P<0.05) when the two groups were compared (Fig. 3). Initial perceived soreness (week 4) was statistically significantly elevated in the NA group (Fig. 4).

Both muscle strength and size increased equally in subjects in both groups. Increases in volume of the quadriceps muscle were significant for both the PT and NA group, respectively (P<0.001, P<0.01). The PT group had a 6.5% increase in muscle volume, and the NA group a 7.5% increase (Table 2), and the difference

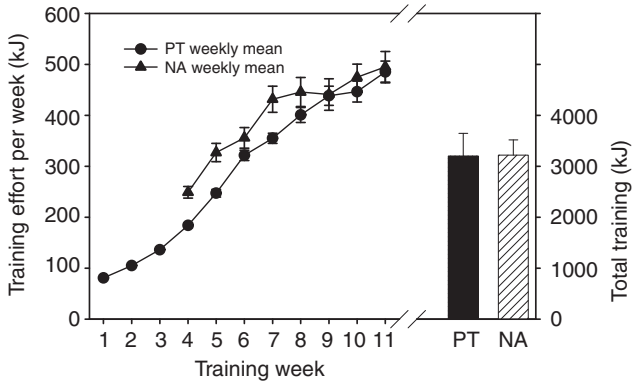

Fig. 2. Work totals throughout eccentric exercise training protocols, expressed as mean weekly work totals (N=7, each group, ±s.e.m.; note that some error bars are hidden by the symbols). Mean weekly work totals doubled for both the pre-trained (PT, circles) and naive group (NA, triangles), while all weekly work totals (weeks 4–11) were not different when comparing the two groups (P>0.05). The histogram on the right shows that the total work performed over the eight-week training session also did not vary statistically between the groups (P>0.05).

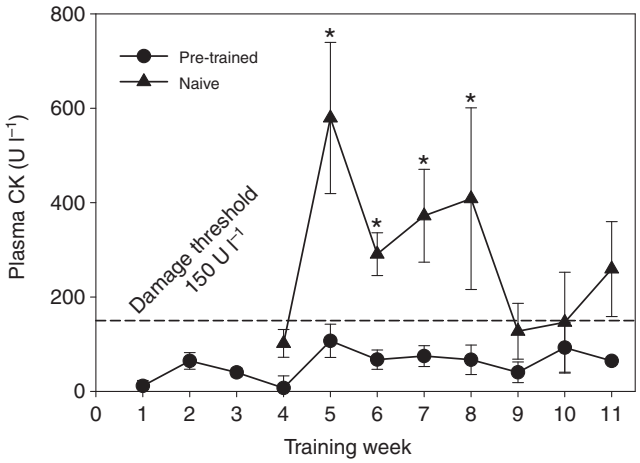

Fig. 3. Plasma creatine kinase (CK) levels were measured in each participant weekly. CK levels increased significantly in the NA group for the weeks 5–8 (*=statistical difference between groups; P<0.05). By contrast, the PT group was never above the control CK level (150 U l^{-1}), representing the muscle damage threshold.

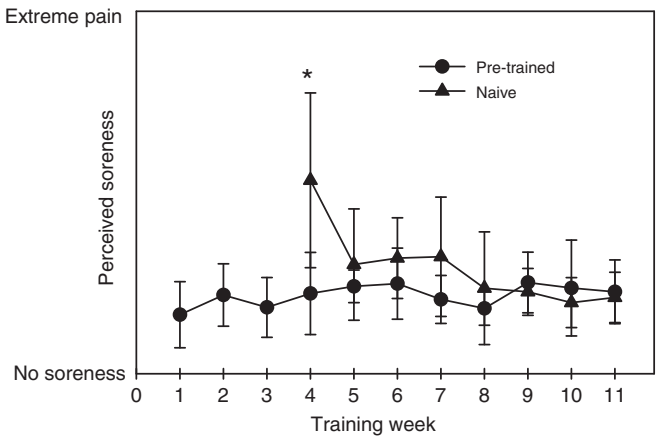

Fig. 4. Values of perceived muscle soreness as measured on a visual analog scale (fixed-length line) assessed before each workout session (N=7 each group; ±s.e.m.). Values remained relatively low throughout the 12-week training period for the PT group, but higher levels of soreness were recorded in weeks 4–7 for the NA group, with a statistical difference (*) in week 4 (P=0.022).

between groups was not statistically significant (P=0.576). Strength increased for all participants in the study – the PT group showed a mean 25% increase, and the NA group a 26% increase; a pairwise comparison of the two groups showed no statistical difference (Table 2).

IGF1-Ea PCR analysis of the biopsies taken in week 8 showed that all 28 (seven pre- and seven post-biopsies for each group) cDNA samples generated correct product with measurable crossing points. Normalized IGF-1Ea mRNA levels increased in all subjects. In the PT group, the pre/post shift was a 55% increase (P=0.003 determined by means of a two-tailed t-test) and 85% increase in the NA group (P=0.002). While these increases were statistically significant within both groups compared with their baseline values, we detected no difference between those shifts comparing groups (P=0.183 determined through a one-tailed, type 1 t-test).

Overall, the total work effort expended over the 11-week training session was identical for both groups. By contrast, muscle damage was statistically different between the groups – with the NA group showing demonstrable symptoms of muscle damage and soreness, whereas the PT group experienced no symptoms of either damage or soreness. Independent of levels of initial damage, the changes in muscle volume, growth factor levels and quadriceps strength were the same for both groups.

DISCUSSION

In this study, one group of participants experienced an initial bout of damaging exercise and the other had no detrimental symptoms of damage. Despite the different initial conditions, both groups experienced the same net increase in muscle size and strength. These results suggest that it is the total work done during training that impacts the final muscle remodeling, apparently independent of an initial 'triggering event'. Thus, increases in muscle volume, quadriceps strength and IGF-1Ea mRNA were all found to occur independent of any muscle soreness or elevated CK – key symptoms of muscle damage.

The suggestion has been made that a damaging event is a necessary initial activating step for muscle hypertrophy (Evans and Cannon, 1991; Folland et al., 2002; Goldspink, 2003; Hawke and Garry, 2001; Smith et al., 1999). While muscle damage can take many forms, there have been abundant studies that have demonstrated that damage is commonly defined by disruption of the extracellular matrix, basal lamina and sarcolemma as well as damage within the muscle fiber to the contractile and cytoskeletal proteins (Newham et al., 1983). Sarcolemma disruption is confirmed by an increase in blood-borne levels of intramuscular proteins such as creatine kinase (CK), which in turn has been linked to production of an inflammatory response (Cannon et al., 1989; Round et al., 1987). The damage to the contractile proteins usually leads to a loss of strength (maximal isometric force) (Clarkson et al., 1992).

In the current study, a damaging initial exercise bout was confirmed by elevated CK levels in the NA group; in weeks 4–7, plasma concentrations were well above the damage threshold and over five times that of the PT group. These elevated CK levels were not intended to quantify the amount of damage but instead to serve as a marker to confirm that ultrastructural damage to the muscle had occurred (Armstrong, 1984; Clarkson, 1997). Another marker of damage was also observed – this being the significant increase in perceived soreness in the NA group, which was absent in the PT group.

An eccentric exercise regime was used in this study specifically because the highest force production in skeletal muscle occurs during lengthening muscle contractions and thus provides the greatest stimulus for muscle growth (LaStayo et al., 2000). Because of these high forces, muscle damage is commonly associated with eccentric contractions. Although damage can be a common manifestation of eccentric exercise, if the magnitude of force is increased gradually and progressively, muscles adapt to any pattern of use, including high eccentric forces, with no detectable muscle injury response (LaStayo et al., 1999; Newham, 1988). The gradual ramp-up protocol used in this study was successful in achieving high training forces while avoiding detectable damage in the PT group.

We observed that the increase in muscle volume for the two groups was equivalent regardless of a damaging bout. From an engineering perspective, damage as a necessary precursor for restructuring would seem to be a poor 'design feature', requiring unnecessary vulnerability (i.e. sarcolemma damage, soreness and weakness) in response to a requirement for additional strength. It seems that a need for added strength to be coupled to a requirement of damage-induced diminished strength would certainly be avoided by natural selection if possible. Indeed, during chronic resistance training, whether an athlete experiences muscle soreness and damage at the onset of training would seem to have no impact whatsoever when training continues uninterrupted over months or

Table 2. Quadriceps muscle volume and isometric strength

	Pre-trained group (PT)			Naive group (NA)		
	Pre-training	Post-training	%Δ	Pre-training	Post-training	%Δ
Quadriceps volume (cm^3)	1651±145	1751±141	6.5*	1906±175	2041±176	7.5*
Quadriceps strength (N)	104.5±64.5	130.5±28.5	24.8*	108.4±81	136.4±118.6	25.8*

Mean values (N=14, ±s.e.m.) of the PT and NA groups before and after the 12-week resistance training. *Significant difference (P<0.05) was seen within the groups for pre- and post-cross volume values as well as pre- and post-strength results. No statistical difference (P>0.05), however, was present between the NA and PT groups for either muscle volume or strength.

years. The addition of muscle size and strength that persists after years of continuous training must be completely uncoupled from any initial damaging bout of muscle damage months or years prior.

There is strong documentation that an acute eccentric muscle damage event results in upregulation of IGF-1Ea (McKay et al., 2008). This study expands this observation to include increases in IGF-1Ea mRNA within the muscle that occur independent of symptomatic damage. The increase in this important myogenic growth factor is consistent with the hypothesis that damage might not be a necessary precursor to muscle hypertrophy.

Thus, our results suggest that muscle hypertrophy can occur independent of any symptoms of muscle damage. In both groups, the high forces produced by lengthening, eccentric contractions provided a powerful stimulus to promote muscle growth and strength. The implications of using high-force lengthening contractions to promote muscle growth, with no detectable (symptomatic) damage, are significant in designing clinical interventions – for example, for those individuals who could benefit from increased muscle strength but are exercise-limited or exercise-intolerant owing to their vulnerability to any inflammatory response. These at-risk individuals include those suffering from, for example, chronic heart failure, obstructive pulmonary disease or even sarcopenia in frail elderly patients (Gosker et al., 2000; Volpi et al., 2004). The loss of muscle mass in this population leads to serious problems, including loss of mobility and often life-threatening falls. Clinically, muscle rebuilding might be impossible in these individuals if damage, and thus an inflammatory response, were a necessary precursor for muscle hypertrophy. What this study did not explore is the issue that some undetected (and perhaps undefined) 'micro-damage' might have occurred. While asymptomatic 'micro-damage' could well accompany each bout of exercise, because it lacks symptoms, it would be non-impacting for this population. However, if increases in muscle size and strength can be achieved independent of any symptoms of damage, chronic eccentric exercise regimes might be perfectly suited for these elderly exercise-intolerant individuals because of the low energy requirements and high force-production abilities of eccentric muscle contractions.

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