Evidence of skeletal muscle damage following electrically stimulated isometric muscle contractions in humans

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Mackey AL, Bojsen-Moller J, Qvortrup K, Langberg H, Suetta C, Kalliokoski KK, Kjaer M, Magnusson SP. Evidence of skeletal muscle damage following electrically stimulated isometric muscle contractions in humans. J Appl Physiol 105: 1620–1627, 2008. First published September 18, 2008; doi:10.1152/japplphysiol.90952.2008.—It is unknown whether muscle damage at the level of the sarcomere can be induced without lengthening contractions. To investigate this, we designed a study where seven young, healthy men underwent 30 min of repeated electrical stimulated contraction of m. gastrocnemius medialis, with the ankle and leg locked in a fixed position. Two muscle biopsies were collected 48 h later: one from the stimulated muscle and one from the contralateral leg as a control. The biopsies were analyzed immunohistochemically for inflammatory cell infiltration and intermediate filament disruption. Ultrastructural changes at the level of the z-lines were investigated by transmission electron microscopy. Blood samples were collected for measurement of creatine kinase activity, and muscle soreness was assessed in the days following stimulation. The biopsies from the stimulated muscle revealed macrophage infiltration and desmin-negative staining in a small percentage of myofibers in five and four individuals, respectively. z-Line disruption was evident at varying magnitudes in all subjects and displayed a trend toward a positive correlation (r = 0.73, P = 0.0663) with the force produced by stimulation. Increased muscle soreness in all subjects, combined with a significant increase in creatine kinase activity (P < 0.05), is indirectly suggestive of muscle damage, and the novel findings of the present study, i.e., 1) macrophages infiltration, 2) lack of desmin staining, and 3) z-line disruption, provide direct evidence of damage at the myofiber and sarcomere levels. These data support the hypothesis that muscle damage at the level of the sarcomere can be induced without lengthening muscle contractions.

EXERCISE INVOLVING UNACCUSTOMED forced-lengthening (eccentric) contractions has conventionally been the most popular model for investigating contraction-induced muscle damage. This is likely due to the finding that this type of muscle contraction appears to result in greater soreness and damage than shortening (concentric) muscle contractions (6, 21, 23, 24). Furthermore, there is evidence for greater damage when the muscle works at longer compared with shorter lengths (5, 22, 26). However, many of the studies comparing these different modes of muscle contraction have relied on indirect markers, such as circulating creatine kinase (CK), soreness, and force loss, to evaluate muscle damage. Morphological assessment of muscle biopsies, on the other hand, leaves little doubt about the occurrence of muscle damage following unaccustomed exercise (7, 14, 23), although this is not always observed (8, 34). With regard to mechanisms leading to such muscle damage, one current theory suggests that instability of sarcomeres on the descending limb of the length-tension relationship during eccentric contractions results in irreversible sarcomere disruption (20a). The ensuing altered calcium concentration in the myofiber could then lead to increased activity of calcium-activated proteases, reactive oxygen species, and phospholipases, the generally accepted active agents involved in structural degradation of the myofiber contractile components and sarcolemma (1, 9). The exact sequence of events remains to be fully elucidated.

The study of skeletal muscle damage and its associated soreness after strenuous muscle loading has provided valuable insight into the degradation/repair processes and regenerative potential of skeletal muscle (8, 13, 27), which are of interest for training and rehabilitation regimens, and in the context of defect repair systems in muscle disorders. While the most widely used exercise model for studying muscle damage/soreness is one involving eccentric contractions, there is emerging evidence for the potential of isometric contractions to also induce muscle damage. This has been studied recently with the aid of electrical stimulation of the knee extensor muscles, where it was reported that stimulated isometric contractions could induce greater damage than voluntary isometric contractions, as assessed by indirect measures (15). Contrary to these findings, another recent study involving the use of electrical stimulation, also of the knee extensor muscles, failed to find any change in muscle soreness, accompanied by only a minor elevation in circulating CK activity (35). The potential of isometric contractions to induce muscle damage is, nonetheless, intriguing, and confirmation of this would be a useful finding in gaining further insight into muscle damage. Furthermore, given the wide range of applications of neuromuscular electrical stimulation, from enhancement of regular exercise training to prevention of atrophy (10), documentation of the influence of stimulated isometric contractions is needed. There are no human studies that we are aware of that have combined both the indirect assessment of damage (CK, soreness) and the direct examination of muscle biopsies to investigate whether skeletal muscle responds to repeated stimulated isometric contractions in a similar manner to lengthening contractions. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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aim of this study, therefore, was to investigate the effects of repeated isometric muscle contractions, induced by electrical stimulation, on direct indexes of myofiber damage, i.e., macrophage infiltration and z-line disruption, in humans. Indirect indexes of damage, such as soreness and CK activity, were also monitored. We hypothesized that our model of electrical stimulation of the muscle in an isometric position would result in direct and indirect evidence of muscle damage, not unlike that reported for contractions involving muscle lengthening.

**METHODS**

**Volunteers**

Seven young, healthy, untrained men [mean age 22 ± 3 (SD) yr; height 1.84 ± 0.05 m; weight 78 ± 6 kg] volunteered for the study and gave written, informed consent. The study was approved by the Ethics Committees of the Municipalities of Copenhagen and Frederiksberg (Ref: H-A-2007-0037) and conformed to the Declaration of Helsinki.

**Experimental Setup**

Stimulation electrodes (Stimtrode, ST32D, Axelgaard manufacturing, Fallbrook, CA; electrode size ~25 × 25 mm, interelectrode center-center distance ~35 mm) were positioned over the middle aspect of the m. gastrocnemius medialis (GM) after shaving, light skin abrasion, and cleaning with ethanol. The electrodes were connected to a stimulator (Elpha II 3000, Dannmeter, Biofina, Odense, Denmark), and the subjects were allowed time for familiarization with electrical stimulation. During the familiarization, the occurrence of actual muscle contraction was visually confirmed. Hereafter, the subjects were positioned in a custom-built test bench designed for measuring unilateral isometric plantar flexor force [described previously (3, 20)]. The subjects were seated with the hip joint flexed to ~60° and the knee joint of the experimental leg flexed to ~5°, and subjects were strapped in firmly to minimize joint movement during muscle contractions. The foot was placed on an adjustable footplate with a mechanical axis of rotation that corresponded to the lateral malleolus (ankle joint position was neutral, i.e., the tibia was perpendicular to the sole of the foot). To register plantar flexion force, the footplate was connected to a strain gauge (Noraxon Inline Force Sensor 320, Scottsdale, AZ) through a rigid steel rod, such that no joint movement was possible during contraction. The strain gauge was connected to a wireless transmitter (Noraxon Telemyo 2400T G2, Scottsdale, AZ) and relayed to a personal computer (TM2400 wireless receiver PC card, Scottsdale, AZ), enabling 500-Hz sampling of the force signal. The system allowed for real-time signal visualization, and, subsequent to the experiments, the force data were evaluated by use of the Noraxon Myoresearch XP1.04 signal analysis software package.

**Exercise Protocol**

Following a series of “warm-up” contractions (2 × ~50% of maximum effort and 2 × ~80% of maximum effort, each separated by 1.5 min), the subjects performed three maximal voluntary contractions (MVC) of the plantar flexors of both legs, separated by 1.5 min. The subjects were instructed to avoid accessory muscle activity during maximal efforts, and attempts with clear muscle activity proximal to the knee joint were immediately discarded. Hereafter, intermittent muscle stimulation was carried out for 30 min (60-Hz stimulation, pulse width 300 μs, rise time 1 s, total stimulation time 4 s, descending time 1 s, rest time 6 s, duty cycle 10 s). Subjects controlled the stimulation intensity and were instructed initially to increase the current to the limit of what was tolerable. The mean stimulation current of the seven participants was 29 ± 10 (SD) mA. During the stimulation period, the subjects were, furthermore, encouraged to increase the intensity, which was possible for all subjects. A clearly visible muscular contraction was observed throughout the stimulation period, which was concurrently confirmed by online visualization of the force signal. The force signal was sampled during MVCs and during 10 stimulation cycles throughout the stimulation protocol and saved for later analysis. The relative stimulation force was determined as the peak plantar flexion force produced by stimulation of the GM (average of 10 contractions), expressed relative to the total force produced by the plantar flexor muscles during MVC.

**Muscle Biopsies**

Muscle biopsies were obtained from the belly of the GM utilizing the percutaneous needle biopsy technique of Bergström (2). The biopsy site was determined after locating major blood vessels in the muscle with the use of ultrasonography. Two biopsies were collected from each individual 48 h after the electrical stimulation: one from the leg that had been stimulated and one from the contralateral leg as a control. On extraction, part of the sample was immersed in 2% glutaraldehyde in 0.05 M sodium phosphate buffer (pH 7.2) for analysis by transmission electron microscopy. The remaining part of the sample was aligned, embedded in optimum cutting temperature, and frozen by immersion in isopentane, precooled by liquid nitrogen.

**Soreness**

Muscle soreness was assessed preexercise (Pre) and on each of the 7 days postexercise with the aid of a visual-analog linear soreness scale, ranging from 0 (normal, no pain) to 10 (worst imaginable leg muscle pain). Soreness was assessed in three different ways: by self-palpation in the belly of the muscle, active muscle contraction (heel-raise), and passive muscle stretch. For self-palpation, subjects were required to palpate the belly of the GM in a seated position, in which they received instruction before the first soreness recordings. This method of self-palpation has been used in many previous studies (19), and while the amount of pressure cannot be standardized using this method, it has proved to be a valuable tool in gross estimation of exercise-induced muscle soreness. An active muscle contraction was performed standing on a step on one leg, and contracting the plantar flexors from a position of dorsiflexion (heel below the level of the step) to full extension (heel above the level of the step), over a period of 3 s. Passive stretch involved standing facing a wall and pressing the ball of the foot up against the wall with a straight knee. The stretch was held for 3 s, and subjects were asked to rate the soreness during this time.

**Blood Sampling**

Blood samples were collected by venepuncture Pre and on days 2, 4, and 7 postexercise. Circulating concentrations of CK, lactate dehydrogenase (LDH), and myoglobin (Mb) were measured as indirect measures of muscle membrane leakage. Plasma CK and LDH levels were measured at the Department of Clinical Biochemistry (Klinisk Biokemisk Afdeling) at Bispebjerg Hospital, Copenhagen, using a multipoint kinetic slide assay, measured on a Vitros 5.1 FS, Ortho Clinical Diagnostics analyzer (Johnson & Johnson, High Wycombe, UK). The Vitros system was subjected to daily internal quality control and reported an intra-assay coefficient of variation (CV) of 2.6% and an interassay CV of 8.4 and 7.4% for low (146 U/l) and high CK (942 U/l) values, respectively. The interassay CV for LDH was 1.4% and interassay CV was 21.8 and 8.4% for low (141 U/l) and high (512 U/l) values, respectively. Plasma Mb levels were analyzed at the Department of Clinical Biochemistry (Klinisk Biokemisk Afdeling) at Frederiksberg Hospital, Copenhagen, using a Mb STAT electrochemiluminescence immunoassay on an Elecsys 2010 (Roche Diagnostics A/S, Hvidovre, Denmark), with an interassay CV of 5%.
**Immunohistochemistry**

Serial transverse sections (10 µm) were cut at −24°C using a cryostat, picked up on SuperFrost Plus slides (Menzel-Gläser, Braunschweig, Germany), and stored at −80°C.

**Inflammatory cells.** Double-staining was performed on one section from each biopsy by simultaneous incubation with primary antibodies for macrophages (mouse anti-CD68, cat. no. M0718, Dako) and the basement membrane component laminin (rabbit, cat. no. Z0097, Dako). Visualization of primary antibody binding was achieved with the following secondary antibodies: Alexa Fluor 568 goat anti-rabbit (Molecular Probes A11036; Invitrogen A/S, Taastrup, Denmark), Alexa Fluor 488 goat anti-mouse (Molecular Probes A11029). 4,6-Diamidino-2-phenylindole in the mounting medium (Molecular Probes ProLong Gold antifade reagent P36931) stained the nuclei, which rendered macrophages green, laminin red, and nuclei blue (see Fig. 3). To semiquantify inflammatory cell infiltration, the number of fibres with one or more CD68+ cells present within the laminin-rich myofiber basement membrane was counted for each section and expressed as a percentage of the total number of fibres in that section. This assessment was carried out by one investigator (A. L. Mackey) in a blinded manner, such that the identity of the sample was not revealed until all of the analysis had been completed.

**Intermediate filament and sarcolemma disruption.** Double-staining was performed in a similar way as described above with primary antibodies for desmin (mouse, cat. no. 18-0016, Zymed, San Francisco, CA) and dystrophin (rabbit, cat. no. ab15277, Abcam, Cambridge, UK), followed by the secondary antibodies Alexa Fluor 568 goat anti-mouse (Molecular Probes A11031) and Alexa Fluor 488 goat anti-rabbit (Molecular Probes A11034), resulting in green staining for dystrophin, red desmin, and blue nuclei (see Fig. 3). Quantification of desmin-negative fibres was performed as described for CD68+ fibres, i.e., [(no. of desmin fibers ÷ total number of fibers) ÷ 100], also in a blinded manner, such that the identity of the sample was concealed until all analyses had been completed.

**Transmission Electron Microscopy**

Following three rinses in 0.15 M sodium phosphate buffer (pH 7.2), the specimens were postfixed in 1% OsO4 in 0.15 M sodium phosphate buffer (pH 7.2) for 2 h. The specimens were dehydrated in graded series of ethanol, transferred to propylene oxide, and embedded in Epon, according to standard procedures. Ultrathin sections were cut with a Reichert-Jung Ultratome E microtome, collected on one-hole copper grids with Formvar supporting membranes, and stained with uranyl acetate and lead citrate. The sections were examined using a Philips CM 100 transmission electron microscope operated at an accelerating voltage of 80 kV. Digital images were obtained with a MegaView II camera and the analySIS software package. The morphology of the z-lines was examined and assessed by a trained laboratory technician, with the identity of each sample concealed until the analysis had been completed. Since the extent of z-line disruption varied widely between individuals, disruption was categorized into three grades, presented in Table 1. The three grades were categorized as follows: +, disrupted (z-line morphology is clearly visible, but altered compared with that observed in the healthy rested state); ++, severely disrupted (z-lines are still visible but the alignment is severely disrupted compared with that observed in the healthy rested state); and ++++, destroyed (z-line alignment has been destroyed to such an extent that it is no longer possible to follow the z-lines, and, in some places, the z-lines are not visible).

**Statistics**

Data were analyzed using GraphPad Prism software (GraphPad Software, CA). Differences were considered significant at P ≤ 0.05. The nonparametric repeated-measures Friedman test was employed to test soreness and CK data for changes over time, and, where overall significance was found, Dunn’s multiple-comparison post hoc test was used to determine where the differences lay. For comparison of two time points, the Wilcoxon signed-rank test was used. Data from immunohistochemical and transmission electron microscopy assessment of control and stimulated muscle were compared using a one-tailed Wilcoxon signed-rank test. The number of fibres used in the analysis in the control and stimulated leg was compared using a two-tailed Wilcoxon signed-rank test. Spearman’s r was used to investigate the relationship between the graded z-line data and the relative stimulation force.

**RESULTS**

All volunteers completed the study. The mean force produced by the electrical stimulation amounted to 5.4 ± 2.7 (SD) % of the total force produced by a MVC of all plantar flexor muscles (see Table 1).

**Soreness**

Delayed-onset muscle soreness (DOMS), as assessed by self-palpation, contraction, and stretch of the muscle, increased in all subjects in the days following the electrical stimulation (P < 0.0001), peaking on day 3 and almost having reached Pre values by day 7 (Fig. 1). In case the muscle biopsy procedure had

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**Table 1. Assessment of muscle biopsies collected 48 h after stimulated contraction**

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>RSF, % MVC</th>
<th>Fibres, no.</th>
<th>CD68+, no.</th>
<th>z-Line Disruption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Con</td>
<td>Stim</td>
<td>Con</td>
</tr>
<tr>
<td>Con</td>
<td>Stim</td>
<td>Desmin−, no.</td>
<td>(0.0)</td>
<td>(0.5)</td>
</tr>
<tr>
<td>Con</td>
<td>Stim</td>
<td>CD68+, no.</td>
<td>(0.0)</td>
<td>(0.0)</td>
</tr>
<tr>
<td>Con</td>
<td>Stim</td>
<td>z-Line Disruption</td>
<td>(0.0)</td>
<td>(0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>N/A</td>
<td>4.621</td>
<td>4.967</td>
<td>0.52</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>5.4 ± 2.7</td>
<td>660 ± 245</td>
<td>710 ± 366</td>
<td>7 ± 17 (±5)</td>
</tr>
<tr>
<td>P</td>
<td>N/A</td>
<td>0.58</td>
<td>0.06</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Data from the control (Con) and stimulated (Stim) leg are shown. The table displays the number of desmin-negative fibres and fibres showing infiltration of CD68+ cells (macrophages), and the number of fibres included in this histological assessment. Values are absolute numbers with percentage of fibres in parentheses. Data in the last column refer to evidence of z-line disruption from transmission electron microscopy analysis. The extent of disruption is graded into categories as follows: +, mild disruption; ++, severe disruption; ++++, destruction of z-line structure (see Fig. 4 for sample images), with a numerical rating of this assessment in parentheses. Relative stimulation force (RSF) is also provided. P values were obtained using a Wilcoxon signed-rank test for matched pairs, comparing the control leg to the stimulated leg. N/A, not applicable; MCV, maximal voluntary contraction.
influenced these measures, the three time points of soreness recordings (Pre, day 1, and day 2) obtained before biopsy sampling were examined further. Analysis using the Friedman test revealed a significant increase in all three modes of soreness assessment (*P < 0.05).

Membrane Permeability

CK, LDH, and Mb were used as a general marker of membrane permeability. No changes were observed in LDH or Mb levels (data not shown), but CK levels increased significantly with time (Friedman test, *P = 0.0008) (Fig. 2). A direct comparison of Pre and day 2 values was also performed using the Wilcoxon signed-rank test to exclude any influence of the muscle biopsies on circulating CK activity. The group mean (n = 7) was observed to increase significantly from 183 ± SD 107 IU/l Pre stimulation to 377 ± SD 447 IU/l on day 2 (*P = 0.0312), confirming enhanced circulating CK activity independent of muscle biopsy sampling.

Immunohistochemistry

Inflammatory cell infiltration and loss of desmin staining were investigated immunohistochemically as evidence of muscle damage (see Fig. 3). These data are presented in Table 1. None of the control biopsies contained any myofibers that were either infiltrated with CD68+ cells or negative for desmin staining. The presence of CD68+ cells (presumed macrophages) was observed in the stimulated muscle in five of the seven participants. Desmin-negative/dystrophin-negative fibers demonstrated a similar pattern, present in four individuals. As can be seen from Table 1, subject 1 was clearly affected by this stimulus to a much greater extent than the other participants, demonstrating CD68+ cell infiltration and lack of desmin staining in 12.0 and 13.6% of myofibers, respectively. Two subjects (no. 6 and no. 7) failed to show any immunohistochemical signs of damage following stimulation.

Transmission Electron Microscopy

z-Line disruption was observed in the biopsy taken from the stimulated leg in all seven individuals (see Fig. 4). Since

![Fig. 1. Self-assessment of muscle soreness in the days following stimulated muscle contraction. Soreness was self-assessed under 3 different conditions: by palpating, stretching, or contracting the muscle. Values are means with SD error bars. Significant compared with preexercise (Pre) (day 0): *P < 0.05, **P < 0.01, ***P < 0.001 (Friedman test). Significant compared with Pre when Pre, day 1, and day 2 were analyzed separately: #P < 0.05, ##P < 0.01 (Friedman test). AU, arbitrary units.](image1)

![Fig. 2. Circulating levels of creatine kinase activity measured before, and in the days following, stimulated muscle contraction. Values are means with SD error bars. *P = 0.0008, Friedman test. *P < 0.05, ***P < 0.001, compared with baseline levels (day 0); †P < 0.05, Wilcoxon signed-rank test comparing day 0 and day 2.](image2)
the extent of z-line disruption varied widely between individuals, disruption was categorized into three grades, presented in Table 1. All control biopsies showed a well-preserved morphology without any evidence of z-line disruption. Spearman’s $r$ value for correlating the graded z-line disruption data with the relative stimulation force data was $0.73 (P = 0.0663)$. This correlation is depicted in Fig. 5.

Fig. 3. Immunohistochemical evidence of muscle damage 48 h after stimulated contraction. Images are from double staining of two serial microthin sections of medial gastrocnemius muscle. Image series 1 (A–D) shows a single fiber lacking staining for desmin (1B) and dystrophin (1A). Image series 2 (A–D) shows that the same fiber, outlined by basement membrane laminin staining (2A) on an adjacent section, has been infiltrated by macrophages (CD68+ cells, 2B). Images 1D and 2D are computer-generated merged images of the individually captured images in A–C. Scale bar = 100 μm.

Fig. 4. Transmission electron microscopy image from control muscle and biopsies taken 48 h after stimulated isometric contractions, showing different grades of disruption to muscle morphology: mild disruption (+), major disruption (++), and destruction (+++) of z-line structure.
DISCUSSION

We report novel data regarding histological and ultrastructural changes in human muscle biopsy samples 2 days after a 30-min bout of stimulated isometric muscle contractions. The presence of macrophages in myofiber cytoplasm, combined with disruption of Z-lines, provides direct evidence of muscle damage. The extent of damage, as assessed by Z-line disruption, appeared to be related to the stimulated force produced by the muscle. Furthermore, DOMS was reported by all participants in this study, confirming that DOMS can be induced by electrically induced isometric muscle contractions.

Our novel finding of myofiber infiltration by macrophages, as assessed by CD68 immunostaining, in a model without muscle lengthening contractions, is interesting in the context of muscle damage and the current theories surrounding this topic. As displayed in Table 1, the proportion of myofiber cross sections demonstrating infiltration in four of the seven participants ranged from 0.1 to 0.7%. One individual clearly responded to a much greater extent with infiltration in 12% of myofibers in a cross section, and two individuals failed to show any macrophage infiltration. While occasional extracellular inflammatory cells are often present in healthy adult muscle that has not been subjected to any special stimulus, inflammatory cell infiltration into the myofiber cytoplasm has traditionally been considered a feature of myopathic conditions and, in healthy muscle, a result of protocols involving forced lengthening contractions (14, 27). While studies in the literature reporting inflammatory cell infiltration in human studies are well outnumbered by animal studies (25, 28), and despite some studies failing to demonstrate an inflammatory response in the muscle with eccentric work (8, 34), it appears that, given the correct dose of mechanical stimulus, the increased presence of inflammatory cells in human skeletal muscle is a normal physiological response where damage and remodeling occur. Inflammatory cells have traditionally been implicated in contributing to muscle damage, but there is growing evidence for a key role in regeneration (17, 30, 31). In light of this, the similarity of the findings in the current study regarding elevated inflammatory activity after stimulated isometric contractions, and those from previous studies employing eccentric contractions (14, 27), suggest similar processes at a cellular level, despite inherent differences between the two types of muscle contraction.

The percentage of myofiber cross sections in the biopsies collected from the stimulated leg that demonstrated a loss in desmin immunoreactivity followed a similar pattern to that observed for macrophage infiltration. Interestingly, a common observation was that the infiltrated fibers were also those fibers that were negative for desmin and dystrophin staining. Desmin is a muscle-specific intermediate filament, surrounding and connecting Z-disks to each other and to costameres, and important for lateral force transmission (4, 11). Disappearance of desmin immunoreactivity from muscle cross sections has been reported in previous animal and human studies, evident immediately after a bout of eccentric contractions (23). While the significance of the disappearance of desmin immunoreactivity is unclear, it is thought to be likely due to either a reorganization in the myofiber, such that the desmin epitope is masked, or to proteolytic digestion of the protein (18). In light of a recent study reporting a peak in lack of desmin immunoreactivity as late as 8 days after a bout of stimulated eccentric muscle contractions in humans (7), it is possible that we would also have observed higher values at a later time point. Nonetheless, our observations pertaining to loss of desmin immunoreactivity...
2 days after stimulus provide strong evidence for remodeling of myofibrillar intermediate filaments following stimulated muscle contractions. This is further supported by the results concerning z-line alterations, as assessed by transmission electron microscopy. Disruption of z-line morphology was evident to varying degrees in all participants in the present study. However, the extent of disruption did not always reflect the magnitude of immunohistochemical findings, such as the presence of inflammatory cells and loss of desmin immunoreactivity (see Table 1). Interestingly though, there was a trend toward a positive correlation \((r = 0.73, P = 0.0663)\) between the graded z-line data and the relative stimulation force values (i.e., the amount of force produced by stimulation of the GM, expressed relative to the total force produced by the plantar flexor muscles), suggesting that force is responsible for disruption of the sarcomere at the z-line. Taken together, the immunohistochemical and electron microscopy data presented here provide direct evidence, for the first time, for the capacity of stimulated isometric contractions to induce muscle damage in humans.

Applications of neuromuscular electrical stimulation include enhancement of regular exercise training and prevention of atrophy during periods of immobilization or during aging [see review by Hainaut and Duchateau (10)]. As the purpose of the stimulated contraction in these applications is to improve muscle strength and prevent loss of muscle mass, induction of muscle damage is undesirable, especially when associated with DOMS. While there appears to be little doubt that electrically stimulated muscle contractions, combined with unaccustomed lengthening contractions, can induce damage (7, 12), there is some discrepancy regarding isometric contractions. Rat muscle subjected to stimulated isometric contractions has been reported to result in nonsignificant minor structural changes (12, 32, 33) and swelling of the sarcoplasmic reticulum (33). Two recent human studies have investigated the effects of stimulated isometric muscle contraction on indirect markers of muscle damage, and both report increased circulating levels of CK activity (15, 35). One of these studies also reported DOMS, peaking 48 h after the stimulation (15), while the other did not observe DOMS (35). The participants in the latter study were, however, well-trained cyclists, in contrast to the relatively untrained volunteers in the study by Jubeau and colleagues (15), which may explain this discrepancy. The participants in the present study reported significant soreness 48 h after the stimulated contractions, supporting the data presented by Jubeau and colleagues (15). Furthermore, our finding of augmented levels of circulating CK is in agreement with these two studies, although somewhat surprising, given the relatively small size of the GM muscle compared with the more popular quadriceps muscles used in these two recent studies (15, 35). Thus the data presented in the present study, taken together with recent work (15, 35), support the hypothesis that stimulated isometric muscle contractions can result in DOMS if the individuals are in an untrained state, since it appears that trained individuals do not experience DOMS when subjected to a similar stimulus (35).

There are several advantages of using the exercise model presented in this study. First, the relatively small size of the GM ensures that the whole muscle can be stimulated to contract. In a recent experiment from our group (16), where subjects underwent a similar stimulation protocol, it was demonstrated by use of positron emission tomography and infusion of \([18\text{F}]\)-fluoro-deoxy-glucose that the GM was uniformly activated by the electrical stimulation (see Fig. 6). It, therefore, seems plausible that the muscle tissue collected from this muscle is likely to be representative of the whole muscle with regard to our observations concerning damage. Second, the GM is a relevant muscle in which to study damage responses in untrained individuals, since it is perhaps not subjected to eccentric contractions on a daily basis to the same extent as the quadriceps, for example, which are highly involved in eccentric-biased daily movement tasks. Furthermore, the experimental setup allows manipulation of muscle length, such that experiments can be performed at muscle lengths that are optimal or suboptimal for contractile force production, thereby investigating the effect of the extent of filament overlap on parameters of muscle damage. A clear limitation, on the other hand, is the size of the muscle, restricting the number of times one can repeatedly biopsy the same muscle without sampling too close to the first biopsy site. Nonetheless, the simplicity of the model, taken together with the presented evidence of its potential to induce myofiber damage, supports and validates our model for further investigation into events induced by stimulation-induced damage and associated soreness.

While there may be some situations in which inducing skeletal muscle damage and regeneration is the goal of a muscle-loading protocol [see Taivassalo and Haller (29)], myofiber damage and its associated soreness are generally unwanted side-effects of training and rehabilitation programs. Study of muscle damage provides valuable insight into the degradation/repair processes and regenerative potential of skeletal muscle (8, 13, 27), which are of particular interest in the context of defect repair systems in muscle disorders. In light of this, the demonstration of the potential for stimulated isometric contractions to induce muscle damage, similar in magnitude to that reported following maximal eccentric contractions, opens up a new avenue for further exploration of this issue. Furthermore, it is likely that these findings would be of interest to those involved in the prescription of electrical stimulation as a means of improving muscle strength or attenuating muscle atrophy during periods of restricted mobility. Further investigations into similarities and differences between different modes of muscle contraction, such as the repeated bout effect, will be interesting to follow and will provide insight into this poorly understood topic.

GRANTS

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REFERENCES


