Sex-based differences in skeletal muscle function and morphology with short-term limb immobilization

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Skeletal muscle atrophy occurs when muscle protein breakdown exceeds synthesis. Atrophy has been shown to occur as a consequence of aging, denervation, disease, non-weight-bearing activity, and spaceflight (1). From an experimental perspective, lower limb suspension (with either a sling or shoe) and immobilization (via a cast or knee brace) have commonly been used as the models to qualitatively and quantitatively assess the progress of, and underlying mechanisms contributing to, skeletal muscle atrophy (1).

Previous studies have shown that voluntary knee extensor peak torque dramatically declines in healthy humans after varying durations (4–6 wk) of lower limb unloading (8, 9, 14). Decrements in knee extensor peak torque were primarily attributed to reductions in muscle and muscle fiber cross-sectional area (CSA) (8, 9, 14). Significantly decreased knee extensor peak torque in both isometric and isokinetic contraction is observed even with shorter term (10–16 days) unilateral leg unweighting interventions (2, 9, 13, 16, 19, 21). Some have concluded that the loss of muscle strength resulting from short-term muscle unloading is primarily due to reduced neural activation of myofibers (8, 14), given that the decrements in muscle strength after shorter term lower limb unloading interventions were not associated with significant muscle morphological changes (13). This assumption, however, may be due to an inability to quantify statistically significant reductions in muscle mass in the short term owing to variability in quantifying muscle mass and/or fiber area that is compounded by small sample size, possibly leading to a type II error. In animal models, gene expression is dramatically altered even after 24 h of hindlimb suspension (35). Furthermore, quadriceps lean mass was significantly reduced by 4.7% after only 2 wk of immobilization (21).

It has been shown that absolute knee extensor peak torque is greater in men than in women during isometric (27) and isokinetic contraction (23). However, some (27, 30), but not all (22, 23), have found no differences between men and women when data are expressed as specific strength (N/cm2), voluntary knee extensor peak torque per unit muscle CSA. Whether these possible sex differences in muscle strength differentially influence the loss of strength consequent to immobilization has, to our knowledge, not been evaluated. One possible sex difference is that women tend to have smaller fibers than men, particularly type II fiber subtypes (34). Furthermore, there appears to be an increase in oxidative stress in atrophying skeletal muscle fibers, and 17β-estradiol acts as an antioxidant and a membrane stabilizer in rats and humans (4, 32, 38). Estradiol can also attenuate creatine kinase appearance (an indicator of muscle membrane damage) from skeletal muscles at rest (4, 32). One study reported a greater degree of atrophy after 2 wk of hindlimb unloading in the soleus and plantaris muscles of ovariotomized animals compared with intact female rats, implicating a protective effect from female sex hormones (15).

Given that there are no data regarding potential sex influences on immobilization-induced atrophy in humans, the purpose of this study was to determine the effects of 14 days of unilateral leg immobilization on muscle function and morphology in men and women. Our a priori hypothesis was that
women would show less atrophy compared with men in response to the immobilization.

METHODS

Subjects. Recreationally active men (n = 13) and women (n = 14) volunteered as subjects (Table 1). All subjects were screened via a questionnaire to exclude those who were smokers, highly trained, injured within the previous 6 mo, or taking regular medications. All female participants were eumenorrheic, and half of the females were taking low-dose estrogen-containing birth control pills. This study was approved by McMaster University and the Research Ethics Board of the Hamilton Health Sciences, and all subjects provided informed consent.

Experimental procedure. Subjects had one leg immobilized by random assignment using a standard knee brace (eP® Knee Control Plus, Smith Orthopedics, Topeka, KS) with a cotton stockinette and were provided with walking crutches. The study period was composed of the Hamilton Health Sciences, and all subjects provided informed consent.

Experimental procedure. Subjects had one leg immobilized by random assignment using a standard knee brace (eP® Knee Control Plus, Smith Orthopedics, Topeka, KS) with a cotton stockinette and were provided with walking crutches. The study period was composed of a total of three testing sessions called Pre (5 days before the immobilization), Day-2 (48 h after the onset of immobilization), and Day-14 (14 days after the onset of immobilization). The Pre time point was chosen to be 5 days in advance of immobilization to allow for recovery from the biopsy before immobilization. The subjects were instructed to refrain from any exercise at least 3 days before the first testing session. Each testing session was identical and included dual-energy X-ray absorptiometry (DEXA), magnetic resonance imaging (MRI), muscle strength, muscle biopsy, and blood sampling, as described below. Body composition and CSA of knee extensor muscles were completed using DEXA and MRI, respectively, on the night before the subsequent testing procedures. The muscle biopsy, blood sampling, and strength testing were completed in the morning after an overnight fast, after a standardized nutritional beverage (500 ml; Boost, Novartis Medical Health, NY) was consumed 2 h before the muscle biopsies in order for each subject to be in a similar postprandial state before each biopsy. Strength testing was carried out immediately after biopsies to avoid influencing biopsy sample results. After the testing, a knee brace was applied with the angle of each knee brace locked at 120° (i.e., 60° from full extension) to provide complete immobilization and yet allow subjects' legs to swing freely during ambulation with crutches, without hitting the ground. A unique identifier was applied to a piece of commercially available standard tape wrapped around to the brace to ensure that the brace was not removed. Compliance and inspection of the immobilized leg and knee brace were monitored via a daily meeting with the subjects. In the presence of this supervision, subjects were allowed to take off their knee braces and stockinette and take a shower in the laboratory without bearing weight on the treatment leg. The knee brace was then reapplied, and new tape was applied. Subjects spent an overall time of 10–15 min with the brace removed.

DEXA. Whole body lean mass, immobilized leg lean mass, and body fat percent were measured at all three time points by using DEXA (model QDR-1000/W, Hologic, Waltham, MA) as described previously (28). The same investigator carried out scanning for all the images.

MRI. MRI was completed in a 1.5-T scanner with superconducting magnets (Symphony Quantum, Siemens, Erlangen, Germany) to determine the CSA of the vastus area (vastus lateralis, vastus intermedius, and vastus medialis) and rectus femoris. MRI was carried out using T1-weighted spin-echo sequences in the axial plane (repetition time/echo time = 400/15; field of view = range from 20 × 10 to 20 × 18 cm; matrix size = range from 180 × 256 to 232 × 256; slice thickness = 5 mm) and was taken at 70% of the distance from the top of the greater trochanter to the lateral joint space of the knee, where a mark was maintained over the course of the study with indelible marker. Imaging was performed with subjects in the supine position with heels fixed on a nonmetallic support to control joint and scan angle.

MRI scans were transferred electronically from the scanner to a personal computer (Windows XP) with eFilm (version 1.5.3 for Windows, Aycan Digitalsystems, Wuerzburg, Germany) and analyzed with Image Pro Plus (V4.0 for Windows, Media Cybernetics, Silver Spring, MD) using manual planimetry. The immobilized leg of each subject was analyzed for CSA. Because it was difficult to distinguish the fascial planes between vastus lateralis, vastus intermedius, and vastus medialis in the images, those three muscle groups were combined as the “vastus area.” The average CSA (cm²) was determined for vastus and rectus femoris and subsequently summed for the “total quadriceps femoris.” The same investigator carried out all measurements on five separate occasions with a low coefficient of variation (<1% for vastus area, <1% for rectus femoris).

Blood sampling. At the three time points, blood samples were taken from an antecubital vein. The samples were immediately put into 10-ml nontreated tubes and allowed to clot for serum. After centrifugation, serum was stored at −80°C for subsequent hormonal assays.

Muscle biopsies. Muscle biopsies were obtained from the vastus lateralis muscle of the immobilized leg under local anesthesia (2% lidocaine) with a modified Bergström biopsy needle with manual suction. Incisions were made at the randomly chosen proximal, distal, or mid (15 cm proximal to lateral joint space) site of the vastus lateralis separated by at 3.5–5.0 cm. There were 7 days between

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Men (n = 13)</th>
<th>Women (n = 14)</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>20.8 ± 1.9</td>
<td>21.3 ± 2.6</td>
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<tr>
<td>Height, cm</td>
<td>178.9 ± 8.2</td>
<td>164.9 ± 5.4*</td>
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<tr>
<td>Body mass index, kg/m²</td>
<td>24.5 ± 3.5</td>
<td>22.8 ± 2.1</td>
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<tr>
<td>Body mass, kg</td>
<td>Pre: 78.8 ± 14.3</td>
<td>62.3 ± 8.7†</td>
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<td></td>
<td>Day-2: 79.4 ± 14.2</td>
<td>62.5 ± 8.9†</td>
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<tr>
<td></td>
<td>Day-14: 78.9 ± 14.0</td>
<td>62.4 ± 9.1†</td>
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<tr>
<td>Body fat, %</td>
<td>Pre: 17.5 ± 4.2</td>
<td>25.7 ± 4.4†</td>
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<tr>
<td></td>
<td>Day-2: 18.4 ± 3.7</td>
<td>26.4 ± 4.1†</td>
</tr>
<tr>
<td></td>
<td>Day-14: 18.0 ± 4.2</td>
<td>26.0 ± 4.2†</td>
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<tr>
<td>Lean body mass, kg</td>
<td>Pre: 64.1 ± 10.7</td>
<td>45.2 ± 5.0†</td>
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<td></td>
<td>Day-2: 63.9 ± 10.5</td>
<td>45.0 ± 5.2†</td>
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<td></td>
<td>Day-14: 63.7 ± 10.0</td>
<td>45.1 ± 5.6†</td>
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<td>Free testosterone, pmol/l</td>
<td>Pre: 51.3 ± 10.2</td>
<td>3.1 ± 1.6†</td>
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<tr>
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<td>Day-2: 48.6 ± 10.6</td>
<td>3.3 ± 1.8†</td>
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<td></td>
<td>Day-14: 50.2 ± 11.8</td>
<td>2.8 ± 1.6†</td>
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<td>Total testosterone, nmol/l</td>
<td>Pre: 14.3 ± 2.5</td>
<td>0.8 ± 0.3†</td>
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<tr>
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<td>Day-2: 14.1 ± 3.0</td>
<td>0.7 ± 0.4†</td>
</tr>
<tr>
<td></td>
<td>Day-14: 14.2 ± 3.9</td>
<td>0.7 ± 0.3†</td>
</tr>
<tr>
<td>Estradiol, pmol/l</td>
<td>Pre: 105.1 ± 22.4</td>
<td>161.7 ± 172.6</td>
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<tr>
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<td>Day-2: 104.4 ± 30.6</td>
<td>163.5 ± 140.8</td>
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<tr>
<td></td>
<td>Day-14: 103.9 ± 30.5</td>
<td>153.7 ± 135.5</td>
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<tr>
<td>Progesterone, nmol/l</td>
<td>Pre: n/a</td>
<td>5.5 ± 8.8</td>
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<td></td>
<td>Day-2: n/a</td>
<td>6.5 ± 10.0</td>
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<td>Day-14: n/a</td>
<td>8.3 ± 14.4</td>
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<tr>
<td>Cortisol, nmol/l</td>
<td>Pre: 585.8 ± 162.2‡</td>
<td>705.2 ± 208.9‡</td>
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<td>Day-2: 502.2 ± 175.6‡</td>
<td>643.8 ± 136.0‡</td>
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<td>Day-14: 457.5 ± 128.6‡</td>
<td>638.8 ± 214.7‡</td>
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Values are means ± SD. Body mass index was calculated based on baseline values (Pre). All values for free and total testosterone, 13 men and 13 women, and for cortisol 12 men and 11 women were assessed. Pre, 5 days before kg immobilization; Day-2, 48 h after onset of immobilization; Day-14, 14 days after onset of immobilization; n/a, not applicable. *Significantly different from men (P < 0.05). †Main effect for gender (P < 0.05). ‡Main effect for time (P < 0.05).
the maximal value after recording for all repetitions. To determine the force production to encourage maximal effort during the exercise subjects were verbally encouraged to voluntarily produce their max-
more than 2 min of recovery time between each exercise mode. All
throughout the complete 65° range of motion. The subjects were given
90 s of rest between the repetitions for the isometric contraction, with
position (37). Each subject performed three repetitions (5 s
testing for each mode was randomly assigned during each testing
session. All subjects had familiarization sessions at least 2 wk before
testing baseline muscle strength measurements. To standardize the
testing, subjects had their shoulders strapped to the chair with adjust-
ments for the back and hips and performed each protocol in the sitting
position (37). Each subject performed three repetitions (5 × 3) with
90 s of rest between the repetitions for the isometric contraction, with
the subject’s knee at an angle of 70°. For concentric-slow and
concentric-fast contractions, subjects carried out 10 repetitions
throughout the complete 65° range of motion. The subjects were given
more than 2 min of recovery time between each exercise mode. All
subjects were verbally encouraged to voluntarily produce their maxi-
mal forces. Furthermore, subjects were given visual feedback of their
force production to encourage maximal effort during the exercise
testing. Subsequently, the highest peak torque value was considered as
the maximal value after recording for all repetitions. To determine the
voluntary force-generating capacity of the muscle, specific strength
was calculated as voluntary peak force per unit CSA of total quadriceps
femoris (from the MRI slice as described above) [N m/ cm² × m], as described previously (29, 30).

Histochromical analysis. Histochromical analyses were conducted as
described by Brooke and Kaiser (12), with the following modific-
tions. The OCT-mounted muscle samples were serially sectioned to
10-µm thickness, and slides were preincubated at a pH value of 4.60
(in 50 mM potassium acetate, 17.5 mM calcium chloride) for 7 min.
Slides were rinsed with distilled, deionized water between each of the
following steps. Slides were incubated in 3 mM ATP using an alkaline
solution (75 mM glycine, 40.5 mM calcium chloride, 75 mM NaCl,
and 67.5 mM NaOH, pH 9.4) for 45 min at 37°C with agitation at
regular intervals. They were incubated consecutively in 1% CaCl₂ and
2% CoCl₂ for 3 min, and then they were incubated in 1% ammonium
sulfide for 30 s at room temperature.

Sections were photographed at ×20 magnification with a micro-
scope (Olympus America, Melville, NY) in conjunction with a SPOT
digital camera (model SP401-115, SPOT Diagnostic Instruments,
MI). On the basis of the staining intensity at pH 4.60 after the
enzymatic reaction, the three fiber types were classified as types I
(dark), IIa (light), and IIx (intermediate). CSAs of the muscle fibers
(µm²) were determined by use of an image analysis program (Image
Pro, V6.0, Media Cybernetics). Total fiber numbers counted to dis-
tinguish the three fiber types and determine CSA were 294 ± 73 for
men and 317 ± 77 for women at Pre and 320 ± 67 for men and 311 ±
92 for women at Day-14. For type I, total circled fiber numbers averaged
126 ± 29 for men and 146 ± 12 for women at Pre and
129 ± 24 for men and 130 ± 42 for women at Day-14. For type IIa,
122 ± 50 for men and 132 ± 52 for women were used at Pre and
140 ± 59 for men and 135 ± 59 for women were taken into account
at Day-14. Finally, for type IIx, 46 ± 10 and 41 ± 14 for men
and women, respectively, at Pre and 48 ± 6 and 46 ± 7 were counted
for men and women, respectively, at Day-14.

Myosin heavy chain analysis. Mixed muscle myosin heavy chain
(MHC) analysis was performed to examine the percentage of MHC
ccontent in a modification of previous studies (3, 34). The muscle samples
(7–8 serial sections) were cut at 20 µm by use of a cryostat and
placed into microcentrifuge tubes including 250 µL of chilled lysis
buffer (10% glycerol (wt/vol), 5% 2-mercaptoethanol (vol/vol), and
2.3% sodium dodecyl sulfate (SDS) (wt/vol) in 62.5 mM tris(hy-
droxymethyl)aminomethane at pH 6.8). The samples were immedi-
ately placed into a 60°C water bath for 10 min and subsequently
stored at −80°C until subsequent histochemical analysis (5).

Muscle strength testing. Muscle strength testing was conducted at
each testing session (Pre, Day-2, and Day-14). Isometric and isoki-
netic (concentric at an angular velocity of 0.52 rad/s: concentric-slow;
concentric at an angular velocity of 5.24 rad/s: concentric-fast) knee
extensor peak torques were determined for all subjects by using a
dynamometer (Biodex-System 3, Biodex Medical Systems, New
York, NY) to evaluate the relative changes in maximal force-gener-
ating capacity over the time course of immobilization. The order of
testing for each mode was randomly assigned during each testing
session. All subjects had familiarization sessions at least 2 wk before
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for men and women, respectively, at Day-14.

Myosin heavy chain analysis. Mixed muscle myosin heavy chain
(MHC) analysis was performed to examine the percentage of MHC

RESULTS

Physical and hormonal characteristics of the subjects. Table
1 shows the physical and hormonal features of the subjects
over 14 days of unilateral leg immobilization. Men were
significantly taller (P < 0.001) and had greater body mass
(P < 0.01) than women, whereas percent body fat was signifi-
cantly greater in women (P < 0.01). The body mass and
fat-free mass were similar in both men and women over three
time points. With respect to free testosterone and total testos-
sterone, men showed a higher concentration compared with
women over 14 days of immobilization (P < 0.001, respec-
tively), whereas no significant changes were found over the
immobilization period in both men and women. Additionally,
no changes were found in estradiol for both men and women
over 14 days immobilization, whereas there was a tendency for
women to have a higher concentration of estradiol compared
with men (P = 0.109). Cortisol significantly decreased be-
were similar with 5.9 ± 5.3, 3.7 ± 7.0, and 5.7 ± 5.0% for men and 6.4 ± 5.6, 2.8 ± 6.0, and 5.9 ± 5.2%, respectively.

Leg lean mass. There were significant decreases between Pre and Day-14 in leg lean mass (kg) in both men and women (P < 0.01, Fig. 2). Furthermore, men showed greater values of leg lean mass compared with women over the time course (P < 0.001, Fig. 2). The percent decreases between Pre and Day-14 for men and women correspond to 3.7 ± 4.2 and 2.7 ± 2.8%, respectively.

Muscle strength. Both men and women showed significant decreases in absolute peak torque (N·m) between Pre and Day-14 and between Day-2 and Day-14 for isometric (P < 0.001, P < 0.01, respectively; Fig. 3A). With respect to concentric-slow and concentric-fast, there were significant decreases between Pre and Day-2, Pre and Day-14, and Day-2 and Day-14 in both men and women (P < 0.01, P < 0.001, P < 0.01 for concentric-slow, P < 0.05, P < 0.001, P < 0.05 for concentric-fast, respectively; Fig. 3, B and C). Men showed significantly greater peak torque values (N·m) compared with women for isometric (P < 0.001; Fig. 3A), concentric-slow (P < 0.001; Fig. 3B), and concentric-fast (P < 0.001; Fig. 3C) contractions, at all times.

When muscle strength was expressed as specific strength [N·m/(cm² × m)], there was a trend for women to show a greater loss in isometric mode between Pre and Day-14 compared with men (interaction; gender × time, P = 0.055, Fig. 3D). With respect to concentric-slow mode, women showed a significant loss between Pre and Day-2 and Pre and Day-14 (P < 0.01, P < 0.001, respectively) but men did not (Fig. 3E). Both men and women showed significant decreases in the concentric-fast mode between Pre and Day-2 and Pre and Day-14 (P < 0.05, P < 0.001, respectively; Fig. 3F). The percent decreases in specific strength at isometric, concentric-slow, and concentric-fast modes between Pre and Day-14 for men and women were 3.1 ± 13.3, 4.7 ± 11.3, and 9.2 ± 18.8% for men, and 17.1 ± 15.9, 16.6 ± 18.4, and 15.6 ± 14.9% for women, respectively.

Histochemical analysis. Both men and women showed significant changes between Pre and Day-14 in CSA of type I, type IIa, and type IIx fibers (P < 0.001, P < 0.01, and P < 0.01, respectively; Fig. 4). Moreover, men showed significantly larger CSAs (μm²) of type IIa and IIx (P < 0.01, P < 0.001, P < 0.05 for type IIx, respectively; Fig. 4A). With respect to concentric-slow mode, women showed a significantly larger CSA of type I, whereas there were no sex-based differences in CSA of rectus femoris (P = 0.149; Fig. 1B).

Fig. 1. Cross-sectional area (CSA, cm²) of vastus (A), rectus femoris (B), and total quadriceps femoris (C) for men (n = 13) and women (n = 14) before (Pre) and after 2 wk (Day-14) of unilateral leg immobilization. Values are means ± SD. Main effects are shown for gender (*) and time (†) (P < 0.05). No interaction (gender × time) was found in CSA of vastus, rectus femoris, and total quadriceps femoris.

Fig. 2. Leg lean mass (kg) for men (n = 11) and women (n = 11) before and after 2 wk of unilateral leg immobilization. Values are means ± SD. Main effects are shown for both gender (*) and time (†) (P < 0.01). No interaction (gender × time) was found.
0.05, respectively; Fig. 4, B and C), but not for type I (P = 0.354; Fig. 4A) at all time points. In terms of fiber-type distribution, no significant changes were found in either men (M) or women (W) for type I (M: 45.2 ± 10.6%, W: 47.8 ± 9.5% for Pre and M: 44.1 ± 9.0%, W: 48.3 ± 7.5% for Day-14, respectively), type IIA (M: 37.0 ± 6.7%, W: 35.0 ± 3.4% for Pre and M: 38.2 ± 11.7%, W: 36.3 ± 7.1% for Day-14, respectively) and type IIX (M: 17.8 ± 8.3%, W: 17.2 ± 10.2% for Pre and M: 17.7 ± 7.7%, W: 15.4 ± 9.4% for Day-14, respectively) during the immobilization. Men had a greater type II (IIa + IIX) fiber-area percent compared with women (P < 0.05; M: 72.8 ± 3.3%, W: 68.6 ± 4.6% for Pre and M: 71.8 ± 2.8%, W: 67.6 ± 4.4% for Day-14, respectively), whereas women showed a higher type I area percent compared with men (P < 0.05; M: 27.2 ± 3.3%, W: 31.4 ± 4.6% for Pre and M: 28.2 ± 2.8%, W: 32.4 ± 4.4% for Day-14, respectively).

The percent atrophy in CSA of type I, IIA, and IIX between Pre and Day-14 for men and women corresponds to 4.8 ± 5.0, 7.9 ± 9.9, and 10.7 ± 10.8% for men and 5.9 ± 3.4, 8.8 ± 8.0, and 10.8 ± 12.1% for women, respectively.

**MHC content.** Both men and women showed a similar percent distribution of MHC I isoforms over 14 days of lower limb immobilization (M: 41.1 ± 11.9%, W: 46.9 ± 11.4% for Pre and M: 38.1 ± 10.4%, W: 44.7 ± 9.7% for Day-14, respectively), IIA (M: 40.1 ± 6.9%, W: 37.5 ± 8.9% for Pre and M: 43.9 ± 14.0%, W: 40.7 ± 9.6% for Day-14, respectively), and IIX (M: 18.8 ± 10.2%, W: 15.6 ± 11.1% for Pre and M: 18.0 ± 9.0%, W: 14.6 ± 11.0% for Day-14, respectively), with no sex-specific differences.

**Correlation coefficients.** Significant correlations were found between the percent loss of isometric and concentric-slow peak torque and the percent atrophy of quadriceps for men (r = 0.727, P < 0.05). However, women did not show any correlation between the percent loss of muscle strength for any mode and the percent atrophy in CSA of quadriceps or fibers.

In terms of correlation coefficients between percent fiber-type distribution and MHC content, men showed significant
DISCUSSION

The novel finding in the present study was that the reduction in specific strength of the knee extensors (concentric-slow and isometric) was attenuated in men compared with women despite similar atrophy in CSA of total quadriceps femoris and myofibers (type I, IIa, and IIx). There were no fiber transformation and similar reductions in leg lean mass for both men and women after 14 days of knee brace-mediated leg immobilization.

There are historically two types of lower limb unweighting models considered to bring about changes in skeletal muscle. One model is regarded as lower limb immobilization via a cast or knee brace, and another is unilateral lower limb suspension with a sling and/or shoe (1). In the present study, subjects did not have to remove the brace for sitting, sleeping, or other daily activities, in contrast to unilateral sling suspension. The knee brace used in the present study was light (~650 g total weight) and well tolerated. It is noteworthy that the unilateral lower limb suspension model may also alter blood flow in the treatment leg (10). For example, several studies with unilateral lower limb suspension have reported the incidence of deep venous thrombosis for a few subjects (8, 10, 16). In the present study, the degree of flexion during bracing (60° from full extension) did not occlude femoral or popliteal artery blood flow (assessed by Doppler ultrasound, results not shown). Thus our lower limb immobilization model and protocol minimized the possibility of developing deep vein thrombosis, as opposed to the sling-suspension model in which the knee is more extensively flexed.

In association with the aforementioned aspects of our immobilization model, it has been reported that the lower limb unloading model may not be a model of complete immobilization because the limb is still able to move at the hip joint (18, 36). Hence, because the rectus femoris is a hip flexor as well as a knee extensor, it is perhaps not surprising that this muscle showed less atrophy because it would likely have been active to a small degree in hip flexion (18, 36). Hortobágyi et al. (20) have also suggested that the model of lower limb immobilization cannot fully be restricted because unintentional isometric muscle tension or reflexive postural adjustments with balance-seeking efforts must occur. In line with those data (18, 36), we had similar results in that the rectus femoris showed less atrophy compared with the vastus in both men and women. Despite the preceding arguments, we still observed significant morphological reductions in the CSA of both the vastus and rectus femoris as well as the total quadriceps femoris as a whole in both men and women with short-term knee brace-mediated immobilization.

Our unweighting model was similar to what Deschenes et al. (13) have reported. In the present study, the angle of knee flexion was set at 60° from full extension, and Deschenes et al. set the angle at 70°. Interestingly, Deschenes et al. did not find statistically significant changes in fiber size or fiber-type distribution of myofibers from the vastus lateralis in a total of eight subjects including men and women after 14 days of leg immobilization with a knee brace. However, Jones et al. (21) have recently shown significant reductions in quadriceps lean mass (patella to groin region) in 9 men after 14 days of lower limb casting. In addition, Hespel et al. (19) showed significant decreases up to ~10% in CSA of quadriceps by MRI (a total of 9 subjects composed of men and women) after 14 days of leg immobilization with a cast, whereas there were no significant decreases in CSA of any fiber type (a total of 8 subjects composed of men and women). The findings in the present study showed significant atrophy of CSA of total quadriceps femoris (a total of 27 subjects), atrophy of myofibers (type I, IIa, and IIx, a total of 17 subjects, respectively), and reductions

Fig. 4. Mean fiber area (μm²) in type I (A), type IIa (B), and type IIx (C) fibers for men (n = 9) and women (n = 8) before and after 2 wk of unilateral leg immobilization. Values are means ± SD. Main effects are shown for gender (*) and time (†) (P < 0.05). No interaction (gender x time) was found in either type I, type IIa, or type IIx.
in leg lean mass (a total of 22 subjects) for men and women after 14 days of immobilization.

Variables such as differences in hormonal profiles, physical activity levels, methodology, and inter- and intraindividual variability may explain why some have not reported significant reductions in the CSA of myofibers (13, 19). Furthermore, the sample size of our study was two- to threefold greater than many of the other studies, which will significantly reduce the risk of a type II error. Subtle differences in study design could also explain why some have not found significant atrophy after 14 days of immobilization. For example, in the study by Deschenes et al. (13), subjects removed their knee braces and performed range-of-motion exercises before retiring at night, whereas the subjects in our study were required to wear the brace while sleeping. Given the potent effect of physical activity on gene expression and protein synthesis (11), it is important to avoid weight-bearing and range-of-motion activity in an immobilization model.

Similarly to previous studies (7, 18), we observed no fiber-type transitions for men or women in either histochemical assessments or in the relative proportion of MHC determined in the same serial sections after short-term immobilization. In accordance with previous data (8), the results of the present study suggest that a fiber-type-specific atrophy is unlikely to alter the relative CSA occupied by a certain fiber type, with unaltered MHC proportions across myofibers over the time course of immobilization. According to Berg et al. (8), specific MHC contents are influenced by not only fiber type but also the relative fiber size. To sum up, it appears unlikely that fiber-type transformations or qualitative changes in MHC proportion in knee extensor muscles occur for men and women in response to 14 days of lower limb immobilization. However, advanced fiber-type classifications in conjunction with histochemical and MHC analyses may reveal the more subtle regulatory pathways and mechanisms that affect the multiple genes that are associated with different modules of fiber-type function (33).

In terms of the functional origin of strength loss in the neuromuscular system, previous studies have shown that changes of motor cortical area size occur without spinal excitability or motor threshold during immobilization, although the reduction in area could be quickly reversed by voluntary muscle contraction after immobilization (24, 39). It has been shown that short-term (10–16 days) as well as relatively long-term lower limb unloading (4–6 wk) cause a reduction in the ability to voluntarily activate a muscle, which indicates that the recruitment of motor units and firing frequencies were altered (9, 14, 20). In association with these phenomena, previous studies have demonstrated that the loss of voluntary muscle strength is greater than that of muscle CSA as a consequence of lower limb unloading (6, 14, 18).

In contrast to the research showing a clear role for neural factors mediating a proportion of the early immobilization-induced strength loss, there are conflicting results regarding whether or not sex-specific differences exist in baseline (i.e., nonimmobilized) values with minimal evidence to support a sex difference during immobilization. For instance, several studies reported sex-specific differences in knee extensor peak torque (22, 23), whereas some studies have shown that there were no sex differences in knee extensor isometric (27) or isokinetic peak torque (30). One reason for the disparity in those studies may in part be due to the different calculations of specific strength (30). For example, some have used leg length to determine specific strength [N·m/(cm² × m)] (23), whereas other group made calculations using body height (m) (30). Although our calculations were also based on body height (30), we found that women showed greater losses of specific strength (concentric-slow and isometric) between Pre and Day-14 compared with men. To our knowledge, there is only one study that has looked at possible sex differences in neural recruitment issues during immobilization (31). This group found that women displayed an intermittent electromyogram recruitment pattern during postimmobilization (4 wk) submaximal contractions that was not apparent before immobilization or after recovery or at all in men (31). Taken together with the data from the present study, it appears that there are minimal to no sex-based differences in specific strength or neural activation patterns at baseline but that women display an immobilization-induced loss of specific strength that is related more to neural vs. atrophy-based factors.

Different fiber-type characteristics between men and women and the contractile properties of the fiber types have been suggested as a potential reason for sex-related discrepancies of muscle strength (17, 25). Our findings demonstrated that men showed a greater percentage of type II fiber (fast-oxidative-glycolytic) area in the vastus lateralis compared with women, and women had a higher percentage of type I fiber (slow-oxidative) area compared with men that was maintained over the immobilization period. Furthermore, a similar fiber-type percent (type I, IIa, and IIx) was found in both men and women throughout the immobilization protocol. Our Pre value findings were consistent with another large study evaluating sex differences in both fiber-type distribution and fiber area percent (34). Some (37) but not all (26) studies have shown that type II fibers may be able to generate a high force, particularly at higher angular velocities (37). Interestingly, in the present study, strength loss in isometric and concentric-slow was highly correlated with the atrophy in CSA of the entire quadriceps muscles for men but not for women. Moreover, even when men had a higher type II area percent compared with women, a similar loss in concentric-fast specific strength was found for both men and women over the 14 days of immobilization. Therefore, sex-related differences in specific loss strength after immobilization cannot fully be explained by sex-specific differences in fiber-type composition.

It has been shown that ovariectomy causes a greater degree of atrophy in the soleus and plantaris muscles after 2 wk of hindlimb unloading compared with control animals (15). The aforementioned observations (15) may have been due to a loss of 17β-estradiol, because it functions as an antioxidant and a membrane stabilizer and protects against exercise-induced muscle damage in rats and humans (4, 32, 38). Although there was a tendency for women to show a higher 17β-estradiol concentration (the synthetic estrogens in oral contraceptives were not detected by the assay and would have yielded an even greater “estrogen” effect) over the immobilization period, both men and women showed a similar extent of muscle atrophy at both the fiber and whole muscle level after 14 days of unilateral immobilization. Therefore, it seems unlikely that 17β-estradiol or synthetic estrogen would have a preventive effect on skeletal muscle atrophy in humans after 14 days of a unilateral leg immobilization.

In conclusion, the findings from the present study indicate that immobilization-induced loss of specific strength at isomet-
ric and slower angular velocity concentric contractions is attenuated in men compared with women. This sex-specific voluntary strength loss occurred despite a similar amount of muscle atrophy at the fiber and whole muscle level, with no fiber-type transitions after only 14 days of unilateral lower leg immobilization. Overall, these findings indicate that the immobilization-induced specific strength loss for women has a proportionately greater neural activation component compared with that observed in men. Future studies will be required to explore whether this sex-based difference is occurring at the level of central recruitment, neuromuscular transmission, or excitation–contraction coupling.

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