Myofibrillar distribution of succinate dehydrogenase activity and lipid stores differs in skeletal muscle tissue of paraplegic subjects

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Submitted 1 June 2011; accepted in final form 2 November 2011

Individuals who are dependent largely on a wheelchair for mobility have an increased risk of developing glucose intolerance and insulin resistance (4, 10, 34, 46). The latter is likely attributed to reduced physical activity, loss of leg muscle mass, and altered body composition (24, 59, 67). Whole body insulin resistance is generally accompanied by ectopic lipid deposition in skeletal muscle tissue (39, 49), which has been associated with muscle mitochondrial dysfunction and a reduced capacity to oxidize fatty acids (41, 45). However, little is known about lipid content and mitochondrial oxidative capacity in deconditioned skeletal muscle tissue in relation to whole body insulin sensitivity in subjects with paraplegia (PAR).

Using nuclear magnetic resonance, previous studies have reported increased intramuscular lipid content in deconditioned leg muscles of PAR individuals (21, 23, 55). In addition, an initial reduction of muscle mitochondrial oxidative activity has been observed directly after spinal cord injury, followed by a recovery to nearly normal levels after 6 mo (13). However, the myofibrillar distribution of mitochondria in deconditioned muscle of PAR subjects has not been evaluated in previous studies. It has been suggested that subsarcolemmal (SS) mitochondria are of more metabolic relevance for fat oxidation, facilitating glucose transport, and the optimal functioning of the insulin-signaling pathway when compared with intermyofibrillar (IMF) mitochondria, which instead support contractile activity (37, 53). In healthy, sedentary, and well-trained males it has been shown that mitochondrial density is two- to threefold higher in the SS area when compared with the IMF area (16). Furthermore, the volume density of intramyocellular lipid (IMCL) droplets in the SS layer is higher when compared with the IMF region (31, 62). Recently, it was shown that in patients with type 2 diabetes, oxidative capacity (47) and electron transport chain activity (53) of SS mitochondria are reduced, whereas IMCL content in the SS layer was threefold higher in diabetic patients when compared with healthy, normoglycemic controls (47). To date, however, it is unknown how subcellular distribution patterns of IMCL and muscle fiber oxidative capacity change in response to muscle deconditioning following paralysis.

The aim of our study was to compare whole body insulin sensitivity, muscle fiber oxidative capacity, and IMCL content between PAR subjects and age-, sex-, and body mass index-matched able-bodied controls. An oral glucose tolerance test was performed to assess glucose tolerance and estimate whole body insulin sensitivity, which is accompanied by reduced SDH activity and increased IMCL deposition in the deconditioned muscle. The latter changes are expected to be visible particularly in the SS location of the muscle fibers.
Table 1. Subjects’ characteristics

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>PAR</th>
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<tbody>
<tr>
<td>Sex (males/females)</td>
<td>7/2</td>
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</tr>
<tr>
<td>Age, yr</td>
<td>31 ± 3</td>
<td>34 ± 4</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.80 ± 0.02</td>
<td>1.81 ± 0.01</td>
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<tr>
<td>Body mass, kg</td>
<td>74.4 ± 3.1</td>
<td>82.1 ± 5.9</td>
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<tr>
<td>BMI, kg/m</td>
<td>23.1 ± 1.2</td>
<td>25.1 ± 1.6</td>
</tr>
<tr>
<td>Energy intake, kcal/day</td>
<td>2,066 ± 131</td>
<td>1,456 ± 141</td>
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<tr>
<td>Exercise frequency, wk</td>
<td>3.9 ± 0.4</td>
<td>3.5 ± 0.8</td>
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<tr>
<td>Exercise duration, min</td>
<td>92 ± 9</td>
<td>101 ± 11</td>
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<td>Time postinjury, yr</td>
<td>NA</td>
<td>10.0 ± 2.6</td>
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<tr>
<td>Whole body insulin sensitivity and glycemic control</td>
<td></td>
<td></td>
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<tr>
<td>Fasting glucose, mmol/l</td>
<td>5.1 ± 0.2</td>
<td>5.7 ± 0.3</td>
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<tr>
<td>Fasting insulin, pmol/l</td>
<td>64 ± 7</td>
<td>78 ± 13</td>
</tr>
<tr>
<td>OGIS120 index, m³/min⁻¹·m⁻²</td>
<td>432 ± 22</td>
<td>361 ± 29</td>
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Plasma lipid profile

<table>
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<tr>
<td>Free fatty acids, μmol/l</td>
<td>499 ± 61</td>
<td>444 ± 49</td>
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<tr>
<td>Triacylglycerols, mmol/l</td>
<td>0.85 ± 0.90</td>
<td>1.30 ± 0.26</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
<td>1.36 ± 0.08</td>
<td>1.21 ± 0.10</td>
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<td>LDL cholesterol, mmol/l</td>
<td>2.62 ± 0.21</td>
<td>2.86 ± 0.41</td>
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<tr>
<td>LDL/HDL ratio</td>
<td>2.05 ± 0.30</td>
<td>2.43 ± 0.37</td>
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<tr>
<td>Total cholesterol, mmol/l</td>
<td>4.37 ± 0.19</td>
<td>4.67 ± 0.44</td>
</tr>
</tbody>
</table>

Values are means ± SE. CON, controls; PAR, subjects with paraplegia; BMI, body mass index; NA, not applicable; OGIS, oral glucose insulin sensitivity (42). *Significantly different from CON (P < 0.01).

MATERIALS AND METHODS

Ethical approval. Nine healthy subjects with PAR and nine able-bodied, lean, age- and sex-matched controls (CON) were recruited (Table 1). Daily energy intake was determined from a food intake questionnaire, which was filled out by the subjects for 2 days prior to the study. Sports participation and exercise frequency and duration in particular were determined from a questionnaire. All subjects were informed about the nature and possible risks of the experimental procedures before their written informed consent was obtained, after approval by the Medical Ethical Committee of the Maastricht University Medical Centre++, The Netherlands.

Diet and physical activity prior to testing. On the evening prior to the measurement, subjects consumed a standardized meal [41.2 kJ/kg body wt, providing 72 energy percent (En%) carbohydrate, 11 En% fat, and 17 En% protein] at 8 PM, after which they remained fasted for 12 h. Subjects were instructed to refrain from any exhaustive physical labor and exercise training for 48 h prior to testing.

Experimental trials. On the day of the experiment, subjects arrived at the laboratory by car or public transport at 8 AM. A Teflon catheter was inserted into an antecubital vein for blood sampling. After 30 min of supine rest, a basal blood sample was collected and a muscle biopsy taken from the m. vastus lateralis. Muscle biopsy samples were obtained from the midbelly region of the vastus lateralis (15 cm above the patella) and ~3 cm below entry through the fascia using the percutaneous needle biopsy technique (6). Muscles samples were dissected, freed from any visible nonmuscle material, embedded in Tissue-Tek (Sakura; Finetek, Zoeterwoude, The Netherlands), and rapidly frozen in liquid nitrogen-cooled isopentane.

Thereafter, a standard oral glucose tolerance test was performed, and blood samples were collected subsequently at t = 30, 60, 90, and 120 min following ingestion of 75 g of glucose (dissolved in 250 ml of water) to monitor plasma glucose and insulin responses.

After completion of the oral glucose tolerance test, localized 1H-MRS experiments were performed on a 1.5-T whole body scanner (Philips Gyrosan S15/ACS; Philips Medical Systems, Best, The Netherlands) to measure lipid levels in the vastus lateralis of the contralateral leg at approximately the same proximo-distal position as for the muscle biopsy.

1H-MRS. IMCL content was measured in subjects in the supine position. Five single-voxel measurements were performed in the vastus lateralis using the point-resolved spectroscopy sequence (repetition time/echo time = 1,500/35 ms), as described previously (17). Briefly, the voxels (10 × 10 × 15 mm³) were placed at different locations within the vastus lateralis, avoiding any subcutaneous fat and minimizing the amount of visible interstitial fat using standard T1-weighted images (Fig. 1). From each voxel, a spectrum without (32 acquisitions) and with (128 acquisitions) chemical shift-selective water suppression was collected. Water, extramyocellular lipid (EMCL) CH₂, and IMCL-CH₂ peak areas were quantified from the unsuppressed and suppressed spectra, respectively, using a nonlinear least-squares algorithm (advanced method for accurate, robust, and efficient spectral fitting) in the jMRUI software package (64), as described previously (17). Both IMCL and EMCL content were expressed as a percentage of the water signal in the unsuppressed spectrum measured in the same voxel, and data from the different voxels of each subject were averaged.

Plasma analyses. Blood samples were collected in EDTA-containing tubes and centrifuged at 1,000 g and 4°C for 10 min. Aliquots of plasma were frozen immediately in liquid nitrogen and stored at −80°C. Samples were analyzed for plasma glucose and insulin to assess whole body insulin sensitivity using the oral glucose insulin sensitivity index (42). Insulin was analyzed by radioimmunoassay (Insulin RIA Kit; Linco Research, St. Charles, MO). Plasma glucose (ABX Pentra Glucose HK CP; Radiometer Nederland, Zoetermeer, The Netherlands), free fatty acids (NEFA-C kit; Wako Chemicals, Neuss, Germany), triglyceride (ABX Pentra Trig CP; Radiometer Nederland), triglyceride (ABX Pentra Trig CP; Radiometer Nederland)
Netherlands), total cholesterol (ABX Pentra Cholesterol CP; Radiometer Nederland), high-density lipoprotein cholesterol (ABX Pentra Cholesterol; Radiometer Nederland), and low-density lipoprotein cholesterol concentrations were analyzed with a COBAS FARA semiautomatic analyzer.

**Muscle biopsy analyses.** Multiple serial cryostat sections (5 μm) from biopsy samples collected from a subject of the CON group were thaw-mounted together with biopsy samples collected from a subject of the PAR group on uncoated, precleaned glass slides.

Slides were stained for muscle fiber composition, as described previously (65). After fixation, cryosections were incubated with primary antibodies directed against laminin (polyclonal rabbit anti-laminin, dilution 1:50; Sigma, Zwijndrecht, The Netherlands), slow type 1 skeletal muscle fibers (A4.840, dilution 1:25; Developmental Studies Hybridoma Bank, Iowa City, IA), and slow and fast type 2a fibers (N2.261, dilution 1:25; Developmental Studies Hybridoma Bank) diluted in 0.05% Tween-phosphate-buffered saline (PBS). Slides were then washed and incubated with the conjugated secondary antibodies goat anti-rabbit IgG Alexa Fluor 350, goat anti-mouse IgM Alexa Fluor 488, and goat anti-mouse IgG Alexa Fluor 555 (dilutions of 1:130, 1:200, and 1:200, respectively; Molecular Probes, Invitrogen, Breda, The Netherlands) diluted in 0.05% Tween-PBS. After a final washing step, all slides were mounted with Mowiol (Calbiochem, Amsterdam, The Netherlands) and coverslips.

An Oil Red O (ORO) staining, combined with a staining for laminin (primary antibody: polyclonal rabbit anti-laminin; secondary antibody: goat anti-rabbit IgG Alexa Fluor 488), was performed as described previously by Koopman et al. (36) and van Loon et al (63) as a measurement for IMCL content. To determine fiber type-specific IMCL content, slides stained for muscle fiber composition were used as a reference.

Muscle fiber type-specific oxidative capacity was estimated by determining maximal SDH activity in the muscle cross-sections (25, 51). To assess intramyocellular SDH activity, a modified SDH staining was used as described previously (25, 56), allowing direct, fiber type-specific determination of SDH activity. The staining procedures were as follows. Freshly prepared cryosections were incubated for 60 min at 37°C in a phosphate buffer at pH 7.6 [13 ml of 0.2 M NaH₂PO₄ (Sigma) + 87 ml of 0.2 M Na₂HPO₄ (Sigma)] containing 0.1 M sodium succinate (Sigma) and 1.2 mM nitro-blue tetrazolium (Sigma). After incubation, the slides were rinsed (3 times in deionized water). The unbound nitro-blue tetrazolium was removed with three exchanges in increasing solutions of acetone in deionized water (30, 60, and 90%, respectively). When the slides were dry, sections were incubated for 60 min with the primary antibodies described above. Slides were then washed (3 × 5 min with PBS), after which the appropriate conjugated secondary antibodies were applied for 30 min. Incubation was followed by a final washing step (3 × 5 min with PBS), and the slides were mounted with Mowiol and coverslips.

After 24 h, glass slides were examined using a Nikon E800 fluorescence microscope (Uvikon, Bunnik, The Netherlands) coupled to a Basler A113 C progressive-scan color charged-coupled device camera with a Bayer color filter. Epifluorescence signal was recorded using a Texas-red excitation filter (540–580 nm) for ORO and A4.840, a fluorescein isothiocyanate excitation filter (465–495 nm) for N2.261 and laminin (for ORO staining), and a 4’-6-diamidino-2-phenylindole UV excitation filter (340–380 nm) for laminin (for fiber type staining). All image recordings and analyses were performed by a researcher blinded for subject coding. Digitally captured images (×240 magnification) were processed and analyzed using Lucia 4.81 software (Nikon, Düsseldorf, Germany).

The signal derived from the antibody against laminin was used to select single muscle fibers, and to differentiate between fiber types within each image, serial cross-section-stained images for fiber type composition were used. The ORO-epifluorescence signal was recorded for each muscle fiber at a ×240 magnification. An intensity threshold representing minimal intensity values corresponding to lipid droplets was set manually and used uniformly for all images. Total cross-sectional area, number of objects, and area emitting ORO-epifluorescence signal were measured. Identical settings were used for all muscle cross-sections, because the recorded ORO signal depends on staining efficiency as well as on the applied image acquisition settings (intensity threshold and integration time). Lipid droplet size was calculated by dividing the area emitting ORO-epifluorescence signal by the number of objects. Fiber type-specific IMCL content is expressed as the fraction of the cross-sectional area that was stained with ORO. Images were analyzed for subcellular localization of IMCL in different consecutive 2-μm layers, as described previously (60, 62). The location of lipid droplets and resultant area fraction within successive bands of 2 μm in width were recorded from the SS region toward the central area of each muscle fiber type-specific cross-section.

In bright-field light microscopy, sections stained for SDH activity were captured at ×120 magnification. A laminin, A4.840, and N2.261 staining on the same tissue was recorded and processed as described above. The bright-field images of the SDH-activity staining were converted post hoc to 8-bit grayscale values. The mean optical density of the SDH-generated signal per individual fiber was determined by averaging the optical density (OD) measured in every pixel in the cell and corrected for the mean OD of the background stain measured in a field of view containing no muscle fibers. The location of SDH activity and resultant OD within successive bands of 2 μm in width was recorded from the SS toward the central region of each muscle fiber type-specific cross-section.

**Statistics.** All data are expressed as means ± SE. Subject, plasma, and mixed-muscle data were compared between groups by means of an independent t-test. Skeletal muscle fiber type-specific variables were analyzed with mixed-model repeated-measures analysis of variance (RM-ANOVA), with fiber type (type 1, type 2a, and type 2x) as the within-subjects factor and group (PAR compared with CON) as the between-subjects factor. Relative differences in both IMCL content and SDH activity within the skeletal muscle cell were calculated and analyzed by ANOVA-RM, with location (SS and IMF) and fiber type (type 1, type 2a, and type 2x) as within-subjects factor and group as between-subjects factor. In case of a significant interaction, independent t-tests were performed to determine group effects, paired t-tests to determine location differences, and RM-ANOVA for fiber type-specific effects. Bonferroni corrections were applied when appropriate. To evaluate the relationships between variables, Pearson correlation coefficients were calculated. Statistical analyses were performed using the SPSS 17.0 software package (SPSS, Chicago, IL). The level of significance was set at P ≤ 0.05.

**RESULTS**

**Subjects’ characteristics.** The characteristics of the CON and PAR subjects are shown in Table 1. Both groups consisted of seven male and two female subjects. No differences in mean age, height, body mass, or body mass index were observed between groups. Energy intake was 31 ± 3% lower in PAR subjects compared with CON subjects (P < 0.01). Sports participation did not differ between PAR and CON subjects. The PAR subjects had been dependent on a wheelchair in daily life for 10.0 ± 2.6 yr due to incomplete or complete spinal cord injury (n = 7; injury levels: C5–C6, C5–C7, C8, T4, T6, T9–T10), spin-bifida (n = 1; injury level: L4–L5), or paralysis due to another cause (n = 1). Mean fasting plasma glucose and insulin concentrations were within the normal range for healthy individuals in both groups (Table 1), although according to the guidelines of the American Diabetes Association (1), two individuals in the PAR group showed impaired glucose tolerance (2-h plasma glucose 8.31 and 8.09 mmol/l). In the
Table 2. Muscle fiber type composition

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<thead>
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<tr>
<td><strong>Myofiber size, μm³</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>6,392</td>
<td>5,359</td>
</tr>
<tr>
<td>Type 2a</td>
<td>6,569</td>
<td>5,044</td>
</tr>
<tr>
<td>Type 2x</td>
<td>6,684</td>
<td>5,447</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly different from CON (P < 0.05); †significantly different from type 1 (P < 0.005); ‡significantly different from type 2a (P < 0.005); #significantly different from CON, independent of fiber type (P < 0.05).

PAR group, fasting plasma glucose concentrations tended to be 12 ± 2% higher (P = 0.06), and whole body insulin sensitivity as determined by the oral glucose insulin sensitivity index tended to be 20 ± 3% lower (P = 0.07) when compared with the CON group. Plasma lipid profiles did not differ between groups.

**Skeletal muscle fiber type composition.** The PAR group had a 43 ± 2% lower percentage of type 1 fibers and a 22 ± 10% higher percentage of type 2a fibers compared with the CON group (P < 0.05, Table 2). The percentage of type 2x fibers tended to be 21 ± 9% higher in the PAR subjects when compared with the CON subjects (P = 0.06). Cross-sectional area of myofibers from PAR subjects was 18 ± 6% smaller when compared with CON subjects independent of fiber type (P < 0.05; Table 2).

**Muscle lipid content and SDH activity.** Examples of 1H-MR spectra from the vastus lateralis of a CON and a PAR subject are shown in Fig. 2, and examples of muscle tissue cross-sections stained for IMCL and SDH activity are shown in Figs. 3 and 4, respectively. The results of the 1H-MRS analyses of mixed-muscle EMCL and IMCL content and the histological analyses of IMCL content and SDH activity are presented in Table 3. EMCL content in vastus lateralis, measured by localized 1H-MRS, was higher in PAR compared with CON subjects (4.58 ± 0.72 vs. 0.99 ± 0.13% of unsuppressed water signal, P = 0.001) and tended to correlate negatively with whole body insulin sensitivity (r = −0.416, P = 0.086). In contrast, IMCL content, analyzed by both localized 1H-MRS and ORO staining, did not significantly differ between groups. The analyses of fiber type-specific IMCL content with ORO staining showed a tendency for a fiber type effect (P = 0.07), independent of group, with greater mean area fractions in type 1 vs. type 2a vs. type 2x fibers. The lipid droplet size was 50 ± 12% larger in PAR individuals compared with CON subjects (P = 0.05) independent of fiber type. Lipid droplet size was inversely correlated with whole body insulin sensitivity (r = −0.815, P < 0.001).

SDH activity in PAR subjects was 57 ± 14% lower compared with CON subjects (P = 0.05) independent of fiber type (Table 3). SDH activity was significantly greater in type 1 fibers than in type 2 fibers (P < 0.05), and SDH activity in type 2a fibers was greater than in type 2x fibers (P = 0.01), all independent of group. SDH activity did not correlate with whole body insulin sensitivity.

**Myofibrillar distribution.** Following the analyses of total IMCL content and SDH activity, the spatial distribution of IMCL and SDH activity inside skeletal muscle fibers is presented in Figs. 5 and 6, respectively. Recordings of successive 2-μm bands were merged into a SS layer (defined as the first 4 μm from the sarcolemma) and an IMF region (defined as the area from the SS layer to the center of the cell).

In the CON group, 62 ± 1% of mixed-muscle IMCL (Fig. 5A) and 60 ± 1% of mixed-muscle SDH activity (Fig. 6A) were located in the SS region, whereas the remaining 38 ± 1 and 40 ± 1% were located in the IMF region (P = 0.01 and P = 0.04, respectively). Similar spatial distributions for IMCL and SDH activity were observed in fiber type-specific analyses for the CON group (Figs. 5, B–D, and 6, B–D). In contrast, in the PAR group, no differences were observed between the SS and the IMF region for either IMCL or SDH activity. In the SS layer, SDH activity was greater in the CON subjects when compared with the PAR subjects (Fig. 6).

**DISCUSSION**

The present study aimed to elucidate the effects of chronic muscle deconditioning on whole body insulin sensitivity, muscle fiber oxidative capacity, and lipid accumulation. We hypothesized that PAR subjects have reduced whole body insulin sensitivity.
accompanied by a lower muscle fiber oxidative capacity and greater IMCL deposition. However, despite the loss of functional ability of the legs, young healthy PAR individuals did not show a substantial decline in glucose tolerance and/or whole body insulin sensitivity. The loss of leg muscle recruitment was accompanied by lower muscle fiber oxidative capacity as determined from SDH activity, whereas mixed-muscle lipid deposition was unaffected. In healthy muscle tissue of controls, both SDH activity and IMCL content were greater in the SS region when compared with the IMF region. The latter myofibrillar distribution pattern was no longer present in the deconditioned muscle of the PAR subjects.

Whole body insulin sensitivity. Insulin sensitivity is lowered following a prolonged period of reduced habitual physical activity (40, 58). Individuals who are dependent largely on a wheelchair are expected to have a reduced physical activity level and, therefore, to be less insulin sensitive. Although fasting plasma glucose and insulin levels in the PAR subjects were within the normal range according to the guidelines of the American Diabetes Association (1), two subjects were diagnosed with impaired glucose tolerance. The tendency toward elevated fasting glucose levels concomitant with the tendency toward reduced insulin sensitivity in the PAR group is in accordance with previous observations of a higher prevalence of glucose intolerance in individuals with spinal cord injury (19, 24). In this study, visceral adipose tissue mass was not assessed, but body mass and body mass index did not differ significantly between PAR and CON subjects.

Muscle fiber oxidative capacity. It is known that with inactivity (14, 50, 52), immobilization (22, 48), and denervation (26, 43), muscle oxidative capacity is reduced. The latter has at least partly been attributed to a change in muscle fiber type composition toward more type 2 fibers (54) and in particular type 2x fibers (12, 13, 57). In accord, we observed a reduced proportion of type 1 fibers and an elevated proportion type 2 muscle fibers in the deconditioned muscle compared with the healthy muscle (Table 2). In addition, besides the substantial changes in muscle fiber type composition, we observed that for all fiber types maximal SDH activity was lower in muscle obtained from the PAR vs. able-bodied subjects.

In addition to muscle fiber type-specific analyses, histology also allows determination of subcellular distribution of muscle fiber oxidative capacity. In healthy muscle tissue of the control group, oxidative capacity as determined from SDH activity was 1.5 times higher in the SS region compared with the IMF...
This is in line with previous reports, which show a greater mitochondrial density and greater lipid oxidative capacity in the SS layer when compared with the IMF area of the muscle fiber (16, 20, 37, 38). Furthermore, endurance-type exercise training has been reported to particularly increase the mitochondrial density in the SS area of the muscle fibers (9, 30, 31). In contrast to the results in healthy muscle, oxidative capacity in the SS region of deconditioned muscle tissue from the PAR subjects was similar to that in the IMF area. Moreover, muscle fiber oxidative capacity in the SS region was substantially lower in PAR subjects when compared with able-bodied subjects. These results are in agreement with a recent study by Nielsen et al. (48), who showed that SS mitochondrial content decreased by 33% after 2 wk of muscle immobilization by whole leg casting, whereas central IMF mitochondrial content remained unchanged. In contrast, Feretti et al. (22) reported a 17% decrease in IMF mitochondria, with no significant decrease in SS mitochondrial content, in young subjects after 37 days of bed rest. The difference in mode of disuse and activity level could explain the observed discrepancies. In our study and the study by Nielsen et al. (48) the muscle disuse was restricted to the legs while the upper body remained active, whereas the subjects in the bed rest study (22) remained refrained from any physical activity during the bed rest period. Our study confirms the hypothesis that SS mitochondria respond to a greater degree to muscle use or disuse than IMF mitochondria (32, 33, 44, 47, 48). In contrast, Chomentowski et al. (15) reported recently that in skeletal muscle tissue of obese, insulin-resistant subjects with or without diabetes, IMF mitochondrial content was reduced, whereas SS mitochondrial content was unaffected compared with sedentary controls. Apparently, adaptive muscle fiber oxidative responses to muscle disuse are markedly different from those in insulin resistance, indicating that the relationship between muscle disuse and insulin resistance is not unambiguous.

### Table 3. Lipid content and SDH activity

<table>
<thead>
<tr>
<th>Lipid content assessed with 1H-MRS</th>
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<th>PAR</th>
</tr>
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<tbody>
<tr>
<td>% unsuppressed water signal</td>
<td>0.99 ± 0.13</td>
<td>4.58 ± 0.72**</td>
</tr>
<tr>
<td>EMCL</td>
<td>1.14 ± 0.10</td>
<td>1.36 ± 0.07</td>
</tr>
<tr>
<td>IMCL content assessed with ORO, area fraction·10⁻³</td>
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<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>23.8 ± 9.1</td>
<td>18.1 ± 7.3</td>
</tr>
<tr>
<td>Type 1</td>
<td>40.1 ± 17.8</td>
<td>35.2 ± 22.2</td>
</tr>
<tr>
<td>Type 2a</td>
<td>15.7 ± 7.1</td>
<td>20.6 ± 7.6</td>
</tr>
<tr>
<td>Type 2x</td>
<td>2.8 ± 1.0</td>
<td>14.6 ± 5.3</td>
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<td>IMCL droplet size, μm²</td>
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<tr>
<td>Mixed</td>
<td>0.38 ± 0.04</td>
<td>0.65 ± 0.08*</td>
</tr>
<tr>
<td>Type 1</td>
<td>0.43 ± 0.10</td>
<td>0.66 ± 0.19</td>
</tr>
<tr>
<td>Type 2a</td>
<td>0.43 ± 0.07</td>
<td>0.64 ± 0.12</td>
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<tr>
<td>Type 2x</td>
<td>0.27 ± 0.05</td>
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<tr>
<td>SDH activity, OD</td>
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<tr>
<td>Mixed</td>
<td>44.0 ± 11.8</td>
<td>18.8 ± 2.5*</td>
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<tr>
<td>Type 1</td>
<td>50.3 ± 14.0</td>
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<tr>
<td>Type 2a†</td>
<td>36.4 ± 8.6</td>
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</tr>
<tr>
<td>Type 2x†‡</td>
<td>29.1 ± 6.6</td>
<td>14.2 ± 1.8</td>
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</table>

Values are means ± SE. 1H-MRS, 1H-magnetic resonance spectroscopy; EMCL, extramyocellular lipids; IMCL, intramyocellular lipids; ORO, Oil Red O; SDH, succinate dehydrogenase; OD, optical density. **Significantly different from CON (P = 0.001); *significantly different from CON, independent of fiber type (P ≤ 0.05); †significantly different from type 1 (P < 0.05); ‡significantly different from type 2a (P < 0.05).
Muscle lipid content. Our study showed a 4.6-fold greater EMCL content in vastus lateralis muscle of PAR subjects compared with controls, which is in line with a previous observation of increased EMCL content in m. soleus of spinal cord injury patients (21, 23, 55). EMCL content tended to correlate negatively with whole body insulin sensitivity, which is in agreement with a study by Elder et al. (21) demonstrating that EMCL is a good predictor of insulin sensitivity in individuals with spinal cord injury.

We hypothesized that, as a result of reduced muscle fiber oxidative capacity and an associated reduced mitochondrial capacity to oxidize fatty acids, IMCL content would be strongly elevated in deconditioned muscle of PAR subjects. However, in contrast to the study of Shah and et al. (55), we observed no significant differences in IMCL content between PAR subjects and able-bodied controls. Our findings are remarkable and suggest that, at least in healthy PAR subjects, negative feedback systems prevent excessive accumulation of IMCL during an extensive period of muscle disuse. One factor that might have contributed to the normal IMCL levels in the PAR group is the fiber type transformation toward faster, less oxidative fibers, which tended to store less IMCL. In addition, it is known that chronic inactivity results in a loss of muscle capillaries (28), potentially limiting lipid deposition in deconditioned muscle of PAR subjects. Another factor that may have played a role in keeping normal IMCL content is that energy intake was 31% lower in PAR subjects compared with CON subjects, whereas sports participation did not differ significantly between PAR and CON subjects. A negative feedback mechanism preventing excess intracellular lipid accumulation also could have occurred at the molecular level. It has been shown that both acute and chronic physical inactivity lead to downregulation of skeletal muscle lipoprotein lipase activity and a reduction of plasma triglyceride uptake into muscle (7, 68). In addition, physical inactivity has been shown to decrease fatty acid translocase CD36 mRNA expression (5), and muscle denervation was shown to decrease long-chain fatty acid transport, which was associated with reductions in plasemallemal CD36 and plasma membrane-associated fatty acid-binding protein (35). Unfortunately, because of the minimal yield of the biopsy samples from PAR subjects, we were not able to determine lipoprotein lipase activity or CD36 and plasma membrane-associated fatty acid-binding protein content in muscle tissue from PAR subjects.

Although no significant differences in IMCL content between PAR and CON groups have been observed in the present study, IMCL droplet size appeared to be greater in deconditioned muscle tissue (Table 3). Droplet size was inversely correlated with whole body insulin sensitivity, which is in accord with previously published data (27). A larger droplet size has been postulated to contribute to an impairment in fatty acid mobilization from the IMCL pool in obese type 2 diabetes patients as a result of an impaired interaction of lipases with triacylglycerols within the droplets due to a reduced surface-to-volume ratio (8, 11, 29, 61). This could eventually lead to an excessive accumulation of IMCL in skeletal muscle and, as such, exacerbate insulin resistance.

In lean muscle tissue, the majority of IMCL droplets are located close to mitochondria (30, 66), and therefore, the distribution pattern of IMCL in lean myofibers is similar to the distribution pattern of oxidative capacity; i.e., IMCL shows an exponential decline from the periphery toward the central region of the
myofiber. This typical IMCL distribution has been observed in both lean sedentary and trained subjects (37, 60). Also in our study, IMCL content in the SS region of muscle fibers of CON subjects was higher than in the IMF area. In contrast, in the deconditioned muscle tissue of PAR subjects, IMCL was homogeneously distributed in the muscle cell in parallel with the uniform distribution of oxidative capacity. It has been suggested that SS mitochondria are of greater metabolic significance for fat oxidation, glucose transport, and the optimal functioning of the insulin-signaling cascade (37, 53). Therefore, the parallel changes in subcellular distribution patterns of SDH activity and IMCL content in deconditioned skeletal muscle point toward an adaptive response to a lower metabolic demand. This is in contrast with findings in type 2 diabetes patients, in whom the decreased muscle oxidative capacity in the SS region was accompanied by an increased IMCL content, indicating a mismatch between lipid demand and supply (47).

Conclusions. In conclusion, deconditioned muscle tissue of PAR subjects shows a large decline in muscle oxidative capacity, which is accompanied by a change in the typical myofibrillar distribution of SDH activity and IMCL toward a more uniform distribution without a substantial rise in total IMCL content. These structural changes in leg muscle tissue are not accompanied by a major decline in whole body insulin sensitivity. These findings suggest that in healthy subjects negative feedback systems prevent excess lipid deposition in muscle tissue during an extensive period of muscle disuse, reflecting an adaptive response to a lower metabolic demand.

ACKNOWLEDGMENTS

We acknowledge all volunteers who participated in this research. We also thank Larry de Graaf for technical support with the MR scanner, Hans Keizer and Milos Beelen for obtaining muscle biopsy samples, and René Koopman and Ralph Manders for assistance with obtaining muscle biopsy samples.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are reported by the authors.

AUTHOR CONTRIBUTIONS


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