Oxidative capacity and glycogen content increase more in arm than leg muscle in sedentary women after intense training

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Submitted 6 February 2015; accepted in final form 18 May 2015

Nordsborg NB, Connolly L, Weihe P, Iuliano E, Krstrup P, Saltin B, Mohr M. Oxidative capacity and glycogen content increase more in arm than leg muscle in sedentary women after intense training. J Appl Physiol 119: 116–123, 2015. First published May 28, 2015; doi:10.1152/japplphysiol.00101.2015.—The hypothesis that the adaptive capacity is higher in human upper- than lower-body skeletal muscle was tested. Furthermore, the hypothesis that more pronounced adaptations in upper-body musculature can be achieved by “low-volume high-intensity” compared with “high-volume low-intensity” exercise training was evaluated. A group of sedentary premenopausal women aged 45 ± 6 yr (± SD) with expected high adaptive potential in both upper- and lower-extremity muscle groups participated. After random allocation to high-intensity swimming (HIS, n = 21), moderate-intensity swimming (MOS, n = 21), soccer (SOC, n = 21) or a nontraining control group (CON, n = 20), the training groups completed three workouts per week for 15 wk. Resting muscle biopsies were obtained from the vastus lateralis muscle and deltoideus muscle before and after the intervention. After the training intervention, a larger (P < 0.05) increase existed in deltoideus muscle of the HIS group compared with vastus lateralis muscle of the SOC group for citrate synthase maximal activity (95 ± 89 vs. 27 ± 34%), citrate synthase protein expression (100 ± 29 vs. 31 ± 44%), 3-hydroxyacyl-CoA dehydrogenase maximal activity (35 ± 43 vs. 3 ± 25%), muscle glycogen content (63 ± 76 vs. 20 ± 51%), and expression of mitochondrial complex II, III, and IV. Additionally, HIS caused higher (P < 0.05) increases than MOS in deltoideus muscle citrate synthase maximal activity, citrate synthase protein expression, and muscle glycogen content. In conclusion, the deltoideus muscle has a higher adaptive potential than the vastus lateralis muscle in sedentary women, and “high-intensity low-volume” training is a more efficient regimen than “low-intensity high-volume” training for increasing the aerobic capacity of the deltoideus muscle.

mitochondria; metabolism; health; performance; swimming; soccer; lean body mass

SINCE THE INITIATION of human muscle biopsy sampling more than 60 years ago (2), exercise training and muscle plasticity have been thoroughly investigated. Frequently, the vastus lateralis muscle is biopsied, and it is often overlooked that the findings do not represent all skeletal muscle groups.

The metabolic response to exercise differs between leg and arm muscle (16). Arm musculature has higher carbohydrate oxidation and lactate release (1) and lower oxygen extraction capacity even in elite cross-country skiers who have performed years of intensive upper-body training (4). Furthermore, arm muscle has a lower oxidative capacity, when comparing the vastus lateralis muscle and deltoideus muscle, despite similar fiber type composition (20) and capillarization (14). The lower initial oxidative capacity is probably related to the nonpostural nature of upper body musculature and may cause the deltoideus muscle to have a higher potential for oxidative adaptation to exercise training.

This idea is supported by a tendency for prolonged 30- to 40-day low-intensity exercise to increase deltoideus muscle but not vastus lateralis muscle β-oxidative capacity evaluated as maximal 3-hydroxyacyl-CoA dehydrogenase (HAD) activity (14). However, deltoid tricarboxylic acid cycle (TCA) capacity is unchanged after prolonged low-intensity exercise when determined as maximal citrate synthase (CS) activity (14). It is likely that exercise intensity is of importance for the observed response to training. In support of this suggestion, high-intensity exercise training increases deltoideus muscle maximal CS activity by ~30%, whereas maximal HAD activity is unchanged as demonstrated by resistance training in elderly men (31). Importantly, the strength training-induced deltoideus muscle adaptations were not more pronounced than interval-based endurance-training adaptations in vastus lateralis muscle (31), leaving open the possibility that other types of training may result in deltoideus muscle adaptations that are not only similar but even greater than in the vastus lateralis muscle.

It is evident that high-intensity training, despite a very low total training volume, enhances aerobic metabolism (12) via upregulation of PGC1α mRNA expression (32) and subsequent mitochondrial biogenesis (3) and respiratory capacity (6) in the vastus lateralis muscle. Thus it appears likely that a high-intensity low-volume interval-based training regime for the upper body could be effective for optimizing muscular oxidative capacity, but this has not previously been investigated.

Exercise-induced muscular adaptations are determinants of the positive health effects of physical training (10, 18, 21). Because the adaptive potential may be higher in upper-body musculature, it is important to evaluate the potential of upper-body training to induce additional training effects.
compared with the often described adaptation of leg muscle-culture.

The purpose of the present study was to test the hypothesis that the oxidative adaptive potential is higher in upper-body than leg muscle-culture in sedentary women. In addition, the hypothesis that “low-volume high-intensity” training increases upper-body muscular oxidative capacity to a greater extent than “high-volume low-intensity” training was investigated.

METHODS

Subjects. Eighty-three sedentary premenopausal women [age 45 ± 6 (± SD) yr; height 165 ± 6 cm; weight 80.0 ± 14.1 kg; body fat 42.6 ± 5.7%] were recruited for the study. The study was designed as a randomized controlled trial. After initial testing of the 262 volunteers, the participants were enrolled in the study based on the selection criteria of a sedentary lifestyle for the last 2 years, mild hypertension (mean arterial pressure 96–110 mmHg), and body mass index >25. The study was approved by the ethical committee of the Faroe Islands as well as the Sport and Health Sciences Research Ethics Committee at the University of Exeter, Exeter, United Kingdom, and conducted in accordance with the Declaration of Helsinki (1964). After being informed verbally and in writing of the experimental procedures and associated risks, all the participants gave their written consent to take part in the study.

Experimental design. The study was designed as a randomized controlled trial. The participants were randomized by successively allocating participants to one of four groups by order of first contact, i.e., the first volunteer would be allocated to group 1, the second to group 2, etc. After closure of recruitment, groups 1–4 were designated by draw as high-intensity intermittent swimming training group (HIS, n = 21; age 44 ± 5 yr; height 164 ± 6.2 cm; weight 76.5 ± 8.8 kg), a moderate-intensity continuous swimming group (MOS, n = 21; age 46 ± 4 yr; height 165 ± 5 cm; weight 83.8 ± 18.8 kg), a soccer training group (SOC, n = 21; age 45 ± 5 yr; height 165 ± 7 cm; weight 79.8 ± 12.8 kg), and a control group (CON, n = 20; age 45 ± 4 yr; height 166 ± 6 cm; weight 77.3 ± 10.4 kg). Three weekly training sessions were performed for 15 wk. CON had no training or lifestyle changes in the same period. Resting muscle biopsies were obtained from vastus lateralis and deltoideus muscle before and after the intervention. Lean body mass was assessed by dual-energy X-ray absorptiometry (DXA) scanning.

Training intervention. HIS completed 44 ± 4 training sessions over the 15-wk intervention period, corresponding to 2.9 ± 0.5 sessions per week. Each session lasted ~15–25 min (3–5 min of effective swimming without any warm-up) and consisted of 6–10 × 30 s of all-out freestyle swimming (front crawl) intervals interspersed by 2 min of passive recovery according to training principles previously described (28). The number of intervals was increased from 6 to 8 after 6 wk and then to 10 after 12 wk. MOS completed a total of 43 ± 4 training sessions in 15 wk, corresponding to 2.9 ± 0.5 training sessions per week. All MOS training sessions lasted 1 h and consisted of continuous front-crawl swimming with the participants encouraged to swim as far as possible during each session. Five trained swimming coaches were present during all training sessions to give technical advice, control the intensity and duration of the training, and ensure a safe training environment. SOC completed 45 ± 3 training sessions over the 15-wk intervention period, corresponding to 3.0 ± 0.4 sessions per week. Each session lasted 1 h and consisted of small-sided soccer games (from 4 vs. 4 to 10 vs. 10) as previously described (35). A trained soccer coach was present during all sessions to control the duration of the training and ensure competitive games. Heart rates were measured during one training session in week 1 and one training session in week 15 in each of the training groups and these data are reported elsewhere (29, 30).

Muscle sampling and analyses. Muscle samples were obtained from the medial part of the vastus lateralis muscle and the posterior (~90% of samples) or anterior (~10% of samples) part of the deltoideus muscle under local anesthesia (1% lidocaine) using the Bergstrom needle biopsy technique with suction (2). Sampling of the anterior deltoideus was performed when palpation of the posterior part was difficult due to adipose tissue despite the possible metabolic differences between the portions. However, it was assumed that both portions are active during swimming and have a high adaptive potential in sedentary women. The muscle samples obtained before and after the intervention were obtained from the same portion. Biopsy samples were immediately frozen in liquid nitrogen and stored at −80°C for subsequent analysis. Due to complications either when obtaining samples or during the analytical process, the number of reported observations may be lower than the included 83 individuals. For all results, the included number of observations is provided (see Fig. 1 for details of participants and analyses). Furthermore, the deltoideus muscle biopsies were generally smaller than the vastus lateralis muscle and analytic priority was made for Western blotting, resulting in fewer available samples for enzymatic activity assays.

Muscle preparation. Wet muscle samples were weighed on scales (XP6U Ultra-Microbalance, Mettler Toledo, Leicester, UK) located in a −20°C cold cabinet before undergoing a freeze-drying process.

Western blotting. Equal amounts of total protein were loaded in each well of precasted gels (Bio-Rad Laboratories). Pre and post samples were diluted with 6 ml glycerol, 0.93 g DTT, 1 g SDS and 1.2 mg bromophenol blue) and protein concentration in each sample was determined using a bovine serum albumin (BSA) standard kit (Pierce, Rockford, IL), and samples were diluted with 6 × Laemmli buffer (7 ml 0.5 M Tris-base, 3 ml glycerol, 0.93 g DTT, 1 g SDS and 1.2 mg bromphenol blue) and ddH2O to reach equal protein concentration before protein expression was determined by Western blotting.

Fig. 1. Illustration of the study recruitment process and number of completed analyses. See text for details. CON, no physical activity; SOC, soccer; MOS, moderate-intensity continuous swimming; HIS, high-intensity interval swimming.
separated according to their molecular weight by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and semi-dry transferred to a polyvinylidene difluoride (PVDF) membrane (BioRad, Denmark). The membranes were blocked in either 2% skimmed milk or 3% BSA in Tris-buffered saline including 0.1% Tween-20 (TBST) before an overnight incubation in primary antibody at 4°C and a subsequent 1-h incubation in horseradish peroxidase (HRP)-conjugated secondary antibody at room temperature. The bands were visualized by enhanced chemiluminescence (ECL, Millipore) and recorded with a digital camera (ChemiDoc MP Imaging System, Bio-Rad Laboratories). Image Lab version 4.0 (Bio-Rad Laboratories) was used for densitometry quantification of the Western blot band intensity and adjustment for background intensity.

Antibodies. It was verified from analyses of a serial dilution of mixed human muscle standard lysates that the loaded amount of protein would result in a signal intensity that was localized on the linear part of the antibody-specific standard curve. Applied antibodies were phosphofructokinase (PFK), 85 kDa, mouse monoclonal (Sc-166722, Santa Cruz Biotechnology) and citrate synthase (CS), 48 kDa, rabbit polyclonal (ab96600, Abcam). Additionally, the Mitoprofile Total OXPHOS Human WB mouse Antibody Cocktail was applied (ab110411, Abcam, UK). This generated results for Mitochondrial Complex I subunit NDUFB8 (CI), 20 kDa (monoclonal ab110242); Mitochondrial Complex II succinate dehydrogenase complex subunit B (CII), 30 kDa (monoclonal ab14714); Mitochondrial Complex III subunit Core 2 (CIII), 45 kDa (monoclonal ab14745); Mitochondrial Complex IV subunit II (CIV), 25 kDa (monoclonal ab110258); and Mitochondrial Complex V ATP synthase subunit alpha (CV), 55 kDa (monoclonal ab14748). The secondary antibodies used were HRP-conjugated rabbit anti-sheep (P-0163), goat anti-mouse (P-0447, DAKO, Denmark), and goat anti-rabbit IgM/IgG (4010-05 Southern Biotech).

Muscle glycogen. Muscle tissue (2 mg dry wt) was extracted in 1 N HCl and hydrolyzed at 100°C for 3 h. Glycogen content was determined by the hexokinase method (26).

DXA scanning. Arm and leg lean body mass (LBM) were evaluated by total body DXA scanning (Norland XR-800, Norland). The body was segmented in accordance with standard procedures to evaluate regional LBM, and all analyses were performed using Illuminatus DXA software (Norland). The effective radiation dose was <0.2 mSv per scan.

Statistical analyses. Data are presented as means ± SD, unless otherwise stated. Statistical analyses were performed using SPSS v.22. Specific statistical analyses were applied to answer the primary hypotheses.

Possible intervention-induced changes in protein expression within a specific muscle and training group were evaluated by calculating the individual after vs. before expression and evaluating whether the training- and muscle group-specific mean was equal to 1 using a one-sample t-test.

Possible differences in protein expression changes between groups within muscle and between muscles within group were evaluated by a mixed-model approach using the SPSS MIXED procedure (5). Muscle and group were specified as fixed effects. Random variation and repeated effects were specified by subject. A significant main effect or interaction was further evaluated by a multiple-comparison approach with Sidak adjustment.

Possible time-specific differences in maximal enzymatic activity (before vs. after intervention) were evaluated by including time as a fixed factor in the mixed model also used for between-groups and muscle protein expression.

Additionally, it was specifically addressed if adaptations in vastus lateralis muscle in SOC were different from adaptations in deltoideus muscle of the MOS and HIS groups and also if the adaptation in deltoideus muscle differed between MOS and HIS. For this hypothesis driven analysis, group and muscle were combined into a single variable ‘GroupMuscle’. A mixed model with GroupMuscle as a fixed factor and subject as a random factor and repeated effect was applied. The following three analyses were evaluated by multiple comparisons with no adjustment to avoid a type II error: SOC vastus lateralis muscle vs. MOS deltoideus muscle; SOC vastus lateralis muscle vs. HIS deltoideus muscle; and MOS vs. HIS deltoideus muscle.

The level of significance was set at P < 0.05, and P < 0.10 is reported as a tendency.

RESULTS

Citrate synthase. Citrate synthase maximal activity was similar between groups at baseline in both deltoideus and vastus lateralis muscle (Fig. 1). In general, the maximal activity was 82 ± 20% higher (P < 0.001; n = 72) in vastus lateralis muscle than in deltoideus muscle, and this difference between muscle groups was evident at all investigated time points and in all training groups (Fig. 2). Specifically, maximal citrate synthase activity was higher (P < 0.001) after training in both muscle groups of the three training groups (deltoideus muscle: 37 ± 24%, 35 ± 52%, 95 ± 89% and vastus lateralis muscle 27 ± 34%, 29 ± 31%, 52 ± 80% for SOC, MOS, and HIS, respectively) with the exception of MOS deltoideus muscle, where a tendency (P = 0.069) was apparent, and CON, where similar levels existed before and after training in both muscle groups. After HIS, the increase was higher (P < 0.05)
in deltoideus muscle than in vastus lateralis muscle. Further, the increase in deltoideus muscle after HIS was higher \((P < 0.05)\) than in deltoideus muscle after MOS, and also higher \((P < 0.05)\) than the increase in vastus lateralis muscle after SOC.

In vastus lateralis muscle, citrate synthase protein expression was increased in SOC, MOS, and HIS but not in CON. In deltoideus muscle, citrate synthase protein expression was increased in SOC and HIS but only tended to increase in MOS and was unaltered in CON (Table 1). The training-induced changes in vastus lateralis muscle were higher in the three training groups than in CON. Further, the increase in deltoideus muscle after HIS was higher \((P < 0.05)\) than in vastus lateralis muscle after SOC and higher \((P < 0.05)\) than in deltoideus muscle after MOS.

3-Hydroxacyl-CoA dehydrogenase. 3-Hydroxacyl-CoA dehydrogenase maximal activity was similar between groups at baseline in both vastus lateralis and deltoideus muscle (Fig. 3). In general, the maximal activity was 28 ± 37% higher \((P < 0.001; n = 40)\) in vastus lateralis than in deltoideus muscle before the training when including all four groups, but this difference was not evident after training when analyzing the three training groups \((5 ± 18\%, P = 0.15, n = 33)\). Specifically, maximal 3-hydroxacyl-CoA dehydrogenase activity after training was increased in deltoideus muscle after SOC, MOS, and HIS \((25 ± 33, 20 ± 31, \text{and} 35 ± 43\%)\). In vastus lateralis muscle, only HIS experienced an increased \((P < 0.05)\) maximal enzymatic activity \((18 ± 30\%)\), while a tendency existed in MOS

### Table 1. Protein expression

<table>
<thead>
<tr>
<th>Intervention Group</th>
<th>Citrate synthase</th>
<th>Phosphofructokinase</th>
<th>Complex I</th>
<th>Complex II</th>
<th>Complex III</th>
<th>Complex IV</th>
<th>Complex V</th>
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<tbody>
<tr>
<td></td>
<td>D</td>
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<td>D</td>
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<tr>
<td>D</td>
<td>22 ± 60%</td>
<td>49 ± 57%</td>
<td>33 ± 58%</td>
<td>12 ± 33%</td>
<td>53 ± 9%</td>
<td>102 ± 34%</td>
<td>9%</td>
</tr>
<tr>
<td>ns; n = 15</td>
<td>ns; n = 13</td>
<td>ns; n = 12</td>
<td>ns; n = 13</td>
<td>ns; n = 14</td>
<td>ns; n = 11</td>
<td>ns</td>
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<tr>
<td>0.13 ± 31%</td>
<td>31 ± 44%</td>
<td>ns</td>
<td>14 ± 58%</td>
<td>ns</td>
<td>12 ± 39%</td>
<td>18 ± 35%</td>
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<td>ns; n = 13</td>
<td>ns; n = 12</td>
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<tr>
<td>P = 0.13, n = 15</td>
<td>P = 0.05, n = 16</td>
<td>P = 0.10; n = 11</td>
<td>ns</td>
<td>ns</td>
<td>P = 0.10; n = 11</td>
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<tr>
<td>D vs. VL vs. CON</td>
<td></td>
<td></td>
<td>P = 0.001</td>
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<td></td>
<td>VL: P &lt; 0.01</td>
<td>VL: P &lt; 0.01</td>
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<tr>
<td>Phosphofructokinase</td>
<td>D</td>
<td>VL</td>
<td>Complex I</td>
<td>Complex II</td>
<td>Complex III</td>
<td>Complex IV</td>
<td>Complex V</td>
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<tr>
<td>D</td>
<td>132 ± 138%</td>
<td>230 ± 319%</td>
<td>218 ± 289%</td>
<td>102 ± 126%</td>
<td>131 ± 121%</td>
<td>161 ± 191%</td>
<td>71 ± 53%</td>
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<td>ns; n = 10</td>
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<td>ns</td>
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<tr>
<td>9 ± 53%</td>
<td>46 ± 23%</td>
<td>ns</td>
<td>124 ± 126%</td>
<td>41 ± 73%</td>
<td>32 ± 45%</td>
<td>161 ± 191%</td>
<td>27 ± 45%</td>
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<td>ns; n = 11</td>
<td>ns; n = 14</td>
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<td>ns</td>
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<tr>
<td>P = 0.08; n = 15</td>
<td>P = 0.06, n = 14</td>
<td>P = 0.006; n = 14</td>
<td>ns</td>
<td>ns</td>
<td>P = 0.001; n = 17</td>
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<tr>
<td>D vs. VL vs. CON</td>
<td></td>
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<td></td>
<td>VL: P = 0.01</td>
<td>VL: P &lt; 0.01</td>
<td>ns</td>
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Results are means ± SD. D, deltoideus muscle; VL, vastus lateralis muscle; CON, no physical activity; SOC, soccer; MOS, moderate-intensity continuous swimming; HIS, high-intensity interval swimming; ns, nonsignificant.
(12 ± 21%, \( P = 0.052 \)) and no changes were detected in SOC (3 ± 25%). Furthermore, no effect of the intervention period was observed in CON. Additionally, the increase in deltoideus muscle after HIS was higher (\( P < 0.05 \)) than in vastus lateralis muscle after SOC but similar to the change in deltoideus muscle after MOS.

**Complex I–V.** In vastus lateralis muscle, complex I and IV expression remained stable with training, complex II increased after MOS and SOC, complex III increased after HIS and SOC, and complex V increased after MOS (Table 1). In deltoideus muscle, complex I, II, III, and IV increased after HIS and MOS and complex V expression increased in all groups (Table 1). The increase in deltoideus muscle was higher than in vastus lateralis muscle for complex II and IV after HIS. Furthermore, the increase in deltoideus muscle after HIS was higher than the increase in vastus lateralis muscle after SOC for complex II, III, and IV (Table 1).

**Phosphofructokinase protein expression.** None of the applied training regimes altered phosphofructokinase expression (Table 1) at a group-specific level. Furthermore, if the three training groups were combined, phosphofructokinase expression was not significantly affected by training in either deltoideus muscle (17 ± 41%, \( P = 0.094, n = 41 \)) or vastus lateralis muscle (3 ± 30%, \( P = 0.82, n = 49 \)). Additionally, no difference between changes after SOC in vastus lateralis muscle and after HIS and MOS in deltoideus muscle was detectable.

**Muscle glycogen.** Muscle glycogen levels were similar between groups at baseline in both vastus lateralis muscle and deltoideus muscle (Fig. 4). In general, the glycogen content was 47 ± 55% higher (\( P < 0.001; n = 25 \)) in vastus lateralis muscle than in deltoideus muscle before the training when including all four groups. Muscle glycogen was only increased in deltoideus muscle after HIS (63 ± 76%, \( P < 0.001, n = 10 \)), whereas deltoideus muscle glycogen levels remained similar after CON, SOC, and MOS (20 ± 77%, \( n = 6; 2 ± 32%, n = 8; -1 ± 24%, n = 6 \)). In vastus lateralis muscle, the glycogen content remained similar after CON, SOC, MOS, and HIS (11 ± 67%, \( n = 13; 20 ± 51%, n = 16; 16 ± 33%, n = 19; 11 ± 34%, n = 13 \), respectively). Additionally, the HIS-induced change in deltoideus muscle was higher (\( P < 0.05 \)) than that in vastus lateralis muscle after SOC, and the change in deltoideus muscle after HIS was higher (\( P < 0.05 \)) than that after MOS.

**Leg and arm lean body mass.** Leg lean mass in SOC, MOS, HIS, and CON was 15.9 ± 2.4, 16.5 ± 3.7, 15.2 ± 2.0, and 16.3 ± 2.6 kg before the training intervention, but increased (\( P < 0.05 \)) by 7 ± 8 and 6 ± 6% after SOC and HIS, respectively, with no change after MOS and CON (3 ± 9%, \( P = 0.17 \) and 1 ± 5%; \( P = 0.34 \), respectively). The increase in SOC and HIS was greater (\( P < 0.05 \)) than in CON. Arm lean mass after SOC, MOS, HIS, and CON was 4.6 ± 1.5, 4.8 ± 1.5, 4.3 ± 0.9, and 4.8 ± 1.1 kg before the training intervention and increased (\( P < 0.05 \)) by 13 ± 15, 17 ± 19, and 18 ± 18% after SOC, MOS, and HIS, respectively, with no change in CON (6 ± 8%, \( P = 0.11 \)). The increase in MOS and HIS was greater (\( P < 0.05 \)) than in CON.
DISCUSSION

The primary finding of the present study was that deltoideus muscle has a higher adaptive potential than vastus lateralis muscle in sedentary women and that “high-intensity low-volume” exercise training increases the aerobic potential of deltoideus muscle more than “low-intensity high-volume” training. Additionally, exercise training increased muscular oxidative capacity both in prime movers as well as in non-primarily-engaged muscle groups.

Adaptive potential of deltoideus muscle vs. vastus lateralis muscle. Citrate synthase maximal activity, citrate synthase protein expression, 3-hydroxyacyl-CoA dehydrogenase maximal activity, complex II, III, IV, and glycogen content increased more in deltoideus muscle after high-intensity swimming training than in vastus lateralis muscle after soccer training. Thus the adaptive potential of deltoideus muscle is higher than that of vastus lateralis muscle in the present population.

The initial ~80% higher maximal citrate synthase activity in vastus lateralis muscle compared with deltoideus muscle is in accordance with previous observations from healthy young males (15) and middle-aged obese subjects (33). The lower initial oxidative capacity of deltoideus muscle is likely to be a primary explanation for the higher adaptability compared with vastus lateralis muscle. Indeed, prolonged bed rest causes a prominent reduction of leg but not arm muscle function (11) illustrating the low initial training status of the arm musculature. Despite the fact that deltoideus muscle adaptability appears higher than that of vastus lateralis muscle and that this may be related to a low initial training status, previous observations suggest that the maximal attainable oxidative potential is also lower. For example, citrate synthase activity is ~33% lower in deltoideus muscle than in vastus lateralis muscle in well-trained triathletes (8), and swimming training for ~8 years results in maximal deltoideus muscle activities of ~29 μmol·g⁻¹·min⁻¹ (7), which is lower than the maximal vastus lateralis muscle activities of ~53 μmol·g⁻¹·min⁻¹ observed in runners (24). Thus years of training may bring deltoideus muscle maximal TCA cycle capacity close to the vastus lateralis muscle untrained level, but not the trained level.

In previous studies, 3-hydroxyacyl-CoA dehydrogenase maximal activity was elevated in deltoideus muscle after prolonged low-intensity exercise (16) but not after strength training (31), which could be interpreted as 3-hydroxyacyl-CoA dehydrogenase expression only improves when the demand for β-oxidation is high. However, in the present study maximal 3-hydroxyacyl-CoA dehydrogenase activity was initially lower in deltoideus muscle than in vastus lateralis muscle, but reached a similar level in the two muscles after the MOS, HIS, and SOC interventions. Thus the adaptability of deltoideus muscle was higher than that of vastus lateralis muscle, but all types of training were able to induce increased deltoideus muscle 3-hydroxyacyl-CoA dehydrogenase maximal activity, including soccer, where the activity in deltoideus muscle is expected to be modest. In vastus lateralis muscle, only the swimming groups experienced an increase, and it can be speculated that this is related to the higher metabolic demand, and thus reduced dependence on β-oxidation, during soccer compared with swimming. Together, the observations allow speculation that when initial 3-hydroxyacyl-CoA dehydrogenase maximal activity is low, as in deltoideus muscle, any type of training will cause an increase. Concurrently, when the initial maximal activity is higher, as in vastus lateralis muscle, only training with a limited total metabolic demand but high β-oxidation will cause further adaptation.

Only limited information exists regarding the adaptability of the capacity of the electron transport chain. The present results demonstrate that, as for citrate synthase, the electron transport chain is also more adaptable in deltoideus muscle than in vastus lateralis muscle. Furthermore, the present results demonstrate that not all respiratory complexes are affected equally by exercise training. For example, in vastus lateralis muscle complex I and IV expression remained stable after training, whereas an increase was observed for several other complexes. This suggests that exercise training not only causes general mitochondrial biogenesis, but also an altered mitochondrial phenotype. Again, it was observed that the increase in deltoideus muscle after HIS was more pronounced than that after SOC for complex II, III and IV in vastus lateralis muscle, which is in line with the observations from the citrate synthase maximal activity determination.

With regard to substrate availability, the result that muscle glycogen levels were initially ~47% higher in vastus lateralis muscle than in deltoideus muscle is even more pronounced than the ~25% observed in healthy young men (20). This is the first study to compare the adaptability of muscle glycogen levels in vastus lateralis muscle and deltoideus muscle in relation to exercise training. In untrained subjects, soccer training has been shown to be a potent exercise mode for increasing glycogen content in the leg (34). The finding that high-intensity swimming training increased muscle glycogen in deltoideus muscle by ~63% whereas no change occurred after soccer training in vastus lateralis muscle clearly demonstrates that not only mitochondrial biogenesis was activated in deltoideus muscle, but also glycogen storage. This may be associated with an increased muscular uptake of glucose via GLUT4 transporters, as reported after prolonged low-intensity exercise for the deltoid muscle (15).

Clearly, the oxidative adaptive potential of deltoideus muscle in the investigated population was higher than that of vastus lateralis muscle, demonstrating that caution should be exercised when extrapolating observations from vastus lateralis muscle to other muscle groups. Additionally, the present results demonstrate that the oxidative capacity and glycogen content of the deltoideus muscle can be brought close to that of untrained vastus lateralis muscle, but not to the level of trained vastus lateralis muscle. This is in accordance with the observation that upper-body oxygen extraction is lower (4) and lactate production higher (16) than in the lower-body musculature, even in elite trained athletes.

Exercise intensity-dependent adaptation in deltoideus muscle. When comparing deltoideus muscle adaptations after MOS and HIS, it was evident that HIS induced more pronounced adaptations in citrate synthase maximal activity, citrate synthase expression, and muscle glycogen content, whereas 3-hydroxyacyl-CoA dehydrogenase and complex I–V adaptations did not differ significantly between muscle groups. Thus it is clear that for deltoideus muscle, high-intensity training, which is expected to cause high relative anaerobic energy production (28), induces adaptations in oxidative capacity as well as substrate availability that surpass those of...
much more prolonged low-intensity training. This is in line with findings for vastus lateralis muscle, where brief high-intensity exercise training causes increased muscular oxidative potential (3) and reduced anaerobic energy production (12). As phosphofructokinase maximal activity was not increased in either deltoideus or vastus lateralis muscle after HIS or SOC, the intense training apparently did not increase the glycolytic enzymatic potential, in contrast to previous observations in untrained young males (25). This observation questions the suggestion of adaptations specific to the prevailing metabolism during training, i.e., “anaerobic training,” at least in untrained women. These findings, in combination with the long duration (15 wk) and relatively high number of participants in the present study compared with other studies, allow the conclusion that “low-volume high-intensity” training not only caused similar but more pronounced adaptations than “high-volume low-intensity” exercise training.

Adaptation in non-primarily-engaged muscle. In freestyle swimming, deltoideus muscle is active during most of the arm stroke, with a strong activation immediately before recovery of the arm, and vastus lateralis muscle is only moderately active as judged from the rectus femoris muscle activation pattern (19). During soccer, vastus lateralis muscle is heavily engaged, as evidenced by analyses of fiber type-specific glycogen depletion patterns, where all fiber types have been shown to be highly activated (22). In contrast, deltoideus muscle contracts against no external resistance in all soccer actions except impacts/tackles between players, which are limited in a soccer game (27). Thus it could be expected that muscle adaptations to swimming would only occur in deltoideus muscle and not in vastus lateralis muscle, and vice versa for the soccer group. However, the observation of increased citrate synthase maximal activity and expression in deltoideus muscle afterSOC and in vastus lateralis muscle after swimming demonstrate that adaptation to exercise training also occurs in muscle groups that are only engaged to a small degree, at least in the present population of untrained women. In contrast, muscle glycogen in deltoideus muscle was only increased after HIS.

Classically, either endurance or sprint training of one leg increases succinate dehydrogenase maximal activity in the trained but not the contralateral untrained leg (17, 36), demonstrating that adaptation to training only occurs in the engaged muscle group, at least in vastus lateralis muscle. The present study is the first time that changes in deltoideus muscle oxidative capacity have been investigated after intermittent ball-game participation. The finding that training increased deltoideus muscle oxidative capacity, in conjunction with the swimming-induced increase of vastus lateralis muscle oxidative capacity, demonstrates that untrained muscle can adapt to even very low degrees of activation such as nonimpact arm swing in soccer.

In numerous recent studies by our research group, soccer training has been shown to induce a broad spectrum of positive health effects in untrained men and women (21). For example, significant cardiovascular (29), muscle metabolic (21), and bone-health-promoting (13) effects have been observed in untrained women. The present study adds novel information regarding the muscular oxidative responses to recreational soccer training, where the oxidative capacity in both leg and arm muscle can be improved in untrained middle-aged women through a period of soccer training.

Limitations. When comparing the adaptive potential of different muscle groups, it is an inherent challenge to match the training stimuli. In the present study, weekly training frequency was matched between the two swimming groups and the soccer group. In addition, the training volume was matched in the moderate-intensity swimming groups and the soccer group using 1-h training sessions. During training, average heart rate was ~80% in SOC (29), ~86% in HIS, and ~73% in MOS (30). To elicit an optimal training response of the vastus lateralis muscle, soccer was played as small-sided games known to cause muscular adaptations similar to or even greater than those produced in continuous running (23). The applied high-intensity swimming intervention was designed to correspond to previous studies of high-intensity exercise proven to be effective for mitochondrial biogenesis (9). The training load of the high-intensity swimming groups was chosen to be much less (total of ~220 min of exercise over the 15 wk) than that of the soccer group (total of ~2,000 min of exercise over the 15 wk) to rule out that a potential higher adaptability of the deltoideus muscle was due to insufficient training stimulation of vastus lateralis muscle. The applied design allows the conclusion that the higher adaptation observed in deltoideus muscle was achieved with a much lower training stimulation compared with vastus lateralis muscle. However, due to the difference in contraction pattern, a quantitative comparison is not possible, so it remains unknown how much more sensitive deltoideus muscle is than vastus lateralis muscle.

Perspectives. The use of exercise models that activate many muscle groups may have additional effects on metabolic fitness, as the adaptive potential of the large lower extremity muscles is less than that of the smaller upper-body musculature.

In conclusion, deltoideus muscle has a higher adaptive potential than vastus lateralis muscle in sedentary women, and “high-intensity low-volume” training is a more efficient regime than “low-intensity high-volume” training for increasing the aerobic potential of deltoideus muscle.

ACKNOWLEDGMENTS

GRANTS
The study was supported by a grant from the Faroese Research Council, as well as by the Faroese Confederation of Sports and Olympic Committee (Ítróttarsamband Føroya) and the Danish Sports Confederation (Danmarks Idrætsforbund). In addition, financial support was obtained from Eik Bank. The Copenhagen Muscle Research Centre is supported by a grant from the Capital Region of Denmark.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
REFERENCES


