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Enzyme activities in the tibialis anterior muscle of young moderately active men and women: relationship with body composition, muscle cross-sectional area and fibre type composition

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ABSTRACT

The aims of this study were (i) to assess the differences between men and women in maximal activities of selected enzymes of aerobic and anaerobic pathways involved in skeletal muscle energy production, and (ii) to assess the relationships between maximal enzyme activities, body composition, muscle cross-sectional area (CSA) and fibre type composition. Muscle biopsies were obtained from the tibialis anterior (TA) muscle of 15 men and 15 women (age 20–31 years) with comparable physical activity levels. The muscle CSA was determined by magnetic resonance imaging (MRI). Maximal activities of lactate dehydrogenase (LDH), phosphofructokinase (PFK), β-hydroxyacyl-coenzyme A dehydrogenase (HAD), succinate dehydrogenase (SDH) and citrate synthase (CS), were assayed spectrophotometrically. The proportion, mean area and relative area (proportion x area) of type 1 and type 2 fibres were determined from muscle biopsies prepared for enzyme histochemistry [myofibrillar adenosine triphosphatase (mATPase)]. The men were significantly taller (+6.6%; P < 0.001) and heavier (+19.1%; P < 0.001), had significantly larger muscle CSA (+19.0%; P < 0.001) and significantly larger areas and relative areas of both type 1 and type 2 fibres (+20.5–31.4%; P = 0.007 to P < 0.001). The men had significantly higher maximal enzyme activities than women for LDH (+27.6%; P = 0.007) and PFK (+25.5%; P = 0.003). There were no significant differences between the men and the women in the activities of HAD (+3.6%; ns), CS (+21.1%; P = 0.084) and SDH (+7.6%; ns). There were significant relationships between height and LDH (r = 0.41; P = 0.023), height and PFK (r = 0.41; P = 0.025), weight and LDH (r = 0.45; P = 0.013), and weight and PFK (r = 0.39; P = 0.032). The relationships were significant between the muscle CSA and the activities of LDH (r = 0.61; P < 0.001) and PFK (r = 0.56; P = 0.001), and between the relative area of type 2 fibres and the activities of LDH (r = 0.49; P = 0.006) and PFK (r = 0.42; P = 0.023). There were no significant relationships between HAD, CS and SDH, and height, weight, muscle CSA and fibre type composition, respectively. These data indicate that the higher maximal activities of LDH and PFK in men are related to the height, weight, muscle CSA and the relative area of type 2 fibres, which are all significantly larger in men than women.

Keywords 3-hydroxyacyl CoA dehydrogenases; citrate (si)-synthase; energy metabolism; enzymes; lactate dehydrogenase; magnetic resonance imaging, muscle fibres; phosphofructokinase; sex factors; succinate dehydrogenase.

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Sex-related differences in muscle metabolism and substrate utilization during exercise have recently received increased attention (Tate & Holtz 1998, Cortright & Koves 2000, Esbjörnsson Liljedahl 2000, Shephard 2000). A variety of techniques, from indirect calorimetry and respiratory exchange ratio (RER) to in vitro measurements of enzyme activities in muscle homogenates, have been used to identify differences between

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men and women during and after submaximal exercise of various intensities, but the results are not conclusive (Tarnopolsky 2000). In several studies it has been reported that women derive proportionally more energy from lipid oxidation, and less from carbohydrate oxidation, compared with men (Froberg & Pedersen 1984, Horton et al. 1998, Tate & Holtz 1998, Tarnopolsky 2000). In other studies no differences between men and women in the relative contribution of fat and carbohydrates to energy supply have been reported (Costill et al. 1979, Romijn et al. 2000). At the cellular level, women seem to have a reduced metabolic efficiency of muscle fibres, i.e. a higher energy cost of contraction, than men (Mattei et al. 1999).

Measurements of muscle enzyme activities in vitro are related to whole-body energy metabolism (Zurlo et al. 1994, Blomstrand et al. 1997) and can provide insight into the relative contribution of aerobic metabolism and glycolytic pathways in skeletal muscle energy production in men and women (Pette & Hofer 1980, Tarnopolsky 2000). Reported maximal activities of glycolytic muscle enzymes are generally higher in men than in women (Komi & Karlsson 1978, Green et al. 1984, Simoneau et al. 1985, Simoneau & Bouchard 1989, Esbjörnsson et al. 1993). Assessments of activities of oxidative enzymes of the Krebs cycle have been contradictory: higher values for men (Green et al. 1984, Simoneau et al. 1985, Borges & Essen-Gustavsson 1989) or no sex differences (Simoneau et al. 1985, Esbjörnsson et al. 1993) have been reported for different enzymes. For enzymes of lipid oxidation, no sex differences have been reported (Green et al. 1984, Simoneau et al. 1985, Esbjörnsson et al. 1993).

The reason for the higher glycolytic activity in men is not clearly understood. Differences in training response and differences in work load relative to body composition could influence enzyme activities. In general, men have larger fibres than women, especially for type 2 fibres, and a higher oxidative and a lower glycolytic capacity has been found in type 1 fibres compared with type 2 fibres (Essen-Gustavsson & Henriksson 1984, Borges & Essen-Gustavsson 1989). The difference between men and women in glycolytic enzyme activities could therefore be the result of a greater absolute (or relative) type 2 fibre area (Simoneau et al. 1985, Esbjörnsson et al. 1993). Body composition and muscle mass could also be determinants of anaerobic capacity (Murphy et al. 1986). The oxidative enzyme activity has been found to be related to the amount of type 1 fibres, which could reflect a difference in physical performance capacity and training status between men and women (Blomstrand et al. 1986).

In most studies on muscle metabolism and sex differences, enzyme activities have been determined in biopsies from the vastus lateralis (VL) muscle, which is used in many activities, ranging from endurance running to high-power activities. The fibre type composition in the VL muscle varies widely (Saltin & Gollnick 1983), and this could influence enzyme activities. The tibialis anterior (TA) muscle, a muscle used for running and walking (Mann & Hagy 1980, Nilsson et al. 1985), has less variability in fibre type composition than the VL muscle (Henriksson-Larsén et al. 1983, Hellwell et al. 1987, Jakobsson et al. 1990).

In this study, the differences between men and women in maximal activities of selected enzymes of aerobic and anaerobic pathways involved in energy production were determined in the TA muscle, and the relationships between maximal enzyme activities, height, weight, muscle cross-sectional area (CSA), and fibre type composition were assessed. By selecting subjects with comparable habitual activity levels, the variability in muscle metabolism because of factors other than the sex can be reduced. The hypotheses were that young men and women with comparable habitual activity levels have similar oxidative enzyme activity levels, and that differences in glycolytic capacity between men and women are the result of differences in their body composition, muscle size and fibre type composition.

**METHODS**

**Subjects**
Fifteen male and 15 female (20–31 years of age) students in the Department of Physical Therapy at Lund University, Sweden, volunteered for the study (Table 1); the men were significantly taller (+6.6%;

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 15) mean ± SD</th>
<th>Women (n = 15) mean ± SD</th>
<th>Difference (%)*</th>
<th>Significance test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.5 ± 3.4</td>
<td>22.7 ± 2.6</td>
<td>+11.0</td>
<td>P = 0.016</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>181 ± 5.4</td>
<td>169 ± 4.5</td>
<td>+6.6</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.0 ± 9.4</td>
<td>63.9 ± 8.2</td>
<td>+19.1</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

*The percentage differences for age, height and weight are the relative amounts by which men exceeded women.
The contractile cross-sectional area (cCSA; cm²) of the ankle dorsiflexor (DF) muscle compartment was assessed using proton T1-weighted spin-echo axial plane imaging in a 1.5-T magnetic resonance imaging (MRI) scanner system (Siemens Magnetom Vision, Siemens Medical Systems, Erlangen, Germany). The DF muscle compartment is to a great extent made up of the tibialis anterior (TA) muscle, and to a much smaller extent of the extensor digitorum longus (EDL) and peroneus tertius (PT) muscles. Anatomical CSA (aCSA; cm²) was selected and a PC based software program (Ps2D, Pallas AB, Sweden) was used to determine the contractile (total CSA corrected for non-contractile components) and non-contractile (fat and connective tissue) components. Each image was analysed five times, and the mean of these five measurements for each subject was recorded. All measurements were carried out by the same person (MMP), who was blinded to the sex of the subjects. Further details of the MRI procedure can be found in Holmba¨ck et al. (2002).

Muscle biopsies

From each subject, one to four biopsy samples (50–80 mg each) were obtained from the right TA muscle at the level of the largest CSA determined from the MRI measurements. All biopsies were obtained with the percutaneous conchotome technique. Biopsies were trimmed, mounted on cork discs, and frozen in isopentane pre-cooled with dry ice and ethanol (−70 °C). Smaller portions of each biopsy were mixed together and frozen separately as one sample in the pre-cooled isopentane, and were later used for the enzyme analyses.

Enzyme histochemistry and analysis of fibre type composition

Serial cryosections of 7 µm thick were cut from each biopsy sample, and all sections were stained for myofibrillar adenosine triphosphatase (mATPase) after pre-incubation at pH 4.3, 4.6 and 10.4 (Brooke & Kaiser 1970). In these 30 subjects, on average only 0.1 ± 0.3% (range 0–1.4%) type 2X myosin (myosin heavy chain, MHC, 2X) was found by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE). Very few type 2B fibres were seen microscopically [the three MHC isoforms 1, 2A and 2X identified by SDS-PAGE gel electrophoresis corresponds to the three main fibre types 1, 2A and 2B, identified by myosin ATPase enzyme histochemistry (Staron 1997)], and so differentiation of the type 2 subtypes was considered unnecessary. Following staining and mounting, sections were viewed in a Leitz Diaplan microscope (Leitz, Wetzlar, Germany) and eight or nine images were captured at a magnification of X100 using a Kappa digital camera (Gleichen, Germany). All image analysis was performed with ImageProPlus 3.1 (MediaCybernnetics, Silver Spring, MD, USA). For each subject, an average 690 fibres (502–991) were counted to estimate the fibre type proportion. To determine the mean areas of type 1 and type 2 fibres, an average of 180 type 1 fibres (108–273) and 81 type 2 fibres (23–194) were measured. All measurements were taken by the same person (MMP), who was blinded to the sex of the subjects.

Measurements of enzyme activities

Muscle samples (one per subject) were weighed (10–20 mg) and diluted 50 times in a cooled 20 mM sodium phosphate buffer (pH 7.4) with 50% glycerol, 0.02% bovine serum albumin, 5 mM mercaptoethanol, and 0.5 mM ethylene diamine tetraacetic acid (EDTA) before homogenization with a glass-on-glass pestle on ice. Absorbance changes for all enzyme assays were measured in an Ultrospec 4000 UV/Visible Spectrophotometer [Pharmacia Biotech (Biochrom), Cambridge, UK] at 25 °C. The molar extinction coefficients used were 6270 L mol⁻¹ cm⁻¹ for nicotinamide–adenine dinucleotide (NADH) at 340 nm [lactate dehydrogenase (LDH), phosphofructokinase (PFK) and β-Hydroxyacyl-coenzyme A dehydrogenase (HAD)]; 16 100 L mol⁻¹ cm⁻¹ for 2,6-dichloroindophenol (DCPIP) at 600 nm [Succinate dehydrogenase (SDH)] and 13 100 L mol⁻¹ cm⁻¹ for DTNB at 410 nm [citrate synthase (CS)]. The maximal enzyme activities were expressed as µmol min⁻¹ g⁻¹...
wet weight. The composition of the assay solutions was as follows:

(i) Lactate dehydrogenase (EC 1.1.1.27): 50 mM imidazol buffer, 2 mM EDTA, 289 μM NADH at pH 7.4 and 2.4 mM pyruvate (Bergmeyer & Bernt 1974).

(ii) Phosphofructokinase (EC 2.7.1.11): 100 μM TRIS-buffer (Trizma Base), 12 mM MgCl₂, 400 mM KCl, 2 mM AMP, 1 mM ATP, 170 μM NADH, 0.0025 mg mL⁻¹ antimycin, 0.05 mg mL⁻¹ aldolase, 0.05 mg mL⁻¹ G-3-PDH-TIM at pH 7.0, and 2.9 mM fructose-6-phosphate (Opie & Newsholme 1967).

(iii) β-Hydroxyacyl-coenzyme A dehydrogenase (EC 1.1.1.35): 200 mM triethanolamine-HCl buffer, 10 mM EDTA, 294 mM NADH at pH 7.0 and 95.6 μM acetoacetyl Coenzyme A (CoA) (Bass et al. 1969).

(iv) Succinate dehydrogenase (EC 1.3.99.1): 10 μL of the homogenate was pre-incubated in a 64 mM phosphate buffer (pH 7.4) with 34 mM succinate for 30 min at 30 °C to stabilize the enzyme (Cooney et al. 1981). Before absorbance measurement, 0.31 mM phenazine methosulphate (PMS), 0.10 mM DCPPIP and 0.85 mM potassium cyanide (KCN) was added.

(v) Citrate synthase (EC 4.1.3.7): 50 mM Tris–HCl buffer, 0.2 mM DTNB, 0.1 mM acetyl CoA, 0.05% Triton X-100 at pH 8.1 and 0.5 mM oxalic acid (Alp et al. 1976).

Protein was measured, modified from Lowry et al. (1951), using a bovine serum albumin standard and Follin–Ciocalteau reagent. The mean protein content for men was 0.25 (±0.11) and for women 0.21 (±0.09) (ns).

All analysis of a given enzyme was performed during 1 day and by the same person (ÅJ), who was blinded to the sex of the subjects. Enzyme activities were measured at least twice on the same homogenate for each subject, after testing for optimal amounts of substrate and homogenate. If the two measurements differed by more than 15%, a third analysis was performed. The recorded maximal enzyme activity for each subject was calculated as the mean of these two or three measurements, and was expressed as micromole converted substrate per minute and gram of tissue wet weight. The average relative variability between the two or three measurements for each subject was 8.9% for LDH, 12.3% for PFK, 12.2% for HAD, 34.1% for CS and 17.4% for SDH. This variability is generally considered to be acceptable and is within the range of those previously reported (Bouchard et al. 1986).

Data and statistical analysis

For each subject, the contractile muscle cross-sectional area (cCSA), the proportion of type 1 fibres, the mean areas of type 1 and type 2 fibres, the relative areas (proportion × area) of type 1 and type 2 fibres, the fibre type ratio (relative area of type 1 fibres divided by the relative area of type 1 plus type 2 fibres; this ratio represents the relative amount of type 1 and type 2 fibres in a given biopsy sample), the ratio of type 2/type 1 fibre area, and the maximal activity of LDH, PFK, HAD, CS and SDH were recorded. From the values of LDH, PFK, HAD, CS and SDH, ‘non-discriminating’ and ‘discriminating’ enzyme activity ratios were calculated for men and women separately. These enzyme activity ratios have been suggested to provide a description of the metabolic characteristics of a muscle at work (Pette & Hofer 1980). Non-discriminating ratios describe activities of enzymes involved in the same, or a functionally related, metabolic pathway, whereas discriminative ratios indicate the relative contribution of not related metabolic pathways to energy supply.

The two-tailed t-test was applied to test the null hypothesis that the difference in the mean value for the men and the women is zero for each recorded variables. The variability in the maximal enzyme activities between subjects was assessed by the coefficient of variation (CV = 100 × SD/mean). The CV is independent of the units of measurement, and is therefore useful for comparing the variability between the men and women for each of the five enzymes. Possible relationships between each of the five enzymes, height, weight, cCSA, fibre type composition and sex were addressed using bivariate Pearson’s correlation coefficient and multivariate analysis (generalized linear models). The fits of the models were assessed using F-statistics.

Throughout, exact significance values are given for values between 0.001 and 0.10, <0.001 is given for smaller values, whereas ns represents significance values >0.10; statistical significance is represented by values <0.05. For all the analyses, SPSS 10.0 software (SPSS, Chicago, IL, USA) was used.

RESULTS

Contractile cross-sectional area and fibre type composition

In Table 2, summary statistics of the cCSA and the fibre type composition, and the relative differences between the 15 men and 15 women, are presented. There were significant differences between the men and the women for cCSA, and the mean areas of type 1 and type 2 fibres, the relative areas of type 1 and type 2 fibres, the
Table 2: Comparison between the men and the women for contractile cross-sectional area of the ankle dorsiflexors and fibre type composition in the tibialis anterior muscle

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 15)</th>
<th>Women (n = 15)</th>
<th>Difference (%)</th>
<th>Significance test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contractile muscle CSA (cm²)</td>
<td>10.0 ± 0.9</td>
<td>8.1 ± 1.4</td>
<td>+19.0</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Proportion of type 1 fibres (%)</td>
<td>77.8 ± 6.8</td>
<td>76.9 ± 8.4</td>
<td>+1.2</td>
<td>ns</td>
</tr>
<tr>
<td>Mean area of type 1 fibres (μm²)</td>
<td>3968 ± 534</td>
<td>3153 ± 606</td>
<td>+20.5</td>
<td>P = 0.001</td>
</tr>
<tr>
<td>Mean area of type 2 fibres (μm²)</td>
<td>6904 ± 1605</td>
<td>4736 ± 1195</td>
<td>+31.4</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Relative area of type 1 fibres (μm²)</td>
<td>3103 ± 614</td>
<td>2435 ± 573</td>
<td>+21.5</td>
<td>P = 0.005</td>
</tr>
<tr>
<td>Relative area of type 2 fibres (μm²)</td>
<td>1505 ± 493</td>
<td>1045 ± 363</td>
<td>+30.6</td>
<td>P = 0.007</td>
</tr>
<tr>
<td>Fibre type ratio (%)</td>
<td>67.4 ± 8.8</td>
<td>69.8 ± 8.9</td>
<td>-3.6</td>
<td>ns</td>
</tr>
<tr>
<td>Type 2/type 1 fibre area ratio</td>
<td>1.79 ± 0.29</td>
<td>1.50 ± 0.26</td>
<td>+16.2</td>
<td>P = 0.029</td>
</tr>
</tbody>
</table>

*The percentage differences for each variable are the relative amounts by which men exceeded women.

Table 3: Comparison between men and women for maximal activities of five enzymes (μmol min⁻¹ g⁻¹ ww) in the tibialis anterior muscle

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 15)</th>
<th>Women (n = 15)</th>
<th>Difference (%)</th>
<th>Significance test</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH</td>
<td>176.1 ± 48.6</td>
<td>127.5 ± 43.2</td>
<td>+27.6</td>
<td>P = 0.007</td>
</tr>
<tr>
<td>PFK</td>
<td>30.6 ± 6.9</td>
<td>22.8 ± 6.2</td>
<td>+25.5</td>
<td>P = 0.003</td>
</tr>
<tr>
<td>HAD</td>
<td>16.9 ± 7.4</td>
<td>16.3 ± 6.7</td>
<td>+3.6</td>
<td>ns</td>
</tr>
<tr>
<td>CS</td>
<td>5.59 ± 2.00</td>
<td>4.41 ± 1.56</td>
<td>+21.1</td>
<td>P = 0.084</td>
</tr>
<tr>
<td>SDH</td>
<td>3.02 ± 1.11</td>
<td>2.79 ± 1.79</td>
<td>+7.6</td>
<td>ns</td>
</tr>
</tbody>
</table>

*The percentage differences for maximal activities of the five enzymes are the relative amounts by which men exceeded women.
†The coefficient of variation, CV, is derived from SD/mean × 100, where SD is the standard deviation of the mean of measurements.

ratio of type 2/type 1 fibres, but not for the proportion of type 1 fibres and the fibre type ratio.

Maximal enzyme activities and enzyme activity ratios

In Table 3, the maximal activities of LDH, PFK, HAD, CS and SDH and the relative differences between men and women are presented. The men had significantly higher maximal enzyme activities for LDH and PFK, whereas differences between men and women in activities of HAD, CS and SDH were not significant.

There were no substantial differences in CV between men and women for LDH, PFK, HAD and CS, whereas the CV for SDH for women was almost twice that for men. This indicates a similar variability between 15 men and 15 women for the measurements of LDH, PFK, HAD and CS, but a larger variability for the measurement of SDH for women than men.

There were significant correlations between LDH and PFK for both men (r = 0.63; P = 0.012) and women (r = 0.67; P = 0.007), between PFK and HAD for men (r = 0.62; P = 0.015), and between SDH and PFK for women (r = 0.51; P = 0.05). There were no significant correlations between the other pairs of enzymes for men and the women separately. When the data on the five enzymes from men and women were combined and analysed together, significant relationships between LDH and PFK (r = 0.73; P < 0.001) and PFK and HAD (r = 0.40; P = 0.027) were found.

Non-discriminating and discriminating enzyme ratios for men and women and the relative difference between men and women are presented in Tables 4 and 5, respectively. The differences between men and women for any of the enzyme ratios were not significant, although the values for the discriminating ratios were consistently larger for men (+12.0 to 21.1%).

Relationships between enzyme activities, height, weight, contractile muscle cross-sectional area and fibre type composition

In the multivariate analysis, each of the five enzymes was considered as a linear combination of sex effect, height, weight, eCSA and each of the variables representing the fibre type composition (cf. Table 2). In all these models, eCSA was the dominant effect: whenever eCSA was included the effects of the other variables were negligible and non-significant.

When men and women were analysed separately, there were weak significant relationships between only a few of the maximal enzyme activities and height,
weight, cCSA and the variables representing the fibre type composition, respectively. For this reason, the data from 15 men and 15 women were combined and analysed together. The relationships between height and LDH (r = 0.41; P = 0.023), height and PFK (r = 0.41; P = 0.025), weight and LDH (r = 0.45; P = 0.013) and weight and PFK (r = 0.39; P = 0.032) were significant, but were not significant between the remaining three enzymes and height and weight. The relationships between cCSA and LDH (r = 0.61; P < 0.001) and between cCSA and PFK (r = 0.56; P = 0.001) were significant, but none of the relationships between the remaining three enzymes and cCSA were significant. In Figure 1a, b, the maximal activities of LDH and PFK are plotted against cCSA for men and women.

A significant relationship was also found between the relative area of type 2 fibres and LDH (r = 0.49; P = 0.006) and PFK (r = 0.42; P = 0.023), respectively, but not between LDH and PFK and any of the other fibre type composition variables. None of the relationships between HAD, CS and SDH and any of the fibre type composition variables were significant. In Figure 2a, b, the maximal activities of LDH and PFK are plotted against the relative area of type 2 fibres for men and women.

The activities of each of the five enzymes were also normalized (in some sense) by dividing the maximal activities of LDH, PFK, HAD, CS and SDH by the cCSA, the relative type 1 and type 2 fibre area, and the fibre type ratio. There was a significant difference between men and women for the LDH/fibre type ratio

### Table 4 Comparison between men and women for non-discriminating enzyme activity ratios in the tibialis anterior muscle

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 15) mean ± SD</th>
<th>Women (n = 15) mean ± SD</th>
<th>Difference (%)</th>
<th>Significance test</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH/SDH</td>
<td>5.82 ± 1.36</td>
<td>5.61 ± 1.40</td>
<td>+3.6</td>
<td>ns</td>
</tr>
<tr>
<td>CS/SDH</td>
<td>2.18 ± 1.23</td>
<td>2.14 ± 1.48</td>
<td>+1.8</td>
<td>ns</td>
</tr>
<tr>
<td>HAD/SDH</td>
<td>6.74 ± 4.68</td>
<td>7.42 ± 4.59</td>
<td>−10.1</td>
<td>ns</td>
</tr>
<tr>
<td>HAD/CS</td>
<td>3.58 ± 2.06</td>
<td>3.90 ± 1.76</td>
<td>−8.9</td>
<td>ns</td>
</tr>
</tbody>
</table>

*The percentage differences of the non-discriminating enzyme activity ratios are the relative amounts by which men exceeded women.

### Table 5 Comparison between men and women for discriminating enzyme activity ratios in the tibialis anterior muscle

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 15) mean ± SD</th>
<th>Women (n = 15) mean ± SD</th>
<th>Difference (%)</th>
<th>Significance test</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH/PFK</td>
<td>68.9 ± 32.2</td>
<td>60.2 ± 35.1</td>
<td>+12.6</td>
<td>ns</td>
</tr>
<tr>
<td>CS/SDH</td>
<td>11.78 ± 5.81</td>
<td>10.36 ± 4.82</td>
<td>+12.1</td>
<td>ns</td>
</tr>
<tr>
<td>LDH/CS</td>
<td>36.6 ± 19.2</td>
<td>30.9 ± 11.0</td>
<td>+15.6</td>
<td>ns</td>
</tr>
<tr>
<td>PFK/CS</td>
<td>6.41 ± 3.20</td>
<td>5.64 ± 2.01</td>
<td>+12.0</td>
<td>ns</td>
</tr>
<tr>
<td>PFK/HAD</td>
<td>1.98 ± 0.58</td>
<td>1.61 ± 0.68</td>
<td>+18.7</td>
<td>ns</td>
</tr>
<tr>
<td>LDH/HAD</td>
<td>11.40 ± 3.69</td>
<td>8.99 ± 4.47</td>
<td>+21.1</td>
<td>ns</td>
</tr>
</tbody>
</table>

*The percentage differences of the discriminating enzyme activity ratios are the relative amounts by which men exceeded women.

Figure 1 (a) and (b) The maximal activities (mmol min⁻¹ g⁻¹ ww) of lactate dehydrogenase, LDH, and phosphofructokinase, PFK, plotted against the contractile muscle cross-sectional area, cCSA (cm²) for the 15 men (●) and the 15 women (○).
P = 0.017) and PFK/fibre type ratio (P = 0.008), but no significant differences between men and women for any of the other ratios. The LDH/fibre type ratio was 29.2% larger and the PFK/fibre type ratio was 26.8% larger for men.

**DISCUSSION**

The overall aim of this study was to assess the differences between men and women in the maximal activities of enzymes of aerobic and anaerobic pathways involved in skeletal muscle energy production and to determine the extent to which any sex-related differences in maximal enzyme activities could be explained by body composition, muscle CSA and fibre type composition. The main findings were that in the TA muscle (i) men had a higher activity of the glycolytic enzymes LDH and PFK than women; (ii) that the activities of the enzymes representing oxidation of fat (HAD) and carbohydrates (CS and SDH) did not differ between men and women; (iii) that the activities of LDH and PFK were related to body composition, CSA of the ankle dorsiflexor muscles and the relative area of type 2 fibres and (iv) when the maximal activities of LDH and PFK were normalized to muscle CSA and fibre type composition, no differences between men and women were found.

Previous studies of muscle enzyme activities in men and women have mainly studied the VL muscle. This is a muscle used in both endurance and high-intensity activities, and the fibre type composition can range from 13 to 73% type 1 fibres (Gollnick et al. 1972, Saltin & Gollnick 1983). The TA muscle is active during basic everyday activities such as walking and maintaining balance, and has a fibre type composition ranging from 65% to above 90% type 1 fibres (cf. Table 1; Henriksson-Larsén et al. 1983, Helliwell et al. 1987, Jakobsson et al. 1990). It is also important, in the study of muscle metabolism and sex differences, to enrol subjects with comparable habitual activity levels to reduce the effects of differences in physical performance capacity and training status. Here, we studied young physiotherapy students who were considered to have comparable activity levels, according to the Grimby Scale of Physical Activity (Grimby 1986). Muscle biopsies from these 30 subjects have also been analysed with regard to capillarization (Porter et al. 2002). Despite sex differences for fibre area, overall, capillarization was not different between these men and women. As the number of capillaries is highly influenced by training and as capillary counts parallel changes in metabolism (for relevant references see Porter et al. 2002), these data indicate that these 30 men and women can be considered to have comparable habitual activity levels and training status. By choosing the TA muscle and selecting individuals based on their activity level, we have tried to minimize the influence of external factors, allowing us to make more detailed inferences regarding muscle metabolism and sex.

The absolute values of maximal enzyme activities, and the relationships between different enzymes in our study, are generally consistent with published values of the human VL muscle (Green et al. 1984, Komi & Karlsson 1978, Simoneau et al. 1985, Essen-Gustavsson & Borges 1986, Simoneau & Bouchard 1989, Esbjörnsson et al. 1993) and the limited number of publications on the human TA muscle (Langhor et al. 1975, Kent-Braun et al. 1997, Borg & Henriksson 1991). None of the studies on the human TA muscle discriminated between men and women, so the results in this study are unique and not directly comparable with those of previous studies.

The maximum in vitro enzyme activity provides a simple way of obtaining information about the flux through a specific metabolic pathway, but only enzymes representing non-equilibrium reactions can give quantitative information about maximum flux through a pathway (Newsholme et al. 1980). Other enzymes can
still be compared in given tissues or under given situations as relative magnitudes of maximal flux rates (Pette & Hofer 1980). The significantly higher activity of the two glycolytic enzymes LDH and PFK in men compared with women in this study, may indicate that men have a higher capacity for anaerobic performance compared with women, and indirectly suggests that women may have lower glycogen utilization during exercise.

Several studies have, indeed, shown that anaerobic, glycolytic performance is lower in women than in men, both in absolute values and in relation to body dimensions (see Esbjörnsson Liljedahl 2000, Mayhew et al. 2001). Although the mechanism(s) is not known, it most likely involves the sex steroids (Cortright & Koves 2000). Animal studies have indicated that testosterone increases the activity of glycolytic enzymes (Ramamani et al. 1999) which would result in a higher LDH-activity in males. Higher oestrogen concentrations in women have been suggested as a reason why women oxidize proportionately more lipid and less carbohydrates during endurance exercise compared with men (Tarnopolsky 2000). The difference in LDH-activity between men and women has also been found to persist after reduced differences in the area of type 2 muscle fibres and of power output (Esbjörnsson Liljedahl 2000), which suggest that factors other than fibre type composition and strength contribute to the difference in LDH-activity between men and women.

As type 1 fibres have higher oxidative and a lower glycolytic capacity than type 2 fibres (Essen-Gustavsson & Henriksen 1984, Borges & Essen-Gustavsson 1989), a high maximal activity of LDH and PFK measured in a given sample can be the result of a difference in the relative amount of type 1 and type 2 fibres in that sample. As expected, there was a significant relationship between the activity of LDH and PFK, and the relative area of type 2 fibres, indicating that subjects with many and large type 2 fibres in their biopsy samples had higher activities of LDH and PFK. However, there was no difference between men and women for the fibre type ratio, a variable describing the relative amount of a given fibre type in a biopsy sample, and no significant relationship between LDH and PFK and the fibre type ratio. This indicates that the difference in glycolytic activity between men and women presented here was not simply the result of a difference in fibre type composition, i.e. the relative amount of type 1 and type 2 myosin, in the analysed samples, between men and women.

The activities of LDH and PFK expressed as a ratio of the relative amount of type 1 and type 2 myosin, i.e. the fibre type ratio, in the analysed samples were significantly larger for men than women, indicating a sex-related difference for the glycolytic enzymes similar to that found by Esbjörnsson Liljedahl (2000). For the same relative amount of type 1 and type 2 fibres in a given biopsy, men had higher maximal activities of LDH and PFK. Interestingly, the activities of the glycolytic enzymes also correlated with body composition and muscle CSA; tall and heavy subjects with large muscles had in general higher maximal activities of LDH and PFK, findings that were also discussed in the thesis work of Esbjörnsson Liljedahl (2000). In the present study, the correlations between muscle CSA and LDH and PFK, respectively, were the strongest of the relationships (also seen in the multivariate analysis), indicating that muscle CSA may have been the strongest determinant of the high glycolytic activity. As men were significantly taller, heavier and had larger muscles than women, they also had higher glycolytic enzyme activities. However, when the activities of the five enzymes were expressed per relative area unit of type 1 and type 2 fibres separately, and per unit muscle CSA, there were no differences between men and women. This implies that for a given muscle size and a given relative fibre area, men and women have the same maximal activities of LDH and PFK. Taken together, these somewhat complex relationships indicate that sex-related differences in glycolytic enzyme activities could partly be an effect of sex and fibre type composition together with effects as a result of body composition and large muscle mass. It is unclear whether body composition and muscle CSA are determined by the same factors that determine the metabolic capacity, and how these variables may affect glycolytic enzyme activity. We cannot, although, completely rule out that the high glycolytic capacity and the large muscle CSA found here are the result of higher intensity exercise in men compared with women. Further studies are undoubtedly needed to clarify these complex relationships.

Our data on the maximal activities of CS and SDH, showed that men and women have similar values of these enzymes, indirectly suggesting that they have a similar capacity for carbohydrate oxidation. In previous studies where the activity level has been carefully monitored and the subjects matched according to their habitual activity level (Esbjörnsson Liljedahl 2000, Kent-Braun & Ng 2000), no differences in oxidative capacity have been found. In some studies using the VL muscle, a positive correlation has been found between type 1 muscle fibres and the activity of oxidative enzymes (Simoneau et al. 1985). The maximal activities of mitochondrial enzymes are highly sensitive to changes in physical activity pattern and readily adapt to alterations in the level of activity (Holloszy & Booth 1976). Thus, a difference in oxidative capacity more likely indicates a difference in...
physical performance of the subjects studied than an effect of sex per se.

The activity of HAD, involved in the oxidation of fat, did not differ between men and women in this study. This finding is in agreement with several studies on younger subjects with comparable habitual activity levels (Bass et al. 1976, Green et al. 1984, Esbjörnsson Liljedahl 2000). The activity of another enzyme involved in oxidation of fat, carnitine palmitoyl transferase (CPT), was also not different between male and female athletes (Costill et al. 1979), or in isolated mitochondria (Berthon et al. 1998) from men and women. In contrast, whole body studies have suggested a higher proportion of lipids as substrate in women (Tarnopolsky et al. 1998), although this might be related to the intensity of exercise performed at the point of investigation.

A complementary description of the metabolic characteristics of a muscle at work is suggested by expressing the relative activity of enzymes representing different metabolic pathways as ‘discriminative’ ratios. ‘Non-discriminating’ ratios reflect that activities of enzymes involved in the same, or in a functionally related, metabolic pathway can vary in absolute values but show a constant relation between each other. ‘Discriminative’ ratios indicate the relative contribution of not related metabolic pathways to energy supply (Pette & Hofer 1980). Expressing enzyme activities in this way, we found no differences between men and women for non-discriminating ratios. Green et al. (1984), found that the ratio of HAD/SDH was significantly higher in women than men. In our study, both ratios of HAD/SDH and HAD/CS were higher in women, but the difference was not significant. Also, the discriminative ratios PFK/HAD and LDH/HAD for men and women were not significantly different, in contrast to the findings by Green et al. (1984).

In summary, our data show differences between men and women for the maximal activities of the glycolytic enzymes LDH and PFK, but not for the activities of muscle enzymes involved in the oxidation of carbohydrates and fat. Our data indicate that the higher maximal activities of LDH and PFK in men are related to the height, weight, muscle CSA and the relative area of type 2 fibres, which are all significantly larger in men than women.

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