The mRNA expression profile of metabolic genes relative to MHC isoform pattern in human skeletal muscles

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The mRNA expression profile of metabolic genes relative to MHC isoform pattern in human skeletal muscles. J Appl Physiol 101: 817–825, 2006. First published June 22, 2006; doi:10.1152/japplphysiol.00183.2006.—The metabolic profile of rodent muscle is generally reflected in the myosin heavy chain (MHC) fiber-type composition. The present study was conducted to test the hypothesis that metabolic gene expression is not tightly coupled with MHC fiber-type composition for all genes in human skeletal muscle. Triceps brachii, vastus lateralis quadriceps, and soleus muscle biopsies were obtained from normally physically active, healthy, young male volunteers, because these muscles are characterized by different fiber-type compositions. As expected, citrate synthase and 3-hydroxyacyl dehydrogenase activity was more than twofold higher in soleus and vastus than in triceps. Contrary, phosphofructokinase and total lactate dehydrogenase (LDH) activity was approximately three- and twofold higher in triceps than in both soleus and vastus. Expression of metabolic genes was assessed by determining the mRNA content of a broad range of metabolic genes. The triceps muscle had two- to fivefold higher MHC Ila, phosphofructokinase, and LDH A mRNA content and two- to fourfold lower MHC I, lipoprotein lipase, CD36, hormone-sensitive lipase, and LDH B and hexokinase II mRNA than vastus lateralis or soleus. Interestingly, such mRNA differences were not evident for any of the genes encoding mitochondrial oxidative proteins, 3-hydroxyacyl dehydrogenase, carnitine palmitoyl transferase I, citrate synthase, α-ketoglutarate dehydrogenase, and cytochrome c, nor for the transcriptional regulators peroxisome proliferator activator receptor gamma coactivator-1α, forkhead box O1, or peroxisome proliferator activator receptor-α. Thus the mRNA expression of genes encoding mitochondrial proteins and transcriptional regulators does not seem to be fiber type specific as the genes encoding glycolytic and lipid metabolism genes, which suggests that basal mRNA regulation of genes encoding mitochondrial proteins does not match the wide differences in mitochondrial content of these muscles.

soleus; triceps; vastus lateralis; metabolic profile

SKELETAL MUSCLE TISSUE CONSISTS of a heterogeneous population of muscle fibers (12). Individual fibers are often classified by the myosin heavy chain (MHC) isoform content. Functionally, the MHC isoform determines the mechanical properties of the fibers with MHC type I fibers characterized by slow-contractile speed and MHC type II fibers by fast-contractile speed (4). The metabolic properties of the MHC fiber subpopulations differ in general with regard to oxidative and glycolytic capacity, with MHC type II fibers primarily being glycolytic and easy to fatigue, and type I fibers more oxidative and fatigue resistant. The higher oxidative capacity of muscles predominantly composed of type I fibers, as the soleus muscle in humans, is reflected in a higher expression of mitochondrial proteins (31), whereas muscles largely composed of type II fibers, as the triceps muscle in humans, have a low mitochondrial content and a high expression of proteins involved in glycolysis (39).

Of note, the metabolic profile of a muscle is highly sensitive to the degree of muscle use and dependent on the associated adaptive changes in metabolic enzymes. Such changes are more marked and rapid than changes between MHC I and II isoforms (15) and clearly underline the basis for great variations in metabolic capacity in different muscle fibers with a given MHC isoform (24). This has been demonstrated for the oxidative activity as measured by succinate dehydrogenase activity using the single-fiber dissection technique (32). Moreover, a discrepancy between fiber-type composition and metabolic profile was demonstrated in the study by Lin et al. (20), where overexpression of peroxisome proliferator activator receptor (PPAR) gamma coactivator (PGC)-1α in mice induced a moderate (10–20%) shift in fiber type toward MHC type I fibers, but a pronounced increase in oxidative enzymes, emphasizing that the metabolic profile is much more labile than the MHC isoform expression. Thus prediction of the metabolic profile solely based on MHC isoform composition may, therefore, be difficult.

The metabolic profile is determined by the expression of metabolic genes. Previous studies have demonstrated that several metabolic genes are regulated at the transcriptional level in response to various acute metabolic challenges; thus fasting (28) and a single bout of exercise (26, 37) induce changes in transcriptional activity and mRNA content for metabolic genes in human skeletal muscle. Accumulated changes in the mRNA content of metabolic genes are also observed by prolonged interventions such as dietary manipulation (40) and training (9, 23, 29).

The purpose of the present study was, therefore, to test the hypothesis that, in human skeletal muscle, 1) a relationship between mRNA profile and MHC composition exists for some metabolic genes in accordance with the functional use of the muscle, whereas 2) for other metabolic genes, the mRNA expression is not tightly related to the MHC fiber-type composition, reflecting that gene regulation in a given MHC fiber type may differ between muscles. This was examined by obtaining muscle biopsies from triceps brachii (characterized by a high proportion of type II fibers) and soleus (characterized by a high proportion of type I fibers) from physically active young men, and measuring mRNA expression for several metabolic genes using single-fiber dissection technique and real-time PCR.

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and had a body mass index of 24.7. Samples were stored at Finetek, Zoeterwoude, The Netherlands) followed by freezing in ical analysis by mounting a small muscle piece in Tissue-Tek (Sakura Denmark). A 5- to 7-mm incision was made, the Bergström needle was introduced into the muscle tissue, suction was applied, and three biopsy was quickly removed, and the biopsy was present, superficial blood was cutaneous, triceps brachii caput medialis, triceps surae pars soleus, and quadriceps pars vastus lateralis by using a Bergström biopsy needle (5). First the skin and the muscle fascia were introduced into the muscle tissue, suction was applied, and three to five cuts were made. The biopsy was split up into two parts. Approximately 50 mg of the biopsy were used for RNA isolation. If present, superficial blood was quickly removed, and the biopsy was frozen in liquid nitrogen. The other part was prepared for histochemical analysis by mounting a small muscle piece in Tissue-Tek (Sakura Finetek, Zoeterwoude, The Netherlands) followed by freezing in 2-methyl-butanate (Acrros Organics) precooled in liquid nitrogen. Both samples were stored at −80°C until analyzed.

**Enzyme activities.** Biopsies were analyzed fluorometrically for citrate synthase (CS), 3-hydroxyacyl dehydrogenase (HAD), phosphofructokinase (PFK), and lactate dehydrogenase (LDH) activities as previously described (8).

**Experimental procedure.** The subjects were instructed not to perform any vigorous exercise 24 h before the experiment and to report to the laboratory after an overnight fast. Biopsies were obtained from three different muscle groups: triceps brachii caput medialis, triceps surae pars soleus, and quadriceps pars vastus lateralis using a Bergström biopsy needle (5). First the skin and the muscle fascia were anesthetized with the use of 20 mg/ml lidocaine (SAD, Copenhagen, Denmark). A 5- to 7-mm incision was made, the Bergström needle was introduced into the muscle tissue, suction was applied, and three to five cuts were made. The biopsy was split up into two parts. Approximately 50 mg of the biopsy were used for RNA isolation. If present, superficial blood was quickly removed, and the biopsy was frozen in liquid nitrogen. The other part was prepared for histochemical analysis by mounting a small muscle piece in Tissue-Tek (Sakura Finetek, Zoeterwoude, The Netherlands) followed by freezing in 2-methyl-butanate (Acrros Organics) precooled in liquid nitrogen. Both samples were stored at −80°C until analyzed.

**Gene 1** and **probe 1** for **MHC IIa** and **GLUT-4** were designed and optimized as previously described (27, 28). All primers and probes (Tag Copenhagen, Copenhagen, Denmark) are presented previously described (8).

**Tissue processing for histology.** For identification of muscle fiber composition, frozen biopsies of the triceps, vastus lateralis, and soleus muscles were cut in 6-μm consecutive, transverse sections on a cryostat at −20°C. All sections were immediately collected on glass slides and processed for morphological staining. Routine ATPase histochemistry was performed after preincubation at pH = 10.30, allowing identification of different fiber types (fiber types I and II), counting on average −450 fibers per biopsy.

**RNA isolation, reverse transcription, and real-time PCR.** Total RNA was extracted from −50-μg muscle tissue using TRizol Reagent (Invitrogen, Carlsbad, CA) following the manufacturer’s instruction. In short, muscle tissue was homogenized in 1-ml TRizol Reagent for 15 s using a Polytron (Kinematica, Luzern, Switzerland) at setting 29. Chloroform was added, and the phases were separated by centrifugation. The aqueous phase with the RNA was transferred to a fresh tube, and the RNA was precipitated by adding isopropanol and left at −20°C for 1 h. After another centrifugation, the RNA pellet was washed in 75% ethanol and finally dissolved in 50-μl diethylpyrocarboxylate-treated water.

The RNA concentration was determined spectrophotometrically, and 2 μg total RNA were reversed transcribed in a total volume of 100 μl using Tagman Reverse Transcription Kit (Applied Biosystems, Foster City, CA) and random hexamers as primers. Real-time PCR was performed using an ABI 7900 Sequence detection system (Applied Biosystems). Primer and probes for MHC I and MHC IIa were adapted from Balagopal et al. (3) and optimized for real-time PCR. Primers and TaqMan probes for amplifying fragments of lipoprotein lipase (LPL), hexokinase (HK) II, carnitine palmitoyl transferase (CPT) I, glycogen synthase (GS) (26), PPAR-α, PGC-1α, HAD, and CS mRNA (29) have previously been described. Primers and probes for CD36, cytochrome (Cyt) c, forkhead box O1 (FoxO1), α-ketoglutarate dehydrogenase (α-KGDH), fatty acid binding protein (FABP), hormone-sensitive lipase (HSL), glucose transporter (GLUT)-4, PFK, LDH A, LDH B, and PPAR-δ mRNA were designed and optimized as previously described (27, 28). All primers and probes (Tag Copenhagen, Copenhagen, Denmark) are presented previously described (8).
The endogenous controls 18S rRNA, β-actin, and GAPDH mRNA were amplified using predeveloped assays (Applied Biosystems). The PCR conditions followed the procedure recommended by the manufacturer with 10-μL reaction volume, and each sample was run in triplicates for 40 cycles. The mRNA content of both target and endogenous control genes was calculated from the cycle threshold values by using a standard curve constructed from a serial dilution of aliquots of cDNA pooled from all the samples. Of the three endogenous controls measured, only β-actin mRNA content was independent of muscle type, and β-actin was therefore used as reference gene. The target gene mRNA content was related to the β-actin mRNA content, and the ratio is presented.

**Statistics.** Together the three muscles covered a broad range of MHC type I fibers (20–83%), and correlation analyses were therefore feasible. The correlation analysis was assessed, applying a general linear model using SYSTAT version 8.0 (SPSS). Furthermore, the differences in mRNA expression between the three muscle groups were assessed by using a one-way ANOVA. If the ANOVA showed a significant difference, Student’s paired t-test with Bonferroni correction was used as post hoc test. The statistical analyses were performed in Excel 2000 (Microsoft). P ≤ 0.05 was considered significant.

**RESULTS**

**MHC mRNA isoforms and fiber types.** Soleus consisted of 68–83% type I fibers, vastus lateralis 40–56%, and triceps only of 20–33% type I fibers. MHC IIa mRNA content correlated negatively (P < 0.05) with the percentage of occurrence of type I fibers (Fig. 1), and MHC I mRNA content correlated positively with percentage of type I fibers (P < 0.05). When the individual muscles were compared, differences in MHC-determined fiber types were also evident at the mRNA level with twofold higher (P < 0.05) MHC I mRNA content in soleus than in the triceps muscle and five- and twofold higher (P < 0.05) MHC IIa mRNA content in triceps and vastus lateralis, respectively, than in the soleus muscle (Table 2).

**Table 2. Comparisons of mRNA content in the triceps, vastus, and soleus muscles**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Triceps</th>
<th>Mean</th>
<th>95% CI</th>
<th>Vastus</th>
<th>Mean</th>
<th>95% CI</th>
<th>Soleus</th>
<th>Mean</th>
<th>95% CI</th>
</tr>
</thead>
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<tr>
<td>MHC I</td>
<td>0.68*</td>
<td>0.45–0.91</td>
<td>1.17</td>
<td>0.74–1.60</td>
<td>1.04</td>
<td>0.78–1.29</td>
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<tr>
<td>MHC Ila</td>
<td>1.65†</td>
<td>0.98–2.31</td>
<td>0.87</td>
<td>0.42–1.31</td>
<td>0.40</td>
<td>0.14–0.66</td>
<td></td>
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<tr>
<td>GLUT-4</td>
<td>1.22</td>
<td>0.90–1.54</td>
<td>0.87</td>
<td>0.55–1.18</td>
<td>0.82</td>
<td>0.45–1.18</td>
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<tr>
<td>HK II</td>
<td>0.69†</td>
<td>0.57–0.80</td>
<td>0.87</td>
<td>0.43–1.32</td>
<td>1.69</td>
<td>0.30–2.58</td>
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<tr>
<td>GS</td>
<td>0.98</td>
<td>0.62–1.35</td>
<td>0.87</td>
<td>0.64–1.10</td>
<td>0.80</td>
<td>0.39–1.02</td>
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<tr>
<td>PFK</td>
<td>1.62†</td>
<td>0.91–2.33</td>
<td>0.81</td>
<td>0.47–1.15</td>
<td>0.52</td>
<td>0.28–0.75</td>
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<tr>
<td>LDH A</td>
<td>0.47†</td>
<td>0.20–0.74</td>
<td>0.22</td>
<td>0.05–0.40</td>
<td>0.12</td>
<td>−0.02–0.26</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LDH B</td>
<td>0.30††</td>
<td>0.22–0.39</td>
<td>1.52</td>
<td>0.68–2.36</td>
<td>1.64</td>
<td>0.69–2.59</td>
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<tr>
<td>LPL</td>
<td>0.55†</td>
<td>0.38–0.72</td>
<td>0.92</td>
<td>0.67–1.18</td>
<td>1.29</td>
<td>0.52–2.06</td>
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<td></td>
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<tr>
<td>CD36</td>
<td>0.73*</td>
<td>0.50–0.95</td>
<td>1.40</td>
<td>0.96–1.81</td>
<td>1.25</td>
<td>0.61–1.89</td>
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<td>HSL</td>
<td>0.40†</td>
<td>0.21–0.59</td>
<td>1.01</td>
<td>0.72–1.30</td>
<td>1.31</td>
<td>0.35–2.26</td>
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<tr>
<td>FABP</td>
<td>0.73</td>
<td>0.49–0.96</td>
<td>0.91</td>
<td>0.57–1.25</td>
<td>1.18</td>
<td>0.34–2.01</td>
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<td>CPT I</td>
<td>0.86</td>
<td>0.52–1.21</td>
<td>0.87</td>
<td>0.61–1.12</td>
<td>0.90</td>
<td>0.39–1.42</td>
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<tr>
<td>HAD</td>
<td>1.27</td>
<td>0.79–1.74</td>
<td>1.19</td>
<td>0.70–1.68</td>
<td>1.11</td>
<td>0.64–1.58</td>
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<td>CS</td>
<td>1.38</td>
<td>1.02–1.74</td>
<td>1.35</td>
<td>0.98–1.71</td>
<td>1.14</td>
<td>0.57–1.72</td>
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<tr>
<td>α-KGDH</td>
<td>1.05</td>
<td>0.76–1.35</td>
<td>1.16</td>
<td>0.87–1.45</td>
<td>0.89</td>
<td>0.71–1.07</td>
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<tr>
<td>Cyt c</td>
<td>0.87</td>
<td>0.64–1.11</td>
<td>0.97</td>
<td>0.61–1.34</td>
<td>1.10</td>
<td>0.58–1.62</td>
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<tr>
<td>PGC-1α</td>
<td>1.02</td>
<td>0.78–1.26</td>
<td>1.28</td>
<td>0.87–1.69</td>
<td>1.20</td>
<td>0.59–1.81</td>
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<tr>
<td>FoxO1</td>
<td>1.06</td>
<td>0.84–1.28</td>
<td>0.78</td>
<td>0.62–0.95</td>
<td>1.13</td>
<td>0.35–1.90</td>
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<tr>
<td>PPAR-α</td>
<td>1.48</td>
<td>1.16–1.81</td>
<td>1.62</td>
<td>1.09–2.14</td>
<td>1.80</td>
<td>0.58–3.02</td>
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<tr>
<td>PPAR-δ</td>
<td>4.21</td>
<td>2.12–6.30</td>
<td>2.24</td>
<td>1.46–3.02</td>
<td>2.31</td>
<td>1.83–2.79</td>
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<tr>
<td>18S rRNA</td>
<td>0.64††</td>
<td>0.57–0.72</td>
<td>0.69</td>
<td>0.61–0.77</td>
<td>0.29</td>
<td>0.10–0.49</td>
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<tr>
<td>GAPDH</td>
<td>1.28†</td>
<td>0.75–1.81</td>
<td>0.88</td>
<td>1.41–0.36</td>
<td>0.50</td>
<td>0.16–0.84</td>
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<tr>
<td>β-actin</td>
<td>0.79</td>
<td>0.60–0.99</td>
<td>1.22</td>
<td>1.90–0.79</td>
<td>0.92</td>
<td>1.70–0.65</td>
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</table>

Values are ratio of target mRNA content to β-actin mRNA content, except for 18S rRNA, GAPDH, and β-actin, which are given per microgram total RNA. Data are presented as arithmetic means with 95% confident intervals. Significant difference (P < 0.05) between muscles are indicated as *triceps vs. vastus; †triceps vs. soleus, and ‡vastus vs. soleus. For abbreviations see legends to Table 1.
**Enzyme activity.** The activity of the two mitochondrial enzymes, CS and HAD, was 2.2-fold higher ($P < 0.05$) in soleus and vastus lateralis than in triceps. The opposite pattern was observed for the glycolytic enzyme PFK and LDH. Thus triceps demonstrated the highest PFK activity with 1.7-fold higher ($P < 0.05$) level than in soleus, and total LDH activity was approximately three- and twofold higher ($P < 0.05$) in triceps than in soleus and vastus lateralis, respectively (Fig. 2).

**The mRNA expression profile.** Several genes encoding proteins involved in carbohydrate metabolism (GLUT-4, HK II, GS, PFK, LDH A, and LDH B) were investigated, and differences in mRNA content were observed between the muscles for all except GLUT-4 and GS. A positive correlation between percentage of occurrences of type I fibers and both HK II and LDH B mRNA content was observed, whereas PFK and LDH A mRNA content correlated negatively and GLUT-4 tended ($P = 0.085$) to correlate negatively with percentage of type I fibers (Fig. 3). This pattern was also evident when the individual muscles were compared and fold differences were calculated. The PFK mRNA was expressed the most in triceps with two- and fivefold lower ($P < 0.05$) levels in vastus lateralis and soleus, respectively, than in triceps. The LDH A mRNA content was threefold higher ($P < 0.05$) in triceps than in soleus, whereas the LDH B mRNA content was threefold higher ($P < 0.05$) in both vastus lateralis and soleus than in triceps. The same pattern was observed for HK II, where the content of HK II mRNA was threefold higher ($P < 0.05$) in soleus than in triceps and vastus lateralis (Table 2).

For genes encoding proteins in lipid metabolism, LPL and HSL mRNA also correlated positively with increasing MHC I fiber-type percentage (Fig. 4), whereas FABP mRNA revealed no correlation with fiber type, and CD36 mRNA only tended ($P = 0.062$) to correlate with occurrence of type I fibers. A difference between the various muscles was evident for LPL, HSL, and CD36 mRNA content. When fold differences were calculated between the three muscles, triceps had the lowest LPL and HSL mRNA content with two- and threefold higher ($P < 0.05$) content in soleus than in triceps, respectively, and the CD36 mRNA content was twofold higher ($P < 0.05$) in vastus lateralis than in the triceps muscle (Table 2). However, no correlation or muscle difference was observed for FABP.

No correlation or differences were observed in the mRNA content of any genes encoding mitochondrial proteins: CPT I, HAD, CS, α-KGDH, and Cyt c (Fig. 4 and Table 2). Furthermore, we investigated the mRNA content of factors involved in transcriptional regulation of metabolic genes: PGC-1α, PPAR-α and δ, and FoxO1. The mRNA content of PGC-1α, PPAR-α, and FoxO1 was equal in triceps, vastus lateralis, and soleus (Table 2) and did not correlate with percentage of occurrence of type I fibers (Fig. 5). However, for PPAR-δ, the difference was close to being significant, with a higher mRNA content in triceps than in vastus ($P = 0.07$) and soleus ($P = 0.10$).

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Fig. 2. Muscle enzyme activities of citrate synthase (CS; A), 3-hydroxyacyl-CoA dehydrogenase (HAD; B), phosphofructokinase (PFK; C), and total lactate dehydrogenase (LDH; D) ($n = 5$). Significant difference ($P < 0.05$) between muscles are indicated as #triceps vs. vastus; Striceps vs. soleus; and £vastus vs. soleus.
The main findings of the present study are that, in human skeletal muscles, some metabolic genes show fiber-type association, whereas others do not. The mRNA expression pattern is for several genes encoding proteins in glucose and lipid metabolism in agreement with the measured enzyme activities and the traditional view of glycolytic/oxidative muscles. Interestingly, none of the genes encoding mitochondrial proteins paralleled the differences in oxidative enzyme activity between the muscles and did not exhibit a fiber-type nor muscle-specific-related mRNA expression.

The first part of the hypothesis that some genes exhibit an mRNA profile/MHC relationship was evident for several genes. The present result that the PFK activity was higher in triceps than in soleus, together with the previous findings that the PFK activity correlated negatively with the percentage of type I fibers in the muscle (8), was well reflected in the relationship between PFK mRNA and percentage of type I fibers and also that the highest PFK mRNA content was in the triceps muscle. Interestingly, the HK II mRNA pattern was inversed with a positive correlation between HK II mRNA content and percentage of occurrence of type I fibers than in type II fibers, and the highest HK II was found in soleus. This is in good agreement with previous studies on muscle-specific LDH isozyme expression. Hence, LDH1 and LDH2 have been shown to be dominant in slow-twitch fibers and LDH5 to be high in fast-twitch fibers (13), and isozyme-specific expression of the LDH H and M chains in skeletal muscles with high- and low-oxidative capacity has been reported (16, 25, 41). Moreover, a typical endurance training adaptation is an increased content of the LDH1 and LDH2 isozymes, reflecting an enhanced capacity for oxidation of lactate in these muscles (33) and emphasizing the relation between LDH isozymes and oxidative capacity of the muscle.

LPL and HSL are involved in delivery of free fatty acid for breakdown within the muscle cell. Although there is a lack of data on comparing human LPL and HSL protein in triceps, vastus, and soleus, studies in rats show higher expression of LPL (14) and HSL (17) protein in soleus than in predominantly glycolytic muscles, which is in accordance with the present findings of both muscle- and fiber-type-related HSL and LPL mRNA expression.

The present finding of only a tendency for a relation between both GLUT-4 and CD36 mRNA and percentage of type I fibers is in contrast to the previous observation that GLUT-4 protein did not correlate with the MHC content (7). Moreover, the lack of a difference between muscles in GLUT-4 mRNA and only

**Fig. 3. Correlations between mRNA content for glycolytic proteins and type I fiber composition.**

A: glucose transporter (GLUT)-4. B: hexokinase (HK) II. C: glycogen synthase (GS). D: PFK. E: LDH A. F: LDH B. The target gene mRNA content has been normalized to the β-actin mRNA content. Biopsies from the same subjects are connected, and the muscle type is marked as follows: triceps (■), vastus (▲), and soleus (○). The bold line represents the result of the linear regression.
a difference between triceps and vastus lateralis for CD36 mRNA levels suggests that the type of muscle with associated activity pattern has a more pronounced effect on the mRNA regulation of these membrane transport proteins than the factors associating the mRNA expression with MHC isoform content.

Taken together, the present results indicate a coupling between the regulation of the expression of PFK, HK II, GLUT-4, LDH A, LDH B, LPL, and HSL mRNA content and MHC isoform composition. These close correlations between mRNA contents and fiber-type composition also suggest that the different daily use of the various muscles exerts a major impact on regulation of both metabolic and MHC genes and may indicate the existence of factors coupling the regulation of these genes. Additionally, the markedly different mRNA expression patterns of PFK, HK II, LDH A, LDH B, HSL, and LPL mRNA in the three muscles are in line with the metabolic profile of the muscles, suggesting that the expression of these genes is regulated at the level of transcription and/or mRNA stabilization.

The hypothesis that, for some metabolic genes, the mRNA expression is not tightly related to the MHC fiber-type composition was true for a number of genes. The previous demonstration that the GS protein amount was equally distributed in human triceps, vastus, and soleus muscles and no correlation was observed between MHC composition and GS protein content (7) is in line with the present findings that GS mRNA content was independent of percentage of type I fibers and independent of muscle type.
(38), higher content of mitochondria with more densely packed cristae in type I fibers (11), and higher activity of oxidative enzymes in type I fibers (8). The higher CS and HAD activity in soleus and vastus lateralis than in triceps in the present study is thus in accordance with the distribution of type I fibers in these muscles. Surprisingly, however, no differences between muscles and no relation with MHC composition were observed in the mRNA expression of any of the investigated genes encoding mitochondrial proteins: CPT I, CS, HAD, and Cyt c. But the present findings are in line with results from studies on rainbow trout showing similar mRNA levels for mitochondrial proteins in white and red muscles (18). A possible explanation for these findings may be that most tissues require mitochondria to maintain energy homeostasis, supporting the need for a constitutive mRNA expression of mitochondrial enzymes. Moreover, the slow turnover of mitochondrial proteins (6) may reduce the differences in basal mRNA levels between muscles. Although the MHC molecules also have a slow turnover (2, 21), differences were evident at the mRNA level for MHC isoforms in the present study, corresponding to the differences in MHC protein expression. This suggests that different transcriptional regulatory pathways are involved in regulating the MHC genes than the investigated genes encoding mitochondrial proteins. A tighter correlation between MHC mRNA content with fiber-type composition was observed for MHC IIa than MHC I. Quantitatively, <4% of muscle fibers show mismatching between MHC mRNA and the corresponding protein (1); however, the tighter correlation for MHC IIa could suggest a greater importance of transcriptional regulation for MHC IIa than MHC I.

As for the genes encoding mitochondrial proteins, similar mRNA levels were evident for the transcriptional regulators PGC-1α, PPAR-α, PPAR-δ, and FoxO1 in the three muscles. This observation is in contrast to a previous human study reporting that type Ila fibers have a higher PGC-1α protein content than type I fibers (34), and another study revealing tremendous differences in PGC-1α expression between muscle types in mice with high levels of PGC-1α mRNA and protein in soleus compared with the levels in the plantaris muscle (mainly in type II fibers) (20). These findings, together with the present observations, underline that caution should be taken when transferring rodent interpretations to a human context and emphasize the importance of human studies. PGC-1α is a transcriptional coactivator thought to play a major role in regulating mitochondrial biogenesis (19, 36), and the present observation of no difference in PGC-1α mRNA between muscles characterized by markedly different mitochondrial content is, therefore, particularly intriguing. It should be noted that a single bout of exercise increases both PGC-1α transcription and mRNA content in human skeletal muscle, but apparently without leading to a training-induced accumulation of PGC-1α mRNA after several weeks of training (29). Together these findings support that PGC-1α expression and activity are regulated at multiple levels.

Taken together, the present results showing that GS, CPT I, CS, HAD, α-KGDH, Cyt c, PGC-1α, PPAR-α, PPAR-δ, and FoxO1 mRNA expression exhibits no relation with neither muscle nor fiber-type composition indicate that these are not tightly regulated with the MHC genes. It may, however, be noticed that PPAR-δ mRNA tended to be negatively related to the occurrence of type I fibers in the muscle, which may be explained by the high IIX fibers content in the triceps muscle (30). The three muscles are characterized by widely different daily activity patterns, with soleus and triceps representing the extremes with a large amount of low-intensity activity and only little activity, but relatively high intensity, respectively. A-
though this is an important factor, both in regulation of the metabolic profile and the expression of MHC isoforms, the mRNA content of genes encoding mitochondrial proteins is not affected by this difference, indicating that the different activity patterns of the three muscles do not result in different basal mRNA expression of these genes. This discrepancy between mRNA level and enzyme activity suggests that these proteins are regulated at the translational level or by posttranslational modifications. However, it should be noted that several of these genes are indeed regulated at the mRNA level in human skeletal muscle, both by acute exercise and by endurance training (26, 29), suggesting that an inducible regulatory pathway has the potential to overrule a constitutive mRNA expression of mitochondrial genes. The possibility that the interpretation of the present data is influenced by acute changes in mRNA content in response to daily activities the day before the biopsies seems unlikely. An effect of transportation to the laboratory in the morning is implausible, because of both the very limited amount of work and the known timing of mRNA responses of the investigated genes (26, 29).

In conclusion, the metabolic properties of muscles are often extrapolated from the MHC-determined fibre-type composition. However, predictions of the metabolic profile solely based on MHC isoform composition are difficult (24, 30), which is further underlined by the present findings.

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