Diversity in levels of intracellular total creatine and triglycerides in human skeletal muscles observed by $^1$H-MRS

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Diversity in levels of intracellular total creatine and triglycerides in human skeletal muscles observed by $^1$H-MRS. J. Appl. Physiol. 87(6): 2068-2072, 1999.—We used $^1$H-magnetic resonance spectroscopy to noninvasively determine total creatine (TCr), choline-containing compounds (Cho), and intracellular (IT) and extracellular (between-muscle fibers) triglycerides (ET) in three human skeletal muscles. Subjects’ (n = 15 men) TCr concentrations in soleus (Sol; 100.2 ± 8.3 (SE) mmol/kg dry wt) were lower (P < 0.05) than those in gastrocnemius (Gast; 125.3 ± 9.2 mmol/kg dry wt) and tibialis anterior (TA; 123.7 ± 8.8 mmol/kg dry wt). The Cho levels in Sol (35.8 ± 3.6 mmol/kg dry wt) and Gast (28.5 ± 3.5 mmol/kg dry wt) were higher (P < 0.001 and P < 0.01, respectively) compared with TA (13.6 ± 2.4 mmol/kg dry wt). The IT values were found to be 44.8 ± 4.6 and 36.5 ± 4.2 mmol/kg dry wt in Sol and Gast, respectively. The ET values of TA (24.5 ± 4.5 mmol/kg dry wt) were lower than those of Sol (P < 0.01) and Gast (P < 0.05). There were no differences in ET (116.0 ± 11.2 (Sol), 119.1 ± 18.5 (Gast), and 91.4 ± 19.2 mmol/kg dry wt (TA)). It is proposed that the differences in metabolite levels may be due to the differences in fiber-type composition and deposition of metabolites due to the adaptation of different muscles during locomotion.

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Table 1. Prior knowledge used to develop the prior knowledge to fit the calf muscle 1H-MR data

<table>
<thead>
<tr>
<th>Prior Knowledge</th>
<th>Expression</th>
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<tbody>
<tr>
<td>$\alpha$ (Cho)</td>
<td>$= \alpha$ (TCr)</td>
</tr>
<tr>
<td>$\alpha$ (ET -CH$_2$)$_n$</td>
<td>$= \alpha$ (IT -CH$_3$)$_n$</td>
</tr>
<tr>
<td>$\alpha$ [ET -CH$_2$)$_n$ - $\alpha$ [IT -CH$_3$)$_n$ ]</td>
<td>$\beta$</td>
</tr>
</tbody>
</table>

MR, magnetic resonance; $\alpha$, damping factor; Cho, choline-containing compounds; TCr, total creatine; ET, extracellular triglycerides; IT, intracellular triglycerides.

The prior knowledge for the triglyceride resonances was obtained from 10 signals with good signal-to-noise ratios (4, 4, and 2 FIDs for Sol, Gast, and TA, respectively). For this purpose, only the signals with clearly separated ET and IT -CH$_2$- and -CH$_3$ resonances were selected (this is possible due to variation in triglyceride content among subjects). After water removal by using HLSVD, these signals were analyzed by VARPRO with the prior knowledge of equal damping factors (Table 1). It should be emphasized here that there were no restrictions on the damping factor ratio between -CH$_2$- and -CH$_3$. Subsequently, frequency shifts between -CH$_2$- and -CH$_3$ resonances for ET and IT signals as well as damping factor ratios between -CH$_2$- and -CH$_3$ resonances were calculated (Table 2). The prior knowledge calculated for the different muscles was found to be the same within the errors, and therefore the final prior knowledge (Table 2) applied to the present in vivo 1H-MR calf muscle data was calculated as an average over 10 signals selected. These values are consistent with the values found earlier (30). The zero-order phase correction was estimated by VARPRO, and the first-order phase correction was fixed to zero.

Water areas were also determined as water was used as the internal standard for each muscle for the absolute quantification of the metabolite and triglyceride resonances (77% of water in muscle (4)). The measured FIDs were first subjected to the HLSVD method (38) to remove all nonwater resonances. This was an important step in the analyses because intense lipid resonances affect the reliability of the VARPRO results. In general, the removal of large resonances, which are not described by a model function, from the signal gives rise to better convergence behavior and more reliable and consistent results (37). The water signals were described by one Lorentzian component each and analyzed by VARPRO. Final concentrations for each metabolite were then corrected by using longitudinal and transverse relaxation times of protons from lipids, water, and nonlipid metabolites in human muscles (30).

Statistics

Differences between each muscle group for TCr, IT, and ET concentrations were determined by repeated-measures ANOVA. A post hoc Scheffé’s F-test was used to analyze any significant difference. The level of significance was chosen at $P < 0.05$. All results are presented as means ± SE.

RESULTS

Figure 1 shows a typical 1H-MR spectrum of soleus muscle fitted by VARPRO. Representative in vivo 1H-MR spectra from the three muscle groups are shown in Fig. 2. Resonances from TCr, Cho, IT, and ET can be clearly observed. Although similar resonances can be observed in each muscle, significant differences in the relative metabolite levels are apparent.

Table 3 shows the levels of metabolites quantified with 1H-MRS. By using muscle water as an internal standard, subjects (n = 15) mean TCr concentrations in Sol were significantly lower ($P < 0.05$) than those in Gast and TA. The concentrations of Cho in TA were lower compared with Sol ($P < 0.001$) and Gast ($P < 0.01$). Also, the IT values of TA were significantly lower than those of Sol ($P < 0.01$) and Gast ($P < 0.05$). There were no significant differences in levels of ET among the three muscle groups. Levels of IT in Gast correlated moderately ($r = 0.52$, $P < 0.05$) with Cho levels. A similar trend was observed in Sol, although it did not reach significance ($P < 0.08$). TCr levels did not correlate significantly with IT concentration, although they...
showed a significant correlation ($r = 0.93; P < 0.001$) with Cho in Sol.

**DISCUSSION**

In this study we have used $^1$H-MRS to noninvasively investigate levels of TCr, Cho, IT, and ET in three different human muscles. The results of the study showed that Gast and TA contain higher concentrations of TCr than does Sol, whereas the levels of IT were lower in TA compared with Sol and Gast. The levels of ET were similar among the three muscles. An additional finding of the present study was the lower level of Cho in TA compared with Sol and Gast.

We are not aware of any study to date that has noninvasively quantified levels of TCr, IT, and ET in different muscle groups in human volunteers. The MRS techniques are of great value as they can be used to determine levels of these metabolites in deep muscles that are difficult to obtain with the biopsy technique. Assessments of IT and ET in different muscles are of significant value for a more in-depth understanding of human lipid metabolism. Also, the noninvasive determinations of TCr can enhance our understanding of Cr metabolism. The concentrations of IT in the three muscles examined in the present study were within the range of IT values obtained from biochemical analyses of biopsies of vastus lateralis muscle by Hurley et al. (24) (59 mmol/kg dry wt), Starling et al. (34) (35 mmol/kg dry wt), Cleroux et al. (14) (28 mmol/kg dry wt), Wendling et al. (40) (26 mmol/kg dry wt), and Kiens et al. (26) (22 mmol/kg dry wt). However, we observed larger IT concentrations in Sol and Gast muscles compared with TA. The levels of TCr in Sol, Gast, or TA observed in the present study were also similar to those obtained from biochemical analyses of muscle biopsies by Edström et al. (18) (Sol 100.6 mmol/kg dry wt), Bangsbo et al. (2) (Gast 119.8 mmol/kg dry wt), and Constantin-Teodosiu et al. (15) (TA 117 mmol/kg dry wt). From the values of these separate studies from different laboratories, it appeared that Sol contained less TCr than did Gast and TA. In the present study, we confirmed a significantly lower TCr content in human Sol compared with in Gast and TA. The differences observed in IT and TCr concentrations among the three muscles examined in the present study are likely to reflect true intermuscular differences.

It has been suggested that the training status of a subject can greatly influence the levels of IT (24–26). However, this does not explain the different levels of IT seen in the three muscles examined, as physical training would generally have a similar effect on all three muscles, unless there was some specific isolated muscle training. This is unlikely for the subjects in the present study. Diet is also a potential factor (34), although its influence should be similar for the three muscles examined. Another possible determinant in the storage of IT and TCr in muscles is the percentage of slow-twitch fibers (19, 27, 28). Interindividual differences in muscle metabolite concentrations can include adaptations to different activity patterns, diet, and fiber-type composition. However, intraindividual metabolite differences in metabolite concentrations of the three muscle observed are possibly due to differences in fiber-type composition.

The differences in metabolite levels due to fiber-type composition of the three muscles examined may be

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Table 3. Concentrations of TCr, Cho-containing compounds, IT, ET determined by $^1$H-MRS in soleus medial gastrocnemius, and tibialis anterior muscles of 15 human volunteers

<table>
<thead>
<tr>
<th></th>
<th>Sol</th>
<th>Gast</th>
<th>TA</th>
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<tbody>
<tr>
<td>TCr</td>
<td>100.2 ± 8.3†</td>
<td>125.3 ± 9.2</td>
<td>123.7 ± 8.8</td>
</tr>
<tr>
<td>Cho</td>
<td>35.8 ± 3.6‡</td>
<td>28.5 ± 3.5‡</td>
<td>13.6 ± 2.4</td>
</tr>
<tr>
<td>IT</td>
<td>44.8 ± 4.6‡</td>
<td>36.5 ± 4.2‡</td>
<td>24.5 ± 4.5</td>
</tr>
<tr>
<td>ET</td>
<td>116.0 ± 11.2</td>
<td>119.1 ± 18.5</td>
<td>91.4 ± 19.2</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as mmol/kg dry wt. MRS, magnetic resonance spectroscopy; Sol, soleus; Gast, gastrocnemius; TA, tibialis anterior. †P < 0.05 Sol vs. TA. ‡P < 0.05 Sol vs. Gast.
reflective of the chronic biological adaptations to locomotion. Some muscles might have developed to be more oxidative and fatigue resistant, whereas others are more glycolytic and less fatigue resistant, as a consequence of the fact that each of the muscles examined has a different functional role during locomotion. Human muscles contain mixtures of these two types of fibers and others that are classified as intermediate (oxidative-glycolytic). The Sol and Gast muscles are primarily involved in plantar flexion of the ankle joint, whereas the TA is used mostly during dorsiflexion, and the three muscles are used during free standing (3). The Gast is more involved in large contractions and in rapid development of tension compared with Sol (3). If chronic physical usage in endurance types of contraction of the different muscles is the primary factor in the end that influences the number of slow-twitch fibers, the oxidative potential, and the levels of IT (24–26), then we can conclude from the findings of this study that the load in aerobic activities in the human TA is less than that of the plantar flexor muscles during normal locomotion.

The higher levels of TCr in Gast and TA may be indicative of the larger percentage of fast-twitch fibers in these muscles as a likely adaptation of these muscles to more powerful contractions compared with Sol. PCR levels are higher in fast-twitch compared with slow-twitch fibers (27, 29, 32, 36). Also, the levels of PCR determine the power generated during muscle contraction (12). Additionally, PCR levels normally are much larger than those of free Cr in human muscle (12, 21, 23). This is thus likely that the difference in TCr levels might be primarily due to larger levels of PCR in Gast and TA compared with Sol. In agreement with this hypothesis, Edström et al. (18) showed higher resting levels of PCR in human vastus lateralis compared with Sol. Vastus lateralis, Gast, and TA muscles have lower percentages of slow-twitch fibers compared with Sol (31).

An additional finding of the present study was the lower level of Cho in TA compared with Sol and Gast. Furthermore, Cho levels showed significant correlation with both IT (in Gast) and TCr (in Sol). Chung et al. (13) have previously shown that Cho resonance is a multi-component peak with contributions from carnitine and glycerophosphorylcholine. The former is closely related to fatty acid metabolism. Therefore, the higher levels of carnitine and IT in Sol and Gast may reflect the higher potential for fat metabolism in theses muscles compared with TA. The significance of the correlations found between different metabolites needs to be explored.

In summary, the results of the present study suggest that IT levels are higher in Sol than in Gast and TA muscles and TCr levels are higher in TA and Gast compared with Sol in humans. On the other hand, the levels of ET are comparable. It is proposed that some of these differences are largely due to the diversity of fiber-type composition as a likely consequence of the selective biological adaptation of each muscle during locomotion.


