Content of intramyocellular lipids derived by electron microscopy, biochemical assays, and $^1$H-MR spectroscopy

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Howald, Hans, Chris Boesch, Roland Kreis, Sibylle Matter, Rudolf Billeter, Birgitta Essen-Gustavsson, and Hans Hoppeler. Content of intramyocellular lipids derived by electron microscopy, biochemical assays, and $^1$H-MR spectroscopy. J Appl Physiol 92: 2264–2272, 2002; 10.1152/japplphysiol.01174.2001.—Three different methods to determine intramyocellular lipid (IMCL) contents in human skeletal muscle have been compared. $^1$H-magnetic resonance spectroscopy (MRS) was evaluated against electron microscopic morphometry and biochemical assays of biopsy samples from m. tibialis anterior of 10 healthy subjects. The results of $^1$H-MRS and morphometry were strongly correlated, proving the validity of the $^1$H-MRS results for the noninvasive determination of IMCL. Biochemical assays yielded results that did not significantly correlate with the results of the other methods. When IMCL levels obtained from the three methods are expressed in common units, it was found that $^1$H-MRS yielded IMCL average levels that were 1.8 times lower than those found by morphometry. Potential reasons for the discrepancy are discussed. It is expected that $^1$H-MRS will be suitable to replace invasive techniques for IMCL determination, whenever noninvasiveness is crucial, e.g., for repeated investigations in studies of substrate recruitment and recovery in exercise.

skeletal muscle; exercise; energy substrates; quantitation; magnetic resonance

The importance of lipid as a substrate for oxidative energy production and long-lasting physical exercise has been reviewed extensively in recent publications (1, 14, 20–22, 29, 33, 40, 45). Lipids are stored in the form of triglycerides in either adipose tissue [extramyocellular lipid (EMCL)] or of lipid droplets in the cytoplasm of muscle cells [intramyocellular lipid (IMCL)]. At both sites, exercise induces lipolysis, resulting in the release of free fatty acids and glycerol. In the case of IMCL, free fatty acids are readily available for oxidation, because lipid droplets are usually located in close contact to muscle mitochondria (41). Free fatty acids from EMCL are complexed with plasma albumin to allow for vascular transport to skeletal muscle capillaries. Specific fatty acid binding proteins then facilitate the transfer through capillary endothelium, sarcolemma, and muscle fiber cytoplasm to the mitochondria (40).

Muscle triglycerides have been measured invasively in biopsies by using biochemical assay methods (11, 13, 18, 25, 37, 44) or electron microscopy (EM) and morphometry (16, 38). However, invasive measurement does not lend itself to repeated measurement, which is necessary to study the kinetics of lipid depletion and repletion. Moreover, the sample is of small size, and biochemical fat determination is technically difficult, because the separation of IMCL and EMCL is critical. EM allows identification of intrafibrillar lipid droplets, but scarcity of the component and small sample size may lead to a large error. Normally, the relevant difference in IMCL has to be >60–100% to be detected with statistical significance in usual study populations (15).

The noninvasive observation of high-energy phosphates by $^{31}$P-magnetic resonance (MR) spectroscopy (MRS) and the investigation of muscular glycogen content by $^{13}$C-MRS are well established (3, 23). Based on the observation of two distinct lipid resonances (34), a method for measuring IMCL by $^1$H-MRS was developed (4). It was based on two facts: 1) one of the two resonances in the lipid CH$_2$ region is dependent on muscle orientation relative to the magnetic field and was, therefore, assigned to IMCL; and 2) the IMCL resonances scale with signal amplitudes of metabolites in the muscle cell (e.g., creatine), when the voxel size is increased, whereas lipid signals of bulk fat show a disproportionate growth. Quantitation of IMCL in animal (39) or human (2) muscle by means of $^1$H-MRS, along with $^{13}$C-MRS determination of glycogen, allows for the noninvasive observation of the complete pattern of intracellular substrate storage and use in human muscle during sports activities or as a result of dietary interventions.

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The purpose of this study was to compare $^1$H-MRS results with those obtained with previously established methods and to estimate variability and expected error associated with each technique.

MATERIALS AND METHODS

Volunteers. Six male and four female subjects gave written, informed consent for participation in the study, which had been approved by the Institutional Review Board of the University of Bern. The subjects averaged $30 \pm 9.7$ yr in age (range 21–48 yr), 69.3 ± 12.5 kg in body weight (range 49–93 kg), and 177.7 ± 8.7 cm in height (range 160–187 cm). Three subjects (two women, one man) were untrained (<2 h of sports/wk). The other seven subjects (two women, five men) had all been involved in regular endurance training (long-distance running, orienteering, triathlon) for several years, averaging 5–15 h of strenuous exercise/wk.

To guarantee a wide spread of IMCL levels in the investigated cohort, the untrained subjects were asked to go for a fast walk of 2 h before the investigations to deplete IMCL. The trained subjects were told to keep their IMCL stores at a high level by abstaining from training for at least 1 day before MRS and biopsy. When the first results were available, it was evident that one of the orienteers had not completely replenished his lipid stores 48 h after his participation in a very demanding competition taking place in a mountain area. Therefore, this particular subject was investigated for a second time after a full week of complete abstinence.

MRS. MR investigations were performed on a SIGNA 1.5-T MR system (General Electric, Milwaukee, WI). MR images were obtained for accurate localization. $^1$H-MR spectra were recorded by using a standard coil for extremities (linear polarized volume coil, diameter 17 cm, length 29 cm) and an optimized PRESS sequence with echo time (TE) of 20 ms, repetition time of 3,000 ms, 128 acquisitions, and 16 phase-rotation steps (4). All measurements were performed in m. tibialis anterior, because this muscle represents the optimal experimental situation for MRS, thanks to the parallel alignment of its fibers and surrounding lipid layers with respect to the static magnetic field.

Imaging parameters had been chosen for optimal visualization of muscles and fasciae (gradient echo sequence, 30° flip angle, repetition time 100 ms, TE 6.8 ms). The voxel position was selected in these $T_2$-weighed images such that the voxel contained as little as possible visible interstitial tissue or fat, to avoid contamination from EMCL (Fig. 1). The first voxel position was marked for subsequent needle biopsy, and a second voxel volume was placed ~5 cm proximal to that location, where experience had shown that less contamination from EMCL can be expected (4). Only MRS data from the biopsy site were used for comparison with EM and biochemical assays. Data from the first and second MRS voxel location were used to estimate the influence of intramuscular differences in IMCL levels. Typical voxel volume was 2.4 ml.

For single-voxel spectroscopy, water presaturation and outer volume suppression were used. Quantitation of spectra was done via the signal of water determined from a fit of the first points in a TE series of free induction decays obtained without water suppression (2). Both IMCL and EMCL peaks were fitted in water-suppressed spectra with four Gaussian lines by using an improved version of a conjugate gradient descent method and prior knowledge restrictions (36). A typical $^1$H-MR spectrum with identification of IMCL and other compounds is shown in Fig. 2.

Initial studies had shown that IMCL in tibialis anterior muscle can be determined with a reproducibility of ~6% (4), which is sufficient to allow for quantitative estimates of biologically relevant changes in IMCL concentrations. Lipid concentrations are given in millimoles per kilogram wet weight of muscle (2).

Muscle biopsy and morphometry. Needle biopsies were taken from the upper part of tibialis anterior muscle at the position at which the first MR spectrum was recorded. A fraction of the tissue sample of each subject was immediately frozen in isopentane cooled in liquid nitrogen and then stored at −80°C for subsequent biochemical analysis. The remainder of the samples were processed for EM by fixation in a 6.25% solution of gluteraldehyde buffered in 0.1 M sodium cacodylate adjusted to 430 mosM with NaCl. Total osmolarity of the fixative was 1,150 mosM, pH 7.4. The blocks were rinsed overnight in 0.1 M sodium cacodylate buffer, postfixed with a 1% solution of osmium tetroxide, and block-

![Image](https://example.com/image.png)

**Fig. 1.** Axial magnetic resonance image for localization through the calf of 1 subject. A voxel of typical size and position is indicated (white square) in tibialis anterior muscle.

![Image](https://example.com/image.png)

**Fig. 2.** $^1$H-magnetic resonance spectrum of tibialis anterior muscle. IMCL-(CH$_2$)$_n$-, intramyocellular lipid with fatty acid chain; EMCL-(CH$_2$)$_n$-, extramyocellular lipid with fatty acid chain; Cr$_2$- creatine CH$_3$ group; Cr$_3$- creatine CH$_2$ group; X3- trimethylammonium; X3, tentatively assigned to taurine.

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contrasted with 0.5% uranyl acetate. After dehydration with increasing ethanol concentrations, six randomly chosen tissue blocks of each subject were embedded in Epon by using moulds with a hemispherical bottom to ensure random orientation. The resulting Epon sticks were reembedded in flat moulds from which blocks were cut out at directions determined by a system of random numbers representing spatial probabilities, thus providing isotopic uniform random sections (42).

Four sections of ~50- to 70-nm thickness were cut from four tissue blocks selected at random and picked up on 200-mesh copper grids covered with a thin carbon-coated Parlodion film. The sections were contrasted with lead citrate and uranyl acetate. Two independent investigators recorded 15 micrographs from each of these four sections in a Philips 300 electron microscope at magnification MP7, resulting in a total of 120 micrographs per biopsy sample and subject.

A final magnification of about ×30,000 was used to estimate the volume density of muscle fiber structures. Systematic sampling was used for all stereological procedures. Point counting was performed by the two independent observers by using a grid containing 100 test points for mitochondria and 400 test points for lipid deposits. All stereological variables were estimated according to standard procedures (43). Statistical analyses were performed for the volume of total mitochondria per fiber volume and volume of total lipid deposits per fiber volume. Initially, volume density of IMCL is expressed as percentage of fiber volume, but conversion to millimoles per kilogram wet weight is possible to allow for comparison with $^1$H-MRS and chemical measurements, applying a multiplication factor of 10.1 (2). The results of the two observers were pooled before comparison with data collected by MRS and biochemical analysis.

Special attention was given to the identification of intramyocellular lipid droplets. By applying the fixation methods described above, these droplets should be spherical in shape and contain slightly shaded, homogeneous material surrounded by a thin borderline. Whereas these criteria apply well for muscle tissue after postexercise repletion of lipid stores (Fig. 3A), identification is more difficult after exhaustive exercise. In the latter case, areas adjacent to mitochondria and those showing dense material of onion-peel

Fig. 3. Electron micrographs of tibialis anterior muscle of the 1 subject examined 1 wk (A) and 48 h after exhaustive exercise (B). Near-longitudinal sections are shown with sarcomeres in total contraction. mf, Myofibrils; mi, mitochondria; IMCL droplets, mostly in close contact to mitochondria. Scale bars = 1 μm.
The appearance (Fig. 3B) were also accepted for point counting of lipid volume density. Biochemical analysis. Freeze-dried muscle tissue was dissected free of visible connective tissue, fat, and blood from 9 of the 10 subjects. In one subject, it was not possible to get pure muscle tissue, as this sample contained almost only visible lipid droplets. Two muscle fiber specimens weighing 1.0–1.5 mg dry wt each were used from eight of the subjects, and from one subject one muscle fiber specimen weighing 0.6 mg dry wt was obtained. The triacylglycerol content of muscle fibers was determined by extraction of neutral fats with a Folch extract (12). The chloroform phase was retained and, after evaporation, hydrolyzed, and the glycerol content was measured (8). Results are given in millimoles per kilogram dry weight. With the assumption that muscle tissue contains 76% water (35), transformation to millimoles per kilogram wet weight is feasible by applying a multiplication factor of 0.24.

Statistics. Results are expressed as means ± SD. Linear regressions, two-sided unpaired t-tests, and correlation among MR, EM, and biochemistry data sets were determined with standard PC based software (Microsoft Excel 7.0 and 97).

RESULTS

Resulting values for the content of intramyocellular lipid determined by 1H-MRS, EM, and biochemical assays are summarized in Table 1.

Table 1 Summary statistics for data determined by 1H-MRS, electron microscopy, and biochemical analysis in original units and converted to millimoles per kilogram wet weight

<table>
<thead>
<tr>
<th></th>
<th>Original Units</th>
<th>mmol/kg wet wt</th>
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<tbody>
<tr>
<td></td>
<td>Mean SD Range</td>
<td>Mean SD Range</td>
</tr>
<tr>
<td>1H-MRS</td>
<td>mmol/kg wet wt</td>
<td>mmol/kg wet wt</td>
</tr>
<tr>
<td>IMCL</td>
<td>2.42 1.57 0.42–5.39</td>
<td>2.42 1.57 0.42–5.39</td>
</tr>
<tr>
<td>Electron microscopy</td>
<td>% = ml/100 ml</td>
<td>% = ml/100 ml</td>
</tr>
<tr>
<td>Vv(int,f)</td>
<td>6.35 1.409 4.42–8.53</td>
<td>4.4 3.1 1.7–10.8</td>
</tr>
<tr>
<td>Vv(lit,f)</td>
<td>0.44 0.308 0.17–1.06</td>
<td>0.6 0.3 0.8–10.1</td>
</tr>
<tr>
<td>Biochemistry</td>
<td>mmol/kg dry wt</td>
<td>mmol/kg dry wt</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>20.5 14.19 3.3–41.9</td>
<td>4.9 3.4 0.8–10.1</td>
</tr>
</tbody>
</table>

n, No. of subjects. From repeated measurements in 1 volunteer, only values after replenishment have been used. MRS, magnetic resonance spectroscopy; IMCL, intramyocellular lipid; Vv(int,f), volume density of total mitochondria; Vv(lit,f), volume density of total intracellular lipid. Conversion of % = ml/100 ml to mmol/kg wet wt required a factor of 10.1; conversion of mmol/kg dry wt to mmol/kg wet wt required a factor of 0.24 (according to 76% water content of total weight). 1H-MRS data are only shown for voxel 1, recorded at the site of biopsy.

IMCL content measured by 1H-MRS in 10 subjects varied over a wide range (Fig. 4). Mean values observed in the voxel positioned at the biopsy site exceeded the ones measured in the proximal voxel by 15% (paired t-test, $P = 0.003$). The IMCL values determined for the two locations were highly correlated ($r = 0.979$, slope $= 0.943 ± 0.065$, $P < 0.001$; intercept $= -0.221 ± 0.180$, $P = 0.25$). The highest IMCL value obtained in a cross-country runner (orienteering) exceeded the lowest one found in a cyclist (triathlon) by a factor of nearly 13. All of the four athletes involved in orienteering reached IMCL values in excess of 2.5 mmol/kg wet wt, whereas the IMCL content in tibialis anterior muscle of the other three trained subjects was not markedly different from that of the three untrained persons (individual values not shown). IMCL of the athlete investigated on two different occasions was 3.25 mmol/kg wet wt after a full week of abstinence from training but only 1.75 mmol/kg wet wt at 48 h after exhaustive exercise.

Determination of IMCL content with EM morphometry yielded values between 0.17 and 1.06% (ml/100 ml). The highest volume density for intramyocellular lipid was detected in the same athlete involved in orienteering who was already mentioned above. His value exceeded that of an untrained subject by a factor of nearly 13. All of the four athletes involved in orienteering reached IMCL values in excess of 2.5 mmol/kg wet wt, whereas the IMCL content in tibialis anterior muscle of the other three trained subjects was not markedly different from that of the three untrained persons (individual values not shown). IMCL of the athlete investigated on two different occasions was 3.25 mmol/kg wet wt after a full week of abstinence from training but only 1.75 mmol/kg wet wt at 48 h after exhaustive exercise.

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Fig. 4. Comparison of IMCL values determined in voxel 1 (site of needle biopsy) and voxel 2 (typical location used in magnetic resonance spectroscopy (MRS) studies (2, 4)). Trained athletes, ◢; untrained subjects. Dotted line, line of identity. A linear regression (solid line) reveals a correlation coefficient of 0.979, with a highly significant slope of 0.94 and an intercept that is not significantly different from the origin ($-0.22 ± 0.18$). wt, Wet weight.
of 6. The rather low lipid volume density of 0.25% of the athlete examined 48 h after competition increased to 0.61% when he had abstained from training for 1 wk. There was a systematic difference between the values measured by the two EM observers (Fig. 5). Nevertheless, the correlation of the individual data was very good ($r = 0.921$, slope of $0.772 \pm 0.109$, $P < 0.001$; intercept of $-0.062 \pm 0.065$, $P = 0.36$). EM morphometry revealed differences in mitochondrial volume density, which was nearly doubled in tibialis anterior muscle of one of the orienteers, compared with the lowest value found in one of the untrained subjects.

Biochemical analysis yielded a low variability in the triglyceride content of duplicate specimens taken from one and the same individual ($r = 0.885$, $P = 0.0015$). Interindividual values varied over a wide range, but, in contrast to $^1$H-MRS and morphometry, there was no indication that this spread was influenced by the training status of the subjects. Biochemically determined triglyceride content in tibialis anterior muscle of the athlete undergoing a biopsy 48 h and 1 wk after exhaustive exercise was not markedly different in the two samples (9.35 and 10.15 mmol/kg dry wt, respectively). Triglyceride content was also analyzed in the subject whose sample did not contain muscle tissue but almost only lipid droplets, and the value in this sample was as high as 245 mmol/kg dry wt. For obvious reasons, this value was excluded from further data processing.

Correlations of the IMCL values determined by the three different methods are displayed in Fig. 6 and evaluated numerically in Table 2. While $^1$H-MRS and morphometry showed a high and significant level of agreement ($r = 0.93$, $P < 0.001$), the correlations of biochemical analysis with the other two methods were much weaker ($r = 0.41$ and 0.47) and statistically not significant (Table 2). IMCL repletion in the athlete investigated 48 h and 1 wk after exhaustive exercise could nicely be documented by both $^1$H-MRS and morphometry but not by the biochemical approach. Moreover, it should be noted that two out of the three highest triglyceride concentrations measured in the biochemical assay originated from two untrained female subjects (open symbols in Fig. 6), a fact that was confirmed neither by $^1$H-MRS nor by EM morphometry.

**DISCUSSION**

Intramyocellular lipid stores in tibialis anterior muscle of trained and untrained subjects have been quantitated by three different techniques: $^1$H-MRS, EM morphometry of biopsy samples, and biochemical assays of biopsy samples. The noninvasive method of $^1$H-MRS could be validated by showing that it produces quantitative tissue contents that scale linearly with those obtained by EM morphometry. Biochemical assays of biopsy samples yielded lipid contents that correlated only weakly with those obtained by the other two methods.

When the subjects for the study were selected and the time points for MRS and muscle biopsy were defined, it was attempted to obtain largely different individual IMCL contents to increase the correlation range. This objective was fulfilled, because large variations in IMCL levels could be demonstrated with all three methods under investigation (Table 1). Conversion of the average values determined by $^1$H-MRS, EM morphometry, and the biochemical assay of freeze-dried samples to common units resulted in IMCL concentrations of 2.4 ± 1.6, 4.4 ± 3.1, and 4.9 ± 3.4 mmol/kg wet wt of muscle tissue, respectively.

In our hands, EM morphometry has been used previously in many studies to measure intramyocellular lipid content successfully. It had been found that it differs according to training status (15, 16), muscle fiber types (17), and diet (Vogt M, Puntschart A, Howard H, Mueller B, Mannhart C, Gfeller-Tuescher L, Mullis P, and Hoppeler H, unpublished observations) and that it decreases as a result of long-lasting muscle work (24, 38). The method depends on an invasive biopsy technique, sophisticated fixation, and sectioning routines for EM, as well as time-consuming analysis of micrographs by point counting. Interobserver compar-
ison in the present study showed that absolute volume contents of IMCL, as determined by EM morphometry, are operator dependent. The good correlation between the results of the two observers, however, indicates that relative IMCL contents can be obtained very accurately and reproducibly by a single observer. Thus elaborated sampling techniques for muscle tissue and clear-cut instructions for identification of lipid deposits in electron micrographs do not completely exclude variation in the measurement of volume densities performed by different observers. Beside observer bias, such differing results may also, in part, be due to uneven distribution of muscle fiber types in the small tissue blocks selected for sectioning.

Tibialis anterior muscle had been chosen because it is the location that has been studied most extensively by ${}^{1}H$-MRS because of the parallel orientation of the muscle fibers and extramuscular lipid sources. However, EM morphometry data for tibialis anterior muscle are not available in the literature. The values measured for volume density of both mitochondria and intracellular lipid in the present study are $\sim 30\%$ lower than those obtained in vastus lateralis muscle of untrained and trained subjects in several earlier experiments (15–17). The highest volume density for intramyocellular lipid was observed in one of the orienteers, but his level of 1.06% was still well below the averages of 2.09 and 1.30% found in vastus lateralis muscle of athletes specialized for marathon and ultramarathon distances, respectively, investigated in earlier studies (24, 30, 38).

The classic biochemical assay was less sensitive in differentiating IMCL from EMCL than both ${}^{1}H$-MRS and EM morphometry. Although freeze-dried muscle biopsy samples were carefully cleaned from visible fat, contamination with triglycerides stemming from adipocytes located between muscle fiber bundles may have taken place. An example of EMCL located in a small cluster of intercellular adipocytes is displayed in Fig. 7. Assuming a 0.5% volume density of intracellular lipid and a fiber diameter of 50 $\mu m$, the volume of triglycerides contained in just the largest of the five fat cells shown in Fig. 7 would be equivalent to the volume of IMCL droplets from $\sim 800$ muscle fibers. Thus it becomes clear why biochemical determination of IMCL must lead to a large variability and to conflicting results with respect to the role of IMCL for energy production during exercise (18, 25, 26, 44).

${}^{1}H$-MRS has recently been used by different authors to measure IMCL content of human tibialis anterior muscle (2, 4, 6, 9, 10, 19, 28, 31, 32). This newly developed approach has the advantage of being observer independent and noninvasive, allowing for frequent investigations in the course of depletion and repletion of IMCL as a consequence of exercise. It has been shown that the reproducibility of ${}^{1}H$-MRS-determined IMCL levels can be as low as 6% (4). The present investigation shows that measured IMCL values can differ systematically if determined in separate locations within the same muscle (slope 0.943 in Fig. 4), meaning that voxel positioning has to be carefully...
standardized in longitudinal studies. For the time being, the disadvantage of the method is that reliable results can only be guaranteed for a limited number of muscles in subjects who are not too obese. The costs per measurement are not negligible but are probably less than what a full cost analysis would yield for a muscle biopsy with follow-up biochemical or EM analysis. The $^1$H-MRS method has already been applied successfully in a number of studies looking into the kinetics of IMCL utilization during, and in recovery from, exercise (4, 7, 9, 10, 28, 31).

The high IMCL concentrations found in the muscle of the four subjects involved in orienteering point to a specific recruiting pattern of tibialis anterior muscle in this particular sport. Competitive cross-country running under difficult ground conditions means heavy stress for stabilization of the ankle and strenuous activation of tibialis anterior muscle for foot dorsiflexion lasting as long as 1–2 h of time. The delayed repletion of intracellular lipid stores described for a marathon run (38) was confirmed by the present observation in one of the subjects, whose IMCL measured by either $^1$H-MRS or morphometry had not recovered 48 h after an orienteering competition but came back to the levels observed in the other trained subjects after 1 wk of complete abstinence from training.

Pair-wise comparison of $^1$H-MRS, morphometry, and biochemically determined IMCL levels shows a strong correlation between $^1$H-MRS and morphometry ($r = 0.934$), however, not for MRS and biochemical assay ($r = 0.413$) or for morphometry and biochemistry ($r = 0.475$). The very strong correlation between MRS and morphometry does not exclude a systematic yet highly linear deviation of the two methods ($^1$H-MRS $/ \text{morphometry} = 0.467 \times \text{morphometry} + 0.367$). The two methods obviously describe the concentration of the same substance, but either the amount of IMCL is systematically overestimated by morphometry, or it is underestimated by $^1$H-MRS, or both. As long as studies are made with the same method, which is typically the case, such an over- or underestimation will not lead to wrong conclusions. However, as soon as results are compared with literature values or as soon as absolute values are used for the calculation of energy expenditure, this discrepancy has to be considered. Up to now, it is not clear which of the methods is closer to the true values. Nevertheless, there are mechanisms that could explain both effects.

The most trivial explanation would be a clerical error or mistake in the quantitation procedure of the $^1$H-MR spectra (2). However, there are several indicators that this can be excluded. 1) IMCL levels are quantified by the signal of the unsuppressed water signal from the

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**Table 2** Correlation statistics for IMCL data determined by $^1$H-MRS, electron microscopy, and biochemical analysis after conversion to common units of millimoles per kilogram wet weight (see Table 1)

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Correlation Coefficient, r</th>
<th>Slope</th>
<th>Significance of Slope ≠ 0, P</th>
<th>Intercept, mmol/kg wet wt</th>
<th>Significance of Intercept ≠ 0, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H-MRS vs. EM morphometry</td>
<td>0.934</td>
<td>0.467 ± 0.059</td>
<td>&lt; 0.001</td>
<td>0.367 ± 0.305</td>
<td>0.26</td>
</tr>
<tr>
<td>$^1$H-MRS vs. biochemical assay</td>
<td>0.413</td>
<td>0.196 ± 0.153</td>
<td>0.24</td>
<td>1.414 ± 0.859</td>
<td>0.14</td>
</tr>
<tr>
<td>EM morphometry vs. biochemical assay</td>
<td>0.475</td>
<td>0.445 ± 0.292</td>
<td>0.17</td>
<td>2.352 ± 1.639</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Slope and intercept values are means ± SD; $n = 11$ for $^1$H-MRS and morphometry $n = 10$ for biochemical assays. From the repeated measurements in 1 volunteer, values both before and after recovery have been used. Only $^1$H-MRS data from voxel 1 (biopsy site) were used. EM, electron microscopy.

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Fig. 7. Light microscopic semithin section of muscle fibers stained with toluidin blue. Magnification, $\times 610$. A cluster of 5 adipocytes of different size (black color) is embedded between 2 muscle fiber bundles, representing an EMCL deposit.
same volume. If the same procedure is used for the splitted (27) creatine-CH₃ signal of the resting muscle, it results in a creatine concentration of ~30 mmol/kg wet wt, a value that is very close to literature values (5, 19). 2) The report of Hwang et al. (19) leads to an IMCL concentration of 1.6 ± 0.9 mmol/kg wet wt for the tibialis anterior muscle in mainly sedentary volunteers. In comparison, Fig. 4 shows that the IMCL concentration of the three sedentary volunteers after weak exercise was 1.33 ± 0.24 mmol/kg wet wt in voxel 1. The ¹H-MRS-determined average IMCL concentration in this voxel for all volunteers (2.42 mmol/kg wet wt, Table 1) is even higher than that in the report of Hwang et al. 3) Our laboratory’s own report on IMCL concentrations in a marathon runner (2) yielded resting IMCL levels of ~4–6 mmol/kg wet wt for the tibialis anterior muscle and 6–12 mmol/kg wet wt for quadriceps muscles. These values point to the higher IMCL levels in trained individuals and the higher values in the quadriceps muscles. Because these values were obtained with the same quantitation procedure, clerical errors in the present study are very unlikely.

Hence ¹H-MRS values reported in this study are consistent with literature and with other parameters that can be determined from the spectrum. In addition, morphometry and ¹H-MRS show a very high correlation. This leads to the conclusion that the remaining discrepancy between morphometry and ¹H-MRS has a systematic and highly reproducible reason. One of the likely explanations could be a borderline around IMCL droplets as seen in EM. This thin line surrounding typical lipid droplets most probably corresponds to a membrane monolayer of phospholipids and a specific protein termed adipophilin enwrapping the hydrophobic core of neutral lipids (46). These membranes would lead to an overestimation of the morphological data, because nonlipid molecules contribute to and enlarge the droplets. ¹H-MRS, on the other hand, does not observe immobilized and rigid molecules when common sequences, as used in the present study (PRESS TE 20 ms), are employed. The short T₂ relaxation time of lipids that are partially immobilized by the adjacent membrane could lead to signal reduction and, subsequently, to a proportional underestimation of IMCL levels. Future experiments will be necessary to clarify these questions.

**Conclusion.** It has been shown that morphometry and ¹H-MRS reveal a very high agreement about IMCL levels, however, with a proportional factor between them. Biochemical analysis, on the other hand, did not correlate well with either morphometry or ¹H-MRS. In addition, biochemical analysis could not show obvious changes in an athlete before and after recovery, differences that have been clearly depicted by ¹H-MRS and morphometry. However, whereas MRS and morphometry can be used without correction for time series and comparison of different volunteers, the constant discrepancy between these two methods needs to be clarified for comparison with literature values and caloricmetric calculations. In conclusion, ¹H-MRS offers a noninvasive method for the determination of IMCL levels, in particular the opportunity to study time series of IMCL levels.

We thank the subjects volunteering for this study for participation and collaboration. The contributions of Eva Wagner, Fraenzi Graber, and Barbara Krieger for electron microscopy procedures and photographic artwork are highly appreciated.

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**REFERENCES**


DETERMINATION OF INTRAMYOCELLULAR LIPID CONTENT

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