Regional differences in intramyocellular lipids in humans observed by in vivo [1]H-MR spectroscopic imaging

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Hwang, Jong-Hee, Jullie W. Pan, S. Heydari, Hoby P. Hetherington, and Daniel T. Stein. Regional differences in intramyocellular lipids in humans observed by in vivo [1]H-MR spectroscopic imaging. *J Appl Physiol* 90: 1267–1274, 2001.—Regional differences in the content of intramyocellular lipids (IMCL), extramyocellular lipids, and total creatine (TCr) were quantified in soleus (S), tibialis posterior (TP), and tibialis anterior (TA) muscles in humans using in vivo ^{1}H proton spectroscopic imaging at 4 T. Improved spatial resolution (0.25-ml nominal voxel resolution) made it feasible to measure IMCL in S, TP, and TA simultaneously in vivo. The most significant regional difference was found in the content of IMCL compared with extramyocellular lipids or TCr. The concentrations of TCr were found to be 29–32 mmol/kg, with little regional variation. IMCL content was measured to be 4.8 ± 1.6 mmol/kg tissue wt in S, 2.8 ± 1.3 mmol/kg tissue wt in TP, and 1.6 ± 0.9 mmol/kg tissue wt in TA in the order of S > TP > TA ($P < 0.05$). It is likely that these IMCL values are consistent with the known fiber types of these muscles, with S having the greatest fraction of type I (slow-twitch, oxidative) fibers and TA having a large fraction of type IIb (fast-twitch, glycolytic) fibers.

magnetic resonance spectroscopy; triglyceride; quantification; phosphocreatine; body composition

BY USING IN VIVO [1]H-magnetic resonance (MR) spectroscopy (MRS) in skeletal muscle, several studies have demonstrated a negative correlation between intramyocellular lipid (IMCL) content and insulin sensitivity in sedentary and Type 2 diabetic subjects (9, 16, 19, 23, 28–30). This is particularly important because decreased insulin sensitivity is known to be a strong predictor for the clinical onset of Type 2 diabetes. However, the interdependence between insulin sensitivity and muscle lipids may be more complex because insulin sensitivity may depend on the fiber type and/or the specific muscle group in humans (15, 21). Nonetheless, this relationship may still be based on lipid content because different fiber types are known to contain varying levels of triglycerides (TG). For example, according to histological analysis, type I fibers (slow twitch) contain higher TG levels than type II fibers (fast twitch, glycolytic or oxidative). Thus to better define the specific relationship between IMCL and insulin sensitivity, spectroscopic studies that are able to measure and resolve IMCL accurately among muscles of different fiber types are important.

The measurement of lipids in muscle by in vivo ^{1}H-MRS is complex because of the presence of two pools of lipids in skeletal muscle, IMCL and extramyocellular lipids (EMCL) (2, 30). These two pools are kinetically different in that the latter is thought to turn over very slowly and serves as a long-term fat depot, whereas the former is thought to be in dynamic and rapid equilibrium with substrate utilization and supply. As reported by Boesch et al. (2, 17, 18), the anisotropic structure of EMCL results in a bulk magnetic susceptibility (30, 31) induced shift of its resonances. The bulk magnetic susceptibility shift is greatest when the muscle fibers' orientation is aligned with the B_0 axis, i.e., the axis of the magnet tube (2, 31). This results in a maximal ~0.2 parts/million (ppm) separation between the EMCL and IMCL resonances, which, although small, allows separate detection of the two pools. Nonetheless, at typical volume resolutions used in single-voxel studies (1.2–8.0 ml) (2, 9, 16–19, 23, 28–30), there is still severe overlap of the IMCL and EMCL components, hampering accurate measurement of IMCL in vivo. Thus in this study we performed high-resolution (0.25 ml) [1]H-MR spectroscopic imaging (SI) at 4 T of the calf to investigate the regional differences in IMCL content in tibialis anterior (TA), tibialis posterior (TP), and soleus (S) muscles.

METHODS

Subjects. MR SI imaging data were collected from 13 nonobese and nondiabetic subjects [7 men: body mass index (BMI) = 23.1 ± 1.6 kg/m^2 (range, 20.7–25.8), age = 35.9 ± 5.2 (range, 25–41) yr old; 6 women: BMI = 22.4 ± 3.7 kg/m^2 (range, 18.1–29.0), age = 26.8 ± 6.6 (range, 22–39) yr old], and the concentrations of total creatine (TCr), IMCL, and EMCL were measured in S, TA, and TP. All subjects were healthy and sedentary except for two female subjects who were trained as collegiate sprinters. All male subjects and

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three female subjects were Caucasians, two female subjects were African-American, and one was Asian. Transverse relaxation time (T2) measurements for quantitative data analysis of concentrations were acquired in four sedentary subjects [1 woman, 3 men, all Caucasians, BMI = 24 ± 3 kg/m^2, age = 37 ± 8 (range, 28–47) yr old]. Subjects were informed of the experimental procedures, and written consent was obtained before the study. The protocol was approved by the Institutional Review Board committees of the Albert Einstein College of Medicine and Brookhaven National Laboratory.

[1]H-MR SI. All data were acquired with a 4-T Varian Inova whole body magnetic resonance system using a volume ^{1}H resonator. The right calf was positioned in the coil, and the left calf was enclosed in a radio-frequency shield. Gradient echo images with repetition time-to-echo time ratio (TR/TE) of 250:18.8 ms were taken for localization within the right calf. The axial slice of interest was positioned at the insertion point of gastrocnemius. Shimming over the entire slice was performed manually (duration, 5 min), resulting in a water line width of ~20 Hz. Localization was achieved by using a slice-selective excitation (10 mm) pulse with a single-spin echo without an additional prelocalization scheme. Two dimensions of in-plane phase encoding (32 × 32) over 16.0 × 16.0 cm^2 resulted in a nominal voxel resolution of 0.25 ml. Water suppression was achieved by using an optimized semiselective refocusing pulse, as described previously (14). The pass band of the refocusing pulse ranged from ~3.0 to 1.0 ppm. For the water-suppressed (metabolite) SI (TR/TE = 1,000:24 ms), the acquisition time was 17 min. To provide an internal concentration reference, a nonsuppressed water SI (TR/TE = 5,000:24 ms) was acquired from the same slice using 16 × 16 phase encodes (acquisition time = 21 min). The water SI was zero filled to 32 × 32 encodes to permit coregistration with the metabolite SI.

To measure the T2 values of the EMCL, IMCL, and TCr resonances, metabolite SIs were acquired at six different echo times, namely, 24, 36, 51, 73, 105, and 150 ms, logarithmically spaced to be linearly fit to an exponential function (26). These SIs were acquired with 24 × 24 phase encodes over a field of view of 16 × 16 cm^2. The data were acquired with a repetition time of 1 s, which resulted in a 1-h acquisition time. To minimize artifacts due to motion, the six different echo times were acquired sequentially before the phase-encoding step was incremented.

Spectral and spatial processing. The data were processed in the spectral domain with the use of a 10-Hz Lorentzian-to-Gaussian transformation and 250-Hz convolution difference. A Hanning filter was applied in both spatial domains. After Fourier transformation, all SI voxels were corrected for B$_0$ shifts by referencing the TCr resonance to 3.0 ppm. For each muscle group (TA, TP, S), three voxels from each muscle were selected using the anatomic images as a guide. The selection criteria for the three voxels in each muscle were as follows: *1*) the voxel was completely within the muscle, and *2*) the voxel was not within visible fat depots or adjacent to the interfaces of muscles, bones, vessels, subcutaneous fat layers, or interfascial fat depots (thereby avoiding artifacts and maintaining good spectral quality). As a consequence of this selection process and the high spatial resolution, the lipid values determined in these studies represent a lower bound, particularly for EMCL. The spectra were phased and baseline corrected by using a cubic spline with fixed baseline points. The metabolite and water SIs were processed identically. The corresponding three water spectra were used to provide an internal reference for quantification.

Data analysis. The metabolite spectral data were analyzed by using a seven-resonance model, including carnitine (trimethyl: 3.2 ppm), TCr (methyl: 3.0 ppm), lipid (C$_2$ methylene: 2.3 ppm), lipid (allylic methylene: 2.1 ppm), EMCL (-CH$_2$-: 1.5 ppm), IMCL (-CH$_2$-: 1.3 ppm), and lipid (-CH$_3$: ~1 ppm). All resonances were fitted assuming a Gaussian line shape. The data were analyzed in a two-step process. Initially, the line width of TCr at 3.0 ppm (-CH$_3$) was determined using a spectral domain-fitting routine. The line width of TCr was then used to fix the line width of the IMCL resonance at 1.3 ppm (-CH$_2$-). The remaining resonances were fitted, allowing the line width and amplitude to vary. A representative spectrum, the calculated fit, and the difference between the fitted and measured data are shown in Fig. 1. The TCr (3.0 ppm) resonance in TA demonstrates a triplet pattern consistent with the dipolar coupling reported by several groups (12, 18); thus a triplet model was used for analysis (12, 18). The unique pattern of dipolar coupling of TCr was not clearly visible in TP and S; thus for these muscles the data were fitted as a single resonance peak. For the water reference spectra, the 4.7-ppm water resonance was fitted by using a Gaussian line shape.

The resonance areas of IMCL, EMCL, and TCr were normalized to the water reference of the corresponding voxel of water SI. A total of nine voxels per subject was analyzed (3 voxels for each muscle). Values from the three voxels were averaged per muscle per subject, and then these data were pooled across all 13 subjects. Comparisons between two data sets were done by using the unpaired Student's *t*-test, and comparisons among three muscles were performed by using ANOVA followed by Student-Newman-Keuls (SNK) testing for significance. The sample sizes of pooled data were 13 subjects for TA and S and 11 for TP. Data from TP of two subjects were not included because of artifacts in the spectra. For the correlation between BMI and muscular lipids, individual values from three different voxels were used for the quantitative analysis using the Pearson product-moment test. All data are presented as means ± SD.

For each subject, the T2 were obtained by measuring peak heights of IMCL (1.3 ppm), EMCL (1.5 ppm), and TCr (3.0 ppm) and by fitting them to a single-exponential function. These T2 values were pooled for all of the volunteers to determine the means ± SD for four subjects.

Absolute quantification using water reference. IMCL, EMCL, and metabolites were quantified relative to muscle
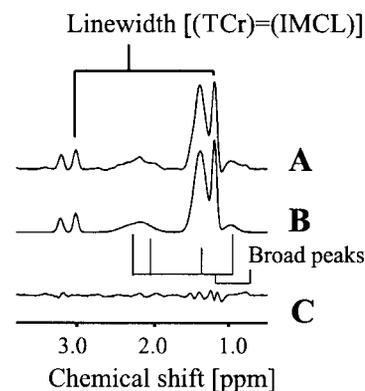

Fig. 1. Spectral fitting using a 7-resonance model. *A*: experimental spectrum from a 0.25-ml voxel. *B*: fitted spectrum using the model. The line width of total creatine (TCr) at 3.0 parts/million (ppm) was used for that of intramyocellular lipid (IMCL) at 1.3 ppm. The broad components were allowed to vary in amplitudes and line widths. *C*: the difference spectrum (*spectrum a − spectrum b*).

water by using units of millimoles per kilogram (28–30). Relaxation corrections [spin lattice, longitudinal relaxation time (T1); and spin-spin, T2] on lipid, water, and TCr were applied. For the lipid and TCr T2, we used our measured values. For the lipid T1 and water T1 and T2, we used the published data reported by Duewell et al. (5) in human muscle at 4 T. For TCr, the T1 of human skeletal muscle at 4 T has not been published. However, T1 of TCr at 4.7 T by Pan et al. (22) in human muscle and that at 1.5 T by Schick et al. (27) were reported to be about the same (1.1 s). The IMCL and EMCL values were corrected for multiple CH_2 groups by using the methods described by Szczepaniak et al. (30). The assumptions are as follows: *1*) methylene (-CH_2-) at 1.3 ppm is a singlet (excluding C_2-C_3 methylene and allylic and diallylic methylene) and *2*) the mean IMCL structure is similar to trioleate (61.0 mmol of ^{1}H/ml TG). Finally, the data were converted to units of millimoles per kilogram wet weight, assuming an 80% tissue water fraction and tissue density of 1.05 (30).

RESULTS

T2 values in three different muscles. The T2 values of TCr, IMCL, and EMCL methylene resonances from the three muscles were measured based on a single-exponential fit. In Fig. 2, data from S are shown, with the relative intensities of each signal and the fit. Unlike the EMCL and IMCL resonances, TCr displays some deviation from a pure single-exponential decay. Several authors have reported a multiexponential behavior of TCr T2, which has been attributed to biochemical heterogeneity, dipolar coupling, or a combination of both (4, 12, 18, 20). However, the detailed analysis of TCr multiexponential behavior was not our objective in this paper; therefore, we employed a single-exponential decay approximation for TCr. Our measured T2 values of IMCL, EMCL, and TCr from the three muscles are given in Table 1.

Enhanced spatial and spectral resolution. The 0.25-ml nominal voxel resolution of the SI made it possible to detect the IMCL in TA, S, and the TP simultaneously, with the latter being a small muscle deep between the tibia and fibula. Representative spectra from S, TP, and TA are shown in Fig. 3 along with an axial image for anatomic reference. In all three muscles, EMCL and IMCL were well resolved (Fig. 3). In the TA (Fig. 3*A*), the EMCL and IMCL resonances were resolved to baseline, allowing an accurate measurement of the IMCL line width. These well-resolved spectra enabled us to *1*) establish that the IMCL line width was similar to TCr, and *2*) use this a priori knowledge in the fitting of the IMCL resonance in all spectra. With the use of the line width of TCr at 3.0 ppm for that of IMCL at 1.3 ppm, the fitted spectrum in Fig. 1*B* was generated. As shown in Fig. 1*C*, there is very little residual coherent signal in the difference spectrum, supporting the validity of our assumptions.

Regional differences of IMCL, EMCL, and TCr. Figure 4 displays the IMCL-to-water values for the three different muscle groups for all 13 subjects. In all subjects, the highest IMCL level was found in S and the lowest in TA ($P < 0.05$ for each of the subjects), including the two trained subjects. The mean intrasubject
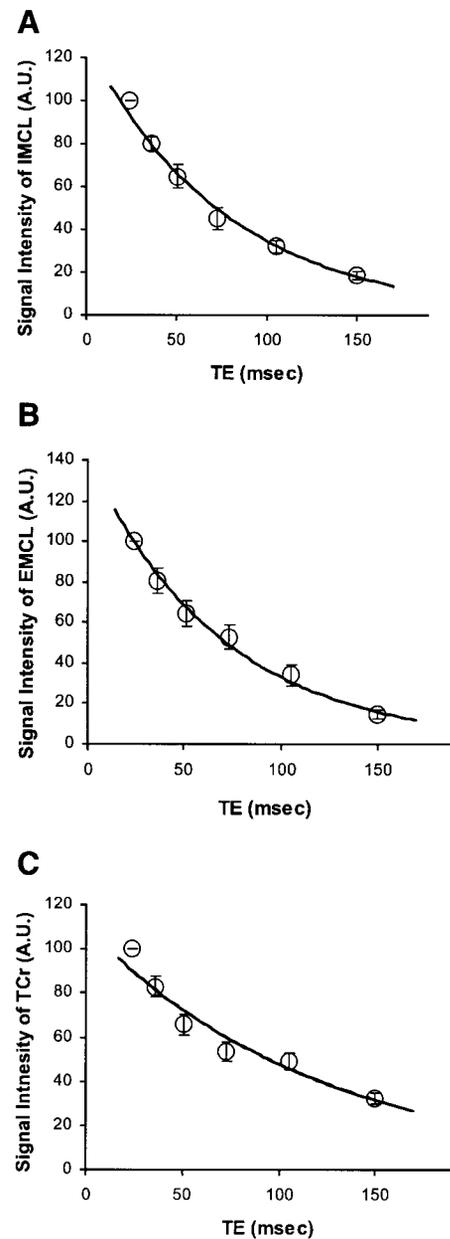

Fig. 2. Transverse relaxation time data for IMCL (*A*), extramyocellular lipid (EMCL; *B*), and TCr (*C*) from the soleus muscle for all 4 volunteers. All data are normalized to the first time point value and are given as means ± SD. *A*: IMCL (1.3 ppm) signal intensities and a single exponential fit; $R^2 = 0.99$ for exponential fit. *B*: EMCL (1.5 ppm) signal intensities and a single exponential fit; $R^2 = 0.99$. *C*: TCr (3.0 ppm) signal intensities and a single exponential fit; $R^2 = 0.95$. TE, echo time; AU, arbitrary units.

coefficients of variation (CVs) for three SI voxels in each muscle were 18.6, 20.1, and 13.0% for S, TP, and TA, respectively.

Figure 5 shows the IMCL content from each muscle group for all subjects ($n = 13$ for S and TA; $n = 11$ for TP), with calculated IMCL concentrations of 4.8 ± 1.6, 2.8 ± 1.3, and 1.6 ± 0.9 mmol TG/kg wet wt for S, TP, and TA, respectively. Using one-way ANOVA SNK testing, S > TP > TA was found in pooled IMCL content ($P < 0.001$ by ANOVA; $P < 0.001$ for S > TP

Table 1. *Relaxation times of IMCL, EMCL, TCr, and water in muscles at 4 T*

	Muscles	T2, ms	T1, s
Muscle water		26 (5)	1.8 (5)
IMCL (1.3 ppm)	S	75 ± 7	
	TP	80 ± 5	0.39 (5)
	TA	100 ± 16	
TCr (3.0 ppm)	S	120 ± 12	
	TP	149 ± 18	1.05 (22), 1.1 (27)
	TA	138 ± 9	
EMCL (1.5 ppm)	S	67 ± 11	
	TP	73 ± 9	0.39 (5)
	TA	71 ± 13	

T2 values are means ± SD. T2, transverse relaxation time; T1, longitudinal relaxation time; ppm, parts/million; IMCL, intramyocellular lipid; TCr, total creatine; EMCL, extramyocellular lipid; S, soleus; TP, tibialis posterior; TA, tibialis anterior. Nos. in parentheses are the reference nos. of the studies from which the relaxation times were extracted.

and S > TA; $P = 0.03$ for TP > TA by SNK). To determine the regional differences without using water as a reference, the relative ratios of peak areas of IMCL in TP/TA and S/TA were measured directly from each spectrum. For the pooled data from 13 subjects, TP/TA and S/TA were significantly different from equality and were 1.8 ± 0.7 and 3.7 ± 1.3, respectively. The S/TA was significantly higher than TP/TA ($n = 13$ and 11, respectively; $P = 0.01$) by Student's t-test. Therefore, by either method of determination (water reference or by ratio), the regional differences of IMCL in three muscles were consistently found in the order of S > TP > TA. Table 2 summarizes the calculated concentrations of IMCL, EMCL, and TCr.

The calculated concentrations of EMCL were similar to the pattern of regional differences seen for IMCL: 26.4 ± 9.9, 21.0 ± 5.2, and 18.7 ± 6.4 mmol TG/kg wet wt in S, TP, and TA, respectively (Fig. 6; $P < 0.05$ by ANOVA). However, although EMCL in S was signifi-
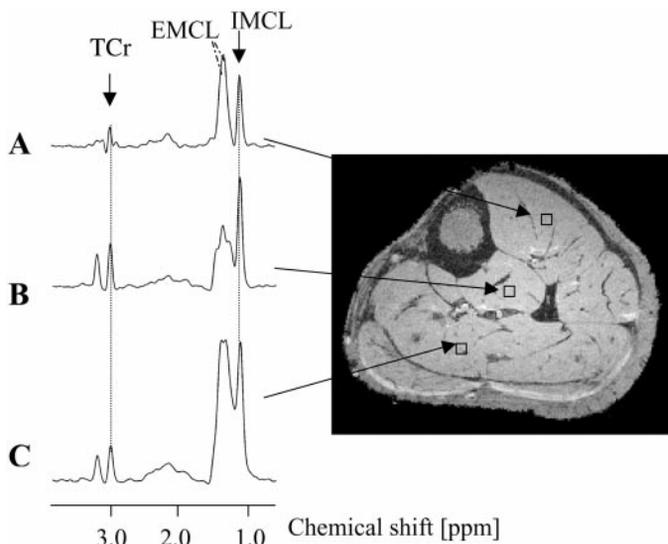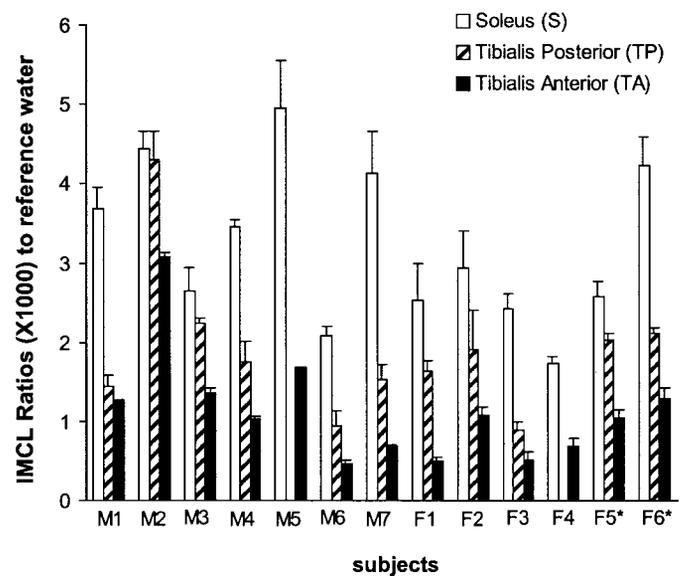

Fig. 4. Mean ± SD of IMCL-to-water ratio values for each muscle and subject ($n = 3$ voxels for each muscle from each subject). M1–M7, male subjects; F1–F6, female subjects. *F5 and F6 are athletically trained subjects.

cantly higher than that in TA ($P = 0.04$), there were no significant differences between S vs. TP and TP vs. TA (SNK).

Figure 7 shows the pooled data of TCr content from three different muscles in 13 subjects. The quantitative values were 31.0 ± 7.1, 31.9 ± 7.7, and 29.2 ± 8.6 mmol/kg wet wt in S, TP, and TA, respectively, and were not statistically significantly different among the three muscles.

Effect of BMI on muscular lipids. Given the accepted metabolic differences in the EMCL and IMCL fat depots (the former representing adipocyte stores and the latter, short-term intramuscular stores), we analyzed their relationship to BMI, an accepted measure of total body adiposity. No significant correlation was

Fig. 3. Representative single-voxel spectra from the tibialis anterior (TA; *A*), tibialis posterior (TP; *B*) and soleus (S; *C*) and anatomic image of the calf.
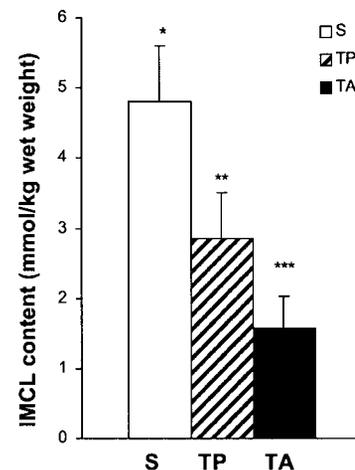

Fig. 5. Pooled IMCL data from all 13 subjects for each muscle group. IMCL content was S > TP > TA. Significant difference by Student-Newman-Keuls test for all comparisons, $P < 0.05$ (*, **, ***). Values are means ± SD; $n = 13$ for S and TA and $n = 11$ for TP.

Table 2. *Results from quantification and comparisons with previous results by MRS and biopsy*

	Muscle	Absolute Values From This Study, mmol/kg wet wt	Reported Values From in vivo NMR, mmol/kg wet wt*	Reported Values From Extracts, mmol/kg wet wt*
IMCL	S	4.8 ± 1.6	10.7 (30), 10.3 (25)	
	TP	2.8 ± 1.3		Muscle lipids by biopsy (10, 11,32) 6–17
	TA	1.6 ± 0.9	5.6 (25)	
EMCL	S	26.4 ± 9.9	29.2 (30), 26.7 (25)	
	TP	21.0 ± 5.2		
	TA	18.7 ± 6.4	21.3 (25)	
TCr	S	31.0 ± 7.1	23 (25), 36.2 (3)	
	TP	31.9 ± 7.7		TCr by biopsy (1, 7) 22–36
	TA	29.2 ± 9.6	28.5 (25)	

Absolute values are means $\pm$ SD. MRS, magnetic resonance spectroscopy; NMR, nuclear magnetic resonance. Nos. in parentheses are reference nos. *If the values were originally reported in mmol/kg dry wt, the values were recalculated based on an assumption of water content of 80% of total weight.

found with either EMCL or IMCL alone vs. BMI except for EMCL in S muscle ($r = 0.34$, $P < 0.03$). The correlation between BMI and total nuclear magnetic resonance visible lipid (EMCL + IMCL) and the EMCL/IMCL were also examined. Although total muscle lipid again showed no significant relationship except in S ($r = 0.31$, $P = 0.05$), the EMCL/IMCL was highly correlated with BMI ($r = 0.40$, 0.60, and 0.55 in S, TP, and TA, respectively; $P \le 0.01$). This relationship remained significant for all muscles studied individually (Table 3).

DISCUSSION

Regional differences of IMCL and EMCL. These regional data demonstrate that IMCL in S and TP are three- and twofold that in TA, respectively. This is in excellent agreement with previous single-voxel ^{1}H-MRS showing two- or threefold higher IMCL content in S relative to that in TA (16, 23, 25) (Table 2). Our data are also consistent with the known fiber-type distribu-
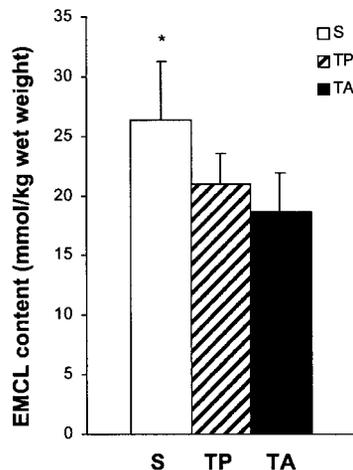

Fig. 6. Pooled EMCL data from all 13 subjects for each muscle group. EMCL content was S > TA (*$P < 0.04$ by Student-Newman-Keuls test). Values are means $\pm$ SD; $n = 13$ for S and TA and $n = 11$ for TP.
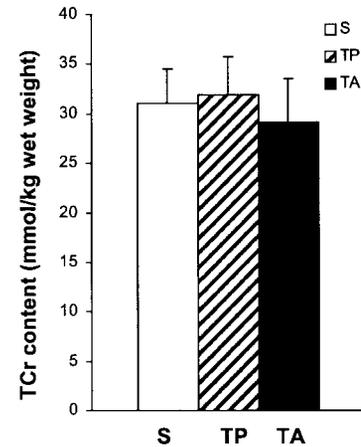

Fig. 7. Pooled TCr data from all 13 subjects for each muscle group. TCr content was not significantly different in the 3 muscles. Values are means $\pm$ SD; $n = 13$ for S and TA and $n = 11$ for TP.

tions of these muscles, with S being primarily (>70%) a type I fiber muscle, whereas TA is relatively higher in type II fibers (6, 24). Fiber type is known to be related to TG content, with histochemical studies showing that type I fibers contain a threefold higher lipid content than type II fibers (8). Notably, biopsy evaluation of the TP muscle has always been difficult because of the size of the muscle and its internal position between the tibia and fibula. Our data suggest that the TP is intermediate in IMCL content to that of S and TA and thus may be interpreted as being intermediate in muscle fiber distribution relative to S and TA.

The mean intrasubject CVs of IMCL from the three muscles (S, 18%; TP, 20%; TA, 13%) can result from two sources: intrinsic variation in the fiber composition within a muscle and methodological variability. Using cross-sectional analyses of TA muscles, Henriksson-Larsen et al. (13) reported significant fiber type II heterogeneity, ranging from 20 to 40%. Our mean IMCL intrasubject CVs (13–20%) from three different voxels in a muscle necessarily include contributions from this fiber heterogeneity. However, the intersubject CVs of IMCL (the IMCL variability in all 13 subjects) were 31% (S), 49% (TP), and 59% (TA), which are much larger than the mean intrasubject CV. This is not unexpected and is most likely related to multiple factors, including exercise, dietary status, and insulin sensitivity. Nonetheless, despite the fiber heterogeneity in a given muscle (13) and intersubject variability,

Table 3. *Mean values of EMCL-to-IMCL ratios and Pearson correlation coefficients (r) with BMI*

	Mean $\pm$ SD	r	P
EMCL/IMCL			
S	5.8 ± 2.4	0.40	0.01
TP	9.3 ± 5.9	0.60	<0.01
TA	14.8 ± 10.6	0.55	<0.01
BMI, kg/m^2	22.8 ± 2.8(19–29)		

BMI, body mass index. $n = 39$ S and TA muscles from 13 subjects; $n = 33$, TP muscles. P values are of Pearson moment correlation test.

the IMCL differences in the order of S > TP > TA were statistically significant ($P < 0.05$).

EMCL was not significantly higher in S vs. TP (24%; $P = 0.10$) or between TP vs. TA (12%; $P = 0.44$) but was significantly greater in S vs. TA (55%; $P = 0.04$). Therefore, although EMCL in S is highest among the three muscles studied, the regional differences in EMCL are not as pronounced as for IMCL content.

Comparisons of IMCL, EMCL, and EMCL/IMCL with previous data. Table 2 summarizes a comparison of the present data with biopsy values and single-voxel measurements acquired at 1.5 T. Compared with biopsy work, our data agree relatively well, with the biopsy values (6–17 mmol/kg wet wt) (10, 11, 32) intermediate to the IMCL and EMCL measurements. Compared with previous 1.5-T data, our S and TA data (there is no equivalent comparison for the TP muscle) also agree reasonably well, particularly with the content of EMCL. However, we found the EMCL/IMCL to be much larger and the IMCL content to be much smaller. At 1.5 T, EMCL/IMCL values have been reported to be ~3:1 in S and 4:1 in TA (25, 30). This is in contrast to the significantly higher ratios measured in this study: 5:1 and 14:1 for S and TA, respectively. The differences may result from *1*) the decreased spectral resolution at 1.5 T, resulting in systematic errors of overestimations of IMCL (most likely related to the assumption of equal line widths of the EMCL and IMCL resonances), or *2*) underestimations of the IMCL T1 relaxation value at 4 T. In our analysis, we assumed that the T1 of IMCL at 4 T is 0.39 s, which has been reported for adipose tissue at 4 T (5). Differences in relaxation times used may also contribute to the differences in water-referenced quantification, for example, the reported values of T2 water have ranged from 30 to 50 ms at 1.5 T.

The relationship between muscle lipids and BMI has been evaluated by several groups (9, 28, 29). Forouhi et al. (9) used long-echo, single-voxel ^{1}H spectroscopy and found that the IMCL content in S correlated with BMI in European men, whereas such a relationship was not seen in South Asian men. This may be compared with the work from Stein et al. (28, 29), who used short echo time, single-voxel ^{1}H spectroscopy and reported a positive correlation of IMCL and total muscular lipids (EMCL + IMCL) to BMI in a racially heterogeneous group with a large range of BMIs. Our results showing the significant correlation of EMCL/IMCL to BMI (seen in all three muscles, $P \leq 0.01$) over a relatively restricted range of BMI in a racially heterogeneous group suggests that both lipid pools are contributory. One perspective in this may be to consider the EMCL/IMCL as a normalized measure of muscle lipid. Thus, as the content of IMCL is viewed as reflecting functional muscle, EMCL may be related to IMCL, whose value depends on the individual's BMI. However, one caveat is also present in this consideration, in that our selection of voxels is likely biased toward regions of low EMCL. Thus it is possible that the "bulk" EMCL and EMCL/IMCL are higher than reported here. However, as noted above, our measures of EMCL/IMCL are

higher than those previously reported. As the objective of this work was accurate measures of IMCL (similar to biopsy data where visible fat is removed from the analysis), we believe that the values obtained reflect the muscular milieu without excessive contribution from interfascial fat depots.

Interestingly, we found no differences in EMCL when the data were segregated by gender in age- and BMI-matched subgroups (4 men and 4 women; men: age = 31.6 ± 5.6 yr old, BMI = 23.7 ± 1.8 kg/m^2; women: 29.8 ± 6.2 yr old, 22.5 ± 5.1 kg/m^2).

The previous finding of higher IMCL in TA in women (23) was not detected in the four women and four men matched for age and BMI nor was it detected in S. In contrast, IMCL contents in women are slightly lower in S and TA than those in men [5.2 ± 1.7 and 1.9 ± 1.4 (men) and 4.0 ± 1.6 and 1.1 ± 0.5 mmol/kg wet wt (women) for S and TA, respectively; $P = 0.05$], whereas EMCL contents are comparable [27.6 ± 11.0 and 17.6 ± 7.1 (men) and 23.3 ± 8.7 and 17.4 ± 6.1 mmol/kg wet wt (women) for S and TA, respectively; $P =$ not significant] in our limited number of subjects. Because IMCL has been linked to insulin sensitivity, and this was not measured in this study, we cannot rule out that there is a gender difference in IMCL when subjects are normalized for insulin action. The differences reported here from previous data on EMCL, IMCL, and EMCL/IMCL may be due to physiological, genetic, and methodological factors. These may include such factors as racial group and athletic activity, as well as technical factors such as the method of spectral analysis and the degree of spectral resolution.

Regional differences of TCr. In contrast to the large variation in IMCL content of the three muscles, the regional differences in TCr were not significant. Previously, Rico-Sanz et al. (25) reported that TCr was 20% higher in TA than in S by treating the TCr resonance at 3.0 ppm as a singlet peak. However, as described by Hanstock et al. (12) and Kreis et al. (18), the TCr resonance in TA shows dipolar coupling. Although we corrected for this by fitting the coupled side peaks for the TA muscle, the complex pattern induced by dipolar/J couplings of carnitine/taurine may have interfered with accurate estimations of TCr at 3.0 ppm. Nonetheless, the TCr content obtained is approximately in the range of 29–32 mmol/kg wet wt, which is in very good agreement with previously reported values of 22–37 mmol/kg wet wt from biochemical essays and in vivo ^{1}H-MRS, as shown in Table 2 (1, 3, 7, 25).

In conclusion, by using high-resolution SI, much clearer detection of the IMCL and EMCL resonances is possible. The improved spatial resolution (nominal voxel size, 0.25 ml) suggests that the line width of IMCL is not the same as that of EMCL and allows the simultaneous measurements of IMCL and EMCL in S, TP, and TA. Across 13 healthy, nonobese volunteers, we have found the IMCL content to be 4.8 ± 1.6 mmol/kg in S, 2.8 ± 1.3 mmol/kg in TP, and 1.6 ± 0.9 mmol/kg in TA. The IMCL contents of the three muscle groups are significantly different in the descending order of S > TP > TA, with $P < 0.05$ for all compari-

sons. We found the concentration of TCr to be 29–32 mmol/kg, with little regional variation, in agreement with previously published values. Finally, despite a small range of BMIs evaluated, we have found there to be a significant correlation between the ratio of EMCL/IMCL to BMI in all three muscles. This may reflect a relationship between EMCL and IMCL, wherein the larger BMI reflects an excess of EMCL in reference to muscular IMCL. It is likely that the observed IMCL values are consistent with the known fiber types of these muscles, with S having the greatest fraction of type I (slow-twitch, oxidative) fibers and TA having a large fraction of type IIb (fast-twitch, glycolytic) fibers. This suggests that IMCL content is related to the oxidative functions of the muscles. With this clearer measure of IMCL, the relationship between IMCL and insulin sensitivity can now be more specifically defined.

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