Skeletal muscle lipid concentration quantified by magnetic resonance imaging$^{1-3}$

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ABSTRACT
Background: Skeletal muscle lipid is associated with obesity and type 2 diabetes and may be altered by diet, physical activity, and weight loss.

Objective: We explored the utility of magnetic resonance imaging (MRI) for quantifying the lipid concentration of muscle tissue in vivo.

Design: Fat-selective MR images of the lower leg were taken in 8 normal-weight [body mass index (in kg/m$^2$) $\leq$ 24.9] and 8 obese (body mass index $> 29.9$) subjects to obtain spatial maps of lipid signal intensity within muscle tissue. Fast-spiral-sequence (echo time $= 5.6–13.8$ ms, repetition time $= 1$ s, 8 interleaves) MRI scans were conducted at 3.0 T by using an extremity transmit-receive coil. Lipid concentrations within muscle were determined from manually drawn regions of interest in the tibialis anterior (TA), soleus, and medial head of the gastrocnemius (MHG) muscle groups.

Results: There was extremely good agreement (mean $R^2 = 0.985$) between the fat signal intensity and the actual lipid concentration of standards containing 2.5, 5.0, and 10.0 g lipid/dL, which were placed on the subject’s leg during each scan. The lipid content of both the soleus (2.99 $\pm$ 0.37 g/dL) and the MHG (3.80 $\pm$ 0.68 g/dL) was higher ($P < 0.05$) than that of the TA (1.83 $\pm$ 0.28 g/dL). Lipid content was more than two-fold higher ($P < 0.05$) in the MHG of obese subjects (5.48 $\pm$ 1.18 g/dL) than in the MHG of normal-weight subjects (2.54 $\pm$ 0.47 g/dL), but did not differ significantly in the TA or soleus.

Conclusions: MRI can be used to quantify lipid within human muscle tissue. MRI can also be used to detect differences in muscle lipid content among various muscle groups and between normal-weight and obese subjects. Am J Clin Nutr 2004;79:748–54.

KEY WORDS Skeletal muscle, triacylglycerol, obesity, body fat, magnetic resonance imaging

INTRODUCTION
In vivo imaging techniques have been used to identify unusual patterns of fatty infiltration within skeletal muscle in certain disease states (1–4). Even the composition of skeletal muscle of otherwise healthy older persons has been reported to be altered with respect to adipose tissue infiltration (5). Many of these early studies, however, only subjectively described fat infiltration within muscle tissue. There is renewed interest in the potential importance of ectopic fat (ie, fat contained within nonadipose tissue or organs) because of its associations with metabolic dysregulation, including type 2 diabetes (6–10), and impaired muscle function (5, 11). This recent attention has stimulated the development of more quantitative approaches to examining the importance of muscle lipid accumulation in health and disease.

Volume-localized magnetic resonance spectroscopy methods have been developed to distinguish intramyocellular from extramyocellular lipid in vivo (12–14). Separation of these 2 lipid components is possible from the separation of the 2 lipid resonances in the lipid-CH$_2$ region. This method has been used extensively by many groups to report associations between intramyocellular lipid and insulin resistance (8, 15, 16) and the effects of exercise on intramyocellular lipid content (17, 18). Absolute quantification of lipid content within muscle with the use of magnetic resonance spectroscopy, however, remains limited. Neither the spatial distribution nor the absolute concentrations of lipid within muscle can be assessed. In addition, the intramyocellular lipid signal is highly dependent on the orientation of the muscle fibers, so that only the lipid of selected voxels (volume elements) within certain muscle groups, specifically, the tibialis anterior (TA) and soleus of the lower leg, can be distinguished reliably (12). Great care must be taken to position the leg so that the muscle fibers are oriented properly (12), and regions of interest containing voxels must be carefully selected to avoid contamination from fatty septa in muscle (ie, intermuscular or extramyocellular lipid).

Magnetic resonance imaging (MRI) methods have been developed to quantify the content of lipid within muscle (19, 20). Standard T1-weighted MR images provide visualization of fatty septa in muscle (intermuscular or extramyocellular lipid) and in subcutaneous adipose tissue. However, small concentrations of lipid within muscle tissue cannot be assessed with T1-weighted images. Fat-selective MRI, with a high sensitivity to lipid signals, as described by Schick et al (19, 20) can produce high-quality spatial maps of lipid contained within muscle. MRI has several potential advantages over other modalities for the noninvasive quantification of lipid contained within muscle.
First, the method is not dependent on the orientation of the muscle fibers, as is the case for magnetic resonance spectroscopy (12). Second, quantification is not limited to certain muscle groups of the lower leg. Furthermore, absolute quantification of skeletal muscle lipid content can be achieved through the use of lipid standards placed during the MRI scan. In the current study, we used a novel MRI approach to examine the hypothesis that MRI can provide quantitative data on the differences in skeletal muscle lipid content among various muscle groups and also between normal-weight and obese subjects in vivo.

SUBJECTS AND METHODS

Fat-selective magnetic resonance imaging

MRI studies were performed on a 3.0-T scanner (Signa; General Electric, Milwaukee) by using a custom-built, quadrature radiofrequency extremity coil. The coil was built by re-tuning a bridge Isotopes, Cambridge, MA) were secured to the midsection of the lower leg parallel to the leg and to the scanner’s main magnetic field. The lower leg and phantoms were centered within the coil precisely at the location of its greatest circumference. No anatomical landmark was used to precisely locate the axial slices, although this would be valuable to standardize the location of the images in future studies using a test-retest paradigm. Triple imaging (T1-weighted MRI, spiral fat-selective MRI, and B1-weighted MRI) was then performed as described above. The entire procedure, including positioning and scanning, was completed within 45 min.

Study protocol

MRI scans of the lower leg were performed in 11 women and 5 men who were classified as being either normal-weight [body mass index (BMI; in kg/m²) ≤ 24.9] or obese (BMI > 29.9). BMI in the normal-weight subjects (2 men and 6 women) ranged from 18.2 to 24.8 (mean ± SD: 21.9 ± 2.1), and BMI in the obese subjects (3 men and 5 women) ranged from 30.0 to 37.1 (mean ± SD: 32.9 ± 3.1). The normal-weight and obese groups were of similar age (44.1 ± 6.9 and 41.4 ± 7.3 y, respectively), and the obese men and women were otherwise healthy. None of the volunteers had type 2 diabetes or engaged in regular exercise, both of which may influence muscle lipid content (21). Subjects provided written informed consent before any study procedures, and the protocol was approved by the University of Pittsburgh Human Subjects Institutional Review Board.

Before each MRI scan, a urine pregnancy test was administered to all women at the General Clinical Research Center to exclude those women who may have been pregnant. The subjects were placed in a supine position within the MRI scanner, with their right leg placed in a transmit-receive extremity coil for image acquisition. Calibration phantoms consisting of 2.5, 5.0, and 10.0 g/dL of purified bovine fat in D₂-chloroform (Cambridge Isotopes, Cambridge, MA) were secured to the midsection of the lower leg parallel to the leg and to the scanner’s main magnetic field. The lower leg and phantoms were centered within the coil precisely at the location of its greatest circumference. No anatomical landmark was used to precisely locate the axial slices, although this would be valuable to standardize the location of the images in future studies using a test-retest paradigm. Triple imaging (T1-weighted MRI, spiral fat-selective MRI, and B1-weighted MRI) was then performed as described above. The entire procedure, including positioning and scanning, was completed within 45 min.

Postimage processing and analysis

MR data were transferred to a remote workstation (O₂; Silicon Graphics, Mountain View, CA) for postprocessing. To eliminate remnant water signals, images acquired with consecutives TEs were used to produce water-corrected images by using a three-point Dixon correction scheme (22, 23). The resulting images (one less than the complete data set) were then extrapolated to TE = 0 to account for T₂* signal loss through the use of a nonlinear, biexponential fit to the signal intensities as a function of the TE. Linear curve fitting was applied to each of the lipid signal intensity data sets derived from the phantoms in the fat-selective images collected at the shortest TE. The concentration of lipid (g/DL) within skeletal muscle corresponded to the mean lipid signal intensity of the manually drawn regions of interest within

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C(x,y,z) = \frac{1}{h} \exp \left( \frac{-I_0(x,y,z)}{h} \right)
\]
specific muscle groups determined from the linear calibration curve for each subject obtained from the phantoms.

Statistical analysis

Simple linear correlation coefficients were obtained from the linear signal intensity–concentration associations within each set of lipid calibration phantoms. Repeated-measures analysis of variance (ANOVA) was used to determine whether the lipid signal within the respective muscle groups differed across the various TEs. Differences in lipid concentration among various muscle groups were determined with one-way ANOVA. Differences in muscle lipid between normal-weight and obese subjects were also determined by using ANOVA. Simple linear bivariate regression analyses were performed to examine whether the lipid contents within the respective muscle groups were correlated and to examine associations between muscle lipid content and BMI. Stepwise multiple regression was used to determine whether age or sex was related to muscle lipid content independently of obesity. The level of significance was set at $P \leq 0.05$ by using a Bonferroni correction for multiple comparisons. All statistics were performed by using JMP version 3.1.6 for the Macintosh (SAS Institute Inc, Cary, NC).

RESULTS

High-resolution T1-weighted images of the lower leg were obtained so that regions of interest could be precisely drawn within the individual muscle groups of each volunteer (Figure 1). In addition to defining the TA, soleus, and medial head of the gastrocnemius (MHG) muscle groups, T1 images were used to visualize and avoid subcutaneous adipose tissue, fatty septa between muscle, and intermuscular adipose tissue in the analysis of skeletal muscle lipid concentrations within the fat-selective images. Fat-selective images of the lower leg (Figure 2) were taken in combination with the lipid phantoms in every subject to produce spatial lipid maps and to quantify the lipid signal within the various muscle groups.
Effects of echo time on lipid-derived proton signals

Because of the nature of the imaging sequence being used, ie, Gradient echo, and the morphologic structure of the muscle tissue, in which tissue fibers are organized along preferential directions, it was important to investigate the potential effect of $T_2^*$ decay on the lipid MRI signal, both in lipid phantoms and in skeletal muscle in vivo. The increase in lipid signal intensity from longer to shorter TEs for a typical subject is shown in Figure 3. From a TE of 13.8 to a TE of 5.0 ms, there was a 20 ± 5%, 40 ± 18%, and 23 ± 7% increase ($P < 0.05$) in lipid signal intensity in the TA, soleus, and MHG, respectively, indicating an effect of a stronger lipid signal at a shorter TE in all muscle groups. To eliminate the $T_2^*$ effects in muscle, we applied curve-fitting algorithms (24) to each data set to determine the lipid signal intensity at TE = 0. This resulted in a negligible change in lipid signal intensity within muscle compared with the lipid signal at the shortest TE (Figure 3).

Analysis of lipid concentrations in chemical phantoms

The lipid signal in fat-selective images was obtained within a set of 3 chemical phantoms in each scan. These phantoms consisted of known concentrations of purified bovine fat dissolved in $D_2$-chloroform so that the proton signal could be attributed solely to lipid within solution. As shown in Figure 4, there was extremely good agreement between the known lipid concentration in solution and the lipid signal intensity on MRI. The correlation coefficients among all 16 subjects ranged from an $R^2$ of 0.951 to an $R^2$ of 1.000 (mean $R^2 = 0.985$). Thus, this method can produce precise lipid quantification. The intercept of the curve was very close to zero (averaging 74 ± 19 intensity units among all scans), which was far below that observed for the 2.5-g/dl lipid phantom, indicating that there was little residual noise in these fat-selective images.

Variability in the lipid content of different muscle groups

The data for lipid concentrations within different muscle groups of all subjects combined are shown in Figure 5. Taking care to avoid any adipose tissue within the muscle visible on the T1-weighted image, the lipid concentration within both the MHG and the soleus was higher ($P < 0.05$) than that in the TA. In addition, the lipid content of the MHG was higher than that of the soleus. For all subjects combined, the variance (SD) in the lipid content of the MHG was higher than the variance in the lipid content of the soleus or TA. The lipid content of the different muscle groups was generally correlated: the lipid content of the MHG was correlated with the lipid content of both the TA (r = 0.66, $P < 0.01$) and the soleus (r = 0.68, $P < 0.01$), and the lipid content of the TA tended to be associated with the lipid content of the soleus (r = 0.46, $P = 0.09$).
Skeletal muscle lipid content was quantified in both normal-weight and obese subjects to examine whether this index of ectopic fat was related to generalized obesity. In addition to the main effect of muscle group, there was a main effect of obesity on muscle lipid content \((P = 0.06)\). There was also a significant interaction \((P < 0.05)\) between the muscle group examined and obesity with respect to muscle lipid content. The lipid concentration within the MHG was approximately two-fold higher \((P < 0.05)\) in obese subjects than in normal-weight subjects \((0.82\) compared with \(2.61\) \(g/dL; P = 0.06)\). Moreover, the lipid concentration within the TA \((0.66\) compared with \(2.61\) \(g/dL; P = 0.05)\) was significantly higher in obese than in normal-weight subjects \((0.13)\) and of the MHG \((4.76\) compared with \(2.61\) \(g/dL; P = 0.05)\) but not with the lipid content of the TA \((r = 0.11)\) or the soleus \((r = 0.12)\). The normal-weight-and-obese groups were matched for sex and age, and the study was not designed to examine the potential confounding influences of age and sex on muscle lipid content. However, we did examine whether differences in muscle lipid content could be detected in normal-weight and obese women. Consistent with the results observed for men and women combined, the lipid content of the MHG was higher in obese than in normal-weight women \((7.10 \pm 0.82\) compared with \(2.61 \pm 0.66\) \(g/dL; P < 0.05)\). The muscle lipid content of the TA \((2.89 \pm 0.55\) and \(1.67 \pm 0.45\) \(g/dL; P = 0.13)\) and of the MHG \((4.76 \pm 0.66\) and \(3.24 \pm 0.46\) \(g/dL; P = 0.10)\) also tended to be higher in obese women than in normal-weight women.

**DISCUSSION**

Lipid accumulation within skeletal muscle has been linked to insulin resistance \((6, 8, 9, 15, 16)\), obesity \((25–27)\) and type 2 diabetes \((27)\). Under certain circumstances, however, lipid contained within muscle may function as an energy substrate \((18, 28)\), and may actually be higher in individuals performing regular exercise \((21)\). These studies highlight the need to develop and refine quantitative methods to better understand the potential role that skeletal muscle lipid plays in both health and disease. This study demonstrates that MRI can be employed to map the 3-dimensional distribution of tissue lipid content over a selected volume of the lower leg in a rapid and quantitative fashion.

The use of fat-selective imaging techniques in combination with chemical calibration standards represents a significant advance over previous methods for characterizing the lipid content of muscle. The tightly coupled associations between the absolute concentration of lipid within the standards and their lipid signal intensity on the fat-selective MR image were consistent for all subjects. Conventional MRI has been used to measure muscle mass \((29–31)\), but fewer attempts have been made to characterize the fat content of muscle. Tsukahara \((32)\) used an \(^1\)H chemical shift technique (Dixon method) to measure the fat and water content of skeletal muscle and found that the lipid content of skeletal muscle increased with age. Kent-Braun et al \((33)\) used MRI to measure the signal intensity in T1-weighted proton images attributable to contractile tissue (ie, muscle) and noncontractile tissue. However, these observations were made without calibration standards (for fat and water signal intensities) and thus could include confounding influences from B1 and Bo inhomogeneity, system gain, slice thickness, and methods of auto-tuning. Fat signal intensities in T1-weighted images are not particularly robust and accordingly require relatively long imaging with conventional MRI. The fast-spiral-sequence, fat-selective imaging in the current study relieves this constraint.

MRI of lower leg muscles showed significant differences in lipid concentration among the various muscle groups quantified. The MHG had more than two-fold the amount of lipid as did the TA, and nearly 30\% more lipid than the soleus, which also contained more lipid than the TA. These results are consistent with those of Schick et al \((19)\), who recently used fat-selective, gradient-echo MRI to quantify lipid content in lower leg muscles. In their MRI studies, however, chemical phantoms were not used to obtain actual lipid concentrations within muscle, and therefore their results were not truly quantitative. Nor did they carefully exclude the intermuscular fat within the specific muscle groups analyzed. The shorter TE used in the current acquisition scheme also provided a stronger lipid signal than did the longer TE used in previous studies. A further advantage of the current quantitative MRI method is the use of a longer TR to achieve T1 saturation. Yet, the studies of others \((19, 20)\) and ours show that MRI can be used to quantify skeletal muscle lipid content in vivo.

Although lipid concentrations were determined in regions of muscle devoid of any macroscopic fat deposits (intermuscular fat), our method, like that of Schick et al \((19, 20)\), is not intended to quantify the lipid signal specifically attributed to intramyocellular lipid as purported in MR spectroscopy studies. Nevertheless, our results agree with those of MR spectroscopy studies showing that the lipid content within soleus muscle is higher than the lipid content within the TA \((15)\). Moreover, MRI, unlike MR spectroscopy, can be used to quantify the actual lipid concentration within muscle, and because, with MRI, muscle lipids are not influenced by their orientation in the magnetic field, MRI of lipid is not constrained to specific muscle groups.

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**FIGURE 6.** Variation in lipid content in the tibialis anterior, soleus, and medial head of the gastrocnemius in normal-weight \([\square]\); body mass index \((\text{BMI})\) \(\leq 24.9\); \(n = 16\) and obese \([\blacklozenge]\); BMI \(> 29.9\); \(n = 16\) subjects. \(*\) Significantly higher lipid content than in normal-weight subjects, \(P < 0.05\). \(†\) Significantly higher lipid content than in the tibialis anterior within the normal-weight and obese groups, \(P < 0.05\). A two-way repeated-measures ANOVA showed main effects of obesity and muscle group with respect to muscle lipid content and also an interaction effect, which led to specific BMI-group analyses by one-way ANOVA with Bonferroni correction for multiple comparisons.
Quantitative differences in the lipid content of various muscles has been attributed to fiber type differences, because direct measures of intramyocellular lipid content in humans showed a higher lipid content of type I, or oxidative, muscle fibers (34, 35). However, considerable variation in lipid content remains within each muscle fiber type (34, 35) and also in each fiber type within specific muscle groups (36). Therefore, muscle group differences in lipid content cannot be attributed solely to fiber type differences.

In addition to variations in the lipid content within distinct muscle groups of the lower leg, fat-selective MRI was able to detect interindividual differences in muscle lipid content. This has particular relevance to the potential use of MRI in quantifying a specific component of regional fat deposition shown to be associated with insulin resistance and type 2 diabetes. Using this fat-selective MRI method, we were able to detect higher amounts of lipid contained within the MHG of obese subjects than in the MHG of normal-weight subjects. The lipid content of the other two muscle groups studied, however, did not differ significantly between the obese and normal-weight subjects, although this does not exclude the possibility that lipid content in these muscles may differ in groups that are more disparate with respect to obesity or total body fat. Nevertheless, these results are generally consistent with prior MRI studies (20) that also found obesity-related differences in MHG but not TA muscle. Furthermore, these MR data agree with the intramyocellular lipid content data derived from muscle biopsy specimens of vastus lateralis (27) showing that obese sedentary subjects with and without type 2 diabetes have significantly more intramyocellular lipid than do normal-weight subjects.

These MRI data further illustrate that interindividual differences in muscle lipid content may be dependent on the muscle group examined. Therefore, MRI may alleviate the constraints of having to restrict the measure of muscle lipid content to one muscle group or one sample, which is often a practical limitation of muscle biopsy. Moreover, noninvasive MRI may be better suited to examining dynamic time-course changes in muscle lipid content, which is also a practical limitation of muscle biopsies. It should be mentioned, however, that the acquisition sequences and postacquisition software used in the present study are not readily available and that a high degree of technical expertise was required to develop these sequences and analyses. In addition, it is not clear whether these quantitative studies can be performed at the magnetic field strength of 1.5 T typical of most clinical MR scanners.

In summary, fat-selective MRI can provide relatively fast and noninvasive measures of skeletal muscle lipid content in multiple muscle groups simultaneously. This method could be used in large-scale, population-based studies or in clinical intervention trials in which it is not practical or feasible to perform muscle biopsies. MRI may help us to better understand the role of muscle fat accumulation in the physiology and pathophysiology of obesity, aging, and metabolic disease.

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All authors were involved in developing the study protocol and the experimental design. VAS and FB developed the pulse sequences and analysis programs for the MRI studies and provided their expertise throughout the project. BHG and TM performed image postprocessing and data analysis. DD provided technical expertise in MR image acquisition and analysis. BHG wrote the draft manuscript with contributions from VAS, FB, TM, DD, RR, and DEK. All authors read, commented on, and contributed to the submitted and revised manuscripts. None of the authors had any financial interests in organizations sponsoring this research.

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