Intramyocellular lipid content is lower with a low-fat diet than with high-fat diets, but that may not be relevant for health.¹–³

Marie-Pierre St-Onge, Bradley R Newcomer, Steven Buchthal, Inmaculada Aban, David B Allison, Aubrey Bosarge, and Barbara Gower

ABSTRACT

Background: Fat deposition in muscle has been found to be related to metabolic risk.

Objective: This study compared soleus intramyocellular lipid (IMCL) concentrations after consumption of weight-maintaining, controlled diets differing in total fat and fat type.

Design: This study consisted of 3 phases of 25 d each in a crossover, controlled feeding design. The low-fat (LF) diet provided 30.8% and 5.2% of energy from fat and polyunsaturated fat (PUFA), respectively. Two higher-fat diets were tested: the high-fat (HF) diet provided 37.9% and 5.8% of energy from fat and PUFA, respectively, and the high-PUFA (HPUFA) diet provided 36.3% and 9.7% of energy from fat and PUFA, respectively. Twenty-four men and women [age range: 19–65 y; body mass index (in kg/m²): 20–35] whose LDL and glucose concentrations were between 130 and 180 mg/dL, respectively, completed all study phases.

Results: IMCL content was 1.88 times as high after the HF diet (P = 0.005) and 1.71 times as high after the HPUFA diet (P = 0.002) as after the LF diet. There was no significant correlation between percentage fat mass or waist circumference and IMCL content. With pooled data from all diets, there was no significant correlation between IMCL content and insulin or glucose concentration. There was no significant difference in IMCL content in subjects with or without the metabolic syndrome or in subjects with LDL particle pattern A or B.

Conclusions: Our results suggest that IMCL content is not modulated by dietary fat type but by total fat intake and that reducing fat intake effectively lowers IMCL. However, the metabolic implications of having lower IMCL concentrations are not clear.

KEY WORDS Snacks, polyunsaturated fat, trans fat, saturated fat, insulin, intramyocellular lipid, metabolic syndrome

INTRODUCTION

Body composition, particularly the distribution of fat mass, has been shown to be closely related to insulin resistance and cardiovascular disease risk. Visceral obesity is a particularly strong correlate of various metabolic disorders. Ectopic fat deposition, such as fat infiltration of liver and muscle tissue, is now increasingly recognized as being associated with a greater risk of insulin resistance and cardiovascular disease. The intramyocellular lipid (IMCL) concentration has been shown to be inversely correlated with whole-body glucose uptake in normal-weight, nondiabetic subjects (1), independent of body mass index (BMI; in kg/m²), fasting plasma glucose, and age. It has been reported that the IMCL concentration correlates positively with obesity and physical fitness and negatively with insulin sensitivity (2, 3). However, the link between insulin resistance and IMCL content is increasingly questioned, and several groups have not found any significant association in healthy normal-weight men (4) or healthy overweight men and women (5).

Recently, more data have become available regarding the effects of diet on IMCL. Short-term very low carbohydrate intakes and starvation led to higher IMCL concentrations than were seen with a mixed carbohydrate diet (6), whereas a 25% caloric restriction, by diet or diet and exercise, has not been shown to affect IMCL stores (5). When macronutrient distribution effects on IMCL have been examined, high-fat diets were shown to more effectively replete IMCL stores after depletion with an exercise intervention (7) and to lead to higher overall IMCL concentrations than did low-fat diets (8). What remains unknown is whether the type of dietary fat—ie, saturated or unsaturated—has a differential effect on IMCL content.

The primary aim of this study was to determine whether IMCL content of the soleus muscle would differ significantly in men and women after a 25-d consumption of a low-fat National Cholesterol Education Program Step I diet (LF diet); a high-fat, high-saturated and -trans fat diet (HF diet); and a high-fat, high-polyunsaturated fat diet (HPUFA diet). We hypothesized that, because unsaturated fatty acids are more easily oxidized than are saturated fatty acids (9), IMCL concentrations would be lowest after consumption of an LF diet, intermediate after consumption of an HPUFA diet, and highest after consumption of an HF diet. The secondary aim of the study was to determine whether IMCL

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content is related to metabolic risk factors, metabolic syndrome, and the pattern of LDL particle density.

SUBJECTS AND METHODS

Subjects and study design

Forty-five subjects with an age range of 19 to 65 y, a BMI of 20 to 35, and mildly elevated LDL-cholesterol concentrations (130–180 mg/dL) were recruited to participate in this randomized, crossover, controlled feeding study. To participate in this study, subjects were required to be free of metabolic disorders such as hypertension, cardiovascular disease (no cholesterol-lowering medication), diabetes (fasting glucose < 126 mg/dL), and hypertriglyceridemia (triacylglycerols < 350 mg/dL); to be weight stable for ≥3 mo; and not to be a smoker. Participants were required to keep any medication taken stable in terms of dosage and type throughout the duration of the study. All subjects scored within the normal range on the Brief Symptom Inventory (10) and agreed to maintain their prestudy physical activity level throughout the study period.

The study consisted of 3 randomly assigned, controlled-feeding phases of 25 d each, which were separated by a 4- or 8-wk washout period. During washout periods, subjects were instructed to resume their usual eating habits and were urged not to consume study foods.

All subjects provided written informed consent. The study was approved by the University of Alabama at Birmingham Institutional Review Board and was conducted at the General Clinical Research Center (GCRC).

Study diets

The 3 study diets contained the same base foods and were designed at 2 energy levels: 1800 and 2500 kcal. Unit foods were added to reach a subject’s daily energy requirement, as estimated by using the Harris-Benedict equation (10). Unit foods consisted of a trail mix designed to have the same macronutrient distribution as the base diet. Dietary provisions were adjusted if a subject’s body weight varied by >1%. Variations in body weight during each phase were kept within 2% of the body weight on day 2 of each phase.

Each diet contained 300 kcal of snacks; the types of snacks differed among the 3 diets. The control (LF) diet contained low-fat snacks, and the overall macronutrient distribution of this diet followed National Cholesterol Education Program Step 1 recommendations: 54.8%, 14.7%, and 30.8% of energy was from carbohydrates, protein, and fat, respectively. The fat breakdown was 5.2% polyunsaturated, 14.2% monounsaturated, and 8.5% saturated fat. The other 2 diets contained high-fat snacks. The HF diet contained snacks that were high in fat and not restricted in saturated or trans fat, whereas the HPUFA diet contained snacks that were free of trans fat and low in saturated fat. The HPUFA diet provided 48.6%, 15.5%, and 36.3% of energy as carbohydrate, protein, and fat, respectively, and the HF diet provided 46%, 16.3%, and 37.9% of energy as carbohydrate, protein, and fat, respectively. Polyunsaturated, monounsaturated, and saturated fat contents were 9.7%, 15.3%, and 8.5%, respectively, for the HPUFA diet and 5.8%, 15.9%, and 11.4%, respectively, for the HF diet. The HPUFA and HF diets were created by removing the snacks of the LF diet and adding HF snacks. Their resulting macronutrient distribution was reflective of this whole-food substitution; we did not design the HF and HPUFA diets to have specific amounts of each dietary fat type.

Breakfast was consumed under supervision at the GCRC each weekday morning. At this time, subjects were weighed and given their food for the rest of the day. All foods to be consumed on the weekend were provided on Friday morning. Subjects were required to consume all study foods and beverages and to consume nothing else except water and noncaloric beverages. Consumption of coffee and tea was not restricted. At each weekday visit, daily report forms were completed to record any symptoms of illness, foods not eaten, beverages consumed, and medication(s) taken during the previous day. If subjects did not consume a study food on a given day, they were instructed to consume the food the following day. Subjects reported that they did not deviate from the protocol and that they complied with the study requirements throughout the study.

Clinical measurements

Blood samples for the measurement of fasting lipid profile, glucose, and insulin were collected at baseline and on days 15 and 25 of each phase. Hip and waist circumferences, taken at the level of largest circumference over the buttocks and at the umbilicus, respectively, were also taken at baseline and on days 15 and 25 of each phase. Blood pressure was measured twice at baseline and again on day 25, and the average of each day’s 2 readings was recorded. Fat mass was assessed by bioelectrical impedance analysis (BC-418; Tanita Corporation, Arlington Heights, IL) at baseline and on days 15 and 25 of each phase.

Complete lipoprotein profiles were analyzed by using the vertical autoprobe technique (Atherotech Inc, Birmingham, AL; 11), which measures concentrations of triacylglycerol, HDL cholesterol (including the subparticles HDL2 and HDL3), LDL cholesterol, VLDL cholesterol, and lipoprotein(a) [Lp(a)] and identifies the LDL particle pattern (pattern A or B) that is present. Triacylglycerols were estimated from the vertical autoprobe. LDL particle pattern A is characterized by large, buoyant LDL particles, and LDL particle pattern B is characterized by smaller, dense particles. Pattern A is considered to be a less atherogenic LDL particle pattern than is pattern B.

The following measurements were performed in the Metabolism Core Laboratory of the GCRC and at the Clinical Nutrition Research Center at the University of Alabama at Birmingham. Glucose was measured in 12 µL serum by using a SIRRUS analyzer (Stanbio Laboratory, Boerne, TX). In the Metabolism Core Laboratory, this analysis has a mean CV of 2.56%. Insulin was assayed in duplicate 100-µL aliquots by using reagents (Linco Research Products Inc, St Charles, MO). In the Metabolism Core Laboratory, this assay has a sensitivity of 3.35 µU/mL, a mean intraassay CV of 3.49%, and a mean interassay CV of 5.57%. Commercial quality-control sera of low, medium, and high insulin concentration were included in every assay to monitor variations over time. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as the product of fasting insulin and fasting glucose divided by 405 (11).

Subjects were characterized as having the metabolic syndrome if they fulfilled 3 of the 5 National Cholesterol Education Program Adult Treatment Panel III criteria for the metabolic syndrome: triacylglycerol ≥ 150 mg/dL, HDL cholesterol < 40 mg/dL in men or < 50 mg/dL in women, blood pressure ≥
Intramyocellular lipid data

Acquisition

IMCL content measurements were conducted on day 25 of each dietary phase. IMCL content reflects the effect of the different diets on IMCL but will not indicate the extent of the difference in the change in IMCL concentrations between diets. Measurements of IMCL content at the start of each phase would have reflected the subject’s previous diet, which would have been variable from phase to phase. By examining differences among diets in endpoint IMCL content after a period during which diet was strictly controlled, we can assess the effect of the consumption of such diets for 25 d on IMCL content.

IMCL content was quantified by using $^1$H magnetic resonance spectroscopy. Two different magnetic resonance scanners were used for this project. Twenty of our 25 subjects were studied with the use of a Philips 3T system (Philips Medical Systems, Bothell, WA). IMCL content was measured in these 20 subjects by using a point-resolved spectroscopy (PRESS), single-voxel acquisition sequence with a commercially provided $^1$H transmit-or-receive phased-array torso coil (Philips Medical Systems). The subjects’ legs were positioned inside this commercial torso coil with the knee in extension and the ankle in a secured neutral position. The water-suppressed PRESS voxels ($1 \times 1 \times 1$–cm voxel size) were positioned in the soleus muscle in areas that avoid fascia, vascular structures, and gross marbling. All PRESS acquisitions used the following variables to collect the IMCL data: repetition time (TR) = 2000 msec, echo time (TE) = 40 ms, and 128 signal averages. As an amplitude reference, separate water-nonsuppressed spectra were also collected with the same TR and TE but with only 16 signal averages. All IMCL and internal water spectra were corrected for T1 and T2 relaxation times and were normalized for PRESS voxel sizes and the number of signal averages collected. As stated previously, the locations of the PRESS voxels were kept constant in each subject at all follow-up visits.

Finally, to ensure that the 4.1T and 3T data were comparable, 3 additional acquisitions were performed on both systems to assess for any systematic differences (eg, receiver gains) between the 2 magnetic resonance system platforms. From these 3 acquisitions, we were able to determine a normalization factor that allowed us to convert the 4.1T-acquired data into comparable 3T data in terms of absolute signal amplitude measurements. This normalization allowed us to combine all of the IMCL measurements into a single data set for subsequent statistical analyses.

Analysis

All spectra were analyzed by fitting the peak positions and areas through the time domain by using the Java-Based Magnetic Resonance User Interface (14). The same fitting procedures were used regardless of the system used for acquiring the spectra. IMCL content in the soleus spectra was fitted by using previously published models and sets of prior knowledge (7, 15–17). In brief, the metabolite signals were analyzed with AMARES, a nonlinear least-squares fitting algorithm operating in the time domain (16). As described previously, our time domain–fitting model was composed of 4 exponentially decaying sinusoids corresponding to the 4 Lorentzian peaks in the frequency domain assigned to the resonances of interest [extramyocellular lipid (EMCL) ($-CH_3$), IMCL ($-CH_3$), EMCL ($-CH_2$), and IMCL ($-CH_2$) peaks corresponding to resonances at 1.6, 1.4, 1.2, and 1.0 ppm, respectively]. Lorentzian decaying sinusoids were also fitted to represent the additional lipid resonances around the 2.5– ppm range, and 2 Gaussian decaying sinusoids were used to represent the total creatine and trimethyl amine resonances. As previously determined, this combination of Lorentzian and Gaussian peak fits proved to be the most reproducible and provided the least amount of residual signal after fitting (7, 17). To account for day-to-day variation in system performance, our protocol normalized the IMCL peak amplitudes to the corresponding internal water peak amplitude in the same muscle location. This is similar to the method described by Krssak et al (1). All peak areas in this study are expressed in arbitrary units per pixel area relative to internal water.

Statistical analyses

To investigate the effect of diet on IMCL concentrations after 25 d of each diet, we implemented a generalized linear model for repeated measures by using PROC MIXED in SAS for WINDOWS software (version 9.1; SAS Institute, Cary, NC). Because of evidence of nonnormally distributed model residuals, the natural log of the IMCL was modeled as the response variable. No particular covariance structure was assumed for the repeated readings. Instead, the covariance values were estimated from the data. If the $P$ value of the overall diet effect was <0.05, pairwise comparisons were performed by using the Bonferroni adjustment. Similar analyses were done to compare participants’ IMCL concentrations on day 25 according to the presence of the metabolic syndrome (yes or no) and the LDL particle pattern (A, A/B, or B).

To investigate the correlation of IMCL to glucose, insulin, HOMA-IR, waist circumference, and fat mass (assessed by using...
TABLE 1
Intramyocellular lipid (IMCL) concentrations in men and women after the consumption of low-fat, high-polyunsaturated fat, and high-fat diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Estimated mean IMCL concentrationa</th>
<th>Log IMCL ± SEMb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-fat (n = 23)c</td>
<td>3.18</td>
<td>1.1555 ± 0.1800</td>
</tr>
<tr>
<td>High-polyunsaturated fat</td>
<td>5.42</td>
<td>1.6912 ± 0.1192</td>
</tr>
<tr>
<td>High-fat</td>
<td>5.96</td>
<td>1.7847 ± 0.1590</td>
</tr>
</tbody>
</table>

a n = 24 subjects who completed IMCL measurements. Values for IMCL are arbitrary units based on water content.
b Estimated means are based on modeling the log(IMCL) with diet as an independent variable via repeated measures with unstructured covariance. Thus, these values are different from the unadjusted sample means per diet.
c Values with different superscript letters are significantly different, P < 0.0001 (P < 0.017 with Bonferroni adjustment).
d One subject was removed from the analyses because of an IMCL value of 0.

BC-418 bioelectrical impedance analysis), we assigned ranks to the values of each variable and applied appropriate analyses to the ranks of these variables. This measure of applying the analyses to the ranks instead of to the original values of the variables was taken to address the issue of nonnormality of the variables. To test the significance of these correlations, a generalized linear model for repeated measures was fitted with ranks of IMCL as the predictor variable and ranks of the other variables (eg, glucose and insulin) as responses—one variable at a time. As in the diet effect, the covariance of the repeated measures was computed on the basis of the data and with the assumption of no particular structure. All data were analyzed by using the SAS for WINDOWS software (version 9.1). A P value of <0.05 was taken as significant, and data are reported as means ± SD when appropriate.

RESULTS
Forty-five subjects were initially enrolled in the study, and 33 (7 M, 26 F) completed all 3 phases of the study. The reasons the subjects did not complete the study included lack of compliance with the diet (n = 2), dislike of the foods (n = 5), loss to follow-up (n = 2), pregnancy (n = 1), work conflict (n = 1), and death in the family (n = 1). We have complete IMCL data on 24 subjects (x ± SD age: 43.6 ± 2.5 y; BMI: 29.4 ± 0.9). Two subjects did not use the scanner because of the presence of metallic fragments in their bodies, and data from 7 subjects could not be used because of issues of compatibility with changes in the magnet during the study. A different acquisition method was used with the 4.1T magnet for those subjects on whom the 3T magnet could not be used. As a result, we have incomplete data from those 7 subjects. One additional subject had nondetectable IMCL in the selected voxel during the LF diet. That data point was removed from the analyses, but that subject’s data from the HF and HPUFA diets are included in the analyses.

IMCL concentrations were lower after the period of LF diet consumption than after the periods of HF diet and HPUFA diet consumption (P < 0.0001 for both; P < 0.017, Bonferroni adjustment; Table 1). In fact, IMCL content was, on average, 1.88 times as high (P = 0.005) after consumption of the HF diet and 1.71 times as high (P = 0.02) after consumption of the HPUFA diet than after consumption of the LF diet.

We also examined the correlation between IMCL concentrations and the absolute values of metabolic and body composition variables on day 25 by using pooled data from all 3 phases. We found no significant correlation between IMCL and any of the variables—ie, fat mass, waist circumference, insulin, HOMA-IR, lipid profile, and systolic and diastolic blood pressures. Similar results were found when we ran the correlations for each diet separately.

We next investigated whether IMCL was correlated with the percentage change from baseline in the metabolic variables. The only significant correlation, when all of the data from the 3 phases were pooled, was with the percentage change in insulin (r = 0.29, P = 0.0139). There was no significant correlation between absolute fasting insulin concentrations and IMCL concentrations (r = 0.157, P = 0.1915).

Data from all 33 subjects who completed the study, regardless of whether they completed the IMCL measurement, showed that 6 subjects were classified as having the metabolic syndrome at the start of the LF diet, 7 subjects were so classified at the start of both the HF and HPUFA diets. At the end of the 25-d feeding period, the number of subjects with the metabolic syndrome was 9 for the LF diet, 7 for the HF diet, and 3 for the HPUFA diet. At the start of the LF diet, 5 subjects had LDL particle pattern B, compared with 9 and 8 for the HF and HPUFA diets, respectively. Ten subjects had LDL particle pattern B at the end of the 25-d feeding period for the LF diet, compared with 7 and 6 subjects at the end of the HF and HPUFA diets, respectively.

IMCL concentrations in subjects with and without the metabolic syndrome are shown for each diet in Table 2. There was no significant difference in IMCL concentrations between subjects with the metabolic syndrome and those without the metabolic syndrome. This lack of difference may be attributed to the small number of participants with the metabolic syndrome. Similarly, there was no significant difference in IMCL between subjects with different LDL particle patterns (Table 3).

DISCUSSION
The results of this study enhance our knowledge of the links among diet, IMCL concentrations, and metabolic disorders. This study is the first to date to examine the effects of different types of dietary fat on IMCL concentrations. In addition, this study is the only one that, to our knowledge, has tested the effects on IMCL content of diets that were not at the extremes of fat content. As may have been expected, the HF and HPUFA diets resulted in significantly greater IMCL concentrations than did the LF diet. It is, however, surprising that a 7-percentage point difference in the percentage of energy derived from fat made such a difference in IMCL content.
Our results suggest that the type of dietary fat does not play a major role in the degree of IMCL deposition. It was hypothesized that the HPUFA diet would lead to lower IMCL than the HF diet because of the degree of unsaturation of the fatty acids that are more easily oxidized than are saturated fatty acids (9). Moreover, contrary to several theories of the link between IMCL and insulin concentrations, we did not find an association between IMCL concentrations and fasting insulin, glucose, or HOMA-IR. In fact, subjects classified as having the metabolic syndrome and those with an atherogenic LDL particle pattern (pattern B) had IMCL concentrations that did not differ significantly from those in subjects without the metabolic syndrome and with LDL particle pattern A.

Our results showing greater IMCL concentrations after periods of high fat consumption are in accordance with previous data from our group (7) showing that IMCL repletion after a period of intense physical activity occurs more rapidly with a moderate-fat diet than with an LF diet (10% of energy from fat). In addition, another study found that, after 7 d of consuming an HF diet (60% of energy from fat), IMCL content was 1.52 times as high as it was after 7 d of consuming a normal-fat diet (30% of energy as fat) (8). These results are similar to the finding in the present study that the consumption for 25 d of high-fat diets, containing 37% of energy as fat, resulted in IMCL concentrations 1.53 times (HPUFA diet) and 1.92 times (HF diet) as high as did consumption of the LF diet, which contained 30% of energy as fat. A limitation of all previous studies examining the effect of fat intake on IMCL concentrations is the lack of reporting of the fatty acid profile of the diets. However, in the present study, we found no effect of dietary fat type on IMCL concentrations. Nonetheless, the HPUFA diet resulted in an intermediate IMCL concentration, and a larger sample may be necessary to distinguish any differential effects of the HF and HPUFA diets.

The lack of correlation between IMCL and percentage body fat was previously observed. In fact, several groups found no association between IMCL concentrations and BMI (18, 19) or fat mass (19). However, in a study of lean and obese adolescents, the IMCL concentration was strongly correlated with both percentage body fat and BMI (20), and, in a cohort of healthy, nondiabetic subjects, it was correlated with waist circumference (3). It is not known why no correlation between IMCL content and percentage body fat was found in the present study and another study (19) when earlier studies found greater muscle lipid concentrations in obese than in lean persons (21, 22). Nevertheless, the results of the present study support the absence of a correlation between IMCL concentrations and percentage body fat and waist circumference.

We also found no significant correlation between IMCL concentrations and markers of metabolic risk. On the basis of previous studies (1, 23), we expected IMCL concentrations to be correlated with fasting insulin and glucose concentrations. Several studies also showed that IMCL content is higher in insulin-resistant than in insulin-nonresistant persons (2, 24–26), which would suggest a link between IMCL concentrations and insulin resistance. It is possible that this link may not be found when assessing surrogate measures for insulin resistance, such as fasting insulin and glucose concentrations and the HOMA-IR. It is also not unreasonable to propose that the relation between IMCL concentrations and insulin resistance cannot be detected in healthy persons who have low concentrations of insulin and glucose. In the present study, subjects were recruited to have fasting glucose concentrations <126 mg/dL. However, other studies in healthy overweight (5, 18) and normal-weight (4) persons have found no correlation between IMCL concentrations and insulin sensitivity. Some studies in rats also found that the IMCL content in the tibialis muscle was a better marker of insulin sensitivity than was that in the soleus muscle (27, 28). However,

### Table 2

<table>
<thead>
<tr>
<th>Metabolic syndrome</th>
<th>Low-fat diet</th>
<th>High-polyunsaturated fat diet</th>
<th>High-fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>3.84 ± 3.24 (8)</td>
<td>5.49 ± 4.88 (2)</td>
<td>7.97 ± 5.94 (4)</td>
</tr>
<tr>
<td>No</td>
<td>4.57 ± 3.84 (15)</td>
<td>6.37 ± 3.31 (22)</td>
<td>7.95 ± 6.99 (20)</td>
</tr>
</tbody>
</table>

1 Values for IMCL are arbitrary units based on water content.
2 Designation of the metabolic syndrome was based on subject characteristics on day 25 of the dietary phase. There was no significant effect of diet on metabolic syndrome status.
3 \( \bar{x} \pm SD; n \) in parentheses (all such values). Subject numbers reflect the number of subjects who completed IMCL measurements.

### Table 3

<table>
<thead>
<tr>
<th>LDL particle pattern</th>
<th>Low-fat diet</th>
<th>High-polyunsaturated fat diet</th>
<th>High-fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pattern A</td>
<td>3.10 ± 1.58 (11)</td>
<td>6.85 ± 3.57 (10)</td>
<td>6.22 ± 3.81 (11)</td>
</tr>
<tr>
<td>Pattern A/B</td>
<td>3.19 ± 2.60 (4)</td>
<td>5.60 ± 1.75 (9)</td>
<td>9.91 ± 9.48 (9)</td>
</tr>
<tr>
<td>Pattern B</td>
<td>6.54 ± 5.02 (8)</td>
<td>6.46 ± 5.21 (5)</td>
<td>8.31 ± 5.85 (4)</td>
</tr>
</tbody>
</table>

1 Values for IMCL are arbitrary units based on water content.
2 Designation of the LDL particle pattern was based on subject characteristics on day 25 of the dietary phase. There was a trend toward a diet effect on LDL particle pattern \( (P = 0.08) \).
3 \( \bar{x} \pm SD; n \) in parentheses (all such values). Subject numbers reflect the number of subjects who completed IMCL measurements.
whereas Jacob et al (26) found that the IMCL content of different muscles varied, they also found that both types of muscle IMCL, the type from tibialis anterior and the type from soleus, were associated with insulin sensitivity. In contrast, Anderwald et al (29) found differences between the correlations of soleus and tibialis anterior IMCL content with whole-body insulin sensitivity by using an insulin clamp method. Kautzky-Willer et al (30) also found correlations between indexes of insulin sensitivity and IMCL from the tibialis anterior but not with IMCL from the soleus. Although the data are inconclusive, it may be that muscle type played a role in the lack of association between IMCL concentrations and fasting markers of metabolic risk in the present study. Future studies should include the investigation of multiple types of muscle for evaluation of IMCL content and its relation to metabolic risk factors.

In addition to the lack of association between IMCL concentrations and metabolic risk factors, we found no difference in the IMCL content of muscle of persons with and without the metabolic syndrome and of persons with LDL particle pattern A rather than pattern B. To our knowledge, only one previous report (31) discussed an association between IMCL concentrations and metabolic syndrome. In that study, IMCL content increased proportionally with the number of metabolic syndrome criteria met by subjects. Furthermore, the author found that IMCL content was higher in persons meeting the cutoff for ≥2 factors of the metabolic syndrome than in those meeting the criterion for ≤1 risk factor for the metabolic syndrome. It is possible that the lack of variability in our subjects (all had fasting glucose <126 mg/dL, normal blood pressure, and triacylglycerol concentrations <350 mg/dL) led to the absence of a difference in IMCL between those with and those without the metabolic syndrome.

Although IMCL has not previously been associated with lipoproteins, we examined its relation with LDL particle pattern. Small, dense LDL particles are characteristic of insulin resistance and type 2 diabetes dyslipidemia and have been reported to induce insulin-secreting β-cell dysfunction (32). This would implicate LDL particle pattern B in the causal pathway for insulin resistance and type 2 diabetes. In line with the absence of a correlation between IMCL concentrations and fasting insulin in the present study, we found no difference in IMCL concentrations between subjects with LDL particle pattern A (large, buoyant particles) or with pattern B (small, dense particles). As with the metabolic syndrome, however, values were numerically higher in all diets in subjects with the more atherogenic LDL particle pattern B than in diets in subjects with LDL particle pattern A.

This study had several limitations. First, because of the shutting down of the 4.1T magnet, we had to use another scanner and a different scanning protocol for some of the subjects. This change led to a reduction in the sample size for IMCL data. However, we were able to conduct multiple scans on the 4.1T and the 3T scanners to obtain a conversion factor for the data, and, therefore, we were able to retain subjects who had undergone the same protocol in the 2 different scanners. Nevertheless, our final sample size may not have been adequate to find differences in IMCL concentrations between the HF and HPUFA diets and to observe differences in IMCL concentrations according to metabolic risk.

Furthermore, although our subjects were instructed to maintain the same level of physical activity during each phase of the study, we did not objectively assess physical fitness in this study, because that was not the primary aim of the study. However, this potential link between IMCL content and insulin resistance has been proposed to be modified by aerobic fitness such that, in highly trained athletes, high IMCL content predicts high insulin sensitivity, whereas, in athletically untrained persons, high IMCL content predicts low insulin sensitivity (2, 3).

Finally, the results of the present study clearly show that consuming an LF diet leads to lower IMCL concentrations. However, the type of fat consumed probably has little or no influence on lipid storage within the muscle. In addition, in the present study, we were unable to link the IMCL concentration to metabolic abnormalities. As a result, we cannot comment on the metabolic implications of lower IMCL concentrations. Nevertheless, the present study does not support the notion that a reduction in the IMCL concentration through the consumption of an LF diet affect metabolic risk.

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