Intramuscular and Liver Triglycerides Are Increased in the Elderly

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Magnetic resonance spectroscopy studies have shown that intramyocellular lipids (IMCL) and liver fat (LFAT) levels vary with insulin sensitivity and obesity, which are common in the elderly. Thus, magnetic resonance spectroscopy was used to investigate the hypothesis that IMCL and LFAT are increased in the elderly. IMCL and LFAT in young (aged 20–32 yr) and elderly (aged 65–74 yr) were measured fasted, and glucose, insulin, total free fatty acids levels, and free fatty acids profiles were measured during a 2-h oral glucose tolerance test. Body fat percentage was determined with dual x-ray absorptiometry. The elderly had significantly greater IMCL (0.12 ± 0.01 vs. 0.08 ± 0.01, mean ± SEM; P = 0.01) and LFAT (0.28 ± 0.06 vs. 0.08 ± 0.01; P = 0.004; expressed as ratios to Intralipid standard) than the young. The elderly had increased insulin resistance as calculated by the Matsuda model compared with the young (5.1 ± 0.9 vs. 9.9 ± 1.4; P = 0.02). Regression analysis of all subjects indicated that the increases in IMCL and LFAT were correlated with insulin sensitivity, glycosylated hemoglobin, plasma lipids, and body fat. Furthermore, the correlation between insulin sensitivity and IMCL and LFAT remained significant, after accounting for the effect of body fat. Increases of IMCL and LFAT occur in elderly individuals and may be related to insulin resistance. (J Clin Endocrinol Metab 89: 3864–3871, 2004)

Recent advances in magnetic resonance spectroscopy (MRS) (1) have enabled measurements of the amount of intramyocellular lipids (IMCL) (1). Studies of IMCL have given rise to new perspectives on the relationship between lipid metabolism and insulin resistance, because IMCL has been found to be associated with insulin resistance and/or obesity (2–5). In healthy men with no family history of diabetes, high levels of IMCL were found to correlate with lower levels of insulin-stimulated glucose uptake during a euglycemic-hyperinsulinemic clamp (6). Women with a history of gestational diabetes had increased levels of IMCL that were associated with body fat mass (7). On the other hand, IMCL was not elevated in obese individuals with normal glucose tolerance (8). Thus, IMCL may be an indicator of dysfunctional muscle lipid metabolism and related to insulin resistance.

The liver plays a primary role in plasma glucose and lipid regulation. As in the case of the muscle, studies conducted in patients with nonalcoholic steatohepatitis and polymetabolic syndrome X have found a correlation between the degree of liver fat (LFAT) and insulin resistance (9, 10). In type 2 diabetes, the amount of insulin taken daily and the ability of iv insulin to suppress endogenous glucose production were significantly correlated with the amount of LFAT (11). Patients with chronic hepatitis C infections develop fatty liver and often type 2 diabetes (12–14).

Insulin resistance increases after the age of 50 yr. Approximately 7 million Americans over the age of 65 yr had type 2 diabetes in 1999, representing 20.1% of this age group (15). Additionally, the National Institutes of Health currently estimates that approximately 16 million people between the ages of 45 and 70 yr are glucose intolerant (15). Thus, nearly 50% of those over the age of 65 yr are estimated to have some degree of insulin resistance in regard to peripheral glucose metabolism. Furthermore, resistance to the action of insulin on muscle protein may be even more widespread in the elderly (16). Additionally, higher rates of obesity in people over the age of 60 yr have been documented since 1960 (17). The latest numbers from the Centers for Disease Control indicate that 25.3% of people aged 60–69 yr are severely obese, with a body mass index (BMI) of greater than 30, whereas only 14% of people aged 18–29 yr are this overweight (18).

In light of the development of insulin resistance and increased adiposity with aging, we have compared IMCL, LFAT, and free fatty acid (FFA) profiles in healthy elderly subjects with corresponding values in their younger counterparts.

Subjects and Methods

Twenty-five volunteers, nine young (five female, four male; ages 20–32 yr) and 16 elderly (11 female, five male; ages 65–74 yr) subjects, were enrolled in the study. Both groups were equally multiracial, with six Caucasians, two Hispanics, and one African-American in the young group and 12 Caucasians, three Hispanics, and one African-American in
the elderly group. One young and two elderly had an immediate family member who had been diagnosed with diabetes, and all others had no family history of diabetes. All subjects read and signed an informed consent. The project was approved by the Institutional Review Board at the University of Texas Medical Branch (Galveston, TX). All volunteers were healthy by history and physical examination, and none were participating in regular aerobic or resistance training routines. Subjects' total cholesterol was less than 250 mg/dl (6.5 mmol/liter), and TSH levels were within the normal range (0.49–4.70 µIU/ml). Further exclusions included palpable liver enlargement; positive hepatitis B, C, or HIV test; and a history of smoking (the latter, or elevation in level of more than one of the following: alkaline phosphatase more than 122 U/liter, alanine aminotransferase; HDL, high-density lipoprotein; M/F, males/females.

Sample analysis

MRS soleus. IMCL was measured with a 1H knee coil on a GE Advantage 1.5Tesla whole-body imager (General Electric, Milwaukee, WI). The calf of the dominant leg was secured within the center of the coil with the subject lying in a supine position and the ankle secured in a neutral position. A tube of 20% Intralipid (iv high-fat total parenteral feeding solution; Baxter Healthcare, Deerfield Park, IL) was placed inside the knee coil to obtain a standard external reference to normalize IMCL concentrations (2). The orientation of the leg in reference to the magnetic field (B0) and the coil position was selected from pilot studies to provide optimal splitting of the IMCL and extracellular fat resonances in the soleus muscle. After a preliminary localization image, three to seven voxels (~7 mm × 7 mm × 10 mm each) were chosen in soleus muscle free from fascia, gross fat marbling, and vessels. The exact voxel volumes were recorded. A voxel was also chosen from the Intralipid external reference. An optimized PRESS (Point RESolved Spectroscopy) sequence with repetition time of 2000 msec and echo time of 35 msec was run. Peak positions and areas of interest [extramuscular (CH3)2 and intramuscular (CH3)2, extramuscular CH3, intramuscular CH3, total creatine, and trimethylamine] were determined by time domain fitting using JMRUI (19, 20). In brief, all water-suppressed free induction decay (FID) metabolite FID) were deconvoluted with the water-unsuppressed FID (water FID) acquired from the same voxel to correct for zero-order phasing and removal of eddy current-induced artifacts (21). The resulting metabolite FIDs were analyzed with AMARES (Method of Accurate, Robust and Efficient Spectral fitting), a nonlinear least-square-fitting algorithm operating in the time domain (22). The time-domain model function was composed of four exponentially decaying sinoids corresponding to the four Lorentzian peaks in the frequency domain assigned to the resonances of interest [i.e. extracellular fat and IMCL-(CH3)2, and -CH3 peaks]. In addition, Lorentzian decaying sinoids were fit to represent the additional lipid resonances, and two Gaussian decaying sinoids were used to represent the total creatine and trimethylamine resonances. The previous knowledge information used for the AMARES fits have been previously published by Rico-Sanz et al. (23).

Spectra from voxels, which did not have optimal thinning or clear intracellular and extracellular lipid peak resolution, were not used in AMARES fitting analysis. This process was repeated for the Intralipid phantom. The triglyceride (TG) levels were computed as a ratio relative to the Intralipid standard using the following formula:

$$TG = \frac{[PM/VM]}{[PI/VI]}$$

TABLE 1. Volunteer baseline measurements

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Elderly</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (M/F)</td>
<td>9 (4/5)</td>
<td>16 (5/11)</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>27 ± 1</td>
<td>69 ± 1</td>
<td></td>
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<tr>
<td>Glucose metabolism</td>
<td></td>
<td></td>
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<tr>
<td>Fasting glucose [mg/dl (mmol/liter)]</td>
<td>88 ± 2 (4.9 ± 0.17)</td>
<td>100 ± 4 (5.56 ± 0.22)</td>
<td>0.015</td>
</tr>
<tr>
<td>Fasting insulin [µU/ml (pmol/liter)]</td>
<td>4.4 ± 0.9 (30.6 ± 6.3)</td>
<td>8.5 ± 1.3 (59 ± 9)</td>
<td>0.041</td>
</tr>
<tr>
<td>HbA1c</td>
<td>5.2 ± 0.1</td>
<td>5.7 ± 0.1a</td>
<td>0.001</td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol [mg/dl (mmol/liter)]</td>
<td>170 ± 7 (4.4 ± 0.18)</td>
<td>207 ± 7 (5.36 ± 0.18)</td>
<td>0.004</td>
</tr>
<tr>
<td>HDL [mg/dl (mmol/liter)]</td>
<td>55 ± 5 (1.42 ± 0.13)</td>
<td>58 ± 4 (1.50 ± 0.10)</td>
<td>0.441</td>
</tr>
<tr>
<td>LDL [mg/dl (mmol/liter)]</td>
<td>95 ± 5 (2.46 ± 0.13)</td>
<td>123 ± 7 (3.18 ± 0.10)</td>
<td>0.009</td>
</tr>
<tr>
<td>Ratio</td>
<td>3.2 ± 0.2</td>
<td>3.8 ± 0.3</td>
<td>0.283</td>
</tr>
<tr>
<td>TGs [mg/dl (mmol/liter)]</td>
<td>97 ± 11 (1.1 ± 0.12)</td>
<td>126 ± 12 (1.42 ± 0.14)</td>
<td>0.144</td>
</tr>
<tr>
<td>Liver function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin [µg/dl (µmol/liter)]</td>
<td>3.9 ± 0.1 (591 ± 15)</td>
<td>3.9 ± 0.1 (591 ± 15)</td>
<td>0.458</td>
</tr>
<tr>
<td>ALK (U/liter)</td>
<td>66 ± 6</td>
<td>77 ± 4</td>
<td>0.149</td>
</tr>
<tr>
<td>ALT (U/liter)</td>
<td>26 ± 5</td>
<td>24 ± 2</td>
<td>0.973</td>
</tr>
<tr>
<td>AST (U/liter)</td>
<td>25 ± 3</td>
<td>26 ± 3</td>
<td>0.475</td>
</tr>
<tr>
<td>Body composition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.73 ± 0.03</td>
<td>1.65 ± 0.02</td>
<td>0.082</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.1 ± 4.8</td>
<td>76.4 ± 4.1</td>
<td>0.778</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>24.4 ± 1.3</td>
<td>27.6 ± 1.2</td>
<td>0.132</td>
</tr>
<tr>
<td>% Total body fat</td>
<td>26.7 ± 1.8</td>
<td>35.9 ± 2.2a</td>
<td>0.006</td>
</tr>
<tr>
<td>% Trunk fat</td>
<td>21.7 ± 1.8</td>
<td>34.8 ± 2.4a</td>
<td>0.0001</td>
</tr>
<tr>
<td>% Extremity fat</td>
<td>28.3 ± 2.1</td>
<td>38.3 ± 2.9a</td>
<td>0.011</td>
</tr>
<tr>
<td>Hip/waist ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>0.89 ± 0.03</td>
<td>0.98 ± 0.03</td>
<td>0.07</td>
</tr>
<tr>
<td>Females</td>
<td>0.71 ± 0.01</td>
<td>0.82 ± 0.01a</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Volunteer baseline measurements, taken from blood drawn during the screening examination, are shown, with the exception of glucose and insulin. Glucose and insulin levels shown are from the study day, after a monitored 12-h fast. Body composition measurements taken during the study day are shown. Percentage of body fat, trunk fat, and extremity fat are calculated from a DEXA whole-body scan. Data are mean ± SEM. P values are calculated from a two-tailed Student’s t test. SI units are in parentheses. ALK, Alkaline; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high-density lipoprotein; M/F, males/females.
where PM is the tissue lipid methylene peak area, VM is the total measured tissue voxel volume, PI is the Intralipid peak area, and VI is the Intralipid voxel volume. The result of this calculation is a ratio and is thus unitless.

MRS liver. The LFAT was measured with a 1H whole-body coil on the same system. Hepatic measurements were performed in the middle right lobe (24). A tube of Intralipid was again used for reference. After a preliminary localization scan, a voxel (30 mm × 30 mm × 20 mm) was chosen at a location free from large vessels. An optimized PRESS sequence was run 256 times without respiratory gating. These spectra represent an average LFAT measurement over the mid-right lobe because respiratory gating was not conducted. By placing the subjects prone, using light restraints, and coaching shallow breathing, the movement induced by respiration was reduced. Spectra were manually phased, and final analysis was then performed with MRUI software, as previously described for muscle.

Glucose analysis. Plasma glucose was measured with a YSI 2300 Stat glucose/lactate analyzer (YSI, Inc., Yellow Springs, OH).

Insulin analysis. Plasma was separated and stored at −80°C until analysis. After thawing, insulin levels were measured using RIA (Diagnostic Laboratories, Los Angeles, CA). The coefficient of variation for these measurements in our lab is less than 10%.

FFA analysis. FFA analysis was done by gas chromatography/FID, by Lipomics Technologies, Inc. (Sacramento, CA).

DEXA. All DEXA scans were performed on a Hologic QDR 4500A system (Hologic, Inc., Bedford, MA) by the same technician.

Waist to hip. The waist was measured at the smallest circumference when facing the standing subject, and the hip was measured at the femoral insertion into the pelvis.

Calculations

Matsuda model. The composite model for insulin sensitivity designed by Matsuda and DeFronzo (25) was used to assess whole-body insulin sensitivity following a 75-g oral glucose load.

\[
\frac{10,000}{\sqrt{\text{FPG}} \times \text{FPI} \times \text{meanOGTTG} \times \text{meanOGTTI}}
\]

The value derived from this equation is an M value of glucose uptake in mg/m²·min, which is approximated to results that would likely have been obtained if a more invasive hyperinsulinemic-euglycemic clamp test had been performed (25). The range of values is 0–14, with 14 being the highest level of insulin sensitivity and zero the lowest.

Statistics

All results were reported as mean ± sem. Differences between the young and elderly subjects were evaluated using a two-tailed Student’s \( t \) test. Differences in glucose and insulin over time between young and elderly subjects were evaluated using a two-way ANOVA with factors time and age, followed by Tukey’s test when appropriate. Pearson correlations between physiological factors were examined. The effect of percentage of body fat was factored out of the correlation by dividing the relevant factor by the percentage of body fat.

Results

Baseline parameters

There were several baseline parameters, which were significantly different between the young and the elderly (Table 1). The elderly had a higher average glycosylated hemoglobin (HbA1c), fasting insulin, and fasting glucose, although these were not in the abnormal range, and no individual was in the diabetic range. The elderly had a higher total cholesterol and low-density lipoprotein (LDL) fraction, and finally, the elderly had a higher percentage of body fat as measured by DEXA, although the BMIs were comparable.

MRS results

The amounts of both IMCL and LFAT were higher in the elderly than in the young. The values for IMCL, expressed as fractions of the signal for Intralipid were 0.12 ± 0.01 vs. 0.08 ± 0.01 (mean ± sem; \( P = 0.015 \)), and for liver 0.27 ± 0.04 vs. 0.08 ± 0.01 (\( P = 0.004 \)) for the elderly and the young, respectively (Fig. 1). Two liver spectra, one young and one elderly, had MRS Intralipid acquisition errors and thus have no external reference and are not included in the analysis.

Insulin sensitivity measures

The plasma glucose levels from the OGTT are shown in Fig. 2A. The glucose concentrations were significantly higher in the elderly at time points −15, 0, 60, 90, and 120 min following the ingestion of glucose. The mean glucose concentrations for the elderly fell into the World Health Orga-
nization and American Diabetes Association classification of glucose intolerant (26), which is defined as 180–200 mg/dl (10–11.1 mmol/liter) at 1 h and 140–200 mg/dl (7.7–11.1 mmol/liter) at 2 h after oral glucose challenge, whereas the young were normal. The plasma insulin concentrations at 15 and 0 min before the ingestion of glucose and 120 min after the ingestion of the glucose were significantly different between the young and the elderly (Fig. 2B). Matsuda insulin sensitivity indices (ISI) scores in the elderly were statistically different from in the young (5.1 ± 0.9 vs. 9.5 ± 1.4; \( P = 0.02 \)), indicating that the elderly were insulin resistant compared with the young.

The plasma FFA levels in the elderly were higher at baseline, although not significantly, yet both young and elderly subjects experienced decreases in plasma FFA after the ingestion of the glucose drink (Fig. 3). Plasma diacylglycerol (DAG) levels were significantly different between the young and the elderly at 60, 90, and 120 min after the ingestions of the dextrose (data not shown).

**Correlations**

Table 2 shows the correlation \( R^2 \) values between IMCL or LFAT and multiple measures of insulin sensitivity and adiposity. Correlations were performed on the combined young and elderly data and with the elderly alone. Correlations were not performed on the young alone, as the homogeneity of the data and the small number of subjects limited the validity of the analyses.

When the young and elderly were analyzed together, LFAT levels correlated with nearly all measures of insulin resistance and obesity. However, when insulin and adiposity measures were normalized for the amount of body fat, HbA1c and ISI were the only variables that still correlated with
HbA1c, and ISI and the elevated LFAT correlated with multievident pathology. The elevations in tissue lipids in the muscle, which is a slow twitch oxidative muscle (type 1), relate strongly with fat content determined by biopsy (28). Quantitatively determined by MRS has been shown to correlate best with whole-body insulin sensitivity (2). Our comparison analysis of age, IMCL, and LFAT. A correlation of LFAT in only the elderly showed a strong relationship with fasting insulin, ISI, total cholesterol, plasma TG, and percentage of body fat.

The IMCL in the entire group correlated with HbA1c, ISI total body fat, truncal body fat, plasma DAG, glucose, and insulin at 120 min and HbA1c, and ISI levels corrected for body fat. Plasma HbA1c and ISI correlated with IMCL when the elderly were considered separately, but significant correlations were eliminated by normalizing the data for body fat.

Discussion

We found that the “healthy” elderly have more LFAT and soleus IMCL than younger subjects as determined by MRS measurements of lipid in liver and muscle. Studies comparing MRS with the traditional biopsy techniques in the soleus have shown the results to be nearly identical (27), and LFAT quantitatively determined by MRS has been shown to correlate strongly with fat content determined by biopsy (28). Whereas MRS measurements of IMCL have been made in both the tibialis anterior and the soleus muscles of the lower leg, studies have indicated that the data from the soleus muscle, which is a slow twitch oxidative muscle (type 1), correlate best with whole-body insulin sensitivity (2). Our subjects were rigorously screened to ensure liver health, so that the increase in fat content does not reflect clinically evident pathology. The elevations in tissue lipids in the elderly in our study occurred concurrently with glucose intolerance. Furthermore, elevated IMCL correlated with HbA1c, and ISI and the elevated LFAT correlated with multiple measures of obesity, HbA1c, ISI, fasting, and 120-min insulin. It is likely that the elevated tissue lipids in otherwise healthy elderly indicate a predisposition for the development of clinically significant insulin resistance.

Previous studies present conflicting results on the relationship among age, IMCL, and LFAT. A study recently published after the completion of our study found nearly identical results, with increases in both LFAT and IMCL correlating with age and insulin resistance, as measured by an insulin clamp (29). However, a study of 30 healthy volunteers between the ages of 22 and 50 yr found a slight but nonsignificant correlation between soleus IMCL and age (30). Their results may reflect an inadequate age span in their population. Tarasow et al. (24) used MRS to measure LFAT in 24 healthy nonobese volunteers aged 45 ± 16 yr (range, 23–68 yr) and found that there was no statistically significant correlation between aging and LFAT. However, the large variation in subjects at the older end of the spectrum indicates that Tarasow et al. may not have had enough subjects, as they included such a wide age range. We studied the same number of subjects yet found a difference with aging, probably because of the older average age of our cohort (52 ± 10 yr) and a clustering of subjects at either end of the spectra.

The increased adiposity of the elderly group could be a possible explanation for the increases in IMCL and LFAT. The prevalence of obesity in the elderly as defined as a BMI more than 30 is double the rate in the young, so in trying to recruit a “normal” population for the study it was not reasonable to match the body fat percentage in the two age groups (17, 18). The elderly with a percentage of body fat low enough to have been matched with that of the young group would likely have been involved in exercise programs. Because exercise is a first-line treatment for improving insulin sensitivity, we opted for regular participation in exercise programs to be an exclusion criterion for this study.

The accumulation of tissue lipid results from an imbalance between the rate of uptake of fatty acids and the rate of fatty acid oxidation and/or, in the case of the liver, the rate of secretion of TG in the form of a very LDL. Both muscle (31) and liver (32, 33) fatty acid uptake is a function of delivery (34). Because the rate of release of fatty acids from adipose tissue into plasma is elevated in obesity (35), it is reasonable to presume that the prevalence of obesity would contribute to the accumulation of tissue lipids. Fasting levels of FFA and TG were higher in the elderly, although neither was significantly increased. Additionally, elderly had higher levels of total cholesterol and LDL, both of which supply potential sources of TG substrate for lipoprotein lipase production of FFA.

Despite the logical link between obesity and tissue lipids, the relationship between body fat and IMCL is unclear in younger subjects. In a study comparing percentage of body fat with IMCL in European men and men of South Asian descent, body fat only correlated with IMCL in the men of European descent (36). Another study found that young, overweight subjects with normal insulin sensitivity had normal IMCL content. These individuals had a compensatory increase in B-oxidation, suggesting that IMCL is not necessarily directly related to percentage of body fat if the oxidative capacity of muscle can be up-regulated (8). Perseghin et al. (2) found that in young subjects, there was strong association between the IMCL and insulin sensitivity that was independent of body fat or gender. Patients with a family history of diabetes had an 84% increase in IMCL compared with 13 controls that were matched for age, BMI, percentage of body fat, and exercise training (37). Thus, whereas increased body fat is a potential contributor to the accumulation of tissue lipid in younger subjects, it is not necessarily directly related. Furthermore, in studies in which there is a dissociation between body fat and IMCL, insulin

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**TABLE 2.** R² values from correlations of LFAT and IMCL to obesity and insulin resistance

<table>
<thead>
<tr>
<th></th>
<th>Young and elderly</th>
<th>Elderly</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(n = 20)</td>
<td>(n = 16)</td>
</tr>
<tr>
<td>Fasting insulin</td>
<td>21.9*</td>
<td>24.2*</td>
</tr>
<tr>
<td>Glucose at 120 min</td>
<td>33.5*</td>
<td>23.9*</td>
</tr>
<tr>
<td>HbA1c</td>
<td>30.0*</td>
<td>39.5*</td>
</tr>
<tr>
<td>ISI</td>
<td>42.6*</td>
<td>19.9*</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>33.5*</td>
<td>5.5</td>
</tr>
<tr>
<td>Fasting plasma TG</td>
<td>56.0*</td>
<td>2.1</td>
</tr>
<tr>
<td>Fasting plasma FFA</td>
<td>1.8</td>
<td>12.7</td>
</tr>
<tr>
<td>Plasma DAG at 120 min</td>
<td>64.9*</td>
<td>40.8*</td>
</tr>
<tr>
<td>% Total body fat</td>
<td>38.5*</td>
<td>24.4*</td>
</tr>
<tr>
<td>Trunk % body fat</td>
<td>45.1*</td>
<td>20.7*</td>
</tr>
<tr>
<td>HbA1c without body fat</td>
<td>19.2*</td>
<td>20.7*</td>
</tr>
<tr>
<td>ISI without body fat</td>
<td>44.7*</td>
<td>27.6*</td>
</tr>
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</table>

Values presented are R² values in percentages from linear regression analysis.

*P < 0.05.
resistance was more closely related to IMCL than to whole-body fat (4, 6, 38). Our findings are consistent with the above studies, because both HbA1c and ISI remained correlated with IMCL after accounting for the percentage of total body fat.

In the current study, there was a correlation between tissue lipids, percentage of body fat, and truncal obesity when both young and elderly groups were analyzed together. However, there was no correlation between body fat and IMCL when only the elderly were considered. Furthermore, although trunk body fat and waist to hip ratios were greater in elderly, neither of these was strongly correlated with IMCL. Thus, although the baseline measurement of body fat was significantly different between the young and elderly, it is unlikely that this alone accounted for the differences in IMCL.

We found a greater relationship between adiposity and LFAT than between adiposity and IMCL. This has also been reported in previous studies. For example, a positive correlation of 37% between LFAT and BMI was found in healthy adults aged 20–68 yr (24). Waist to hip ratio measurements have also been correlated with LFAT (39). However, the correlations of body fat percentage only account for 38% of the increase in LFAT seen in the elderly. Furthermore, neither BMI nor waist to hip ratio was correlated with LFAT in the elderly. Finally, in the study by Petersen et al. (29), body fat measures were matched in the young and elderly, and a significant increase in LFAT was still present in the elderly compared with the young. Thus, although it appears that in the elderly obesity may play more of a role in the elevation of LFAT, as opposed to IMCL, obesity alone cannot explain the elevated tissue lipids in either case.

We found that all indices of insulin resistance correlated with IMCL measurements when all the subjects were analyzed together. These findings agree with those from several other studies that have found that individuals with high IMCL levels are also insulin resistant (2–4, 6, 8, 37). We found glucose intolerance and elevations in the average values of indices of insulin resistance in the elderly. The plasma insulin and glucose levels of the elderly were significantly elevated compared with the young at 120 min after the glucose load. A delay in the clearance of glucose after the glucose drink, indicating a delayed clearance of glucose despite higher levels of insulin, and both of these measures correlated significantly with IMCL and LFAT. HbA1c and ISI were correlated with either IMCL or LFAT, even after correcting for differences in body fat.

Several theories have been proposed for the regulation of IMCL. A few studies have found that the combination of hyperinsulinemia and elevated FFAs was necessary to increase IMCL in young adults (1, 40). A likely mechanism to explain this relationship is that elevated insulin levels increase glucose uptake and oxidation in muscle. Glucose metabolism increases malonyl coenzyme A, which inhibits carnitine palmitoyl transferase-1 and thus entry of long-chain fatty acids in the mitochondria for oxidation (41). In this circumstance, accumulated fatty acid uptake due to increased plasma concentrations of fatty acids would be channeled into tissue TG. Because both insulin and plasma FFA were elevated in the fasted state in the elderly, the insulin significantly so, limited oxidation of FFA uptake was likely in part responsible for the accumulation of IMCL in the elderly. This notion is supported by previous reports indicating that fatty acid oxidative capacity is impaired in the elderly (42–44).

It is unclear whether increases in IMCL cause insulin resistance or are simply a reflection of its development. The general relationship between plasma FFAs and insulin sensitivity has been recognized for many years. For example, infusion of heparin or solutions of lipids, which increase the plasma lipids, increase insulin resistance (45, 46). Administration of Acipimox, a nicotinic acid derivative that lowers plasma FFAs, has been shown to improve insulin resistance (47). More recent studies have found that IMCL levels also change with plasma FFA levels and insulin resistance. A 72-h water-only fast caused a decrease in serum insulin concentrations with an increase in plasma FFAs and IMCL levels in the vastus lateralis muscle of healthy men (48). Infusion of heparin and Intralipid caused increases in IMCL as well as a 40% increase in whole-body insulin resistance (49). The mechanism whereby FFA causes insulin resistance is not by inhibiting glucose oxidation (50). It is more likely that active products of fatty acids, such as DAG or ceramides (51), inhibit the insulin signaling pathway. These compounds have been shown to cause chronic activation of protein kinase C in animals (52, 53). Although the relationship between intracellular and plasma levels of DAG is not known, it is interesting to note that plasma DAG concentration increased in the elderly after the glucose drink, whereas the corresponding values in the young were suppressed. Also, the plasma levels of DAG 120 min after the glucose load correlated more strongly with the tissue lipids than any other parameter measured. This increase in DAG could be a reflection of incomplete action of lipoprotein lipase on plasma TGs, or could represent an increased release of DAG from muscle and adipose cells. Thus, it may be that the accumulation of IMCL reflects an imbalance between fatty acid uptake and disposal, and it is the resulting accumulations of active products of fatty acids that actually mediate the insulin resistance. Whether or not IMCL directly inhibits insulin action or is related to impaired insulin action by the common pathway of elevated tissue levels of fatty acids and potentially active products, the increased IMCL in elderly reflects a propensity for peripheral insulin resistance.

In conclusion, our results indicate that the elderly have an increase in LFAT and IMCL when compared with the young. These increased tissue stores reflect an imbalance in the normal relationship between tissue uptake and disposal of fatty acids in the liver and muscle, respectively. These increases in tissue lipid may be related to decreased sensitivity to the action of insulin in both liver and muscle. The similarity of the increase in LFAT and IMCL seen in normal, otherwise healthy elderly individuals to the elderly with diseases such as diabetes, hepatitis C infections, and nonalcoholic steatohepatitis suggests increased tissue lipids as a mechanism for increased susceptibility of the elderly to a variety of metabolic problems.

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