Fatty liver in type 2 diabetes mellitus: relation to regional adiposity, fatty acids, and insulin resistance

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Kelley, David E., Therese M. McKolanis, Refaat A. F. Hegazi, Lewis H. Kuller, and Satish C. Kalhan. Fatty liver in type 2 diabetes mellitus: relation to regional adiposity, fatty acids, and insulin resistance. Am J Physiol Endocrinol Metab 285: E906–E916, 2003; 10.1152/ajpendo.00117.2003.—The current study was undertaken to examine metabolic and body composition correlates of fatty liver in type 2 diabetes mellitus (DM). Eighty-three men and women with type 2 DM [mean body mass index (BMI) 34 ± 0.5 kg/m²] and without clinical or laboratory evidence of liver dysfunction had body composition assessments of fat mass (FM), visceral adipose tissue (VAT), liver and spleen computed tomography (CT) attenuation (ratio of liver to spleen), muscle CT attenuation, and thigh adiposity; these assessments were also performed in 12 lean and 15 obese nondiabetic volunteers. Insulin sensitivity was measured with a euglycemic insulin infusion (40 mU·m⁻²·min⁻¹) combined with systemic indirect calorimetry to assess glucose and lipid oxidation, and with infusions of [²H₂]glucose for assessment of endogenous glucose production. A majority of those with type 2 DM (63%) met CT criteria for fatty liver, compared with 20% of obese and none of the lean nondiabetic volunteers. Fatty liver was most strongly correlated with VAT (r = −0.57, P < 0.0001) and less strongly but significantly associated with BMI (r = −0.42, P < 0.001) and FM (r = −0.37, P < 0.001), but only weakly associated with subcutaneous adiposity (r = −0.29; P < 0.01). Fatty liver was also correlated with subfascial adiposity of skeletal muscle (r = −0.44; P < 0.01). Volunteers with type 2 DM and fatty liver were substantially more insulin resistant those with type 2 DM but without fatty liver (P < 0.001) and had higher levels of plasma free fatty acids (P < 0.01) and more severe dyslipidemia (P < 0.01), a pattern observed in both genders. Plasma levels of cytokines were increased in relation to fatty liver (r = −0.34; P < 0.01). In summary, fatty liver is relatively common in overweight and obese volunteers with type 2 DM and is an aspect of body composition related to severity of insulin resistance, dyslipidemia, and inflammatory markers.-Fatty liver is considered to occur commonly in type 2 diabetes mellitus (DM), with estimates of prevalence ranging from 21 to 78% (2, 5, 25). Obesity, insulin resistance, and increased concentrations of plasma fatty acids are considered to increase the risk for fatty liver (7, 22), and each of these metabolic factors is also characteristic of type 2 DM. In some individuals, fatty liver can lead to steatohepatitis and progress further to end-stage liver disease; however, many clinical symptoms of fatty liver are nonspecific or silent (2). Nevertheless, even for those in whom there is not progression to inflammation and fibrosis in the liver, this does not mean that fatty liver is benign. It has been reported that fatty liver influences severity of hepatic insulin resistance in type 2 DM. Ryysy et al. (37) observed that hepatic fat content in patients with type 2 DM predicted the amount of daily insulin needed to maintain glycemic control; also, among nonobese men without type 2 DM, fatty liver was found to correlate with hepatic insulin resistance independently of obesity and intra-abdominal adiposity (38).

Obesity is an important factor in determining the severity of insulin resistance in type 2 DM (21). One of the potential mechanisms by which obesity may trigger insulin resistance is through the accumulation of excess lipid within liver and skeletal muscle (41, 44). Evidence for this concept has been found with respect to fat content within skeletal muscle (19, 42). There are also clinical data and animal studies suggesting that fat accumulation in heart, liver, and pancreas also contributes to the pathophysiology of insulin resistance and type 2 DM (44). Fatty acid flux to the liver has been postulated as an important factor in the pathogenesis of fatty liver (7) and is also an important determinant of the synthesis and secretion of triglyceride-rich lipoproteins. It is possible, therefore, that hepatic steatosis may influence the severity of dyslipidemia in type 2 DM. In this context, it has recently been reported that intra-abdominal fat is a determinant of the severity of dyslipidemia in type 2 DM (32), and it is recognized that hypertriglyceridemia is more severe in individuals with fatty liver (7, 14, 22).

In the current study, we have assessed the prevalence and severity of hepatic steatosis in overweight and obese patients with type 2 DM among a cohort without clinical manifestations of liver disease, and we...
have compared these characteristics with those of lean and obese individuals without type 2 DM. Computed tomography (CT) imaging was used to assess hepatic steatosis on the basis of previously established criteria that have been validated in relation to liver biopsy (27, 35, 36). Our purpose was to test the hypothesis that physiological phenotypes could be identified by relation to the presence or absence (and in relation to severity) of fatty liver disease of type 2 DM with respect to systemic and hepatic insulin resistance and in relation to plasma fatty acids and dyslipidemia. We also wanted to test the hypothesis that fatty liver disease of diabetes is a component of lipotoxicity in type 2 DM (44), and we therefore examined whether fatty liver is correlated with excess fat in skeletal muscle, with increased inflammatory markers, and with a pattern of body composition that entails a relative preponderance of visceral adiposity.

Methods

Research volunteers. The participants in this investigation were recruited in response to an advertisement for one of three separate clinical investigations, each of which involved participants with type 2 DM and entailed use of identical body composition procedures and a determination of insulin sensitivity by the glucose clamp method at the same rate of insulin infusion. Individuals interested in participating received a medical examination. For research volunteers with type 2 DM, the inclusion criteria included a body mass index (BMI) >27 kg/m²; current diabetes treatment by diet alone, sulfonylurea, or metformin; good general health other than diabetes; and stable weight (not more than 2 mo). Current treatment with insulin or thiazolidinedione was given at 40 mg·m⁻²·min⁻¹. Plasma glucose was measured every 5 min and was allowed to decrease until euglycemia (100 mg/dl) was achieved, which was then maintained with an adjustable infusion of 20% dextrose. Samples for insulin, fatty acids, and enrichment of [6,6-²H₂]glucose were obtained at least hourly during the midportion of the clamp and at 15-min intervals during the final hour. The dextrose infusion was prelabeled with [6,6-²H₂]glucose to maintain fasting rates of glucose and lipid oxidation. Protein oxidation was estimated on the basis of urine urea nitrogen excretion measured in an overnight timed urine collection.

To measure insulin sensitivity, the glucose clamp method was used (9), in which a 4-h continuous infusion of insulin was given at 40 mU·m⁻²·min⁻¹. Plasma glucose was measured every 5 min and was allowed to decrease until euglycemia (100 mg/dl) was achieved, which was then maintained with an adjustable infusion of 20% dextrose. Samples for insulin, fatty acids, and enrichment of [6,6-²H₂]glucose were obtained at least hourly during the midportion of the clamp and at 15-min intervals during the final hour. The dextrose infusion was prelabeled with [6,6-²H₂]glucose to maintain fasting rates of glucose and lipid oxidation. Protein oxidation was estimated on the basis of urine urea nitrogen excretion measured in an overnight timed urine collection.

Body composition assessments. Weight and height were measured using a calibrated scale after volunteers removed shoes and heavy clothing. To measure fat mass (FM) and fat-free mass, dual-energy X-ray absorptiometry (DEXA) was performed, as previously described (20). CT was used to assess the degree of liver steatosis, measure the cross-sectional area of adipose tissue in the abdomen and midhigh, and evaluate thigh muscle attenuation. Prior studies have shown a strong correlation between CT attenuation values in the liver and fatty infiltration measured by biopsy (27, 35, 36). The ratio of liver to spleen (L/S ratio) for CT attenuation values is another index, with a L/S ratio <1 considered to represent fatty liver (27). A 9800 CT scanner (GE Medical Systems, Milwaukee, WI) was used to obtain a scout film of the lower thoracic and lumbar spine for localization of two axial scans. A cross-sectional scan of 10-mm thickness was centered at T₁₁–₁₂ to image liver and spleen, and a second scan was centered at the L₄–₅ vertebral disc space to image abdominal adipose tissue (AT). Each image was obtained at end of inspiration by use of 120 kVp, 170 mA, and a 2-s scan time. As shown in Fig. 1, liver CT attenuations were determined by calculating the mean Hounsfield unit (HU) of three regions of interest (ROI) −120 mm² in the liver (2 right lobe, 1 left lobe), and that of spleen also, on the basis of the mean HU of three ROI of ~75 mm². ROI values in the liver and spleen were selected in peripheral areas away from major portal, arterial, and venous vessels; hepatic fatty infiltration is generally diffuse for fatty liver (27, 35). The total cross-sectional abdominal AT was calculated as the area occupied by tissue in the fat density range (~190 to ~30 HU) on an Advantage GE work station (GE Medical Systems). A cursor was used to manually define the boundary between the visceral and subcutaneous AT by following the abdominal wall musculature in continuity with the deep fascia of the paraspinal muscles. The subcutaneous AT was further divided into superficial subcutaneous AT and deep subcutaneous AT by manually tracing the circumferential superficial fascia, as previously described (20). With the same cutaneous settings as for the abdomen, a 10-mm axial image of both midhighs was obtained. The location of this scan was determined from external measurements as the distance between the anterior superior iliac crest and the inferior margin of the patella divided by one-half. Muscle attenuation was calcu-
Liver and Spleen CT with Regions of Interest (ROI)

L/S Ratio = mean Hounsfield Unit (HU) of Liver ROI = mean HU of Spleen ROI

![Liver and Spleen CT with Regions of Interest (ROI)](image)

Fig. 1. Cross-sectional computed tomography (CT) of the abdomen is shown with 3 regions of interest (ROI) placed on liver and spleen for measurement of attenuation values, or ratio of liver to spleen (L/S ratio) in Hounsfield units (HU).

lated for both legs as the mean HU of the pixels in the muscle density range (0–100 HU). Muscle area was calculated for muscle tissue in the low muscle density range (0–34 HU), high muscle density range (35–100 HU), and total muscle range (0–100 HU). The thigh fascia lata was used to subdivide the AT of one leg into superficial AT and subfascial AT (15).

Analyses. Insulin was measured by RIA (Pharmacia) with a double-antibody separation, as was glucagon (Diagnostic Products). Plasma free fatty acids (FFA) were measured with an enzymatic method (Wako). Glucose was measured by the glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH). Plasma samples for analysis of [6,6-2H2]glucose were first deproteinized by aceton (4:1, vol/vol), evaporated to dryness, and derivatized with pyridine-acetic anhydride to form a pentaacetate derivative. An aliquot was diluted in ethyl acetate and injected onto a GC (Agilent 6890 Series) coupled to a Mass Selective Detector (Agilent 5973 Network). Selective ion monitoring was used to measure fragments at 200.1 and 202.1, with enrichment calculated by a percentage ratio of the 202.1 to the 200.1 ions. All samples were run in triplicate.

Measures of levels of IL-6, TNF-α, and CRP were obtained from frozen stored plasma collected on the morning of the clamp studies after an overnight fast. The specimens were frozen at −70°C and measured in duplicate by an enzyme-linked immunosorbent assay (ELISA) kit from R&D Systems (Minneapolis, MN). The detectable limit for IL-6 (by HS6000 Quantikine kit) was 0.10 pg/ml and for TNF-α (by HSTA60 kit) was 0.18 pg/ml. Serum levels of CRP were also measured in duplicate by ELISA on the basis of purified protein and polyclonal anti-CRP antibodies (Calbiochem). The CRP assay was standardized according to World Health Organization First International Reference Standard with a sensitivity of 0.08 μg/ml. Total cholesterol was determined using an enzymatic method of Allain et al. (1). HDL cholesterol was determined after selective precipitation by heparin-manganese chloride and removal by centrifugation of VLDL and LDL (45). Triglycerides were determined enzymatically using the procedure of Buocolo and David (4). Proton nuclear magnetic resonance (NMR) spectroscopy was used to quantify subclasses of VLDL, LDL, and HDL in plasma specimens obtained during fasting conditions on the morning of the clamp and kept frozen (−70°C) until analysis with a commercial laboratory NMR Lipoprofile (Raleigh, NC).

Calculations. To calculate rates of glucose appearance and R4 during fasting and insulin infusion conditions, steady-state and non-steady-state equations, respectively, were used and modified to include the use of a labeled exogenous dextrose infusion (46). During insulin infusions, the rate of EGP was calculated as the difference between the overall rate of glucose appearance, measured isotopically, and the corresponding rate of exogenous glucose infusion. Rates of glucose and lipid oxidation were calculated using the indirect calorimetry equations of Fryn (11).

Statistics. Data are presented as means ± SE, unless otherwise indicated. Analysis of variance, with use of non-parametric analyses where appropriate, was used to examine for the effects of group and gender. Correlations and linear regression were used to examine associations between variables. A P value <0.05 was considered significant.

RESULTS

Prevalence of fatty liver in type 2 DM. Values for the attenuation of the liver, as a measurement of hepatic fat content, determined by CT and expressed in HU and as the L/S ratio of these attenuation values, are shown in Table 1 for lean (n = 12) and obese (n = 15) nondiabetic volunteers and for those with type 2 DM (n = 83). Mean values for CT attenuation of the spleen differed only nominally across groups, but the mean CT attenuation value for liver was lowest in type 2 DM, intermediate in obese, and highest in lean subjects. Despite clear differences in adiposity between lean and obese nondiabetic volunteers, the obese group being >10 BMI units higher, mean L/S ratio was not significantly different between these groups. None of the lean and 20% of the obese volunteers had values for the L/S ratio <1, consistent with fatty liver. Mean values for weight, BMI, and FM were similar in obese nondiabetic volunteers and for those with type 2 DM.

Table 1. Fatty liver and other aspects of body composition in lean and obese nondiabetic volunteers and in those with type 2 DM

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Obese</th>
<th>Type 2 DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>L/S ratio</td>
<td>1.27 ± 0.03*</td>
<td>1.13 ± 0.07*</td>
<td>0.90 ± 0.03†</td>
</tr>
<tr>
<td>Liver attenuation</td>
<td>66.7 ± 1.1*</td>
<td>54.6 ± 3.7*,†</td>
<td>45.4 ± 1.4‡</td>
</tr>
<tr>
<td>Spleen attenuation</td>
<td>52.8 ± 1.0</td>
<td>48.6 ± 0.8</td>
<td>50.5 ± 0.4</td>
</tr>
<tr>
<td>% Fatty liver</td>
<td>0*</td>
<td>20†</td>
<td>65†</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.6 ± 0.6*</td>
<td>33.7 ± 1.0†</td>
<td>34.0 ± 0.5</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>66.8 ± 3.8*</td>
<td>93.3 ± 3.7†</td>
<td>96.6 ± 1.9</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>12.6 ± 1.1*</td>
<td>38.8 ± 2.4†</td>
<td>37.2 ± 1.2</td>
</tr>
<tr>
<td>VAT, cm²</td>
<td>64 ± 12*</td>
<td>144 ± 10†</td>
<td>236 ± 9</td>
</tr>
<tr>
<td>Ab SAT, cm²</td>
<td>151 ± 21*</td>
<td>490 ± 29†</td>
<td>427 ± 14</td>
</tr>
<tr>
<td>Deep</td>
<td>76 ± 12*</td>
<td>227 ± 13†</td>
<td>214 ± 8</td>
</tr>
<tr>
<td>Superficial</td>
<td>68 ± 9*</td>
<td>247 ± 20†</td>
<td>197 ± 9</td>
</tr>
<tr>
<td>Muscle Attenuation, HU</td>
<td>52.0 ± 0.7*</td>
<td>ND</td>
<td>45.7 ± 0.5</td>
</tr>
<tr>
<td>Muscle area, cm²</td>
<td>302 ± 23*</td>
<td>ND</td>
<td>283 ± 7</td>
</tr>
<tr>
<td>% Low density</td>
<td>9.5 ± 0.9*</td>
<td>ND</td>
<td>22.1 ± 0.9</td>
</tr>
<tr>
<td>VAT/Ab SAT</td>
<td>0.45 ± 0.06*</td>
<td>0.32 ± 0.04†</td>
<td>0.59 ± 0.03</td>
</tr>
<tr>
<td>VAT/superficial Ab AT</td>
<td>1.04 ± 0.20*</td>
<td>0.66 ± 0.09†</td>
<td>1.48 ± 0.13</td>
</tr>
<tr>
<td>VAT/Ab SAT</td>
<td>0.96 ± 0.13</td>
<td>0.60 ± 0.13*,†</td>
<td>1.17 ± 0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE. L/S ratio, ratio of liver to spleen; BMI, body mass index; VAT, visceral adipose tissue; SAT, subcutaneous AT; HU, Hounsfield units; Ab, abdominal; DM, diabetes mellitus; ND, not determined. * Lean or obese vs. type 2 DM, P < 0.05; † obese vs. lean, P < 0.05.
abietic and obese type 2 DM volunteers, yet there was a significant difference between the mean values for L/S ratio, being lower in type 2 DM. A majority of the obese DM subjects (63%) had a value for the L/S ratio <1.0, consistent with a fatty liver.

Relation of fatty liver to obesity, abdominal adiposity, and muscle lipid. Other parameters of body composition, including BMI, abdominal and peripheral AT distribution, and muscle CT attenuation values, are also presented in Table 1. Age was similar in volunteers with type 2 DM and the non-diabetic obesity, but the lean, non-diabetic group were ~10 yr younger than those with type 2 DM (51.5 ± 0.9, 45.7 ± 1.6, and 42.1 ± 1.8 yr; P < 0.05). Among the study cohort of 110 research volunteers, 22 were African-American, 2 were Asian-American, 3 were Hispanic, and the remainder were white, non-Hispanic. Separate analyses were not performed by ethnicity, but within the cohort for type 2 DM, gender-based analyses are included.

Among all volunteers, there was a significant correlation between BMI and the L/S ratio (r = −0.42, P < 0.001) and between FM and the L/S ratio (r = −0.37, P < 0.001). However, the correlation was stronger between L/S ratio and visceral AT (VAT; r = −0.57, P < 0.0001), as shown in Fig. 2. If these analyses are limited to the cohort with type 2 DM, the correlation between VAT and L/S ratio remains significant (r = −0.64, P < 0.0001), as also shown in Fig. 2. Among all volunteers, the correlation between subcutaneous abdominal AT (SAT) and the L/S ratio was significant (r = −0.29, P < 0.01), but substantially less robust than the correlation with VAT. In regression analyses restricted to the cohort with type 2 DM, the correlations between the L/S ratio and FM (r = −0.18; P = 0.1) and SAT (r = −0.04; P = 0.7) were not significant. The correlation between the L/S ratio and the ratio of VAT to SAT was significant and similar in the entire cohort (r = −0.32; P < 0.001) and in analysis restricted to those with type 2 DM (r = −0.50; P < 0.001).

Data on the CT attenuation values of muscle were available for volunteers with type 2 DM and for lean volunteers, and values were significantly lower in those with type 2 DM (52 ± 0.7 vs. 45.7 ± 0.5 HU; P < 0.01). Among volunteers with type 2 DM, muscle CT attenuation was significantly correlated with VAT (r = −0.38; P < 0.001) and with BMI (r = −0.30; P < 0.01) but was not significantly correlated with FM or SAT. Although these patterns of associations were similar to the pattern observed between the L/S ratio and adiposity, we did not find a significant correlation within the cohort with type 2 DM between muscle CT attenuation and the L/S ratio (r = 0.12, P = 0.33). However, there was a significant correlation between the L/S ratio and subfascial AT (SFAT) in the thigh (r = −0.44, P < 0.001), but there was not a significant correlation between thigh SAT, a much larger depot of AT in the lower extremity, and the L/S ratio (r = −0.09; P = 0.2).

Body composition in type 2 DM associated with vs. without fatty liver. To further examine the relationship between body composition and fatty liver in those with type 2 DM, values for body composition are shown in Table 2, separated by gender and by values for the L/S ratio (≤1.0 vs. >1.0). Age was nearly identical in those with fatty liver compared with those without fatty liver.

Table 2. Body composition in women and men with type 2 DM and fatty liver

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th></th>
<th>Men</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal L/S</td>
<td>Fatty Liver</td>
<td>Normal L/S</td>
<td>Fatty Liver</td>
</tr>
<tr>
<td>L/S ratio</td>
<td>1.16 ± 0.02</td>
<td>0.73 ± 0.04*</td>
<td>1.13 ± 0.02</td>
<td>0.79 ± 0.03*</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>91.9 ± 5.2</td>
<td>91.6 ± 4.7</td>
<td>81.1 ± 6.4</td>
<td>87.5 ± 4.8</td>
</tr>
<tr>
<td>AST</td>
<td>21.0 ± 1.9</td>
<td>25.5 ± 1.5</td>
<td>26.5 ± 2.2</td>
<td>30.1 ± 4.0</td>
</tr>
<tr>
<td>ALT</td>
<td>26.2 ± 1.8†</td>
<td>31.9 ± 1.9 †, †</td>
<td>37.1 ± 3.7</td>
<td>44.2 ± 3.6†</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>33.7 ± 0.8†</td>
<td>36.0 ± 0.9†</td>
<td>28.7 ± 1.0</td>
<td>33.9 ± 1.2‡</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>38.0 ± 1.8†</td>
<td>41.2 ± 1.5 †</td>
<td>23.4 ± 1.9</td>
<td>37.3 ± 3.5‡</td>
</tr>
<tr>
<td>VAT, cm³</td>
<td>195 ± 20</td>
<td>255 ± 14 †</td>
<td>168 ± 16</td>
<td>289 ± 16</td>
</tr>
<tr>
<td>Ab SAT, cm²</td>
<td>499 ± 19 †</td>
<td>469 ± 14 †</td>
<td>270 ± 26</td>
<td>375 ± 22</td>
</tr>
<tr>
<td>VAT/Ab SAT</td>
<td>0.40 ± 0.06†</td>
<td>0.54 ± 0.03 †, †</td>
<td>0.65 ± 0.06</td>
<td>0.84 ± 0.08*</td>
</tr>
<tr>
<td>Muscle CT attenuation</td>
<td>44.9 ± 1.1 †</td>
<td>43.9 ± 0.7 †</td>
<td>48.0 ± 0.7</td>
<td>47.9 ± 0.7</td>
</tr>
<tr>
<td>SFAT, cm²</td>
<td>18.5 ± 1.6 †</td>
<td>20.9 ± 1.7</td>
<td>9.7 ± 0.7</td>
<td>17.2 ± 2.1†</td>
</tr>
<tr>
<td>Thigh SAT, cm²</td>
<td>126 ± 10.4†</td>
<td>140.3 ± 10.0†</td>
<td>61.7 ± 8.1</td>
<td>77.1 ± 9.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. Values for alkaline phosphatase, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) are in units of enzyme activity/100 ml plasma. SFAT, subfascial AT. * Normal L/S vs. fatty liver, P < 0.05; † women vs. men, P < 0.05.
(51.8 ± 1.1 vs. 50.9 ± 1.6 yr). There were higher values of the liver enzyme alanine aminotransferase (ALT) in those with fatty liver. Among women with type 2 DM, in comparison of those with fatty liver vs. those without, there was not a significant group difference for BMI, FM, SAT, or thigh SAT. There was, however, significantly greater VAT in women with fatty liver. Values for skeletal muscle CT attenuation were similar in women with fatty liver compared with those without fatty liver. Among men with type 2 DM, those with fatty liver were considerably more obese than those without fatty liver, by an average of ~5 BMI units. This difference between groups in the severity of obesity was also evident in differences in VAT and SAT. In men, muscle CT attenuation was similar in those with fatty liver vs. those without fatty liver, although there was significant difference in thigh SFAT, being higher in men with fatty liver.

Another difference of body composition that was observed in comparing individuals with type 2 DM and fatty liver vs. either nondiabetic volunteers or those with type 2 DM but without fatty liver concerned the presence of a gradient of CT attenuation values in the liver of those with fatty liver. In volunteers with type 2 DM and fatty liver, there was a statistically significant gradient of CT attenuation values, with the right hepatic lobe having a lower value than the left (L) hepatic lobe (n = 32, 35 ± 2 vs. 40 ± 2 HU, R vs. L; P < 0.001). However, in nondiabetic volunteers [66 ± 2 vs. 67 ± 1 HU, not significant (NS)] and in those with type 2 DM but without fatty liver (n = 21; 56 ± 1 vs. 59 ± 1 HU, NS), there was not a gradient from right to left for hepatic density.

**Insulin sensitivity in relation to fatty liver.** Values for fasting metabolism are shown in Table 3, with values in those with type 2 DM separated according to values for the L/S ratio (=1.0 vs. >1.0, as used above) and separated by gender. As would be expected, volunteers with type 2 DM had FPG and an increased value for hepatic density. Fasting rates of EGP did not differ significantly in type 2 DM with respect to presence of fatty liver, although there was a modest effect for higher values in women (P < 0.05) and a nearly significant interaction with the presence of fatty liver (P = 0.052). Also, fasting concentrations of plasma glucagon did not differ in those with type 2 DM with respect to fatty liver (74 ± 6 vs. 84 ± 4 pg/ml; NS; normal L/S vs. fatty liver). Fasting values for systemic respiratory quotient (RQ) and resting energy expenditure were also similar in type 2 DM with vs. without fatty liver and were higher among women. Among a subset of these volunteers (n = 33), fasting values for ketones were available, and they did not differ in those with vs. without fatty liver (2.1 ± 0.4 vs. 1.8 ± 0.3 mg/dl). There was a significant correlation between fasting ketones and fasting values for plasma FFA (r = 0.52; P < 0.001), and this relationship was not affected by fatty liver. Thus values for fasting RQ and ketones did not identify differences in fasting patterns of fatty acid oxidation in relation to fatty liver in type 2 DM.

Table 3. Fasting metabolism in women and men with type 2 DM and fatty liver

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Lean</th>
<th>Normal L/S</th>
<th>Fatty Liver</th>
<th>Normal L/S</th>
<th>Fatty Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPG, mg/dl</td>
<td>82 ± 1†</td>
<td>174 ± 10</td>
<td>166 ± 7</td>
<td>173 ± 14</td>
<td>205 ± 13</td>
</tr>
<tr>
<td>Hb A1c, %</td>
<td>4.9 ± 0.1†</td>
<td>8.2 ± 0.3</td>
<td>7.8 ± 0.3</td>
<td>8.2 ± 0.4</td>
<td>8.4 ± 0.4</td>
</tr>
<tr>
<td>Fasting insulin, μU/ml</td>
<td>4.7 ± 2†</td>
<td>13.5 ± 1.5</td>
<td>17.8 ± 1.3*</td>
<td>14.9 ± 5.7</td>
<td>19.0 ± 2.3*</td>
</tr>
<tr>
<td>FFA, μmol/l</td>
<td>448 ± 2†</td>
<td>667 ± 222</td>
<td>750 ± 190‡</td>
<td>547 ± 31</td>
<td>623 ± 50*</td>
</tr>
<tr>
<td>RQ</td>
<td>0.80 ± 0.01</td>
<td>0.80 ± 0.01</td>
<td>0.78 ± 0.01</td>
<td>0.79 ± 0.01</td>
<td>0.78 ± 0.01</td>
</tr>
<tr>
<td>REE, kcal·min⁻¹·kg FFM⁻¹</td>
<td>31.8 ± 0.9</td>
<td>36.1 ± 1.32</td>
<td>37.6 ± 0.6‡</td>
<td>30.5 ± 1.0</td>
<td>31.9 ± 0.8</td>
</tr>
<tr>
<td>EGP, mg·min⁻¹·kg FFM⁻¹</td>
<td>2.85 ± 0.13</td>
<td>3.26 ± 0.1</td>
<td>2.97 ± 0.1</td>
<td>2.75 ± 0.1</td>
<td>2.97 ± 0.1</td>
</tr>
</tbody>
</table>

| **Men**     |      |            |             |            |            |

Values are means ± SE. FPG, fasting hyperglycemia; Hb A1c, hemoglobin type A1c; FFA, free fatty acid; RQ, respiratory quotient; REE, resting energy expenditure; FFM, fat-free mass; EGP, endogenous glucose production; *Normal L/S vs. fatty liver, P < 0.05; †Lean vs. DM, P < 0.05; ‡Women vs. men, P < 0.05.
Table 4. Insulin-stimulated metabolism in women and men with type 2 DM and fatty liver

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Normal L/S</th>
<th>Fatty Liver</th>
<th>Lean</th>
<th>Normal L/S</th>
<th>Fatty Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dl</td>
<td>86 ± 1.1†</td>
<td>90 ± 2</td>
<td>92 ± 2</td>
<td>95 ± 5</td>
<td>101 ± 4</td>
<td></td>
</tr>
<tr>
<td>Insulin, pmol</td>
<td>47 ± 1.7†</td>
<td>58 ± 3</td>
<td>60 ± 2</td>
<td>63 ± 4</td>
<td>65 ± 4</td>
<td></td>
</tr>
<tr>
<td>FFA, μmol/l</td>
<td>49 ± 1†</td>
<td>151 ± 26</td>
<td>251 ± 27*</td>
<td>142 ± 26</td>
<td>237 ± 30*</td>
<td></td>
</tr>
<tr>
<td>R4, mg·min⁻¹·kg FFM⁻¹</td>
<td>11.33 ± 0.73</td>
<td>4.89 ± 0.73</td>
<td>3.19 ± 0.19*</td>
<td>5.03 ± 0.81</td>
<td>2.74 ± 2.25*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. R4, glucose disposal; ox, oxidation; nonox, nonoxidative. *Normal L/S vs. fatty liver, P < 0.05; †women vs. men, P < 0.05.

−0.57; P < 0.001), an index of fat content in muscle (18). As shown in Fig. 5, suppression of EGP was blunted in men, and there was some relation to fatty liver. In regression analysis, steady-state values for the suppression of EGP were significantly and negatively correlated with VAT (r = −0.33, P < 0.01) and with insulin-suppressed plasma FFA (r = −0.36, P < 0.01), but were not significantly correlated with the L/S ratio.

Lipoproteins and cytokines in relation to fatty liver. The group with type 2 DM manifested a dyslipidemic profile compared with either lean or obese nondiabetic volunteers, having a twofold increase in plasma triglyceride (82 ± 1, 91 ± 3, and 177 ± 5 mg/dl; P < 0.001), lower mean plasma HDL-C (55 ± 5, 55 ± 3, and 43 ± 2 mg/dl; P < 0.01), and similar LDL-C (110 ± 14, 143 ± 9, and 128 ± 4 mg/dl; NS). Across all volunteers, plasma triglyceride and HDL-C were inversely but similarly correlated with the L/S ratio (r = −0.34 and 0.34, respectively, both P < 0.001). Values for plasma lipoproteins in volunteers with type 2 DM, divided according to those with or without fatty liver and by gender, are shown in Table 5. Both men and women with type 2 DM and fatty liver had a significant elevation of plasma triglycerides compared with those without fatty liver, and a significant reduction in plasma HDL-C.

Also shown in Table 5 are values for plasma CRP, TNF-α, and IL-6. Plasma CRP was higher in women than in men with type 2 DM, but among men, those with fatty liver had significantly higher plasma CRP. Plasma levels of TNF-α were increased in men and women with fatty liver compared with those without fatty liver, and the same trend was observed for IL-6, although these differences were not statistically significant. Both fatty liver (L/S ratio) and fatty muscle (CT attenuation values) correlated significantly, and to a similar degree, with plasma levels of TNF-α and CRP. The L/S ratio, but not muscle CT attenuation, was correlated with IL-6 (r = −0.34, P < 0.01).

Fasting profiles of lipoproteins were assessed by NMR spectroscopy to assess the distributions of particle sizes in a subset of volunteers with type 2 DM (n = 24) and in lean nondiabetic volunteers (n = 12); these data are shown in Table 6. Type 2 DM volunteers who had fatty liver had higher concentrations of large VLDL, but similar concentrations of intermediate and small VLDL, as those without fatty liver. Those with fatty liver had a significantly lower concentration of large LDL but a significantly and substantially higher concentration of small LDL. Also, those volunteers with type 2 DM and fatty liver had a significantly lower concentration of large HDL particles. Triglyceride concentration in VLDL particles was approximately twofold greater in volunteers with type 2 DM and fatty liver compared with those without fatty liver.

Multivariate correlates of insulin resistance and of fatty liver in type 2 DM. On the basis of results of simple correlations, a stepwise regression analysis was performed using the cohort with type 2 DM, with the L/S ratio as the dependent variable and with VAT, FM, BMI, muscle CT attenuation, SFAT of the lower extremity, IL-6, and plasma FFA as the significant independent variables. VAT was the strongest single correlate (r = −0.64; P < 0.001), and after adjustment for this variable, the residual variance for the L/S ratio was significant, with additional correlation with IL-6 and SFAT of the lower extremity as sequentially added steps; these three variables accounted for 49% of the variance in the L/S ratio (P < 0.001), and the remaining variables did not add further significance.

Similarly, on the basis of significant simple correlations, a stepwise regression analysis was performed to examine the relation between aspects of body composition and insulin resistance (IR) in type 2 DM, using insulin-stimulated glucose R4 as the dependent variable and including the L/S ratio, VAT, BMI, muscle CT attenuation, and SFAT of the lower extremity as independent variables. VAT was the strongest single correlate (r = −0.74; P < 0.001), and after adjustment for VAT, residual variance was significantly correlated (in a step-wise manner) with BMI and the L/S ratio; together, these variables accounted for 63% of the variance in insulin-stimulated glucose R4 among this cohort with type 2 DM. If fatty acid levels during fasting and after insulin infusion are added as independent

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variables, then the significant factors that emerge are VAT and mid-clamp levels of plasma fatty acids, and there is no longer a significant independent contribution from fatty liver.

**DISCUSSION**

In recent years, there has been growing interest in understanding the mechanisms leading to the accumulation of excessive fat within skeletal muscle and liver in those with obesity and type 2 DM and in the role that this might have in the pathogenesis of insulin resistance (30). A number of investigations indicate that increased lipid content within skeletal muscle fibers can be associated with IR (19, 42); our group has further reported that adipose tissue accumulating in proximity to skeletal muscle and beneath muscle fascia is also related to IR (15). Similar interest has arisen with regard to increased fat deposition within the liver and the role that hepatic steatosis might have in IR (22). A high prevalence of fatty liver in association with type 2 DM has been reported (2, 5). In the current study, nearly two-thirds of the 83 volunteers with type 2 DM had fatty liver.

Fig. 3. A: rates of glucose utilization (Rd) in mg·min⁻¹·kg fat-free mass⁻¹ (FFM) are plotted against time during basal and insulin-stimulated conditions in volunteers with type 2 DM without fatty liver (■), in those with fatty liver (▲), and lean controls (dotted circles). Insulin infusions were started at time 0. Rates of Rd were significantly lower in type 2 DM with fatty liver at all time points after 60 min compared with those without fatty liver, and values for Rd did not change significantly from baseline in type 2 DM with fatty liver. B: values for Rd plotted against individual values for L/S ratio in lean and obese nondiabetic volunteers (○) and in those with type 2 DM (■). There was statistically a highly significant curvilinear fit (r = 0.77; P < 0.001), with a cubic function (F = 38). Higher values for L/S ratio are indicative of lower fat content in the liver.

Fig. 4. Values for fasting and insulin-suppressed plasma free fatty acids (FFA), with values at 120 min representing the midpoint of 4-h insulin infusion. The 4 groups are lean, nondiabetic volunteers (●), obese nondiabetic volunteers (○), type 2 DM without fatty liver (■), and type 2 DM with fatty liver (▲). Fasting and insulin-suppressed values for plasma FFA were significantly greater in volunteers with type 2 DM and fatty liver than in the other 3 groups.

Fig. 5. Values for endogenous glucose production (EGP), measured by isotope dilution methods, in volunteers with type 2 DM are plotted against time during fasting conditions (−30 min to 0 min) and in response to a 4-h insulin infusion. Because of both gender and fatty liver effects, respective curves are shown for women with fatty liver (●), women without fatty liver (○), men with fatty liver (▲), and men without fatty liver (○). Also, 2 additional curves are shown for nondiabetic volunteers (men: dotted squares; women: dotted circles). At baseline there was a significant effect of gender, with values higher in women, whereas at steady-state insulin infusion, rates of EGP were higher in men. Effect of fatty liver was significant only at 180 min of insulin infusion.
with type 2 DM. As a group, those with type 2 DM, 
ering all volunteers and in analyses restricted to those 
was correlated with the amount of VAT both in consid-
_between fatty liver and visceral adiposity. The L/S ratio 
(37, 38). We observed a quite strong association be-
nd a relation between fatty liver and central obesity 
men, and the other in lean nondiabetic men, did not 
without waist circumference or central abdominal adiposity (7, 
rent study is the use of noninvasive imaging, although 
postulated (7, 28, 29). A relative limitation of the cur-
clusion of fatty liver with VAT was more robust 
than with numerous other indexes of obesity, such as 
statistically significant and is the use of noninvasive imaging, although

A number of prior studies have related fatty liver to 
waist circumference or central abdominal adiposity (7, 
14, 17, 22, 23, 28). On the other hand, two studies, one 
in 20 individuals with type 2 DM, 17 of whom were 
men, and the other in lean nondiabetic men, did not 
find a relation between fatty liver and central obesity 
(37, 38). We observed a quite strong association be-
tween fatty liver and visceral adiposity. The L/S ratio 
was correlated with the amount of VAT both in consid-
ering all volunteers and in analyses restricted to those 
with type 2 DM. As a group, those with type 2 DM, 
particularly women, had significantly higher amounts of 
VAT than did obese nondiabetic volunteers. The correlation of fatty liver with VAT was more robust 
than with numerous other indexes of obesity, such as systemic FM or BMI, and fatty liver was not correlated 
with the amount of subcutaneous adiposity. These 
findings of the current study indicate that, among 
various aspects of adiposity, VAT is the pivotal factor 
related to fatty liver in type 2 DM.

One potential explanation for this association be-
tween VAT and fatty liver might be portal delivery of 
fatty acids to the liver (31). In the current study, there 
was a significant association between the severity of 
fatty liver and plasma fatty acids, whether measured 
during fasting or during insulin clamp conditions. This 
certainly suggests that fatty liver is related to 
increased delivery of fatty acids, as has been previously 
postulated (7, 28, 29). A relative limitation of the current 
study is the use of noninvasive imaging, although

### Table 5. Lipoproteins and inflammatory markers in women and men with type 2 DM and fatty liver

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Normal L/S</th>
<th>Fatty Liver</th>
<th>Normal L/S</th>
<th>Fatty Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides, mg/dl</td>
<td>130 ± 11</td>
<td>217 ± 22*</td>
<td>172 ± 29</td>
<td>216 ± 13*</td>
</tr>
<tr>
<td>HDL-C, mg/dl</td>
<td>50 ± 5†</td>
<td>43 ± 1*,†</td>
<td>45 ± 2</td>
<td>37 ± 2*</td>
</tr>
<tr>
<td>LDL-C, mg/dl</td>
<td>125 ± 7</td>
<td>131 ± 6</td>
<td>128 ± 9</td>
<td>125 ± 7</td>
</tr>
<tr>
<td>CRP, μg/ml</td>
<td>3.8 ± 0.6†</td>
<td>4.0 ± 0.5†</td>
<td>0.9 ± 0.2</td>
<td>2.2 ± 0.4*</td>
</tr>
<tr>
<td>TNF-α, pg/ml</td>
<td>3.4 ± 0.3</td>
<td>3.9 ± 0.2*</td>
<td>3.3 ± 0.2</td>
<td>4.2 ± 0.3*</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>2.2 ± 0.4</td>
<td>3.3 ± 0.6*</td>
<td>1.5 ± 0.3</td>
<td>2.4 ± 0.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE. CRP, C-reactive protein; LDL-C, LDL cholesterol. * Normal L/S vs. fatty liver, P < 0.05; †women vs. men, P < 0.05.

There is good correlation with histological assessments (36); at present, NMR might be considered the most precise noninvasive approach (34). From an anatomic perspective, it is attractive to postulate that mesenteric adipocytes might be a crucial source of fatty acids entering portal circulation and causing fatty liver, but this explanation must be considered speculative. Even though higher rates of lipolysis have been noted for mesenteric adipocytes, mesenteric AT constitutes ~10% of overall FM (31). Nevertheless, in the current study, there is the intriguing observation that, in those with fatty liver, the right lobe of the liver had lower CT attenuation values, indicative of greater fat content, than did the left lobe, and this is a pattern consistent with known patterns of portal flow (13) and prior patterns of hepatic steatosis (35). Nevertheless, delivery of systemic fatty acids from the much larger FM cannot be easily discounted.

An unanticipated finding relating fatty liver to other aspects of body composition was the correlation observed with SFAT in the lower extremity. SFAT in the lower extremity is identifiable on CT imaging of the mid thigh, and although previously observed by Goodpaster et al. (15) to have a correlation with IR, to our knowledge this is the first report of an association with fatty liver. SFAT represents a relatively minor proportion of lower extremity AT and may contribute to IR by regional release of either fatty acids or cytokines; insulin regulation of lipolysis differs from subcutaneous adipose; in subcutaneous adipose. IL-6, pg/ml 3.4

### Table 6. Lipoprotein subclasses in relation to fatty liver in type 2 DM

<table>
<thead>
<tr>
<th>Lipoprotein Class</th>
<th>Lean Non Diabetic Volunteers (n = 12)</th>
<th>Type 2 DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal L/S (n = 9)</td>
<td>Fatty liver (n = 13)</td>
</tr>
</tbody>
</table>

- **VLDL-TG, mg/dl**
  - Large: 6 ± 2, 38 ± 13, 89 ± 11*  
  - Intermediate: 34 ± 8, 28 ± 4, 47 ± 7  
  - Small: 15 ± 2, 20 ± 4, 22 ± 3

- **LDL-C, mg/dl**
  - Large: 76 ± 13, 51 ± 12, 32 ± 6*  
  - Intermediate: 22 ± 8, 40 ± 12, 18 ± 8  
  - Small: 10 ± 4, 22 ± 9, 76 ± 12*  

- **HDL-C, mg/dl**
  - Large: 39 ± 6, 20 ± 4, 11 ± 2*  
  - Small: 24 ± 2, 21 ± 2, 18 ± 1

Values are means ± SE. * Normal L/S vs. fatty liver, P < 0.05.
nisms leading to fat accumulation in liver and muscle, and of the interrelationship of excess lipid within these two organs, would prove to be an interesting area of body composition research with implications for understanding the pathogenesis of IR. To summarize, our findings indicate that selected depots of lipid accumulation, such as VAT, fatty liver, and SFAT of the lower extremities, although each accounts for a relatively small absolute amount of adiposity, contribute in an interactive manner to the severity of obesity-related IR in type 2 DM.

In the current study, we also examined the effect of gender on body composition in type 2 DM. Among nondiabetic individuals, gender is recognized to have several clear effects on body composition, among which is that, at any BMI, women generally have greater FM than men and less VAT (16). It is interesting, therefore, to note that, among those with type 2 DM examined in the current study, women had higher subcutaneous adiposity and very similar amounts of VAT as found in men, revealing perhaps some blunting of the usual gender effect on VAT. Also, men and women with type 2 DM did not differ in regard to prevalence of fatty liver.

IR is typically quite pronounced in type 2 DM (8, 24), and one of the main goals of the current study was to examine the potential relation of IR to fatty liver. We observed a strong association between severity of fatty liver and systemic IR. As clearly shown in Fig. 3, those with fatty liver had marked IR, more severe than those with type 2 DM without fatty liver. This is consistent with the prior study of Giulio et al. (14). In multivariate analysis of aspects of body composition, fatty liver was an independent correlate of severity of IR but was of essentially equivalent significance as VAT. We interpret these findings to suggest that fatty liver chiefly influences IR as a component of an overall axis of central adiposity, as has been recently also reported by Gastaldelli et al. (12). Also, we find that severity of fatty liver in type 2 DM is related to higher levels of fatty acids, during both fasting and insulin-stimulated conditions. It is well recognized that fatty acid concentrations modulate insulin action in health and in type 2 DM (39). In the multivariate models, if fasting or insulin-suppressed levels of plasma FFA are entered along with the above components of body composition, then the two significant factors are VAT and fatty acids. Thus, although we cannot fully discount the possibility that fatty liver might act in some manner to directly cause peripheral tissue IR, a reasonable interpretation is that the association between IR and fatty liver is part of a larger relationship among fatty acids, central adiposity, and IR.

There was also a strong association of fatty liver with dyslipidemia. It is characteristic for individuals with type 2 DM to manifest a dyslipidemia of elevated triglyceride, reduced HDL-C, and small, dense LDL-C composition, a pattern that has also been related to obesity and IR (43). Recently, Nievels et al. (32) reported that the dyslipidemia profile is specifically related to VAT and IR (32). In the current study, in volunteers with type 2 DM with fatty liver, VLDL particles were larger, reflecting higher triglyceride content, and HDL particles were smaller and so were LDL particles. Thus these findings support the concept that fatty liver in type 2 DM may have a role in aggravating dyslipidemia and, accordingly, in directly modulating risk for cardiovascular disease (CVD). Nevertheless, we also observed that, in type 2 DM, severity of the dyslipidemic profile was related not only to the presence of fatty liver but also to greater amounts of VAT, although there was no effect of subcutaneous or overall adiposity. These findings in a group with type 2 DM are consistent with the data of Nievels et al., obtained in a cohort of nondiabetic men and women. Although it is not possible from the current data to separate the effects of fatty liver and VAT, it is clear that the liver is the site of triglyceride synthesis for VLDL and that fatty liver is associated with hypertriglyceridemia (7, 29). An important role for the enzyme hepatic lipase can also be postulated in determining the severity of the dyslipidemic profile, because this enzyme converts large buoyant LDL particles to smaller, dense LDL (47). What is striking in the current data is that, among those with type 2 DM without fatty liver, abnormalities of the lipoprotein profile are relatively modest.

Obesity is associated with increased plasma levels of inflammatory cytokines, a finding attributed in part to secretion by adipocytes (41). Kupffer cells of the liver can also secrete cytokines, and it has been found that factors such as TNF-α and IL-6 can influence fatty acid metabolism in the liver and dispose to formation of fatty liver (3, 7, 10). These inflammatory markers can also contribute to risk for CVD (26). In the current study, inflammatory markers of CRP, TNF-α, and IL-6 were higher in those with fatty liver. Several of these cytokines are known to alter fatty acid metabolism by the liver and might contribute in a causative manner to the formation or maintenance of fatty liver (6). The association of fatty liver and inflammatory markers is a further indication that, in those with type 2 DM, hepatic steatosis is associated with a number of risk factors for CVD.

In summary, among a cohort of overweight and obese research volunteers with type 2 DM and without clinical evidence of steatohepatitis, a high prevalence of fatty liver was observed and was found to correlate with visceral adiposity, more severe insulin resistance, higher levels of inflammatory markers, and a more pronounced dyslipidemic lipoprotein profile. Fatty liver appears to be manifest along an axis that also includes visceral adiposity and increased plasma fatty acids, and in this context we conclude that fatty liver is a body composition manifestation of obesity in type 2 DM that portends adverse metabolic risk.

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REFERENCES


