Effects of Identical Weight Loss on Body Composition and Features of Insulin Resistance in Obese Women With High and Low Liver Fat Content

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Our objective was to determine how 8% weight loss influences subcutaneous, intra-abdominal, and liver fat (LFAT), as well as features of insulin resistance, in obese women with high versus low LFAT. A total of 23 women with previous gestational diabetes were divided into groups of high (9.4 ± 1.4%) and low (3.3 ± 0.4%) LFAT based on their median LFAT (5%) measured with proton spectroscopy. Both groups were similar with respect to age, BMI, and intra-abdominal and subcutaneous fat. Before weight loss, women with high LFAT had higher fasting serum insulin and triglyceride concentrations than women with low LFAT. At baseline, LFAT correlated with the percent of fat ($r = 0.44$, $P < 0.05$) and saturated fat ($r = 0.45$, $P < 0.05$) of total caloric intake but not intra-abdominal or subcutaneous fat or fasting serum free fatty acids. Weight loss was similar between the groups (high LFAT −7.4 ± 0.2 vs. low LFAT −7.7 ± 0.3 kg). LFAT decreased from 9.4 ± 1.4 to 4.8 ± 0.7% ($P < 0.001$) in women with high LFAT and from 3.3 ± 0.4 to 2.0 ± 0.2% ($P < 0.001$) in women with low LFAT. The absolute decrease in LFAT was significantly higher in women with high than low LFAT (−4.6 ± 1.0 vs. −1.3 ± 0.3%, $P < 0.005$). The decrease in LFAT was closely correlated with baseline LFAT ($r = -0.85$, $P < 0.001$) but not with changes in the volumes of intra-abdominal or subcutaneous fat depots, which decreased similarly in both groups. LFAT appears to be related to the amount of fat in the diet rather than the size of endogenous fat depots in obese women. Women with initially high LFAT lost more LFAT by similar weight loss than those with low LFAT, although both groups lost similar amounts of subcutaneous and intra-abdominal fat. These data suggest that LFAT is regulated by factors other than intra-abdominal and subcutaneous fat. Therefore, LFAT does not appear to simply reflect the size of endogenous fat stores. *Diabetes* 52:701–707, 2003

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ALT, alanine aminotransferase; FFA, free fatty acid; IMCL, intramyocellular lipid; LFAT, liver fat; MRI, magnetic resonance imaging.

Recent studies in both fatless mice and humans have suggested that the amount of fat deposited in insulin-sensitive tissues, such as skeletal muscle (1–9) and liver (10,11), is correlated with features of insulin resistance independent of the amount of subcutaneous fat. Fatless mice also do not have any intra-abdominal fat despite being insulin resistant. Treatment of lipodystrophy by subcutaneous fat transplantation normalizes fat content in insulin-sensitive tissues and reverses insulin resistance (12). These data imply that insulin resistance is caused by fat accumulation in insulin-sensitive tissues independent of intra-abdominal fat.

According to the "Portal theory," visceral fat perturbs metabolism by exposing the liver to high concentrations of free fatty acids (FFAs) (13). This theory is, however, presently poorly supported by experimental mechanistic data, although there are abundant epidemiological data linking android-type of fat distribution and cardiovascular risk (14). For example, catheterization studies have suggested that fatty acids released by the splanchnic bed contribute only ~10% of total FFA delivery to the liver (15) and that increased delivery of FFAs to the liver in upper body obesity is due to excessive release by upper body subcutaneous rather than visceral adipose tissue (16,17). Although the latter data do not exclude other mechanisms via which intra-abdominal fat could induce insulin resistance, they cast some doubt on the validity of the portal theory.

One possibility to test whether liver fat (LFAT) and intra-abdominal fat are linked is to determine whether the size of the two depots change in parallel in response to a therapeutic maneuver. Weight loss decreases the volume of intra-abdominal fat (18–22). Early studies in massively obese subjects who were treated surgically using gastroplasty (23–26), gastric bypass (25,27), gastric banding (28), or with low-calorie diets (29,30) reported a reduction in the amount of hepatic steatosis. However, in these studies, assessment of hepatic steatosis was usually semiquantitative and the sizes of other fat depots were not quantitated. These studies therefore do not answer the question of whether weight loss–induced changes in intra-abdominal and LFAT depots are interrelated. There are no data on the possible contribution of exogenous fat intake on the amount of fat in the liver.

In the present study, we studied a homogenous group of

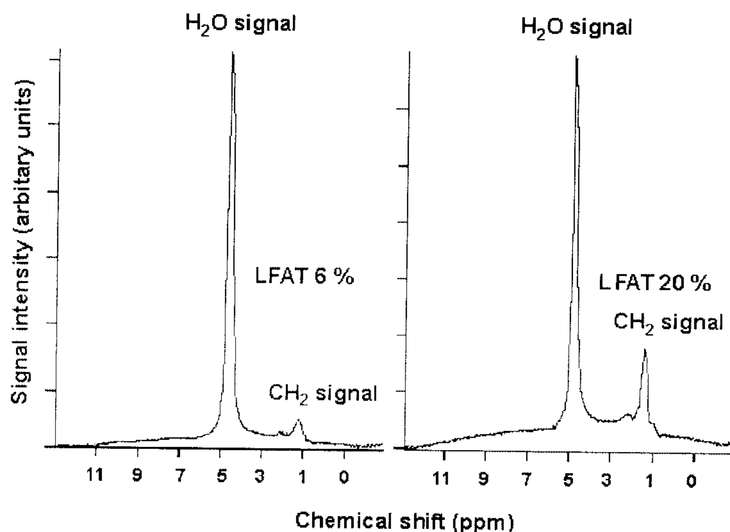

FIG. 1. Proton magnetic resonance spectra from two patients with fat percents (percent LFAT is a ratio of the area under the methylene peak to that under methylene and water peaks × 100) of 6 and 20%. The water peak has a chemical shift of 4.8 ppm, and the methylene (CH_2) signal of fat has a chemical shift of 1.4 ppm relative to the water peak. The height of the signal (*y*-axis) is in arbitrary units.

obese women, all of whom participated in a weight loss program designed to induce moderate 8% weight loss. The amount of LFAT was quantitated by proton spectroscopy and intra-abdominal and subcutaneous fat by magnetic resonance imaging (MRI). This design allowed us to analyze how features of insulin resistance are related to LFAT and other fat depots and whether changes in the size of various fat depots are interrelated.

RESEARCH DESIGN AND METHODS

Subjects and study design. The participants of the study were recruited using hospital records of women who had been treated during 1987–1999 at the Department of Obstetrics and Gynecology at Helsinki University Central Hospital because of gestational diabetes. They had to fulfill the following inclusion criteria: *1*) previous gestational diabetes, *2*) current age 20–50 years, *3*) BMI after delivery between 28 and 35 kg/m^2, and *4*) no known acute or chronic disease based on history and physical examination and standard laboratory tests (blood counts, serum creatinine, thyroid-stimulating hormone, electrolyte concentrations, liver function tests, and electrocardiogram). A 2-h oral glucose tolerance test with 75 g glucose was performed to exclude women with diabetes (31). Other exclusion criteria included treatment with drugs, which may alter glucose tolerance, pregnancy, lack of reliable contraception, postmenopausal status, or clinical or biochemical evidence of any significant disease other than obesity. A total of 27 women met the inclusion and exclusion criteria and were included in the study. LFAT was measured by proton spectroscopy, intra-abdominal and subcutaneous fat by MRI, and various features of insulin sensitivity before and after weight loss as detailed below.

After baseline measurements, the women participated in a weight loss program in which the goal was to achieve 8% weight loss within 3–6 months. The women were placed on a nutritionally balanced hypocaloric diet that consisted of 30% of energy as fat (10% saturated, 10% monounsaturated, and 10% polyunsaturated fat), 50% kcals as carbohydrate, and 20% as protein. Cholesterol was limited to 300 mg/day. Patient age and body weight were used to determine the estimate of their basal metabolic rate, which was multiplied by a correction factor to allow for activity to determine total daily energy requirement. Based on the estimate of initial caloric needs, a prescribed diet caused a caloric deficit of ~600–800 kcal/day, based on body weight. Patients weighing <100 kg were prescribed a diet causing a caloric deficit of 600 kcal/day, and patients weighing >100 kg used a diet that caused a caloric deficit of 800 kcal/day. During the weight loss period, the patients met with a dietitian every 2 weeks. Four women in the high and seven women in the low LFAT group used 120 mg orlistat t.i.d. as an adjunct to the hypocaloric diet. Use of orlistat did not affect conclusions of the study, since a comparable number of subjects in both groups used orlistat (data not shown). Before the metabolic studies and the weight loss period, all women were asked to keep a 3-day diary of food intake. Alcohol consumption and composition of daily fat intake was calculated from these food diaries using Nutrica software (Research Center of the Social Insurance Institution, Helsinki, Finland). The metabolic studies were performed after a 2-week period of weight maintaining to avoid any confounding effects of caloric restriction. Four of 27 women did not achieve the required 8% weight loss and were withdrawn from the study. The remaining women were divided into two groups with high (9.4 ± 1.4%) and low (3.3 ± 0.4%) LFAT based on their median LFAT content (5%). The term "high" merely denotes LFAT above the median.

The nature and potential risks of the study were explained to all subjects before written informed consent was obtained. The Ethics Committee of Helsinki University Central Hospital approved the experimental protocol.

Methods

LFAT content. Localized single voxel (2 × 2 × 2 cm^3) proton spectra were acquired using a 1.5-T whole-body system (Magnetom Vision; Siemens, Erlangen, Germany) that consisted of a combination of whole-body and loop surface coils for radiofrequency transmitting and signal receiving. T1-weighted spin-echo magnetic resonance images were used for localization of the voxel of interest within the right lobe of the liver. Vascular structures and subcutaneous fat tissue were avoided in localization of the voxel. Subjects were lying on their stomach on the surface coil, which was embedded in a mattress to minimize abdominal movement due to breathing. The single voxel spectra were recorded by using the stimulated-echo acquisition mode sequence with an echo time of 20 ms, a repetition time of 3,000 ms, and a mixing time of 30 ms. A total of 1,024 data points over 1,000 kHz spectral width with 32 acquisitions were collected. Water-suppressed spectra with 128 acquisitions were also recorded to detect weak lipid signals. The short echo time and long repetition time were chosen to ensure a fully relaxed water signal, which was used as an internal standard. Chemical shifts were measured relative to water at 4.80 ppm. The methylene signal, which represents intracellular trigyceride, was measured at 1.4 ppm. The spectra were fitted in the time domain using the variable projection method (32,33). Spectroscopic intracellular triglyceride content (in percent) was expressed as a ratio of the area under the methylene peak to that under the sum of methylene and water peaks × 100 (LFAT). This measurement has been validated against histologically determined lipid content of liver biopsies in humans (34) and animals (35) and against estimates of fatty degeneration or infiltration by X-ray computed-assisted tomography (36). The magnetic resonance spectroscopy percent is not, however, identical to the percent determined histologically or biochemically from liver biopsies. Examples of two spectra of women with a low and high LFAT are shown in Fig. 1. The reproducibility of LFAT measurements performed on two separate occasions in nondiabetic subjects (n = 10) is 11% in our laboratory (37).

Intra-abdominal and subcutaneous fat (MRI). A series of T1-weighted trans-axial scans for the determination of intra-abdominal and subcutaneous fat were acquired from a region extending from 8 cm above to 8 cm below the fourth and fifth lumbar interspace (16 slices, field of view 375 × 500 mm^2, slice thickness 10 mm, breath-hold repetition time 138.9 ms, echo time 4.1 ms). Intra-abdominal and subcutaneous fat areas were measured using an image analysis program (Alice 3.0; Parexel, Waltham, MA). A histogram of pixel intensity in the intra-abdominal region was displayed, and the intensity corresponding to the nadir between the lean and fat peaks was used as a cut point. Intra-abdominal adipose tissue was defined as the area of pixels in the intra-abdominal region above this cut point. For calculation of subcutaneous adipose tissue area, a region of interest was first manually drawn at the demarcation of subcutaneous adipose tissue and intra-abdominal adipose

tissue as previously described (38). The reproducibility of intra-abdominal and subcutaneous fat measurements performed on two separate occasions in nondiabetic subjects (n = 10) is 5 and 3% in our laboratory (37).

Ambulatory blood pressure monitoring. Noninvasive 24-h blood pressure monitoring was performed on a normal weekday with an automatic ambulatory blood pressure monitoring device (Diasys Integra; Novacor SA, Rueil-Malmaison, France). The device was set to record blood pressure and heart rate every 15 min during the day and every 30 min during the night. Day and night were defined from awake and sleeping periods in the patient's diary.

Other measurements. Blood samples were taken after an overnight fast for measurement of fasting plasma glucose, HbA_{1c}, liver enzymes, serum triglyceride, total and HDL cholesterol, and serum free insulin. The percent of body fat was determined by using bioelectrical impedance analysis (BioElectrical Impedance Analyzer System, model no. BIA-101A; RJL Systems, Detroit, MI) (39). Waist circumference was measured midway between spina iliaca superior and the lower rib margin and hip circumference at the level of the greater trochanters (40).

Analytical procedures. Plasma glucose concentrations were measured in duplicate with the glucose oxidase method using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA) (41). Serum free insulin concentrations were measured by radioimmunoassay (Phadeseph Insulin RIA; Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) after precipitation with polyethylene glycol (42). HbA_{1c} was measured by high-pressure liquid chromatography using the fully automated Glycosylated Hemoglobin Analyzer System (BioRad, Richmond, CA) (43). Serum FFAs were measured using a fluorometric method (44). Serum total cholesterol, HDL cholesterol, and triglyceride concentrations were measured with respective enzymatic kits from Roche Diagnostics using an autoanalyzer (Roche Diagnostics Hitachi 917; Hitachi, Tokyo). LDL cholesterol concentration was calculated using the formula of Friedewald (45). Serum aspartate aminotransferase, alanine aminotransferase (ALT), and γ-glutamyl transferase activities were determined as recommended by the European Committee for Clinical Laboratory Standards.

Statistical analyses. The unpaired t test was used to compare mean values between low and high LFAT groups after logarithmic transformation, if necessary. Spearman's rank correlation coefficient was used to calculate correlation coefficients between selected variables. All calculations were made by GraphPad Prism version 3.0 (GraphPad, San Diego, CA). Data are shown as means ± SE. A P value <0.05 was considered statistically significant.

RESULTS

Baseline characteristics. The groups with high and low LFAT were almost identical with respect to age (37 ± 1 vs. 37 ± 2 years, respectively, NS), BMI (33 ± 1 vs. 32 ± 1 kg/m^2, NS), waist-to-hip ratio (0.96 ± 0.02 vs. 0.94 ± 0.02, NS), and the amounts of intra-abdominal (1,665 ± 141 vs. 1,497 ± 167 cm^3, NS), subcutaneous (6,363 ± 408 vs. 5,850 ± 261 cm^3, NS), and whole-body fat (37 ± 1 vs. 36 ± 1%, NS). Fasting plasma glucose (5.7 ± 0.2 vs. 5.9 ± 0.2 mmol/l, NS) and HbA_{1c} (5.5 ± 0.1 vs. 5.6 ± 0.1%, NS) concentrations were also similar. The women with high LFAT had higher fasting serum insulin (15 ± 2 vs. 9 ± 1 mU/l, $P < 0.01$) and triglyceride (1.73 ± 0.18 vs. 1.27 ± 0.14 mmol/l, P = 0.06) concentrations than women with low LFAT. Alcohol consumption was minimal and similar within the groups (3 ± 1 vs. 7 ± 3 g/day, high versus low LFAT, NS). Before weight loss, LFAT did not correlate with BMI (r = 0.17, NS), percent whole-body fat (r = 0.12, NS), fat mass (r = 0.15, NS), the waist-to-hip ratio (r = 0.24, NS), the volume of intra-abdominal (r = 0.24, NS) or subcutaneous (r = 0.007, NS) fat, or the concentrations of fasting FFA (r = 0.06, NS). Of the components of diet before weight loss, LFAT was significantly correlated with the percent of total energy that consisted of fat (r = 0.43, $P < 0.05$) and the percent of saturated fat (r = 0.45, $P < 0.05$) (Fig. 2).

Effects of weight loss on body composition and liver enzymes. The women in the high and low LFAT groups lost weight over comparable time periods (18 ± 1 vs. 19 ± 2 weeks, respectively, NS), a similar percent of their body

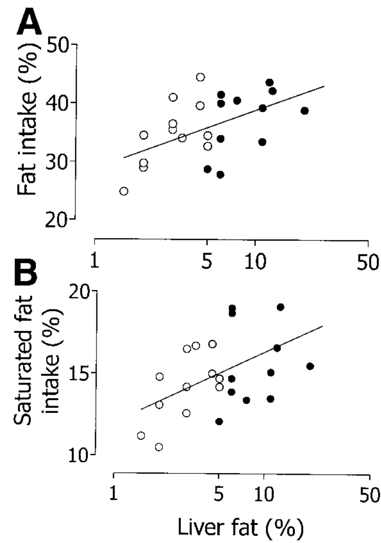

FIG. 2. The relationship between LFAT and total fat intake (percent fat of total daily energy intake) (r = 0.43, P < 0.05) (A) and saturated fat intake (percent saturated fat of total daily energy intake) (r = 0.45, P < 0.05) (B) in women with low (○) and high (●) LFAT.

weight (−8.4 ± 0.2% vs. −8.3 ± 0.2%, NS), and similar kilograms of body weight and units of BMI (Fig. 3).

Weight loss decreased LFAT from 9.4 ± 1.4 to 4.8 ± 0.7% ($P < 0.001$) in women with high LFAT and from 3.3 ± 0.4 to 2.0 ± 0.2% ($P < 0.001$) in women with low LFAT (Fig. 4). The absolute decrease in LFAT was significantly higher in women with high than low LFAT (−4.6 ± 1.0 vs. −1.3 ± 0.3%, $P < 0.005$), and after weight loss, LFAT was still significantly higher in women with initially high than low LFAT ($P < 0.001$). The change of LFAT correlated closely with LFAT before weight loss ($r = -0.85$, $P < 0.001$) (Fig. 5). The slopes of the regression lines relating LFAT to its change by weight loss were similar, showing that there was no difference in the relative decrease in LFAT between the groups. In the entire group, the percent of LFAT lost was 39 ± 5%, which was much higher than the percent of whole-body fat mass lost (14 ± 1%, $P < 0.001$). The groups lost comparable amounts of fat from subcutaneous (−841 ± 75 vs. −830 ± 73 cm^3, high versus low LFAT, NS)

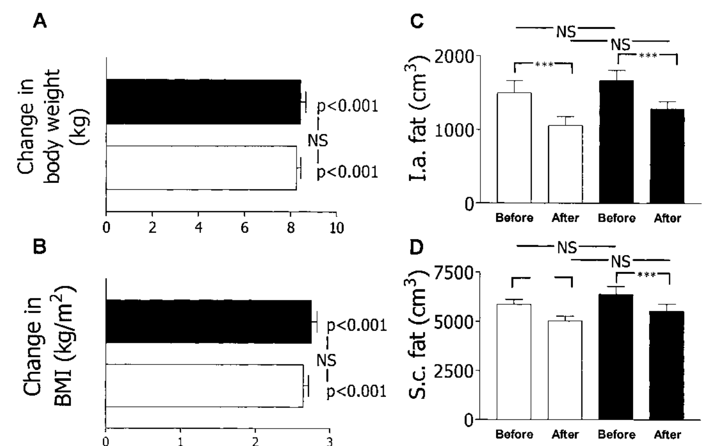

FIG. 3. Effects of weight loss on measures of body composition in women with low (□, n = 12) and high (■, n = 11) LFAT. Change in body weight (A) and BMI (B) by weight loss. Intra-abdominal (I.a.) (C) and subcutaneous (S.c.) (D) fat volumes before and after weight loss. ***P < 0.001 before vs. after weight loss.

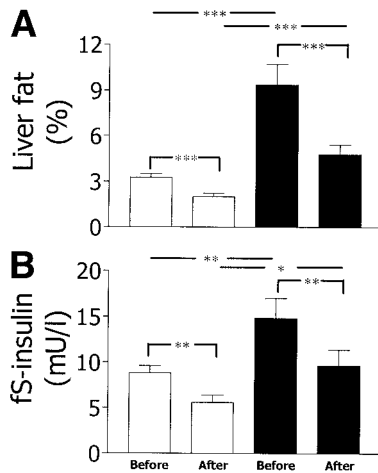

FIG. 4. Effects of weight loss on LFAT (*A*) and fasting serum insulin concentrations (*B*) in women with low (□) and high (■) LFAT. *P < 0.05, **P < 0.01, ***P < 0.001.

and intra-abdominal regions (−383 ± 67 vs. −441 ± 122 cm^3, NS). The change in LFAT did not correlate with the change in intra-abdominal fat (r = −0.17, NS) or changes in other measures of body composition (data not shown). Fasting serum FFA concentrations were not different at baseline (680 ± 45 vs. 732 ± 44 μmol/l, low versus high LFAT, NS) and were decreased by weight loss (−79 ± 37 μmol/l, $P < 0.05$), with no differences between the groups (−81 ± 56 vs. −78 ± 51 μmol/l, low versus high LFAT).

Before weight loss, serum ALT was correlated with LFAT (r = 0.41, P = 0.05). Serum ALT concentrations decreased significantly by weight loss in both women with high (32 ± 4 vs. 25 ± 4 units/l, before versus after, $P <$ 0.05) and low LFAT (26 ± 6 vs. 23 ± 6 units/l, $P < 0.05$). The change in serum ALT correlated with that in LFAT (r = 0.58, $P < 0.005$). Serum γ-glutamyl transferase concentrations decreased in women with high LFAT (31 ± 5 vs. 27 ± 4 units/l, before versus after, $P < 0.05$) but not in those with low LFAT (26 ± 5 vs. 23 ± 5 units/l, NS). Serum AFOS or bilirubin concentrations did not differ between the groups before or after weight loss (data not shown).

Effect of weight loss on metabolic parameters and 24-h blood pressure. Weight loss resulted in a decrease in serum fasting insulin concentrations in both groups (15 ± 2 to 10 ± 2 units/l, before versus after, $P < 0.01$ vs. 9 ± 1 to 6 ± 1 unit/l, $P < 0.001$, high versus low LFAT). After weight loss, serum insulin concentrations were significantly higher in women with high than low LFAT (Fig. 4). Serum triglyceride concentrations slightly decreased in women with high LFAT (1.73 ± 0.18 to 1.40 ± 0.13 mmol/l, before versus after, P = 0.09) but not in women with low LFAT (1.27 ± 0.14 to 1.20 ± 0.14 mmol/l, low LFAT, NS). Serum LDL cholesterol decreased significantly in both groups (3.25 ± 0.2 to 2.86 ± 0.2 mmol/l, high LFAT, $P <$ 0.01 and 3.36 ± 0.2 to 2.90 ± 0.1 mmol/l, low LFAT, $P <$ 0.05, before versus after) with no differences between the groups. Serum HDL cholesterol concentrations were slightly but not significantly (1.35 ± 0.09 vs. 1.17 ± 0.08 mmol/l, P = 0.14) higher before weight loss in women with high versus low LFAT and did not change significantly by weight loss (data not shown). Mean 24-h systolic blood pressure decreased in the low LFAT group, from 116 ± 2 to 110 ± 1 mmHg ($P < 0.005$) and from 125 ± 7 to 118 ± 4 mmHg ($P < 0.05$) in the high LFAT group. After weight loss, systolic 24-h blood pressure was significantly higher in women with high than low LFAT ($P < 0.05$). Mean diastolic 24-h blood pressures did not differ significantly between the groups and did not change by weight loss (data not shown). The change in LFAT did not correlate with changes in serum fasting insulin (r = 0.25, NS) or triglyceride (r = 0.02, NS) concentrations or with the change in 24-h systolic blood pressure (r = 0.09, NS).

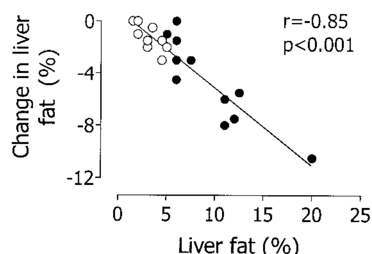

FIG. 5. The relationship between baseline LFAT and its absolute change by weight loss in women with low (○) and high (●) LFAT.

DISCUSSION

The aim of the present study was to determine how similar degrees of weight loss affect LFAT in a homogenous group of obese women divided into two groups based on their median LFAT. Before weight loss, the two groups of women had similar body compositions, judging from the percent of total body fat as well as subcutaneous and intra-abdominal fat. Despite this, women with high LFAT had higher serum fasting insulin and triglyceride concentrations than those with low LFAT. LFAT also correlated significantly with the percent of fat and the percent of calories derived from fat in the diet before weight loss. Moderate weight loss decreased LFAT, and this decrease was closely correlated with baseline LFAT. The absolute but not relative decrease was greater in women with initially high than low LFAT. The absolute decreases in intra-abdominal and subcutaneous fat volumes and fasting serum FFAs were similar between the groups and did not correlate with the change in LFAT.

Both subcutaneous and intra-abdominal fat decreased by weight loss, confirming previous observations (18,19, 22). The change in LFAT by the 8% weight loss was closely correlated with baseline LFAT but not with changes in either subcutaneous or intra-abdominal fat volumes. The failure of the change of LFAT to correlate with the change in intra-abdominal or subcutaneous fat was not due to the methodology used, since the interindividual coefficient of variation of LFAT was higher (72%) than that of intra-abdominal (32%) or subcutaneous (19%) fat and the coefficient of variation of repeated measurements of LFAT (11%) was higher than that of intra-abdominal (5%) or subcutaneous (3%) fat. These data should not be interpreted to suggest that the magnitude of weight loss does not influence the amount of fat lost from the liver, since weight loss was deliberately kept as constant as possible. Indeed, in older studies, the decrease in LFAT, as determined by liver biopsies, has been significantly related with the magnitude of weight loss (30). However, the correla-

tion with baseline LFAT rather than those in subcutaneous and intra-abdominal depots suggests that the amount of fat in the liver may not simply reflect the flux of endogenous FFA from peripheral and intra-abdominal sites to the liver. This interpretation assumes that change in size of a fat depot correlates with its metabolic activity, which is uncertain in the absence of FFA turnover data. Also, these data do not exclude the possibility that intra-abdominal fat causes insulin resistance in humans (46).

The liver can derive its fatty acids via de novo lipogenesis, from endogenous FFA, or from remnant lipoproteins. The significant positive relationship before weight loss between saturated fat intake and LFAT provides some support for the possibility that exogenous fat contributed to hepatic fat stores, although the pathways contributing to hepatic triglyceride storage under postprandial conditions have not been precisely defined (47). In animals, the liver has a high capacity for triglyceride storage and the size of this pool can change several-fold within hours (48,49). There is evidence that triglyceride storage occurs in the liver whenever FFA availability exceeds hepatic disposal via secretion and oxidation (50). If the reverse is true, then a decrease in the contribution of exogenous fatty acids by caloric restriction could preferentially mobilize hepatic rather than subcutaneous or intra-abdominal triglycerides. In the present study, serum fasting FFAs decreased, which, based on previous studies where endogenous FFA kinetics have been determined with tracer techniques (16,51), is likely to reflect a decrease in FFA availability. This change could stimulate mobilization of hepatic triglycerides and could explain why a greater percent of LFAT (39% by 8% weight loss) than total body fat (14%) was lost during weight loss.

Although caloric restriction could have contributed to the greater mobilization of liver than peripheral fat, it cannot explain why women with initially high LFAT lost more fat from their liver than women with low LFAT. We did not assess diet composition in detail during or after weight loss and we therefore do not know whether women with high LFAT decreased their initially high fat intake more than women with low LFAT. However, at least the women were advised to use a diet with a similar composition by the dieticians. If the women consumed the prescribed diet (those who successfully completed the study did based on the amount of weight loss), then a greater decrease in the contribution of exogenous fatty acids to hepatic triglycerides in the women with high than low LFAT could explain why the absolute decrease in LFAT was greater in the women with initially high than low LFAT. Obviously, this explanation needs to be tested more directly, provided methods can be developed to quantitate the contribution of dietary fatty acids to hepatic fat storage.

Data are rapidly accumulating in mice and men to suggest that fat accumulation in tissues that either produce glucose and triglycerides (the liver) or utilize glucose (skeletal muscle) is sufficient to cause insulin resistance in these tissues independent of the size of intra-abdominal or adipose tissue depots. Mice that are unable to store adipose tissue either paradoxically because of overexpression of $SREBP_{1c}$ in adipose tissue (52) or because of a lack of peroxisome proliferator–activated receptor-γ (3), mutation in the mouse fatty liver dystrophy (fld) gene (53), fat-specific expression of diphtheria toxin (2), or overexpression of a dominant negative protein that prevents DNA binding of transcription factors (the A-ZIP fatless mouse) (54) all have fatty livers but no visceral or subcutaneous fat and all are insulin resistant. In the A-ZIP mouse, treatment of lipodystrophy with subcutaneous fat transplantation reverses insulin resistance in both the liver and in skeletal muscle, from which excessive triglyceride stores are simultaneously depleted (1). In humans, we have previously shown that fat accumulation in the liver is closely correlated with, specifically, hepatic sensitivity to insulin in middle-aged men independent of the size of intra-abdominal or subcutaneous fat depots (10) and in patients with type 2 diabetes (38). Consistent with these data, in the present study, a high LFAT content was associated with features of insulin resistance and these abnormalities persisted after weight loss, although weight loss did correct some of the metabolic abnormalities.

We did not observe a correlation between changes in LFAT and those in markers of insulin resistance by weight loss. Several factors could contribute to the failure to observe such a relationship. We did not specifically quantitate hepatic sensitivity but used the serum fasting insulin concentration as a surrogate marker of insulin action. We also merely measured the percent LFAT rather than the total hepatic fat content. In previous biopsy studies, liver size has been mostly increased or unchanged in individuals with a fatty liver (55), which suggests that measurement of the percent of fat in the liver may have underestimated the amount of fat in those with a high LFAT percent. On the other hand, the change in LFAT was significantly ($r = 0.58$, $P < 0.005$) correlated with that change in serum ALT, which is a marker of hepatic steatosis, irrespective of liver size (26,56). It is also possible that weight loss resulted in loss of intramyocellular lipid (IMCL), as was recently shown in the study of Greco et al. (57). However, in their study, a significant decrease in IMCL was observed in patients who lost 24% of their body weight by biliopancretic diversion but not in the group treated with a hypocaloric diet, which lost only 9% of their body weight (57). In the present study, we attempted to determine IMCL but this was only successful in some of the obese women because of difficulties in distinguishing between intra- and extramyocellular fat peaks with a 1.5-T magnet (5). In the women in whom IMCL could be quantitated, it remained unchanged (data not shown). Changes in muscle insulin sensitivity also appear to not be of importance for regulation of fasting serum insulin concentrations, at least in mice that selectively lack the insulin receptor in their skeletal muscle (58). This is in contrast with mice, in which the insulin receptor has been selectively deleted from the liver, that develop marked hyperinsulinemia and hepatic insulin resistance (59).

To conclude, LFAT content before weight loss in a relatively homogenous group of obese women was unrelated to the volume of intra-abdominal and subcutaneous fat. We found, however, a significant correlation between total fat and saturated fat intake and LFAT, raising the possibility that fatty acids derived from the diet may influence LFAT content. Taken together these data dem-

onstrate, in keeping with previous cross-sectional data showing that LFAT can vary independent of overall and intra-abdominal fat (10), that the change in LFAT by an intervention, in this case weight loss, does not simply reflect changes in endogenous fat stores. Clinically, this study supports current recommendations to use a diet with a low saturated fat and overall fat content in individuals with features of insulin resistance.

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