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Fat Accumulation in the Liver Is Associated with Defects in Insulin Suppression of Glucose Production and Serum Free Fatty Acids Independent of Obesity in Normal Men

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We determined whether interindividual variation in hepatic insulin sensitivity could be attributed to variation in liver fat content (LFAT) independent of obesity. We recruited 30 healthy nondiabetic men whose LFAT (determined by proton spectroscopy); intraabdominal, sc, and total (determined by magnetic resonance imaging) fat; and insulin sensitivity of endogenous glucose rate of production (R_a) and suppression of serum FFA [euglycemic insulin clamp combined with [3-^{3}H]glucose (0–300 min); insulin infusion rate, 0.3 mU/kg·min, 120–300 min] were measured. The men were divided into groups of low (mean ± SD, 1.7 ± 0.2%) and high (10.5 ± 2.0%) LFAT based on their median fat content. The low and high LFAT groups were comparable with respect to age (44 ± 2 *vs.* 42 ± 2 yr), body mass index (25 ± 1 *vs.* 26 ± 1 kg/m^2), waist to hip ratio (0.953 ± 0.013 *vs.* 0.953 ± 0.013), maximal oxygen uptake (35.6 ± 1.5 *vs.* 33.5 ± 1.5 ml/kg·min), and intraabdominal, sc, and total fat. The high compared with the low LFAT group had several features of insulin resistance, including fasting hyperinsulinemia (7.3 ± 0.6 *vs.* 5.3 ± 0.6 mU/liter; $P < 0.02$, high *vs.* low LFAT) hypertriglyceridemia (1.4 ± 0.2 *vs.* 0.9 ± 0.1 mmol/liter; $P < 0.02$), a low high density lipoprotein (HDL) cholesterol concentration (1.4 ± 0.1 *vs.* 1.6 ± 0.1 mmol/liter; $P < 0.05$), and a higher ambulatory 24-h systolic blood pressure (130 ± 3 *vs.* 122 ± 3 mm Hg; $P < 0.05$). Basal glucose R_a and serum FFA were comparable between the groups, whereas insulin suppression of glucose R_a [51 ± 8 *vs.* 20 ± 12 mg/m^2·min during 240–300 min ($P < 0.05$) or −55 ± 7 *vs.* −85 ± 12% below basal ($P < 0.05$, high *vs.* low LFAT)] and of serum FFA (299 ± 33 *vs.* 212 ± 13 μmol/liter; 240–300 min; $P < 0.02$) were impaired in the high compared with the low LFAT group. Insulin stimulation of glucose Rd were comparable in the men with high LFAT (141 ± 12 mg/m^2·min) and those with low LFAT (156 ± 14 mg/m^2·min; P = NS).

Fat accumulation in the liver is, independent of body mass index and intraabdominal and overall obesity, characterized by several features of insulin resistance in normal weight and moderately overweight subjects. (*J Clin Endocrinol Metab* 87: 3023–3028, 2002)

STUDIES IN FATLESS mice have demonstrated that fat accumulation in liver and skeletal muscle is associated with severe insulin resistance and signaling defects such as those in insulin-stimulated insulin receptor substrate-1 (IRS-1)- and IRS-2-associated phosphatidylinositol 3-kinase activities (1–5). Treatment of lipodystrophy in fatless mice with fat transplantation completely reverses insulin resistance (6). In humans, several forms of lipodystrophy are accompanied by insulin resistance (7). Also, accumulation of fat within skeletal muscle fibers has recently been shown to be associated with whole body insulin resistance and a defect in activation of IRS-1-associated phosphatidylinositol 3-kinase by insulin in normal men, independent of body mass index (BMI) and body fat content (8).

We have recently demonstrated that hepatic fat content, measured with proton spectroscopy and within a range below that clinically considered a fatty liver, varies considerably in type 2 diabetic patients and is closely correlated with hepatic insulin sensitivity (9). In this study the patients with high liver content were, however, also more obese than those with a low liver fat content (LFAT) (9). In nondiabetic individuals a fatty liver has been associated with causes of insulin resistance such as obesity (10–13) and with consequences of insulin resistance such as hypertriglyceridemia and hyperinsulinemia (13, 14). In patients referred for investigation of abnormal liver function tests who were subsequently shown to have nonalcoholic steatohepatitis, Bacon *et al.* (15) found only 39% to be 10% over ideal body weight. It is, however, unknown, whether fat accumulation in the liver is associated with insulin resistance specifically in this tissue, independent of overall obesity. In the present study we examined whether variation in hepatic fat content is related to insulin sensitivity of endogenous glucose production and other features of insulin resistance independent of overall obesity and fat distribution in nondiabetic men.

Abbreviations: ALT, Alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HDL, high density lipoprotein; IRS, insulin receptor substrate; LFAT, liver fat content (percentage); NAFLD, nonalcoholic fatty liver disease; R_a, rate of production; R_d, rate of utilization; VO_2max, maximal oxygen uptake.

Subjects and Methods

Subjects and study design

Thirty men, aged 30–55 yr, were recruited from occupational health services in Helsinki. The subjects were healthy, as judged by history and physical examination, and did not use any drugs. The men also did not have serological evidence of hepatitis A, B, or C or autoimmune hepatitis nor did they have clinical signs or symptoms of inborn errors of metabolism or a history of use of toxins, other than less than moderate amounts of alcohol (<20 g/d), or drugs associated with steatosis (16). Alcohol consumption was assessed by detailed history and laboratory markers [serum γ-glutamyltranspeptidase, the aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio, and mean corpus-

cular volume]. The subjects were divided into two groups based on their median LFAT (3%). Their physical and biochemical characteristics are shown in Table 1. The subjects underwent measurements of 1) *in vivo* insulin sensitivity of glucose rate of production (R_a) and rate of utilization (R_d) using the euglycemic insulin clamp technique combined with infusion of [3-^{3}H]glucose; 2) liver fat content by proton spectroscopy; 3) sc, intraabdominal, and total abdominal fat volumes by magnetic resonance imaging; 4) maximal oxygen uptake (VO_2max); and 5) 24-h ambulatory blood pressure as detailed below.

The purpose, nature, and potential risks of the studies were explained to the patients before their written informed consent was obtained. The experimental protocol was approved by the ethical committee of the Helsinki University Hospital.

In vivo insulin sensitivity of glucose production and utilization

At 0800 h after an overnight fast, two indwelling catheters placed in an antecubital vein and retrogradely in a heated hand vein for infusion of glucose, insulin, and [3-^{3}H]glucose and for sampling of arterialized venous blood. To determine R_a and R_d under basal and hyperinsulinemic conditions, [3-^{3}H]glucose was infused in a primed (20 μCi) continuous (0.2 μCi/min) fashion for a total of 300 min (9, 17). Baseline blood samples were taken for measurement of fasting serum insulin and glucose concentrations and for the biochemical measurements listed in Table 1. After 120 min, insulin was infused in a primed continuous (0.3 mU/kg·min) fashion as previously described (9). Plasma glucose was maintained at 5 mmol/liter (90 mg/dl) until 300 min using a variable rate infusion of 20% glucose (17). Blood samples for measurement of glucose specific activity were taken at 90, 100, 110, and 120 min and at 15- to 30-min intervals between 120 and 300 min. Serum free insulin concentrations were measured every 60 min during the insulin infusion.

LFAT (determined by proton spectroscopy)

Image-guided proton magnetic resonance spectroscopy was performed on a 1.5 T whole body system device (Magnetom Vision, Siemens, Erlangen, Germany) using a combination of whole body and loop surface coils for radiofrequency transmitting and receiving of signals. T1 weighted high resolution magnetic resonance images (repetition time = 250 msec; echo time = 5 msec) were used for determination of the volume of interest for further spectroscopic localization using the STEAM sequence (repetition time = 3000 msec; echo time = 20 msec; 1024 data points over a spectral width of 1000 kHz; number of acquisitions, 32 and 128 to ensure good signal to noise ratio). Volumes of interest (8 cc) in the liver were placed, avoiding vascular structures and sc fat tissue. These areas were typically within the right lobe. Chemical shifts were measured relative to water signal intensity at 4.8 ppm (S_{water}). Methylene signal intensity, which represents intracellular triglycerides in the liver (9), was measured at 1.4 ppm (S_{fat}). Signal intensities were obtained by a time domain fitting routine VAPRO-MRUI (www.mrui.uab.es/mruiHomePage.html). This measurement of percent hepatic fat by proton spectroscopy has been validated against the lipid content of liver biopsies in humans (18). It has also been validated against liver density measurements performed by computed tomography. The latter validation has also been performed by us previously (9). The percent liver fat was calculated by dividing 100 times S_{fat} by the sum of S_{fat} and S_{water}.

Intraabdominal and sc fat (determined by magnetic resonance imaging)

A series of T1-weighted trans-axial scans for the determination of intraabdominal and sc fat were acquired from a region extending from 8 cm above to 8 cm below the fourth and fifth lumbar interspaces (16 slices; field of view, 375 × 500 mm^2; slice thickness, 10 mm; breath-hold repetition time divided by the echo time, 138.9 msec/4.1 msec). Intraabdominal and sc fat areas were measured using an image analysis program (www.perceptive.com/ALICE.HTM). A histogram of pixel intensity in the intraabdominal region was displayed, and the intensity corresponding to the nadir between the lean and fat peaks was used as a cut-off point. Intraabdominal adipose tissue was defined as the area of pixels in the intraabdominal region above this cut-point. For calculation of sc adipose tissue area, a region of interest was first manually drawn at the demarcation of sc adipose tissue and intraabdominal tissue as previously described (9).

Maximal aerobic power (VO_2max)

Maximal aerobic power was measured directly using an incremental work-conducted upright exercise test with an electrically braked cycle ergometer (Ergometer Ergoline 900ERG, Ergoline GmbH, Bitz, Germany) combined with continuous analysis of expiratory gases and minute ventilation (V_{max}229 series, SensorMedics, Yorba Linda, CA). Exercise was started at a work load of 50 watts. The work load was then increased by 50 watts every 3 min until perceived exhaustion or a

TABLE 1. Physical and biochemical characteristics of men with liver fat content below (low LFAT) and above (high LFAT) the median

	Low LFAT	High LFAT	Significance (P)
No. of subjects	15	15	
Liver fat content (%)	1.7 ± 0.2	10.5 ± 2.0[a]	
Age (yr)	44 ± 2	42 ± 2	NS (0.57)
BMI (kg/m^2)	25 ± 1	26 ± 1	NS (0.37)
Waist to hip ratio	0.953 ± 0.013	0.953 ± 0.013	NS (1.00)
Body fat (%)	19 ± 1	21 ± 1	NS (0.27)
Intraabdominal fat (cm^3 × 10^{-3})	3075 ± 340	3208 ± 261	NS (0.76)
Sc fat (cm^3 × 10^{-3})	2421 ± 253	2867 ± 250	NS (0.32)
Total abdominal fat (cm^3 × 10^{-3})	5496 ± 490	6076 ± 439	NS (0.39)
Intraabdominal/sc ratio	1.36 ± 0.12	1.19 ± 0.11	NS (0.32)
VO_2max (ml/kg · min)	35.6 ± 1.5	33.5 ± 1.5	NS (0.32)
Fasting plasma glucose (mmol/liter)	5.5 ± 0.1	5.5 ± 0.1	NS (0.85)
Fasting serum insulin (pmol/liter)	32 ± 4	44 ± 3[b]	0.016
Fasting serum C peptide (nmol/liter)	0.47 ± 0.06	0.78 ± 0.09[b]	0.009
Serum triglycerides (mmol/liter)	0.9 ± 0.09	1.4 ± 0.2[c]	0.027
Serum cholesterol (mmol/liter)	5.2 ± 0.3	5.4 ± 0.2	NS (0.44)
Serum HDL cholesterol (mmol/liter)	1.6 ± 0.09	1.4 ± 0.08[c]	0.042
Alanine aminotransferase (U/liter)	27 ± 3	39 ± 4[c]	0.035
Aspartate aminotransferase (U/liter)	28 ± 3	30 ± 2	NS (0.59)
γ-Glutamyltransferase (U/liter)	20 ± 2	41 ± 5[b]	0.001
Alcohol intake (g/wk)	70 ± 12	92 ± 20	NS (0.45)

All data are shown as the mean ± SE.
[a] $P < 0.001$ for low *vs.* high LFAT.
[b] $P < 0.02$ for low *vs.* high LFAT.
[c] $P < 0.05$ for low *vs.* high LFAT.

respiratory quotient of 1.10 was reached. Maximal aerobic power was defined as the VO_2max of the last 30 sec of exercise.

Twenty-four-hour ambulatory blood pressure

Noninvasive ambulatory blood pressure monitoring was performed on a normal weekday with an automatic ambulatory blood pressure-monitoring device (Diasys Integra, Novacor, Rveil-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 the waking and sleeping periods in the patient's diary.

Analytical procedures and calculations

Glucose specific activity was determined as previously described (19). Glucose R_a and R_d were calculated using the Steele equation, assuming a pool fraction of 0.65 for glucose and a distribution volume of 200 ml/kg for glucose (20). The endogenous glucose R_a was calculated by subtracting the exogenous glucose infusion rate required to maintain euglycemia during hyperinsulinemia from the rate of total glucose R_a. The percent suppression of basal endogenous glucose R_a during the last hour (240–300 min) by insulin was used as an index of the sensitivity of endogenous glucose production to insulin (percent suppression).

Other measurements

Plasma glucose concentrations were measured in duplicate with the glucose oxidase method using a Glucose Analyzer II (Beckman Coulter, Inc. Instruments, Fullerton, CA) (21). Serum C-peptide concentrations were determined by RIA (22). Serum free insulin was measured using RIA (insulin RIA kit, Pharmacia Biotech, Uppsala, Sweden) after precipitation with polyethylene glycol (23). Serum FFA were measured using a fluorometric method (24). Hemoglobin A_{1c} was measured by high pressure liquid chromatography (25) using the fully automated Glycosylated Hemoglobin Analyzer System (Bio-Rad Laboratories, Inc., Richmond, CA). Total cholesterol, HDL cholesterol, and triglycerides were measured as previously described (26). Total body fat was determined by bioimpedance analysis (BioElectrical Impedance Analyzer System model BIA-101A, RJA Systems, Detroit, MI).

Statistical analyses

The unpaired *t* test was used to compare mean values between low and high LFAT groups. Simple correlation analyses were performed using Spearman's nonparametric rank correlation coefficient. Multiple linear regression analysis was used to determine factors explaining interindividual variation in features of insulin resistance. The calculations were made using the Systat statistical package, version 10.0 (Systat, Evanston, IL) and PRISM version 2.01 (GraphPad Software, Inc., San Diego, CA). All data are shown as the mean ± SEM. $P < 0.05$ was considered statistically significant.

Results

Subject characteristics (Table 1)

The men with a high compared with those with a low LFAT were similar with respect to age, physical fitness, and alcohol intake. All measures of obesity, including BMI, waist to hip ratio, and intraabdominal, sc, and total fat measured with magnetic resonance imaging, were comparable between the two groups. Fasting plasma glucose concentrations were comparable, but both fasting serum insulin and C-peptide concentrations were significantly higher in the men with high LFAT compared with those low LFAT (Table 1). If analyzed by multiple linear regression analysis with fasting insulin as the dependent variable, LFAT was significant when unadjusted (R^2 = 31.2%; $P < 0.001$) or adjusted for intraabdominal fat ($P = 0.002$ for LFAT, $P = 0.108$ for intraabdominal fat; R^2 = 37.6%, $P < 0.001$), sc fat ($P = 0.001$ for LFAT, $P = 0.022$ for sc fat; R^2 = 43.6%, $P < 0.001$), total fat ($P = 0.001$ for LFAT, $P = 0.02$ for total fat; R^2 = 44.0%, $P < 0.001$), or alcohol consumption alone ($P = 0.005$ for LFAT, $P = 0.35$ for alcohol consumption/wk, R^2 = 33.5%, $P = 0.004$) or for alcohol consumption and intraabdominal fat (LFAT, $P = 0.005$; alcohol consumption, $P = 0.45$; intraabdominal fat, $P = 0.14$; R^2 = 39%, $P = 0.004$). The men with high LFAT also had 53% higher concentrations of fasting serum triglycerides and significantly lower HDL cholesterol concentrations than the men with low LFAT. Of liver enzymes, ALT and γ-glutamyltransferase, but not AST, were slightly higher in the high compared with the low LFAT group (Table 1). The AST/ALT ratios averaged 1.08 ± 0.07 and 0.88 ± 0.09 in groups with low and high LFAT (P = NS), and mean corpuscular volumes averaged 90 ± 1 and 91 ± 1 fl (P = NS).

Insulin action in vivo on endogenous glucose production and disposal

During the insulin infusion, serum insulin concentrations were significantly higher in the men with a high LFAT (138 ± 6 pmol/liter) compared with those with a low LFAT (114 ± 6 pmol/liter; $P < 0.01$). This difference was, however, due to the slightly higher basal insulin concentrations in the men with high compared with low LFAT because the increment in serum insulin concentrations above basal during the insulin infusion was comparable between the groups (99 ± 8 and 85 ± 4 pmol/liter, respectively; $P = 0.11$). Rates of basal endogenous glucose R_a were comparable between the groups (102 ± 6 *vs.* ± 111 ± 6 mg/m^2·min, low *vs.* high LFAT). During the last hour of the insulin infusion, endogenous glucose R_a was significantly higher in the men with high (51 ± 8 mg/m^2·min, 1.22 ± 0.19 mg/kg·min, or 1.56 ± 0.24 mg/kg fat-free mass·min) compared with those with low (20 ± 12 mg/m^2·min, 0.47 ± 0.32 mg/kg·min, or 0.62 ± 0.38 mg/kg fat-free mass·min; $P < 0.05$ for all) LFAT. The percent suppression of endogenous glucose R_a below basal averaged 56 ± 7% *vs.* 85 ± 12% (high *vs.* low LFAT, $P < 0.05$; Fig. 1). If analyzed by multiple linear regression analysis with the percent suppression of endogenous glucose R_a below basal

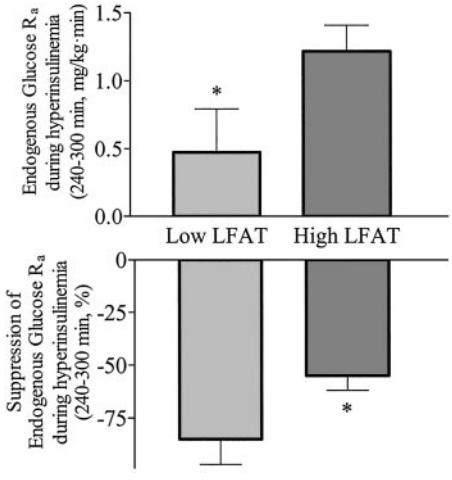

FIG. 1. Endogenous glucose R_a during the last hour of hyperinsulinemia (240–300 min) in men with low and high LFAT. *, $P < 0.05$ for low *vs.* high LFAT.

as the dependent variable, LFAT was significant when unadjusted (r = 0.40; $P = 0.036$) or adjusted for BMI ($P = 0.043$ for LFAT, $P = 0.56$ for BMI, $P = 0.098$ for model), sc fat ($P = 0.041$ for LFAT, $P = 0.55$ for sc fat, $P = 0.07$ for model), and waist/hip ratio ($P = 0.031$ for LFAT, $P = 0.64$ for waist/hip ratio, $P = 0.075$ for model) and was marginally significant if adjusted for intraabdominal fat ($P = 0.064$ for LFAT and $P = 0.153$ for intraabdominal fat; $P = 0.041$ for model). Rates of glucose R_d were equal to production basally and were not different during hyperinsulinemia (141 ± 12 *vs.* 156 ± 1 mg/m^2·min, respectively; P = NS).

Insulin action on serum FFA concentrations

Fasting serum FFA concentrations were comparable basally (606 ± 173 *vs.* 647 ± 232 μmol/liter; P = NS, low *vs.* high LFAT) and during the first 2 h of the insulin infusion (Fig. 2). During the last hour of the insulin infusion, serum FFA remained 41% higher in the group with a high LFAT than in that with a low LFAT (299 ± 33 *vs.* 212 ± 13 μmol/liter; $P < 0.02$).

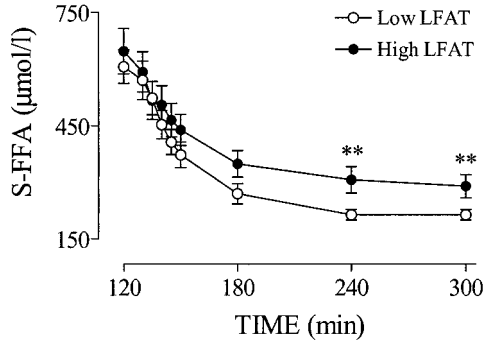

FIG. 2. Serum FFA concentrations at baseline before the start of the insulin infusion (120 min) and during the last hour of the insulin infusion (240–300 min) in men with low and high LFAT. **, $P < 0.02$ for men with low *vs.* high LFAT.

Relationships between LFAT and features of insulin resistance

As predicted from the significant differences between means of fasting insulin, lipids, and 24-h systolic blood pressure (Table 1), these parameters were significantly correlated with LFAT. These relationships and lack of a relationship between intraabdominal and liver fat are depicted in Fig. 3.

Discussion

In the present study men with a relatively normal body weight were studied to determine whether LFAT, as measured using proton spectroscopy, is associated with features of insulin resistance independent of body weight. We found, as previously in a group of type 2 diabetic patients treated with insulin (9), that LFAT is an independent determinant of the sensitivity of endogenous glucose production to insulin. A high LFAT was also associated with several facets of insulin resistance, including hyperinsulinemia, hypertriglyceridemia, a low HDL cholesterol concentration, and a slightly increased ambulatory systolic blood pressure. Suppression of serum FFA was also impaired in men with high compared with those with low LFAT. Although these data do not exclude the possibility that LFAT is, on the average, increased in markedly obese subjects, they support the idea that fat accumulation in an insulin-sensitive tissue is an important determinant of its sensitivity to insulin.

To our knowledge, hepatic sensitivity to insulin has not been previously quantitated in connection with quantification of hepatic fat in nondiabetic subjects. This turned out to

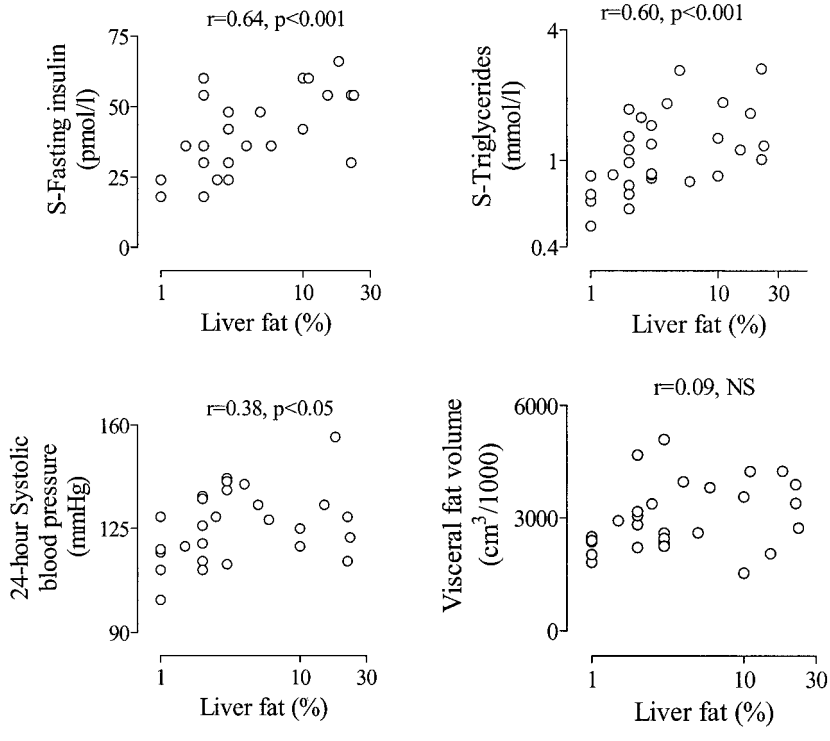

FIG. 3. Relationships between liver fat and serum fasting insulin (*upper panel* on the *left*), serum triglycerides (*upper panel* on the *right*), 24-h ambulatory systolic blood pressure (*lower panel* on the *left*), and intraabdominal fat volume determined with magnetic resonance imaging (*lower panel* on the *right*) in 30 apparently healthy men.

be of interest, because in the present study insulin stimulation of glucose uptake did not differ between the groups differing with respect to hepatic fat sensitivity content or insulin sensitivity. Consistent with the lack of a difference in peripheral glucose disposal, physical fitness, as determined directly by measuring VO_2max, was similar between the groups. We used a low dose insulin infusion to avoid completely suppressing glucose production and to allow quantification of the sensitivity of glucose production to insulin (27). These conditions were therefore not ideal for detecting interindividual differences in muscle insulin sensitivity. Keeping this precaution in mind, these human data support recent data in tissue-specific knockout mice, in which the phenotype varies depending on the tissue from which insulin action has been eliminated. For example, selective deletion of the insulin receptor from muscle results in slight increases in serum FFA and triglycerides, but not in hyperinsulinemia, glucose intolerance, or diabetes (28), whereas a similar maneuver in the liver leads to marked hyperinsulinemia under fasting conditions and in the fed state and diabetic glucose tolerance (29). In the present study the parameter best correlated with LFAT was fasting insulin, which is consistent with the liver being the key target for insulin action after an overnight fast (30). In humans, abnormal liver function is common among nondiabetic subjects and is a possible risk factor for type 2 diabetes. In the Third National Health and Nutrition Examination Survey, nonalcoholic fatty liver disease (NAFLD) was defined as in the present study and was found in 23% of the population (31). Adults with NAFLD were more than twice as likely to have diabetes than those without NAFLD, even after adjustment for BMI, age, gender, and race using logistic regression (31).

The finding of a significant association between LFAT and components of the metabolic syndrome is consistent with previous data, although overall adiposity has not been stringently controlled for. Thus, men with steatosis (14) or elevated serum aminotransferase levels (11) have been found to have higher triglyceride (11, 14) and insulin (14) and lower HDL cholesterol (11) concentrations, but in these studies the men with compared with those without steatosis or elevated liver enzymes also had markedly higher BMIs (11, 14) and waist to hip ratios (11). Alternatively, the studies have been descriptive without a control group and defined abnormal based on laboratory reference ranges (32). The study by Marceau *et al.* (13) included severely overweight patients, in whom intraoperative routine knife biopsies were taken in patients undergoing antiobesity surgery. In this study the correlation coefficients between liver steatosis and markers of insulin resistance (serum triglycerides and low HDL cholesterol, fasting blood glucose) were approximately 2-fold higher than those between steatosis and BMI ($r = 0.15$).

In the present study intraabdominal fat was not correlated with LFAT. This does not necessarily exclude the possibility that release of FFA by visceral (*i.e.* ip) fat contributes to fat accumulation in the liver, because intraabdominal fat includes not only visceral, but also retroperitoneal, fat. On the other hand, as recently reviewed by Frayn (33), the portal theory, *i.e.* that visceral fat perturbs metabolism by exposing the liver to high concentrations of FFA, is currently no more than a hypothesis lacking experimental support. For example, FFA released from the splanchnic bed account for only about 10% of the FFA reaching the liver (34). More recent studies using selective catheterization of the hepatic vein to determine the maximal contribution of visceral and mesenteric fat depots to increased fatty acid release in upper body obese women have concluded that the major source is not the visceral depot, but rather upper body nonsplanchnic sc tissue (35, 36). It is therefore unclear whether visceral fat is a "major culprit" or an "innocent bystander."

The flux of FFA to the liver could originate from three sources: hydrolysis of dietary chylomicrons, FFA from endogenous stores, especially adipose tissue, and *de novo* lipogenesis (37). In the present study we did not attempt to quantitate fat intake between the two groups, partly because in a relatively small group a negative result using food questionnaires could be subject to a type 2 statistical error. We did find, however, that the ability of insulin to suppress serum FFA was impaired in those with high *vs.* low LFAT. In several studies failure of insulin to normally lower serum FFA has been shown to reflect impaired suppression of lipolysis (38), but in the absence of turnover data this cannot be concluded with certainty. Impaired suppression of FFA could reflect not only resistance of lipolysis in adipose tissue, but also excessive intravascular lipolysis of triglyceride-rich lipoproteins or impaired FFA clearance (39).

Although the features of insulin resistance observed in the present study could, based on available mechanistic information, be consequences of hepatic insulin resistance, this study is cross-sectional and does not prove cause and effect. The present data do, however, support the emerging concept that fat accumulation in insulin-sensitive tissues is deleterious for insulin action and that this can occur independent of overall adiposity. If hepatic insulin resistance indeed was a consequence of increased hepatic fat, its reduction might be a new therapeutic target. In this respect it is of interest that the main mechanism by which metformin lowers blood glucose concentrations involves inhibition of steroid-responsive element-binding protein 1 expression and hepatic steatosis (40).

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