Effect of liver fat on insulin clearance

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Kotronen A, Vehkavaara S, Seppälä-Lindroos A, Bergholm R, Yki-Järvinen H. Effect of liver fat on insulin clearance. Am J Physiol Endocrinol Metab 293: E1709–E1715, 2007.—A fatty liver is associated with fasting hyperinsulinemia, which could reflect either impaired insulin clearance or hepatic insulin action. We determined the effect of liver fat on insulin clearance and hepatic insulin sensitivity in 80 nondiabetic subjects [age 43 ± 1 yr, body mass index (BMI) 26.3 ± 0.5 kg/m2]. Insulin clearance and hepatic insulin resistance were measured by the euglycemic hyperinsulinemic (insulin infusion rate 0.3 mU·kg⁻¹·min⁻¹ for 240 min) clamp technique combined with the infusion of [3-3H]glucose and liver fat by proton magnetic resonance spectroscopy. During hyperinsulinemia, both serum insulin concentrations and increments above basal remained ~40% higher (P < 0.0001) in the high (15.0 ± 1.5%) compared with the low (1.8 ± 0.2%) liver fat group, independent of age, sex, and BMI. Insulin clearance (ml·kg fat free mass⁻¹·min⁻¹) was inversely related to liver fat content (r = −0.52, P < 0.0001), independent of age, sex, and BMI (r = −0.37, P = 0.0001). The variation in insulin clearance due to that in liver fat (range 0–41%) explained on the average 27% of the variation in fasting serum insulin concentrations. The contribution of impaired insulin clearance to IS-insulin concentrations increased as a function of liver fat. This implies that indirect indexes of insulin sensitivity, such as homeostatic model assessment, overestimate insulin resistance in subjects with high liver fat content. Liver fat content correlated significantly with IS-insulin concentrations adjusted for insulin clearance (r = 0.43, P < 0.0001) and with directly measured hepatic insulin sensitivity (r = −0.40, P = 0.0002). We conclude that increased liver fat is associated with both impaired insulin clearance and hepatic insulin resistance. Hepatic insulin sensitivity associates with liver fat content, independent of insulin clearance.

hepatic insulin resistance; homeostasis model assessment; fasting serum insulin

THE LIVER IS THE PRIMARY SITE of insulin clearance (6). In nondiabetic subjects, 50–70% of the insulin secreted into the portal system is removed by the liver during first-pass transit measured either by hepatic catheterization techniques (2, 31, 41) or by calculating the ratio of the total production of insulin, determined by peripheral C-peptide concentrations, to the area under the peripheral insulin curve (27). In advanced liver disease, insulin clearance is decreased (10, 17, 25, 45), which is considered to be one of the main causes of hyperinsulinemia in liver cirrhosis (10, 17).

Intrahepatic fat content, when measured by histology, can vary from virtually 0 to 100%. The amount of liver fat due to nonalcoholic causes increases in parallel with increasing obe-

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glucose concentrations and for the biochemical measurements [serum (S)-alanine aminotransferase, S-aspartate aminotransferase, S-triglycerides, S-high density lipoprotein (HDL) concentrations, S-C-peptide, and S-free fatty acid (FFA)]. After 120 min, insulin was infused in a primed, continuous, (0.3 mU·kg⁻¹·min⁻¹) fashion, as previously described (30). Plasma glucose was maintained at 5 mmol/l (90 mg/dl) until 360 min using a variable rate infusion of 20% glucose (5). Blood samples for measurement of glucose-specific activity and FFA concentrations were taken at 90, 100, 110, and 120 min and at 15- to 30-min intervals between 120 and 360 min. Serum-free insulin was measured at 0, 120, 150, and 180 min, and at 60-min intervals between 240 and 360 min. Glucose-specific activity was determined as previously described (29). Glucose rate of appearance was calculated using the Steele equation, assuming a pool fraction of 0.65 for glucose and a distribution volume of 200 ml/kg for glucose (34).

**Insulin clearance.** Insulin clearance was calculated by dividing the rate of insulin infusion [mU·kg⁻¹·fat-free mass (FFM)⁻¹·min⁻¹] by the steady-state serum insulin concentration measured between 150 and 360 min (12). Insulin sensitivity to serum insulin was calculated by subtracting S-insulin concentration from the steady-state serum insulin concentration (150–360 min).

**Liver fat content (proton spectroscopy).** Localized single-voxel (2 × 2 × 2 cm³) proton spectra were acquired using a 1.5-T whole body system (Siemens Magnetom Vision, Erlangen, Germany), which consisted of a combination of whole body and loop surface coils for radio-frequency transmitting and signal receiving, as previously described (30). T1-weighted high-resolution MRI scans were used for localization of the voxel of interest within the right lobe of the liver. Magnetic resonance spectroscopy measurements of the liver fat were performed in the middle of the right lobe of the liver at a location that was individually determined for each subject; vascular structures and subcutaneous fat tissue were avoided when selecting the voxel. Subjects lay on their stomachs on the surface coil, which was embedded in a mattress to minimize abdominal movement due to breathing. The single-voxel spectra were recorded using the stimulated echo acquisition mode sequence, with an echo time of 20 ms, a repetition time of 3,000 ms, a mixing time of 30 ms, 1,024 data points over 1,000-KHz spectral width, with 32 averages. Water-suppressed spectra with 128 averages were also recorded to detect weak lipid signals. A 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 (H 2 O). The methylene signal, which represents intracellular triglyceride, was measured at 1.4 parts/million. Signal intensities were quantified by using an analysis program, VAPRO-MRUI (http://www.mrui.uab.es/mrui/). Spectroscopic intracellular triglyceride content (liver fat) was expressed as a ratio of the area under the methylene peak to that under the methylene and water peaks (×100 = liver fat %). This measurement has been validated against histologically determined lipid content (38) and against estimates of fatty degeneration or infiltration by X-ray computer-assisted tomography (30). All liver fat results other than the liver fat measured by histology (vide infra) are given in the actual units of measurement, i.e., using the spectroscopy data. When measured by proton spectroscopy, normal liver fat is ~5% (37, 42). All spectra were analyzed by physicians who were unaware of any of the clinical data. The reproducibility of repeated measurements of liver fat in nondiabetic subjects studied on two separate occasions by the same reader in our laboratory is 11% [derived from the coefficient of variability (CV) between measurements, CV = SD/mean × 100%] (36). In the low very low range of LFAT (~3%), values were reported as integers (except for two subjects).

**Histological vs. spectroscopic liver fat.** For the present study, a liver biopsy was taken in 13 patients with suspected nonalcoholic steatosis under ultrasound control. Fat content of the liver biopsy specimens (%fat-laden hepatocytes) was analyzed by an experienced liver pathologist in a blinded fashion.

**Analytic procedures, calculations, and other measurements.** Plasma glucose concentrations were measured in duplicate with the glucose oxidase method using Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA) (13). Serum free insulin concentrations were measured with the Auto-DELFIA kit (Wallac, Turku, Finland), and C-peptide concentrations by radioimmunoassay (16). S-HDL cholesterol and S-triglyceride concentrations were measured with the enzymatic kits from Roche Diagnostics (Roche Diagnostics Hitachi, Hitachi, Tokyo, Japan). S-alanine aminotransferase and S-aspartate aminotransferase activities were determined as recommended by the European Committee for Clinical Laboratory Standards. S-FFA concentrations were measured using a fluorometric method (24).

The percent body fat was determined using bioelectrical impedance analysis (BioElectrical Impedance Analyzer System, model number BIA-101A, RJL Systems, Detroit, MI) (19). Waist circumference was measured midway between spinia iliaca superior and the lower rib margin, and hip circumference at the level of the greater trochanters (21).

**Statistical analyses.** Nonnormally distributed data were used after logarithmic transformation. The unpaired Student’s t-test was used to compare mean values between groups. Analysis of covariance was used to adjust for age, sex, and body mass index (BMI). Correlation analyses were performed using Spearman’s nonparametric rank correlation coefficient. Analysis of covariance was used to compare slopes of regression lines between liver fat and S-insulin and S-insulin adjusted for insulin clearance. If neither the slopes nor the intercepts differed between women and men, a common regression equation was calculated for all data. Multiple linear regression analyses were used to determine the independent determinants of the variation in IS-insulin concentrations. For calculation of the percent variation in fasting insulin concentrations attributed to impaired insulin clearance due to liver fat, the difference between the percent derived from the multiple-regression analyses (insulin clearance and liver fat content) and the percent derived from simple regression analyses of one of the variables (e.g., insulin clearance) was subtracted from the percent derived from simple regression analyses of the other variable (e.g., liver fat). All data are shown as means ± SE. Calculations were made using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA) and SPSS 14.0 for Windows (SPSS, Chicago, IL). A P value of <0.05 was considered statistically significant.

**RESULTS**

**Histologically determined vs. spectroscopic liver fat.** The relationship between the percent liver fat determined by proton spectroscopy (calculated from the areas under the water and methylene peaks) and the percent fat in the liver biopsy (percentage of fat-laden hepatocytes) is shown in Fig. 1. The units of liver fat determined by these approaches differ (by definition), but histological liver fat can be calculated based on spectroscopy from the following equation: Liver fat by histology (%) = 3.5 ± 10.1 + 2.2 ± 0.5 × Liver fat by spectroscopy (%).

**Subject characteristics.** Liver fat averaged 2 and 15% in the low- and high-LFAT groups, respectively (Table 1). The low- and high-LFAT groups were similar with respect to age, systolic and diastolic blood pressure, and S-FFA concentrations. The high-LFAT group was more obese and had higher fasting plasma glucose and S-triglyceride, and lower S-HDL cholesterol concentrations than the low-LFAT group. S-insulin, S-C-peptide, and serum liver enzyme concentrations were higher in the high- compared with the low-LFAT group, independent of age, sex, and BMI.
Liver fat content was inversely related to the rate of insulin clearance (r = 0.81, P = 0.00008). Liver fat content did not correlate with fS-FFA (r = 0.11, not significant (NS)) but was significantly correlated with S-FFA concentrations during the insulin infusion (r = 0.55, P < 0.0001), independent of age, sex, and BMI (r = 0.37, P = 0.001). Insulin clearance did not correlate with S-FFA in the basal state (r = −0.07, NS) or during the insulin infusion (r = −0.18, NS). Both BMI (P = −0.25, P = 0.025) and waist (r = −0.30, P = 0.01) were significantly inversely correlated with insulin clearance, but these relationships became nonsignificant if adjusted for liver fat. Whole body fat percentage was not related to insulin clearance (r = −0.07, NS).

**Determinants of fS-insulin concentrations.** Liver fat content correlated significantly with fS-insulin concentrations (r = 0.62, P < 0.0001), independent of age, sex, and obesity (r = 0.34, P = 0.002). In simple linear regression analyses, liver fat content and insulin clearance explained 38 and 42% of the variation in fS-insulin concentrations, respectively. To determine the extent to which liver fat content contributed to the variation of fS-insulin concentrations due to impaired insulin clearance, multiple linear regression analyses were employed. Both liver fat (P < 0.0001) and insulin clearance (P < 0.0001) were independent determinants of fS-insulin concentrations and together explained 53% of their variation. Thus, on the average, 27% of the variation in fS-insulin concentrations were attributed to impaired insulin clearance due to liver fat content.

After adjusting for the rates of insulin clearance, liver fat content correlated significantly with fS-insulin (r = 0.43, P < 0.0001) and explained 22% of its variation (Fig. 4). The slopes of the regression lines were significantly different (P = 0.002).

### Hepatic insulin resistance.
Rates of endogenous glucose production did not differ between the groups in the basal state.

![Fig. 1](http://ajpendo.physiology.org/)
percent suppression of endogenous glucose production during the last hour of insulin infusion was significantly correlated with liver fat content ($r = -0.40, P = 0.0002$, Fig. 5) but was unrelated to insulin clearance ($r = 0.19$, NS). In multiple linear regression analysis, both hepatic insulin resistance ($P < 0.0001$) and insulin clearance ($P < 0.0001$) were independent determinants of fasting insulin concentrations and together explained 52% of their variation ($P < 0.0001$ for ANOVA). If liver fat content was incorporated into these analyses as an independent variable, hepatic insulin resistance ($P < 0.0001$), insulin clearance ($P < 0.0001$), and liver fat content ($P < 0.0001$) were independent determinants of the fasting insulin concentration ($r^2 = 59\%, P < 0.0001$ for ANOVA). Additional inclusion of fasting C-peptide did not abolish these significances (data not shown).

**DISCUSSION**

The liver is a major site of insulin action, clearance, and degradation (6). The majority (80%) of endogenously secreted
insulin is cleared by the liver, 15% by the kidney, and 5% by muscle (8). Of intravenously infused insulin, 50, 30, and 10%, respectively, are cleared by these tissues (8). Liver fat is closely correlated with fS-insulin concentrations (15, 42), but the extent to which impaired insulin clearance due to hepatic fat accumulation contributes to hyperinsulinemia has not previously been determined. In the present study, we found hepatic fat accumulation to be an important and independent regulator of insulin clearance, as measured by the euglycemic hyperinsulinemic clamp technique, i.e., after intravenous insulin administration. Impaired insulin clearance due to increased liver fat content explained on the average 27% of the variation in fS-insulin concentrations. The slopes of the regression lines relating liver fat and fS-insulin and fS-insulin corrected for insulin clearance differed significantly, implying that the contribution of impaired insulin clearance to fS-insulin concentrations increases with increasing liver fat content. Direct measurement of hepatic insulin sensitivity showed that liver fat was also associated with hepatic insulin resistance.

Hepatocytes loaded with triglycerides exhibit impaired insulin clearance in vitro (35). In a study of 46 nondiabetic subjects with a wide range of adiposity, the liver-to-spleen attenuation ratio, a qualitative marker of liver fat, has been found to be inversely related to insulin clearance (9). However, in this study, insulin clearance was not adjusted for percent body fat (9), and it thus remained unclear whether the apparent decrease in insulin clearance in those with high liver fat was due to liver fat or to differences in the insulin distribution space between the obese subjects with high liver fat compared with the nonobese subjects with less liver fat. Since fat is essentially water free, the plasma volume per body weight unit decreases with increasing percent body fat (1). Thus, when insulin is infused at a rate that is calculated based on kilograms of body weight or body surface area (9), insulin clearance will be lower in obese than in nonobese subjects (44). In the present study, we found that, during a prolonged low-dose insulin infusion, serum insulin concentrations and insulin increments were ~40% higher in subjects with high liver fat, independent of age, sex, and BMI. Consequently, insulin clearance was considerably impaired in the high- compared with the low-LFAT group. Although it would have been ideal to study weight-matched groups with high and low liver fat, the present data suggest that liver fat impairs insulin clearance independent of BMI.

It has been proposed that FFA-mediated reduction in insulin clearance is an adaptive mechanism that exacerbates hyperinsulinemia to overcome peripheral insulin resistance (18). In a previous study, Wiesenthal et al. (43) showed that, in dogs, hepatic insulin extraction is impaired when serum FFA concentrations are elevated to supraphysiological concentrations by infusions of a soy-based lipid emulsion and heparin during euglycemic hyperinsulinemia. In humans, a day-long infusion of a soy-based lipid emulsion and heparin (S-FFA ~500–800 μmol/l) has been shown to reduce insulin clearance in subjects with a family history of type 2 diabetes (14). In the present study, where no exogenous FFA were infused, fasting FFA were in this range but were uncorrelated with both liver fat and insulin clearance. However, the fS-insulin concentrations were twofold higher in the high- compared with the low-LFAT group. This difference in insulin levels in the face of similar FFA concentration most likely reflects adipose tissue insulin resistance and may make it difficult to observe a relationship between fasting FFA and insulin clearance.

Previous studies have shown that insulin clearance is decreased in obesity (23, 33). It has also been suggested that intra-abdominal rather than subcutaneous fat influences splanchnic insulin clearance (26). Moreover, insulin clearance increases by weight loss (28). The decrease in insulin clearance has also been observed in insulin-resistant compared with age- and BMI-matched insulin-sensitive subjects (12). However, the causes of the variation in insulin clearance have not been determined in these studies. Our data suggest that liver fat content is an important contributor to the variation in insulin clearance and could contribute to decreased insulin clearance in obesity (23, 33). Liver fat could also explain why weight-matched groups differing with respect to insulin sensitivity also may exhibit differences in insulin clearance. Furthermore, liver fat content is better correlated with intra-abdominal than subcutaneous fat (15, 42). Our laboratory has previously shown that a decrease in liver fat content, achieved by rosiglitazone therapy, increases insulin clearance and enhances hepatic insulin sensitivity significantly independent of body weight in type 2 diabetic patients (40). In addition, liver fat can be considerably reduced by weight loss (4, 39). Thus interventions that change liver fat also appear to change insulin clearance.

In the present study, on the average, 27% of the variation in fS-insulin concentrations could be explained by those in insulin clearance when fasting insulin concentrations were measured in subjects whose liver fat content varied over a wide range. The contribution of impaired insulin clearance to fasting hyperinsulinemia increased as a function of liver fat (Fig. 4). This implies that indirect measures of insulin sensitivity, such as the homeostatic model assessment of insulin resistance (22), overestimate insulin sensitivity in individuals in whom the insulin resistance is associated with, or perhaps due to, a fatty liver rather than, e.g., skeletal muscle. Note that the C-peptide-to-insulin ratio differed only marginally between the groups, possibly because C-peptide clearance is also subject to interindividual variation (7), which was not quantitated in this study. Nevertheless, we also showed by direct measurement of insulin sensitivity that hepatic insulin sensitivity decreases as a function of liver fat. This insulin resistance represented an underestimate of hepatic insulin resistance, as insulin concentrations increased with increasing liver fat content.

In conclusion, a fatty liver is associated with both hepatic insulin resistance and impaired insulin clearance. The impact of liver fat on insulin clearance increases as a function of liver fat. This implies that indirect measures of insulin sensitivity, such as fasting insulin, overestimate hepatic insulin resistance the more fat the liver contains.

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