Effects of insulin therapy on liver fat content and hepatic insulin sensitivity in patients with type 2 diabetes

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Juurinen L, Tiikkainen M, Häkkinen A-M, Hakkarainen A, Yki-Järvinen H. Effects of insulin therapy on liver fat content and hepatic insulin sensitivity in patients with type 2 diabetes. Am J Physiol Endocrinol Metab 292: E829–E835, 2007. First published November 7, 2006; doi:10.1152/ajpendo.00133.2006.—We determined whether insulin therapy changes liver fat content (L FAT) or hepatic insulin sensitivity in type 2 diabetes. Fourteen patients with type 2 diabetes (age 51 ± 2 yr, body mass index 33.1 ± 1.4 kg/m²) treated with metformin alone received additional basal insulin for 7 mo. Liver fat (proton magnetic resonance spectroscopy), fat distribution (MRI), fat-free and fat mass, and whole body and hepatic insulin sensitivity (6-h euglycemic hyperinsulinemic clamp combined with infusion of [3-3H]glucose) were measured. The insulin dose averaged 75 ± 10 IU/day (0.69 ± 0.08 IU/kg, range 24–132 IU/day). Glyco-sylated hemoglobin A1c (Hb A1c) decreased from 8.9 ± 0.3 to 7.4 ± 0.2% (P < 0.001). Whole body insulin sensitivity increased from 2.21 ± 0.38 to 3.08 ± 0.40 mg/kg fat-free mass (FFM)·min (P < 0.05). This improvement could be attributed to enhanced suppression of hepatic glucose production (HGP) by insulin (HGP 1.04 ± 0.28 vs. 0.21 ± 0.19 mg/kg FFM·min, P < 0.01). The percent suppression of HGP by insulin increased from 72 ± 8 to 105 ± 11% (P < 0.01). LFAT decreased from 17 ± 3 to 14 ± 3% (P < 0.05). The change in LFAT was significantly correlated with that in hepatic insulin sensitivity (r = 0.56, P < 0.05). Body weight increased by 3.0 ± 1.1 kg (P < 0.05). Of this, 83% was due to an increase in fat-free mass (P < 0.01). Fat distribution and serum adiponectin concentrations remained unchanged while serum free fatty acids decreased significantly. Conclusions: insulin therapy improves hepatic insulin sensitivity and slightly but significantly reduces liver fat content, independent of serum adiponectin.

IN THE BASAL STATE after an overnight fast, rates of glucose production and utilization are increased in patients with type 2 diabetes (15, 51). The increase in glucose utilization is due to the stimulatory effect of hyperglycemia, i.e., glucose mass action (53). Lowering of fasting glucose with insulin decreases rates of both glucose production and utilization (13). This implies that hepatic and not peripheral insulin sensitivity predicts insulin requirements during insulin therapy, and indeed hepatic insulin sensitivity, measured directly using the euglycemic clamp technique and an infusion of [3-3H]glucose, has been shown to be the best correlate of insulin requirements in type 2 diabetic patients (34). Hepatic glucose production is more sensitive to insulin than is peripheral glucose uptake in both normal subjects (53) and patients with type 2 diabetes (7).

If insulin sensitivity is measured using an insulin infusion rate of 1 mU·kg⁻¹·min⁻¹, hepatic glucose production is maximally suppressed, even in most type 2 diabetic patients, and therefore interindividual variation in hepatic insulin sensitivity is missed (7, 13, 14, 17, 18, 22, 29, 31, 35, 50). Therefore, direct measurement of hepatic insulin sensitivity necessitates use of a prolonged low-dose insulin infusion.

Hepatic insulin sensitivity, when measured directly or using fasting insulin as a marker, is closely correlated with liver fat content in normal men and women (45), patients with lipodystrophy (37), and type 2 diabetic patients (4, 21, 34). It is, however, unclear whether fat in the liver is a cause or a consequence of hyperinsulinemia. A short-term 67-h insulin infusion was found not to affect insulin sensitivity but to slightly increase hepatic lipid content (1). Although insulin inhibits lipolysis and stimulates lipogenesis in adipose tissue and lowers serum free fatty acids (FFA), which might reduce liver fat content, it also stimulates fatty acid and VLDL synthesis in the liver (23). Body weight also increases during insulin therapy in proportion to improved glycemic control (47), while weight loss reduces liver fat content (43). We have recently shown that peroxisome proliferator-activated receptor-γ (PPARγ) agonism but not metformin decreases liver fat content in type 2 diabetes (44). PPARγ agonism has also been shown to reduce liver fat content in uncontrolled (3, 4, 26) and placebo-controlled (8, 38) studies. The effect of PPARγ agonism may be mediated via increases in serum adiponectin, an adipokine exclusively produced in adipose tissue (3, 20, 44). Insulin has been shown acutely to increase adiponectin release but also to reduce adiponectin gene expression in vitro and to decrease serum adiponectin concentrations during acute infusions in vivo (20, 28, 54). Effects of chronic insulin therapy on serum adiponectin concentrations are unknown. In the present study, we wished to determine the effects of 7 mo of basal insulin therapy on hepatic insulin sensitivity, liver fat content, and serum adiponectin concentrations in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Subjects and Study Design

This study was an investigator-initiated study not financially supported by the industry. We recruited 14 patients with type 2 diabetes who were poorly controlled with metformin alone to be treated with additional basal insulin for 7 mo. We have previously shown, using techniques identical to those used in the present study, that treatment...
with metformin does not change liver fat content (44). Additional inclusion criteria were stable body weight and glycemic control for 6 mo before participation. Exclusion criteria were clinical evidence of cardiovascular or liver or other disease, treatment with drugs that may alter glucose tolerance, abnormal serum creatinine, macroalbuminuria, proliferative retinopathy, excessive alcohol consumption (>20 g/day), and drug abuse. The inclusion and exclusion criteria were reviewed at a screening visit, where the patients underwent a history and physical examination, and blood samples were taken for measurement of the blood count, serum creatinine, electrolytes, fasting plasma glucose, glycosylated hemoglobin (Hb A1c), liver enzymes, and lipids. An ECG was recorded, and a urine sample was taken to exclude patients with infections and macroalbuminuria. If a patient was considered eligible after the screening visit, metabolic studies [measurement of liver fat, insulin sensitivity of glucose rate of appearance (Rg) and rate of disappearance (Rd), body composition] were performed before and after 7 mo of treatment with basal insulin as detailed below. Baseline characteristics of the patients are shown in Table 1. The nature and potential risks of the study were explained to all subjects before obtaining their written, informed consent. The experimental protocol was approved by the ethical committee of the Helsinki University Central Hospital.

Methods

In vivo insulin action on glucose production and utilization. Patients were admitted to the hospital on the evening before the study. At 6 PM, an indwelling 18-gauge catheter (Venflon, Viggo-Spectramed, Helsingborg, Sweden) equipped with an obturator was inserted in an antecubital vein. On this evening before the study, the patients did not take their bedtime insulin injection. To determine glucose Rg and Rd, a primed continuous intravenous infusion of [3-3H]glucose was started at 4 AM and continued for a total of 11 h. The priming dose of [3-3H]glucose was adjusted according to the fasting blood glucose concentration measured at 4 AM as follows: priming dose = [glucose (mmol/l) at 4 AM/5] × 20 μCi/min. This dose was infused intravenously over 10 min and was followed by a continuous-rate infusion of [3-3H]glucose at a rate of 0.2 μCi/min as previously described (44). Before the start of the insulin infusion, another catheter was inserted in a retrograde position in a heated dorsal hand vein for withdrawal of arterialized venous blood. Baseline blood samples were taken for measurement of fasting plasma glucose; glucose specific activity (SA); Hb A1c; triglycerides; total, HDL, and LDL cholesterol; adiponectin, FFA; and serum free insulin concentrations. At 9 AM, after a 300-min equilibrium period, a primed continuous (0.3 mU/kg/min) infusion of insulin (Actrapid Human, Novo Nordisk, Bagsvaerd, Denmark) was started, as previously described. Because hepatic glucose production is more sensitive to suppression by insulin than muscle glucose uptake (53), we chose a low insulin infusion rate to be able to accurately assess hepatic insulin sensitivity. Plasma glucose was adjusted to and then maintained at ~8 mmol/l (144 mg/dl) for 360 min. This was done using a variable-rate infusion of 20% glucose based on plasma glucose measurements, which were made from arterialized venous blood every 5–10 min. Blood samples for measurement of glucose SA were taken basally at 270, 280, and 300 min and at 420, 480, 540, 580, 600, 630, and 660 min during the insulin infusion. Serum free insulin concentrations were measured every 60 min and serum FFA at 300, 310, 315, 320, 325, and 330 min and then at 30- to 60-min intervals during the insulin infusion. 

[3-3H]glucose SA and calculation of glucose kinetics. To determine glucose SA, plasma was deproteinized with Ba(OH)2 and ZnSO4 and evaporated as described (33). The dried glucose residue was resuspended and counted in a double-channel liquid scintillation counter (Rackbeta 1215, Wallac, Turku, Finland) after addition of 10 ml of Aquasol liquid scintillation fluid (NEN-DuPont, Boston, MA) and correction for quenching. The [3-3H]glucose SA (dpm/μmol) was calculated by dividing the disintegrations per minute in 0.3 ml of plasma by the plasma glucose concentration (μmol/ml). The infusion was diluted 1:100 and 1:1,000, and duplicates were counted to determine the infused [3-3H] concentration. Glucose Rg and Rd were calculated using the Steele equation, assuming a pool fraction of 0.65 for glucose and distribution volume of 200 ml/kg for glucose. Endogenous glucose Rg was calculated by subtracting the exogenous glucose infusion rate required to maintain euglycemia during hyperinsulinemia (300–660 min) from the rate of total glucose Rg. The percent suppression of basal endogenous glucose Rg during the last 2 h (540–660 min) by insulin was used as a measure of hepatic insulin sensitivity, i.e., the sensitivity of endogenous glucose production to insulin (%suppression of endogenous Rg).

Substrate Oxidation Rates

Glucose and lipid oxidation rates were measured with indirect calorimetry using the Deltatrac Metabolic Monitor (Datex, Helsinki, Finland) as previously described (12, 33). The measurements were performed between 210 and 250 min during the basal period and between 540 and 580 min during hyperinsulinemia. Samples of inspired and expired air, which were suctioned at 40 l/min, were analyzed for O2 and CO2 concentration differences using paramagnetic O2 and CO2 analyzers, respectively. The hood was placed on the subject’s head 10 min before the measurement was started. Urine was collected during the study, and the protein oxidation rate was estimated from urea nitrogen excretion (1 g nitrogen = 6.25 g protein). The following constants were used for calculation of glucose and lipid oxidation rates from gas exchange data: oxidation of 1 g of protein requires 966 ml of O2 and produces 782 ml of CO2, of 1 g of glucose requires 746 ml of O2 and produces 746 ml of CO2, and of 1 g of lipid requires 2,029 ml of O2 and produces 1,430 ml of CO2. The rate of nonoxidative glucose disposal was calculated by subtracting the rate of glucose oxidation from the rate of total glucose disposal.

Liver Fat Content (Proton Magnetic Resonance Spectroscopy)

Localized single-voxel (2 × 2 × 2 cm3) proton spectra were recorded using a 1.5-T whole body system (Siemens Magnetom Vision, Erlangen, Germany), consisting of the combination of whole body and loop surface coils for radiofrequency transmitting and signal receiving. T1-weighted high-resolution magnetic resonance images were used for localization of the voxel of interest within the right lobe of the liver. Vascular structures and contaminating signals from

Table 1. Physical and biochemical characteristics of the 14 type 2 diabetic patients before and after 7 mo of insulin therapy

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb A1c, %</td>
<td>8.9±0.3</td>
<td>7.4±0.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/l*</td>
<td>10.7±0.7</td>
<td>8.4±0.6</td>
<td>0.001</td>
</tr>
<tr>
<td>S-ALT, U/l</td>
<td>51±10</td>
<td>40±5</td>
<td>NS</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>101±6</td>
<td>104±6</td>
<td>0.02</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>33.1±1.4</td>
<td>34.0±1.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>1.01±0.02</td>
<td>1.02±0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>69.5±3.8</td>
<td>72.0±4.0</td>
<td>0.004</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>31.8±2.6</td>
<td>32.3±2.6</td>
<td>NS</td>
</tr>
<tr>
<td>Intra-abdominal fat, dm³</td>
<td>3.5±0.3</td>
<td>3.5±0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Abdominal subcutaneous fat, dm³</td>
<td>5.3±0.3</td>
<td>5.4±0.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are means ± SE. Physical and biochemical characteristics of the 14 type 2 diabetic patients (11 men, 3 women; age, 51 ± 2 yr; duration of diabetes, 7 ± 1 yr) before and after 7 mo of insulin therapy. Hb A1c, glycosylated hemoglobin (Hb A1c); S-ALT, serum alanine aminotransferase; NS, not significant. *Single measurement of fasting plasma glucose before the insulin clamp study.
subcutaneous fat and the gallbladder were avoided in localization of the voxel. Subjects were lying 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 by 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, and 1,024 data points over 1,000-Hz spectral width with 32 averages. Water-suppressed spectra with 128 averages were also recorded to detect weak lipid signals. The short echo time and the 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 parts/million (ppm). The methylene signal, which represents intracellular triglyceride, was measured at 1.4 ppm. Signal intensities were quantified by using the analysis program Magnetic Resonance User Interface (MRUI; http://www.mrui.uab.es/mrui/). Spectroscopic intracellular triglyceride content was expressed as methylene/(water + methylene) signal area ratio × 100. This measurement of liver fat by proton magnetic resonance spectroscopy has been validated against chemically determined lipid content of liver biopsies in humans and against estimates of fatty degeneration or infiltration by computed tomography (24, 39, 42). All spectra were analyzed by a physicist who was unaware of any of the clinical data. The measurements were performed in the evening at 8 PM after a 4-h fast. Previous studies have shown that liver fat content is not influenced acutely when measured 4 h after a high-fat meal (40). The reproducibility of repeated measurement of liver fat in nondiabetic subjects studied on two occasions in our laboratory is 11%.

Measurements of Body Composition

Intra-abdominal and subcutaneous fat (MRI). A series of T1-weighted transaxial 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 mm², 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 (37).

Fat mass and fat-free mass. Fat-free and fat mass were determined by bioelectrical impedance analysis (BioElectrical Impedance Analyzer System model no. BIA-101A, RJL Systems, Detroit, MI) (25). Waist circumference was measured midway between the lower rib margin and the iliac crest, and hip circumference over the great trochanters.

Other Analytic Procedures

Plasma glucose concentrations were measured in duplicate by the glucose oxidase method using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Serum free insulin concentrations were measured using the Auto-DELFIA kit from Wallac. Hb A1c was measured by high-pressure liquid chromatography using the fully automated Glycosylated Hemoglobin Analyzer System (Bio-Rad, Richmond, CA). 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, Japan). LDL cholesterol was calculated using the formula of Friedewald. Serum alanine aminotransferase (ALT) activity was determined by a method recommended by the European Committee for Clinical Laboratory Standards. Serum adiponectin concentrations were measured by an ELISA kit from Bridge International (San Jose, CA) and serum FFA concentrations by a fluorometric method (27).

Statistical Analyses

The paired t-test was used to compare changes before and after insulin treatment for normally distributed parameters. Correlation analyses were performed using the Spearman nonparametric correlation coefficient. All calculations were made using 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

The insulin dose averaged 75 ± 10 IU/day (0.69 ± 0.08 IU/kg, range 24–132 IU/day). Body weight increased by 3.0 ± 1.1 kg (P < 0.05; Table 1). This was mainly fat-free mass, which increased by 2.5 ± 0.7 kg. Fat distribution and fat mass remained unchanged (Table 1).

Liver Fat Content and Serum ALT

Liver fat content (LFAT) decreased slightly but significantly by 20% from 17 ± 3 to 14 ± 3% (P = 0.04; Fig. 1). LFAT and the insulin dose at 7 mo were significantly correlated (r = 0.63, P < 0.05; Fig. 1). LFAT correlated significantly with serum ALT concentrations (r = 0.77, P < 0.01) and with the amount of intra-abdominal fat (r = 0.74, P < 0.01) and fasting insulin (r = 0.57, P < 0.05). The volume of intra-abdominal fat also correlated with serum ALT concentrations (r = 0.77, P < 0.01).

![Fig. 1. Top: individual values of liver fat content before and after 7 mo of insulin therapy. Bottom: relationship between insulin dose at 7 mo and liver fat content. The hatched line is the line of identity.](http://ajpendo.physiology.org/DownloadedFrom/10.2307/3099062)
Glucose Metabolism, FFA Concentrations, and Substrate Oxidation Rates in the Basal State

Basal Rₜ decreased from 3.42 ± 0.20 to 2.66 ± 0.33 mg·kg⁻¹·min⁻¹ (P < 0.05) and basal Rₐ from 3.28 ± 0.27 to 2.40 ± 0.31 mg·kg⁻¹·min⁻¹ (P < 0.01; Fig. 2). Serum fasting FFA decreased from 970 ± 75 to 751 ± 51 μmol/l (P < 0.05) and serum triglyceride concentrations from 2.7 ± 0.4 to 2.0 ± 0.2 mmol/l (P < 0.05). Serum LDL [2.5 ± 0.3 vs. 2.2 ± 0.2 mmol/l, before vs. after, not significant (NS)] and HDL (1.1 ± 0.1 vs. 1.2 ± 0.1 mmol/l) cholesterol concentrations remained unchanged.

Rates of substrate oxidation in the basal state showed a trend to change toward less lipid oxidation (1.56 ± 0.14 vs. 1.24 ± 0.20 mg·kg⁻¹·min⁻¹, P = 0.15) and increased carbohydrate oxidation (0.49 ± 0.20 vs. 1.28 ± 0.47 mg·kg⁻¹·min⁻¹, P = 0.09). The rate of protein oxidation remained unchanged (0.058 ± 0.004 vs. 0.063 ± 0.006 mg·kg⁻¹·min⁻¹, NS).

Insulin Sensitivity

Before the insulin infusion (no insulin injection the previous evening), serum free insulin concentrations averaged 11 ± 2 vs. 14 ± 4 mU/l for before vs. after insulin therapy (NS). During the insulin infusion, serum free insulin concentrations averaged 33 ± 3 and 41 ± 6 mU/l before and after insulin therapy, respectively (NS). Plasma glucose concentrations during the last 2 h of the insulin infusion (540–660 min) averaged 145 ± 2 and 144 ± 1 mg/dl (NS). Whole body insulin sensitivity increased from 2.2 ± 0.4 to 3.1 ± 0.4 mg·kg⁻¹·min⁻¹ (P < 0.05). This improvement was due to enhanced hepatic insulin sensitivity, since hepatic glucose production was better suppressed by insulin after than before insulin therapy (0.21 ± 0.19 vs. 1.04 ± 0.28 mg·kg⁻¹·min⁻¹, respectively, P < 0.01), and the percent suppression of hepatic glucose production (HGP) by insulin increased from 72 ± 8 to 105 ± 11% (P = 0.001; Fig. 2). The change in LFAT was significantly correlated with the change in HGP suppression by insulin (difference in mg·kg⁻¹·min⁻¹, r = 0.56, P < 0.05). Serum FFA concentrations during the insulin infusion were significantly lower after (439 ± 42 μmol/l) than before (563 ± 66 μmol/l, P < 0.05) insulin therapy. The percent suppression of serum FFA by insulin remained unchanged (42 ± 12 vs. 42 ± 17%, respectively, NS).

Insulin-stimulated Rₜ remained unchanged (3.3 ± 0.4 vs. 3.2 ± 0.4 mg·kg⁻¹·min⁻¹, NS), but the fraction of glucose Rₜ directed toward oxidation increased significantly from −7 ± 6 (−0.40 ± 0.28 mg·kg⁻¹·min⁻¹) to 30 ± 3% (1.24 ± 0.12 mg·kg⁻¹·min⁻¹, P < 0.001). The rate of lipid oxidation decreased significantly from 1.88 ± 0.15 to 1.09 ± 0.06 mg·kg⁻¹·min⁻¹ (P < 0.001). Rates of total energy expenditure remained unchanged (data not shown), as did serum adiponectin concentrations (4.6 ± 0.5 vs. 4.2 ± 0.4 mg/l, NS).

DISCUSSION

These data are the first to examine effects of long-term insulin therapy on liver fat content. We demonstrate that insulin, at doses high enough to achieve reasonably good glycemic control, does not adversely influence liver fat content in patients with type 2 diabetes. Indeed, a small but significant decrease in liver fat content was observed. Hepatic insulin sensitivity, as measured directly by the ability of insulin to suppress hepatic glucose production, increased significantly. Serum FFA concentrations and rates of lipid oxidation decreased, while serum adiponectin concentrations remained unchanged.

A total of 18 studies have examined effects of insulin therapy on insulin sensitivity in type 2 diabetes. In 14 of these 18 studies, a significant improvement in whole body or peripheral glucose uptake (5, 6, 9, 13, 14, 16, 18, 22, 31, 32, 35, 41, 50, 55) or in the metabolic clearance rate of glucose (2, 17) was observed. The median duration of these studies was 4 wk and number of patients 10. In all except two studies (13, 29), the insulin infusion rate was higher than that used in the present study. With regard to the studies finding no improvement in peripheral glucose uptake, either a low insulin infusion rate similar to that in the present study was used (13, 29), the
duration of insulin infusion was longer before than after insulin therapy (leading to underestimation of glucose uptake after insulin therapy) (19), or glucose concentrations were higher during the insulin infusion before than after insulin therapy (also underestimates glucose uptake after insulin therapy) (29).

The finding of no change in insulin-stimulated glucose uptake in the present study is consistent with the studies of Nankervis et al. (29) and Firth et al. (13). It does not exclude an improvement at higher insulin concentrations, but a low physiological insulin infusion rate was specifically chosen to achieve as physiological an insulin concentration as possible and to maximize the possibility of observing an improvement in hepatic insulin sensitivity. In the basal state, before the start of insulin infusion, the rate of glucose utilization decreased significantly by insulin therapy, in keeping with other studies (13, 14, 17, 18, 22, 29, 31, 35, 50, 55), as did the ability of glucose itself to promote glucose utilization at a fixed insulin concentration (the mass-action effect of glucose) (53).

As discussed above, although effects of insulin therapy on whole body insulin sensitivity have been extensively investigated, most studies used supraphysiological insulin concentrations that completely suppressed hepatic glucose production and thereby ignored changes in hepatic insulin sensitivity. In keeping with present data, enhanced suppression of hepatic glucose production by insulin was observed by Firth et al. (13), Pisu et al. (31), and Yu et al. (55). We now extend these data by showing that hepatic fat content decreases during chronic insulin therapy. The 20% decrease (from 17.4 to 13.9%) in liver fat content is one-half of what has been observed during treatment with PPARγ agonists in studies of patients with type 2 diabetes (mean 40%, range 32–51%) (52) or what is observed during a moderate weight loss of 7 kg (43). Since the normal value for liver fat is maximally 5% (40, 46), the liver was still fatty and therefore also insulin resistant at the end of insulin therapy. We found a significant correlation in the present study between changes in liver fat and hepatic insulin sensitivity, in keeping with our previous cross-sectional studies, which included 20 type 2 diabetic patients in one study (34) and 30 middle-aged healthy men in another (36).

With regard to factors enhancing hepatic insulin sensitivity during insulin therapy, adiponectin was not responsible, since at least total serum adiponectin levels remained unchanged. Serum FFA concentrations were decreased by insulin therapy. Assuming this was due to insulin inhibition of lipolysis, the reduction in liver fat and enhanced hepatic insulin sensitivity could in part have been due to reduced FFA flux to the liver. Fasting serum free insulin concentrations were similar before and after insulin therapy, since insulin was not administered in the evening before the repeat metabolic measurements. We have, however, previously measured diurnal insulin profiles before and during basal insulin therapy (48) and shown that insulin therapy does induce hyperinsulinemia, which in turn would be expected to stimulate rather than inhibit lipogenesis in the liver. Since liver fat content decreased, the metabolic alterations favoring inhibition of lipogenesis or other alterations resulting in reduced hepatic triglyceride stores must have dominated. Recently, quantification of the relative contribution of various sources to hepatic triglycerides in patients with nonalcoholic fatty liver disease revealed that, even under fasting conditions, a significant proportion of liver triglycerides originated from de novo lipogenesis (11). A decrease in glucose concentrations could therefore have reduced hepatic triglyceride stores by limiting the availability of two-carbon precursors for de novo lipogenesis. However, since neither changes FFA, insulin or glucose correlated with those in liver fat or in hepatic insulin sensitivity, all these possibilities are speculative and remain to be proven in humans by direct measurements.

As discussed above, when measured under conditions of everyday living rather than a clamp, glucose disposal decreases basally and remains unchanged postprandially by insulin therapy (30). This implies that inhibition of hepatic glucose production is the mechanism via which insulin lowers glucose. We have previously found a close correlation between liver fat and insulin requirements in type 2 diabetic patients, independent of body weight (34). S-ALT, a marker of liver fat (Fig. 2), also predicts insulin requirements independent of body weight (49). In the present study, we found a positive correlation between fasting insulin before insulin therapy and liver fat. This is consistent with hepatic insulin resistance increasing the need for endogenous insulin secretion to control hepatic glucose production. During insulin therapy, the need to use more insulin in those with an insulin-resistant fatty liver does not disappear. Consistent with these data, there is correlation between liver fat measured by proton magnetic resonance spectroscopy and the insulin dose (Fig. 1).

In conclusion, the present study shows that insulin therapy increases hepatic insulin sensitivity and slightly decreases liver fat content in obese type 2 diabetic patients. Importantly, chronic insulin therapy does not increase liver fat content. This supports the view that the hyperinsulinemia that accompanies a fatty liver is a consequence rather than cause of the fatty liver. On the other hand, during insulin therapy, FFA concentrations decrease, while in individuals with a fatty liver, hyperinsulinemia and liver fat content are positively correlated with each other, i.e., the higher the insulin concentration, the higher the FFA. We cannot determine whether liver fat was decreased by insulin because FFA were lowered.

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