Effect of Weight Loss on Liver Free Fatty Acid Uptake and Hepatic Insulin Resistance

Antti P. M. Viljanen, Patricia Iozzo, Ronald Borra, Mikko Kankaanpää, Anna Karmi, Riikka Lautamäki, Mikko Järvisalo, Riitta Parkkola, Tapani Rönnemaa, Letizia Guiducci, Terho Lehtimäki, Olli T. Raitakari, Andrea Mari, and Pirjo Nuutila

Turku Positron Emission Tomography (PET) Centre (A.P.M.V., P.I., R.B., M.K., A.K., R.L., M.J., L.G., P.N.), and Departments of Radiology (R.P., P.N.), Medicine (T.R.), and Clinical Physiology (O.T.R.), University of Turku and Turku University Hospital, Turku, Finland; PET Centre (P.I., L.G.), Institute of Clinical Physiology, Consiglio Nazionale delle Ricerche National Research Council, Pisa, Italy; Department of Clinical Chemistry (T.L.), University of Tampere and Tampere University Hospital; and Institute of Biomedical Engineering (A.M.), National Research Council, Padua, Italy

Objective: Weight loss has been shown to decrease liver fat content and whole-body insulin resistance. The current study was conducted to investigate the simultaneous effects of rapid weight reduction with a very-low-calorie diet on liver glucose and fatty acid metabolism and liver adiposity.

Hypothesis: We hypothesized that liver insulin resistance and free fatty acid uptake would decrease after weight loss and that they are associated with reduction of liver fat content.

Design: Thirty-four healthy obese subjects (body mass index, 33.7 $\pm$ 8.0 kg/m^2) were studied before and after a very-low-calorie diet for 6 wk. Hepatic glucose uptake and endogenous glucose production were measured with [^{18}F]fluorodeoxyglucose during hyperinsulinemic euglycemia and fasting hepatic fatty acid uptake with [^{18}F]fluoro-6-thia-heptadecanoic acid and positron emission tomography. Liver volume and fat content were measured using magnetic resonance imaging and spectroscopy.

Results: Subjects lost weight (11.2 $\pm$ 2.9 kg; $P < 0.0001$). Liver volume decreased by 11% ($P < 0.002$), which was partly explained by decreased liver fat content ($P < 0.0001$). Liver free fatty acid uptake was 26% lower after weight loss ($P < 0.003$) and correlated with the decrement in liver fat content ($r = 0.54$; $P < 0.03$). Hepatic glucose uptake during insulin stimulation was unchanged, but the endogenous glucose production decreased by 40% ($P < 0.04$), and hepatic insulin resistance by 40% ($P < 0.05$).

Conclusions: The liver responds to a 6-wk period of calorie restriction with a parallel reduction in lipid uptake and storage, accompanied by enhancement of hepatic insulin sensitivity and clearance. **(*J Clin Endocrinol Metab* 94: 50–55, 2009)**

Obesity is commonly associated with an accumulation of fat in the liver (1, 2), and liver adiposity correlates with insulin resistance in whole-body (3, 4) and hepatic insulin resistance (5). Low-calorie diets have been shown to reduce hepatic steatosis (6, 7) and improve insulin sensitivity (8). Liver fat content can be measured noninvasively with magnetic resonance spectroscopy (MRS) (9). Positron emission tomography (PET) has proved to be a superior tool for the direct assessment of liver glucose and free fatty acid (FFA) metabolism noninvasively (10, 11).

Increased flux in FFA via the portal vein from the visceral fat to the liver in obesity is proposed to be one of the mechanisms to cause accumulation of fat in the liver (12). Whether FFA uptake can be decreased by reduced energy intake and whether it induces changes in liver fat content has not been studied in obese humans.

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Abbreviations: BMI, Body mass index; [^{18}F]FDG, [^{18}F]fluorodeoxyglucose; FFA, free fatty acid; Ki, fractional rate of tracer uptake; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; OGIS, oral glucose insulin sensitivity; PET, positron emission tomography.

Current evidence suggests an inverse relationship between hepatic fat content and insulin-mediated hepatic glucose uptake because in patients with type 2 diabetes, insulin-mediated hepatic glucose uptake is reduced (13). Therefore, we hypothesized that liver fatty acid uptake is associated with liver fat content in obese individuals, and that the reduction in liver fat content induced by a very-low-calorie regimen parallels that of fatty acid uptake by the organ. We also assumed that weight reduction enhances insulin-stimulated hepatic glucose uptake and suppression of glucose output, resulting in an improvement in hepatic insulin sensitivity. PET and MRS were used as tools in the investigation.

Subjects and Methods

Subjects

The study included 34 obese [body mass index (BMI) >27 kg/m^2] subjects who were recruited from an occupational health service clinic. They were healthy except for overweight, as judged by medical history and physical examination, including anthropometric measurements and blood pressure measurement. Laboratory tests included an oral glucose tolerance test to exclude diabetes. No smoking was allowed, and use of alcohol was prohibited. Furthermore, patients with an eating disorder, known cardiovascular disease, hypertension or medication for hypertension, previous or present abnormal hepatic or renal function, or oral corticosteroid treatments were excluded. Written informed consent was obtained after the purpose and potential risks of the study had been explained to the subjects. The study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland and conducted according to the principles of the Declaration of Helsinki.

Study design

Insulin-stimulated glucose uptake was measured using [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) and euglycemic clamp from 16 consecutive subjects, and liver fatty acid uptake was measured from 18 consecutive subjects postprandially with [^{18}F] fluoro-6-thia-heptadecanoic acid ([^{18}F]FTHA). The study included 10 males and 24 females. One subject withdrew from the study, so 33 were finally included in the analyses. Because of radiation dose limits for scientific work, the same subjects were not studied with both radiotracers. Liver triglyceride content was studied in all subjects with MRS. After the screening phase, subjects participated in the first PET and MRS and magnetic resonance imaging (MRI) study. Thereafter, they were prescribed a very-low-calorie diet. All daily meals were replaced by dietary products for a period of 6 wk (Nutrifast; Leiras Finland, Novartis Medical Nutrition AB, Sweden; 2.3 MJ, 4.5 g fat, 59 g protein, and 72 g carbohydrate per day). At the end of the 6 wk of dieting, a 1-wk recovery period with a eucaloric diet was allowed to reverse the catabolic state. The assessments were repeated after this recovery period.

Image acquisition and processing

The positron emitting tracers, [^{18}F]FDG and [^{18}F]FTHA, were produced as previously described (14, 15). In [^{18}F]FDG studies, an eight-ring ECAT 931/08-tomograph was used for image acquisition (Siemens/CTI, Knoxville, TN). A 5-min transmission scan was performed with a removable ring source containing ^{68}Ge before the emission scan to correct for the tissue attenuation of γ photons. A bolus of [^{18}F]FDG was injected iv 50 min after the start of the euglycemic clamp, and dynamic scans were performed using 5×180 sec frames in liver and in skeletal muscle. In [^{18}F]FTHA studies, an integrated PET/CT, GE Discovery ST System (General Electric Medical Systems, Milwaukee, WI) was used for image acquisition. A bolus of [^{18}F]FTHA was injected iv, and a dynamic scan was performed using 4×300 sec frames in the fasting liver. An arterialized blood sample was drawn once during each time frame for measurement of plasma radioactivity. All data were corrected for dead time, decay, and photon attenuation.

Measurement of liver and muscle glucose uptake

Plasma and tissue [^{18}F]FDG time-activity curves were analyzed graphically (16) to quantify the fractional rate of tracer uptake (Ki). Lumped constants of 1.2 and 1.0 were used in the muscle and liver glucose uptake analysis, as previously validated (10, 17). The rate of glucose uptake was obtained by multiplying Ki by the plasma glucose concentration (10). Fractional uptake constant of [^{18}F]-FTHA (Ki) was calculated according to the graphical analysis of Patlak and Blasberg (16). Tissue FFA uptake was calculated by multiplying the Ki with the mean serum FFA concentration during the corresponding PET scan, and liver FFA uptake was calculated by multiplying tissue FFA uptake with the liver volume.For determination of the rate of whole-body glucose uptake, the euglycemic insulin clamp technique was used (18). Serum insulin was increased for 120 min using a primed-continuous (1 mU $\cdot$ kg^{-1} $\cdot$ min^{-1}) infusion of insulin (Actrapid; Novo Nordisk A/S, Bagsvaerd, Denmark). Normoglycemia was based on plasma glucose measurements performed every 5 min from arterialized blood; whole-body glucose uptake was calculated from the glucose infusion rate during the period of PET scanning and expressed per kilogram of body weight (μmol $\cdot$ min^{-1} $\cdot$ kg^{-1}).

An oral glucose tolerance test was done after an overnight fast within 3 d of the imaging studies. A commercial liquid product of 75 g of glucose was given orally, and venous blood sampling was done at -15, 0, 15, 30, 45, 60, 90, and 120 min. The oral glucose insulin sensitivity (OGIS) index was calculated as previously described (19). Endogenous glucose production was calculated as previously described (20).

Hepatic insulin resistance and fractional extraction

Hepatic insulin resistance was calculated as a product of endogenous glucose production and insulin levels (21). Insulin clearance was calculated from the oral glucose tolerance test as mean insulin secretion divided by mean insulin concentration during the oral glucose tolerance test. Insulin clearance from the euglycemic clamp was calculated as the insulin infusion divided by the steady-state insulin concentration during the clamp. Hepatic insulin fractional extraction was calculated as 1 $-$ (clamp insulin clearance)/(oral glucose tolerance test insulin clearance).

1H MRS and MRI studies

Patients were instructed to fast for 12 h before the 1H MRS examination. In addition to the 1H MRS measurement of hepatic triglyceride content, MRI was performed to obtain an anatomical reference for PET. Axial T1-weighted dual fast field echo images (echo time, 2.3 and 4.6 msec; repetition time, 120 msec; slice thickness, 10 mm without gap) covering the area of the liver was acquired during standardized breath-hold instructions. A 1.5 T magnetic resonance imager (Gyroscan Intera CV Nova Dual; Philips Medical Systems, Eindhoven, The Netherlands) with a flexible surface coil and body coil was used for MRI and MRS. A single voxel with a volume of 27 cm^3 was positioned in the liver outside the area of the great vessels. To ensure similar voxel placement before and after the intervention, the voxel location was recorded in each patient. A PRESS 1H MRS sequence was used with the following parameters: repetition time = 3000 msec, echo time = 25 msec, with data acquired during breath-hold intervals. 1H MRS findings of the liver have been validated in both animal and human studies (22, 23). Using a local workstation, liver margins were outlined manually on each individual image. Total liver volume was calculated by multiplying the measured surface areas of each slice by the slice thickness, as previously described (24). Additionally, a single T-1 weighted fast field echo image was obtained at the level of the intervertebral disc L2–L3 for analysis of abdominal adipose tissue masses as previously described (25). An adipose tissue density of 0.9196 g/ml was used for converting the measured volumes into weight.

Biochemical analyses

Arterialized plasma glucose was determined in duplicate by the glucose oxidase method (Analox GM9 Analyzer; Analox Instruments, London, UK). Glycosylated hemoglobin was determined by HPLC (Variant II; Bio-Rad, Hercules, CA). Plasma cholesterols and triglycerides were determined by the photometric enzymatic method (Modular P800; Roche Diagnostics GmbH, Mannheim, Germany). Plasma γ-glutamyl transferase was determined by photometric method (IFCC 2002, Modular P800; Roche Diagnostics GmbH). Serum C-peptide was determined by time-resolved immunofluorometric assay (AutoDELFIA, PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland). Serum insulin was determined by time-resolved immunofluorometric assay (AutoDELFIA, PerkinElmer Life and Analytical Sciences). Serum FFAs were determined by photometric enzymatic assay (NEFA C, ACS-ACOD; Wako Chemicals GmbH, Neuss, Germany; and Modular P800, Roche Diagnostics GmbH). Serum high-sensitivity C-reactive protein was analyzed with the sandwich immunoassay method using an Innotrac Aio1 immunoanalyzer (Innotrac Diagnostic, Turku, Finland).

Statistical analysis

Results are given as mean ± SD, unless stated otherwise. Effects of treatment were examined by comparing pretreatment and posttreatment values using the nonparametric Wilcoxon signed rank test. Differences between subgroups were calculated with the nonparametric Wilcoxon two-sample two-sided exact test. Univariate associations between the study variables were analyzed by calculating the Pearson's and Spearman's correlation coefficients when appropriate. All statistical analyses were performed using the statistical analysis system, SAS version 8.02 (SAS Institute Inc., Cary, NC).

Results

Whole-body and metabolic characteristics

Subjects lost 11.2 ± 2.9 kg of body weight ($P < 0.001$) after the very-low-calorie diet. Blood pressure was reduced by 12/9 mm Hg ($P < 0.001$ for both) (Table 1). Fasting glucose, insulin, and C-peptide decreased, whereas fasting serum FFA remained unchanged. Serum lipids, γ-glutamyl transferase, and high-sensitivity C-reactive protein also decreased (Table 2). Skeletal muscle insulin sensitivity increased by 35% ($P < 0.005$) and in line with this, whole-body insulin sensitivity improved by 32% ($P < 0.02$) (Table 3). There was no significant change in oral glucose tolerance test 2-h values.

TABLE 1. Effects of weight loss on anthropometrics (n = 33)

	Baseline	After weight loss	*P* value
Weight (kg)	98.2 ± 12.5	87.0 ± 11.4	<0.0001
BMI (kg/m^2)	33.7 ± 4.1	29.9 ± 4.0	<0.0001
Visceral fat mass (kg)	2.1 ± 0.7	1.5 ± 0.5	<0.0001
Waist circumference (cm)	105 ± 8.5	94 ± 7.8	<0.0001
Hip circumference (cm)	114 ± 7.4	106 ± 6.5	<0.0001
Waist to hip ratio	0.923 ± 0.065	0.920 ± 0.193	<0.0001
Systolic blood pressure (mm Hg)	140 ± 16.7	128 ± 16.7	<0.0001
Diastolic blood pressure (mm Hg)	93 ± 9.7	84 ± 10.2	<0.0001

Data are mean ± SD. *P* values compare pretreatment and posttreatment values using nonparametric Wilcoxon signed rank test.

TABLE 2. Effects of weight loss on laboratory parameters (n = 33)

	Baseline	After weight loss	*P* value
FP-glucose (mmol/liter)	5.8 ± 0.6	5.6 ± 0.5	<0.04
B-Hb_{A1C} (%)	5.6 ± 0.3	5.4 ± 0.3	<0.0001
FS-insulin (pmol/liter)	62.7 ± 39.5	55.3 ± 44.2	0.06
FS-C-peptide (nmol/liter)	0.74 ± 0.26	0.63 ± 0.22	<0.003
FS-FFA (mmol/liter)	0.74 ± 0.25	0.71 ± 0.30	0.21
Total cholesterol (mmol/liter)	4.7 ± 0.8	3.7 ± 0.6	<0.0001
HDL cholesterol (mmol/liter)	1.4 ± 0.3	1.3 ± 0.3	<0.01
Triglycerides (mmol/liter)	1.1 ± 0.5	0.9 ± 0.4	<0.0003
LDL cholesterol (mmol/liter)	2.8 ± 0.8	2.1 ± 0.6	<0.0001
γ−GT (U/liter)	27.1 ± 18.8	19.1 ± 12.7	<0.0001
C-reactive protein (mg/liter) (n = 16)	2.9 ± 2.2	1.4 ± 1.4	<0.001

Data are mean ± SD. *P* values compare pretreatment and posttreatment values using nonparametric Wilcoxon signed rank test. FP, Fasting plasma; FS, fasting serum; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Hb_{A1C}, glycosylated hemoglobin.

Liver metabolism and fat content

Liver volume decreased by 11% (from 1600 ± 340 ml to 1420 ± 300 ml; $P < 0.002$). This was partly explained by liver triglyceride content, which decreased by 60% ($P < 0.0001$). Although serum FFA concentrations did not differ, liver FFA uptake was 26% lower after the dieting period ($P < 0.003$), whereas insulin-mediated hepatic glucose uptake was nonsignificantly affected (Fig. 1). The endogenous glucose production, as determined from the FDG plasma radioactivity curves of 15 subjects, was decreased by 40% ($P < 0.04$). Consequently, the net balance of glucose across the liver (*i.e.* difference between

TABLE 3. Effects of weight loss on glucose uptake and insulin clearance rates (n = 16)

	Baseline	After weight loss	*P* value
Liver insulin-mediated glucose uptake (μmol/min · 100 ml)	2.3 ± 0.8	2.2 ± 0.7	0.86
Skeletal muscle glucose uptake (μmol/min · 100 ml)	4.8 ± 3.0	6.5 ± 3.1	<0.005
Whole-body insulin sensitivity (μmol/min · kg)	24.2 ± 10.1	31.9 ± 15.7	<0.02
Plasma glucose during clamp (mmol/liter)	5.1 ± 0.2	5.2 ± 0.3	0.14
Serum insulin during clamp (pmol/liter)	458 ± 96.8	441 ± 72.3	0.25
Endogenous glucose production (mmol/min)	0.65 ± 0.25	0.43 ± 0.30	<0.04
Insulin clearance rate in OGTT (liters/min · m^2)	1.27 ± 0.37	1.49 ± 0.37	<0.006
Insulin clearance rate in clamp (liters/min · m^2)	0.55 ± 0.11	0.63 ± 0.28	0.45

Data are mean ± SD. *P* values compare pretreatment and posttreatment values using nonparametric Wilcoxon signed rank test. OGTT, Oral glucose tolerance test.

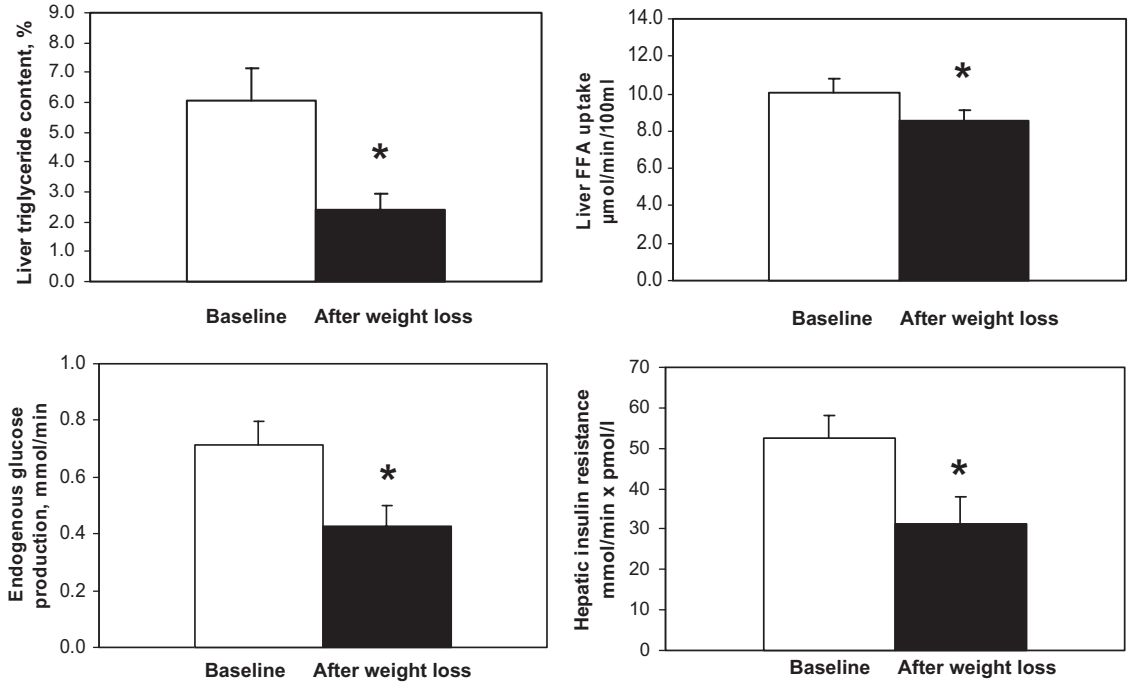

FIG. 1. Effects of weight loss on liver metabolism. *White bar*, Before weight loss; *black bar*, after weight loss. Data are mean ± SE. *, $P < 0.05$ compared with baseline.

uptake and release) was changed in favor of glucose retention as the organ demonstrated an improvement in hepatic insulin resistance ($P < 0.05$). In addition, hepatic insulin fractional extraction (n = 16) increased by 9% ($P < 0.05$).

Associations of liver fat

In univariate analysis, liver fat content was positively associated with whole-liver FFA uptake, visceral fat mass, fasting serum FFA levels, fasting blood glycosylated hemoglobin and fasting plasma triglyceride levels (Table 4).

Liver fat content was negatively associated with skeletal muscle insulin-stimulated glucose uptake both before and after the intervention ($r = -0.60$, $P < 0.02$; and $r = -0.68$, $P < 0.01$) and with the OGIS index ($r = -0.43$, $P < 0.04$; and $r = -0.57$, $P < 0.003$, respectively). Baseline values and changes in hepatic insulin fractional extraction were associated with those in skeletal muscle glucose uptake ($r = 0.72$, $P < 0.002$; and $r = 0.75$, $P < 0.002$, respectively) and the OGIS index ($r = 0.60$, $P < 0.02$; and $r = 0.56$, $P < 0.03$ respectively).

TABLE 4. Correlations of liver triglyceride content

	Liver triglyceride content	
	Baseline value	Changes after weight loss
Whole liver FFA uptake	$r = 0.50$, $P < 0.05$	$r = 0.54$, $P < 0.03$
Visceral fat mass	$r = 0.53$, $P < 0.002$	$r = 0.39$, $P < 0.04$
Serum FFA levels	$r = 0.36$, $P < 0.04$	$r = 0.41$, $P < 0.02$
Blood Hb_{A1C}	$r = 0.50$, $P < 0.003$	$r = 0.37$, $P < 0.04$
Serum triglycerides	$r = 0.40$, $P < 0.02$	$r = 0.36$, $P < 0.05$

Hb_{A1C}, Glycosylated hemoglobin.

Discussion

To our knowledge, this is the first study where liver glucose and fatty acid metabolism, together with liver triglyceride content, were evaluated simultaneously before and after weight loss induced by a very-low-calorie diet. As could be expected, liver triglyceride content per volume decreased by 60%, and liver volume decreased by 11% with dieting. When the metabolic rates before and after the 6-wk calorie restriction were compared, liver insulin resistance and especially hepatic fatty acid uptake were significantly decreased. These findings are accompanied by improved skeletal muscle insulin sensitivity and amelioration of all measured metabolic and hemodynamic variables.

Liver triglyceride content was on average 6.1% at baseline, with a mean BMI of 33.7 kg/m^2, and almost half of the subjects had values in excess of 5%, *i.e.* the diagnostic cutoff of steatosis (26, 27). After a very-low-calorie-diet of 6 wk, mean triglyceride content was 2.4% with a BMI of 29.9 kg/m^2, and only one eighth of the subjects had levels within steatotic range. Triglyceride content decreased after 6 wk of very-low-calorie diet in parallel with a decrease in visceral fat mass. This was consistent with the observation that 80% of the total loss in hepatic triglycerides induced by a very-low-calorie regimen occurs within 2 wk from the start of caloric restriction, as compared with a progressive, and slower decline in the visceral adipose tissue mass (28). The decrease in liver volume with dieting has previously been reported in the severely obese (24) but was also demonstrated here. Interestingly, it could only partly be explained by reduced triglyceride content. This might suggest that the volume is also decreased due to reduced inflammation of the liver. In agreement with previous studies, we found lower circulating concentrations

of high-sensitivity C-reactive protein, as well as γ-glutamyl transferase levels (29).

Liver FFA uptake was decreased in parallel with a decrease in liver fat when measured after a 1-wk normocaloric diet following the very-low-calorie diet period. The circulating fatty triglyceride levels correlated significantly with liver fat content, indicating excess dietary fat and increased substrate delivery in the development of hepatic steatosis (30), thus supporting this mechanistic link in the association of insulin resistance with liver fat content. Because fasting serum fatty acid concentrations were similar after the dieting period, the change in the uptake was due to direct effects on the intrinsic regulation of fatty acid uptake by the liver. The findings of a reduced fatty acid uptake, together with a lower lipid content in the liver, resemble those recently ascribed to physical fitness by Hannukainen *et al.* (31). In our current study, participants were instructed not to change their physical activity to avoid the overlapping effects of exercise; thus caloric restriction or physical activity alone may share similar outcomes in terms of hepatic lipid storage.

The rapid loss of body weight after 6 wk of very-low-calorie diet leads to the elevation of whole-body insulin sensitivity and skeletal muscle glucose uptake, which is in line with the literature (8). Mean fasting C-peptide levels decreased, and fasting insulin levels tended to decrease ($P = 0.06$) with the diet intervention in parallel with increased hepatic insulin fractional extraction. Importantly, the endogenous glucose production and liver insulin sensitivity were improved by more than 20% after the intervention, consistent with the described relationship linking liver steatosis and hepatic glucose production (32), and with the significant decline in endogenous glucose production induced by a 2-d very-low-calorie regimen in patients with diabetes (33). Insulin-mediated hepatic glucose uptake was not changed. This evidence is compatible with our previous findings (34) showing that insulin-mediated glucose uptake in the liver is much less dependent on insulin resistance than it is in the whole body and does not differ between nondiabetic insulin-resistant and insulin-sensitive subjects. Liver glucose uptake is modulated by plasma glucose and fatty acid concentrations (13, 35), neither of which changed in our study. Altogether, the above observations can be summarized as follows: the insulin-mediated retention of glucose by the liver—accounting for the balance between output and uptake of glucose—is enhanced due to caloric restriction, and this effect is probably mediated, at least in part, through the lowering of fatty acid uptake and accumulation in the organ. This may be the mechanism explaining the association between changes in glycosylated hemoglobin and liver fat content observed in this study.

Hepatic insulin fractional extraction was associated with insulin sensitivity. The liver removes approximately 50% of secreted insulin during the first portal passage (36, 37), and reduced hepatic insulin clearance is a typical finding in insulin resistance (38). Although a decrease in insulin clearance through saturation of the insulin removal processes may be a consequence of the increased insulin levels after insulin resistance, a primary role of the liver in controlling insulin action by modulating the supply of insulin to the systemic circulation has also been theorized (37). In support of this hypothesis, implicating visceral fat as the regulatory element, either a selective elevation of fatty acid levels in the portal vein or the accumulation of triglycerides in the liver has been shown to negatively affect hepatic insulin clearance, causing hyperinsulinemia (39, 40), whereas chronic hyperinsulinemia is a known down-regulator of whole-body insulin sensitivity (41). Our data may support either of these alternative hypotheses, altogether suggesting that they may be simultaneously operative.

In conclusion, the liver responds to a 6-wk period of calorie restriction with a parallel reduction in lipid uptake and storage, accompanied by enhancement of hepatic insulin sensitivity and clearance and a decrease in circulating inflammatory markers, glycosylated hemoglobin, and liver enzymes.

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Address all correspondence and requests for reprints to: Dr. Pirjo Nuutila, Turku PET Centre, Turku University Hospital, P.O. Box 52, FIN-20521, Turku, Finland. E-mail: pirjo.nuutila@utu.fi.

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