Hepatic Fat Content and Insulin Action on Free Fatty Acids and Glucose Metabolism Rather Than Insulin Absorption Are Associated With Insulin Requirements During Insulin Therapy in Type 2 Diabetic Patients

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To determine causes of interindividual variation in insulin requirements, we recruited 20 type 2 diabetic patients with stable glucose control and insulin doses for >1 year on combination therapy with bedtime NPH insulin and metformin. Insulin absorption (increase in free and total insulin over 8 h after a subcutaneous dose of regular insulin) and actions of intravenous (6-h 0.3 mU · kg^{-1} · min^{-1} euglycemic insulin clamp combined with [3-^{3}H]glucose) and subcutaneous (glucose infusion rate required to maintain isoglycemia and suppression of free fatty acids [FFAs]) insulin, liver fat content (proton spectroscopy), visceral fat (magnetic resonance imaging), weight, and body composition were determined. We found the following variation in parameters: insulin dose range 10–176 U (mean 42 U, fold variation 17.6×) or 0.13–1.39 U/kg (0.44 U/kg, 10.7×), absorbed insulin 10.6×, action of subcutaneous insulin to suppress FFAs 7.5× and to stimulate glucose metabolism (*M* value) 11.5×, body weight 67–127 kg (91 kg, 1.9×), liver fat 2–28% (12%, 14×), and visceral fat 179–2,053 ml (1,114 ml, 11.5×). The amount of insulin absorbed, measured as either free or total insulin, was significantly correlated with its ability to suppress FFAs and stimulate glucose metabolism but not with the insulin dose per se. The actions of absorbed insulin were, on the other hand, significantly correlated with the daily insulin dose (r = 0.70 for action on FFAs, P < 0.001, and r = –0.61 for *M* value, P < 0.005). Actions of subcutaneous and intravenous insulin to suppress FFAs were significantly correlated (r = 0.82, P < 0.001, R^2 = 67%). Of the measures of adiposity, the percent hepatic fat was the parameter best correlated with the daily insulin dose (r = 0.76, P < 0.001). The percent hepatic fat was also significantly correlated with the ability of intravenous insulin to suppress endogenous glucose production (r = 0.72, P < 0.005). We conclude that the major reason for interindividual variation in insulin requirements in type 2 diabetes is the variation in insulin action. Variation in hepatic fat content may influence insulin requirements via an effect on the sensitivity of endogenous glucose production to insulin. *Diabetes* 49:749–758, 2000

Studies comparing different insulin regimens in patients with type 2 diabetes have suggested that satisfactory glycemic control can be achieved by the use of combination regimens with a single injection of bedtime NPH insulin and oral agents (1–3). The combination of metformin and bedtime insulin may be particularly advantageous with respect to glycemic control, weight gain, and the frequency of hypoglycemia compared with other bedtime regimens (3). However, success with any regimen depends on careful adjustment of the insulin dose based on results of home glucose monitoring. In 2 of our previous studies, each of which lasted 1 year, the bedtime insulin dose required to achieve reasonable glycemic control varied 20-fold, from 8 to 170 U/day (1,3). The causes of this large interindividual variation have not been determined.

Theoretically, insulin requirements could depend on *1*) the amount of insulin absorbed, *2*) the action of absorbed insulin, and *3*) possibly other factors such as insulin antibodies. Previous insulin absorption studies have been performed almost exclusively in patients with type 1 diabetes using iodinated insulin ([^{125}I]-labeled regular insulin) to trace insulin absorption (4–9). Those studies have shown that insulin absorption, at constant temperature under resting conditions, is slowed by increases in subcutaneous fat thickness (10,11), total dose injected (9), decrease in subcutaneous blood flow (6,12,13), and injection site (10). Insulin antibodies have not been found to influence insulin requirements (5), nor has the amount of insulin absorbed been shown to correlate with insulin action in normal subjects (14). Causes of variation in insulin absorption have been sparsely studied in patients with type 2 diabetes. In 1 study, subcutaneous fat

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ALT, alanine aminotransferase; FFA, free fatty acid; FFM, fat-free mass; MRI, magnetic resonance imaging; NASH, nonalcoholic steatohepatitis; R_a, rate of glucose production; R_d, rate of glucose utilization; RIA, radioimmunoassay; SA, specific activity; S_{fat}, methylene signal intensity; S_{water}, water signal intensity; TE, echo time; TR, repetition time; WHR, waist-to-hip ratio.

thickness was found not to influence the disappearance rate of iodinated insulin (15). Whether interindividual variation in insulin sensitivity modulates insulin requirements in type 2 diabetes has not been studied.

In the present study, we wished first to quantify the extent to which insulin absorption and action determine insulin requirements in patients with type 2 diabetes. On separate days, using [3-^{3}H]glucose and the euglycemic insulin clamp technique, we determined the amount of absorbed insulin from the increase in free and total insulin concentrations after a subcutaneous injection of regular insulin and the action of intravenous insulin on suppression of serum free fatty acid (FFA) and glucose production and utilization. The action of subcutaneous insulin was also quantitated by measuring the ability of subcutaneous insulin to suppress FFAs. Second, we searched for causes of variation in insulin absorption and action by determining various parameters characterizing body size, fat content, and fat distribution. These parameters included measurement of hepatic fat content by proton spectroscopy, visceral fat by magnetic resonance imaging (MRI), body fat content by bioimpedance plethysmography, and subcutaneous fat thickness by ultrasound.

RESEARCH DESIGN AND METHODS

Patients. We recruited 20 type 2 diabetic patients (17 men and 3 women, mean age [± SE] 57.8 ± 2.3 years, weight 90.8 ± 3.1 kg, BMI 28.9 ± 0.7 kg/m^2, HbA_{1c} 7.6 ± 0.2%, fasting C-peptide 1.1 ± 0.1 nmol/l, duration of diabetes 9 ± 1 years, serum triglycerides 2.1 ± 0.2 mmol/l, HDL cholesterol 1.06 ± 0.04 mmol/l, and total cholesterol 5.1 ± 0.2 mmol/l) for the studies. All patients had been treated with the combination of bedtime NPH insulin and metformin (2 g/day) for at least 1 year. Additional inclusion criteria included stable body weight (change of <3%) and insulin dose (change of <5%) during the previous 6 months. Glycemic control during the previous 6 months also had to be stable and acceptable as defined by an HbA_{1c} <8.5%. Exclusion criteria were congestive heart failure, myocardial infarction, or stroke during the previous year; liver disease (>2-fold elevation in transaminases); serum creatinine concentration >120 µmol/l; macroalbuminuria; proliferative retinopathy or severe maculopathy; and epilepsy or other severe disease.

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 ethics committee of the Helsinki University Hospital.

Study design. The patients were admitted to the hospital 1 day before the studies and placed on a weight-maintaining diet that contained 25 kcal/kg, with 50% of calories from carbohydrates, 30% from fat, and 20% from protein. The patients participated in the following studies: *1*) measurement of action of intravenous insulin on endogenous glucose production and utilization, *2*) measurement of absorption and action of subcutaneous insulin, and *3*) measurement of liver and intra-abdominal fat contents, as detailed below. In addition, body weight, body composition, and the thickness of subcutaneous abdominal fat were determined as described below.

Action of intravenous insulin. At 8:00 P.M. on the evening before the study, an indwelling catheter, equipped with an obturator, was placed in an antecubital vein. To determine total rates of glucose production (R_a)—i.e., the sum of hepatic and renal glucose production (16) and utilization (R_d)—a primed continuous intravenous infusion of [3-^{3}H]glucose was started at 3:00 A.M. and continued for a total of 660 min. The priming dose of [3-^{3}H]glucose was adjusted according to the fasting blood glucose concentration measured at 3:00 A.M. as follows: priming dose = [glucose (mmol/l) at 3:00 A.M./5] × 20 µCi. This dose was infused intravenously over 10 min. The continuous rate infusion of [3-^{3}H]glucose was thereafter started at a rate of 0.2 µCi/min. At 7:30 A.M., another catheter was placed retrogradely in a heated hand vein to obtain arterialized venous blood for sampling. Baseline blood samples were taken for measurement of insulin antibodies, total and HDL cholesterol, triglyceride, and C-peptide concentrations. At 8:00 A.M., after a 300-min equilibrium period, a primed continuous (0.3 mU · kg^{-1} · min^{-1}) infusion of insulin was started, as previously described (17,18). Plasma glucose was allowed to decrease to 8 mmol/l (144 mg/dl) and was then maintained at that level for 360 min using a variable rate infusion of 20% glucose based on plasma glucose measurements, which were made from arterialized venous blood every 5 min. Blood samples for measurement of glucose specific activity (SA) were taken at –30, –20, –10, and 0 min and at 15- to 30-min intervals between 120 and 360 min. Serum free insulin concentrations were measured every 60 min and serum FFAs at 0, 10, 15, 20, 25, and 30 min and then at 30- to 60-min intervals during the insulin infusion.

Absorption and action of subcutaneous insulin. The patients did not take their bedtime NPH insulin injection before the insulin absorption study, which was performed after a 10- to 12-h overnight fast starting at 7:30–8:00 A.M. Two indwelling catheters were inserted, 1 retrogradely in a heated hand vein for withdrawal of arterialized venous blood and 1 for infusion of 20% glucose. A fixed dose of 36 U regular insulin was injected subcutaneously by the same investigator (L.R.) at a 45° angle with a 29-gauge needle 4 cm left of the umbilicus. The temperature in the room was recorded in each study and was between 24 and 25°C. We chose a large dose for 2 reasons. First, we wished to quantitate insulin absorption from the increment in circulating free insulin concentrations, which can only be done reliably if a relatively large dose is used. Second, we also wished to quantitate the action of the subcutaneous insulin from the glucose infusion rate needed to maintain glucose at its fasting concentration during an 8-h period. We also chose to use a fixed dose of insulin and related the increment in circulating insulin concentrations to the estimated distribution space of insulin. The latter is roughly equal to the extracellular space and therefore closely correlated with fat-free mass (FFM) (19). A fixed rather than a variable dose of insulin adjusted to body size was chosen, since the absolute insulin dose injected influences the profile of absorbed insulin (9). Serum free and total insulin concentrations were measured before and for 480 min after the insulin injection (at 30-min intervals until 270 min and at 330, 420, and 480 min). Insulin absorption was calculated from the increase in free and total insulin concentrations above basal during the 480-min period and expressed as area above basal divided by FFM (insulin absorption [mU/l] × min/kg FFM). Of note, the correlation coefficient between a fixed dose of insulin divided by FFM and increment in serum insulin concentrations is equal to that relating a fixed insulin dose to the increment in serum insulin concentrations divided by FFM.

The action of subcutaneous insulin on glucose metabolism was assessed by determining the glucose infusion rate needed to maintain isoglycemia during the 480-min period. The glucose infusion rate was adjusted based on plasma glucose measurements, which were measured from arterialized venous blood every 5 min for the entire 480 min. The action of subcutaneous insulin on FFA metabolism was assessed by measuring how serum FFAs were suppressed by the insulin injection. Serum FFAs were measured basally, at 10-min intervals between 0 and 60 min, at 30-min intervals between and 60 and 270 min, and at 330, 420, and 480 min.

Liver fat content (proton spectroscopy). Single-voxel (2 × 2 × 2 cm) proton spectra from the liver were acquired using 32 excitations, a loop surface coil, and a 1.5 T magnetic resonance device (Magnetom Vision; Siemens, Erlangen, Germany). Spatial location was achieved using a stimulated echo acquisition mode applied with a repetition time (TR) of 3,000 ms and an echo time (TE) of 20 ms. A long TR and short TE were chosen to minimize effects of T1 and T2 relaxation, respectively, on signal intensities. Chemical shifts were measured relative to water signal intensity (S_{water}) at 4.8 ppm. Methylene signal intensity (S_{fat}), which represents intracellular triglyceride in the liver (20), was measured at 1.4 ppm. Signal intensities were obtained by time domain fitting routine VARPRO-MRUI (carbon.uab.es/mrui). This measurement of percent hepatic fat by proton spectroscopy has been validated against the lipid content of liver biopsies in humans (21) and animals (20). It has also been validated against liver density measurements performed by computed tomography (22). The latter validation was repeated by quantifying liver density in 8 of the patients in the present study with the HiQ computed tomography device (Siemens). The entire liver was scanned with a 10-mm collimator. By using a standard region-of-interest method, the density of normal liver parenchyma was calculated in Hounsfield units. The percent liver fat measured by proton spectroscopy correlated closely (r = –0.85, P < 0.01) with liver density measured with computed tomography (Fig. 1). Hepatic fat percentage was calculated by dividing (100 × S_{fat}) by the sum of S_{fat} and S_{water}. Examples of 2 patients with low and high percent hepatic fat are shown in Fig. 1.

Intra-abdominal fat (MRI). A series of T1-weighted transaxial scans for the determination of visceral and subcutaneous fat were acquired from a region extending from 4 cm above to 4 cm below the fourth and fifth lumbar interspace (16 slices, field of view 375 × 500 mm^2, slice thickness 10 mm, breath-hold TR/TE 138.9 ms/4.1 ms). Visceral and subcutaneous fat areas were measured using image analysis program Alice (www.perceptive.com/alice.htm). 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. Visceral adipose tissue was defined as the area of pixels in the

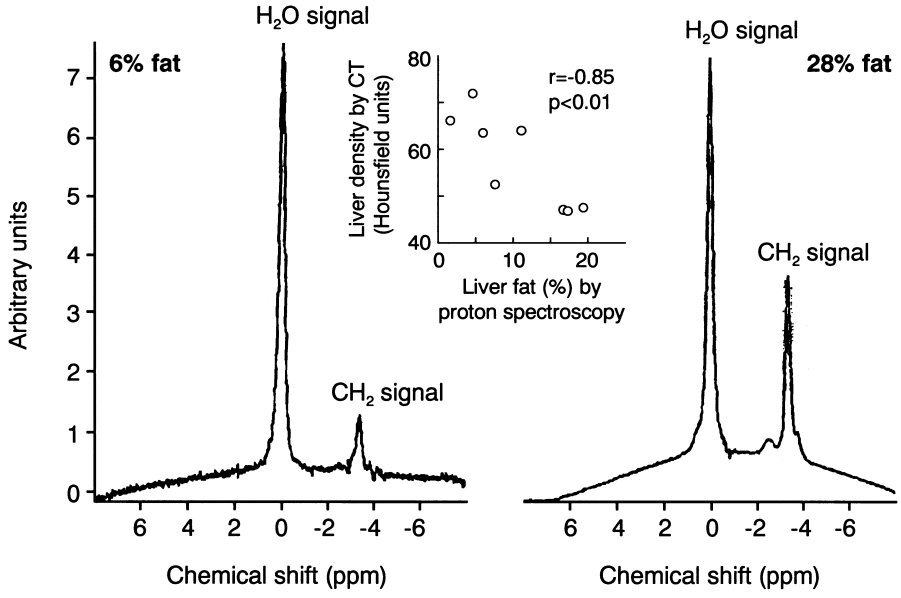

FIG. 1. Proton magnetic resonance spectra from 2 patients with fat percentages of 6 and 28%. The water peak has a chemical shift of 0 ppm, and the methylene (CH_2) signal of fat has the chemical shift of 3.4 ppm relative to the water peak. The height of the signal (*y*-axis) is in arbitrary units.

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 visceral tissues. The measurement could not be performed in 3 subjects because of their large body size.

Subcutaneous fat thickness (ultrasound). The thickness (in millimeters) of the subcutaneous fat layer at the injection site was determined by high-frequency (10 MHz) ultrasound using ImagePoint Multispecialty Ultrasound System (Hewlett Packard, Andover, MA).

Analytical procedures and calculations

[3-^{3}H]glucose SA and calculation of glucose kinetics. Plasma was deproteinized with $Ba(OH)_2$ and $ZnSO_4$ and evaporated as described previously (23). The dried glucose residue was resuspended, counted in a double-channel liquid scintillation counter (Rackbeta 1215; Wallac, Turku, Finland) after adding 10 ml Aquasol liquid scintillation fluid (NEN-DuPont, Boston, MA), and corrected for quenching. The [3-^{3}H]glucose SA (in disintegrations per minute per micromole) was calculated by dividing the disintegrations per minute in 0.3 ml plasma by the plasma glucose concentration (in micromoles per milliliter). The infusate was diluted 1:100 and 1:1,000, and duplicates were counted to determine the infusate [3-^{3}H] concentration. 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 (18). Endogenous glucose R_a was calculated by subtracting the exogenous glucose infusion rate required to maintain isoglycemia during hyperinsulinemia (0–360 min) from the rate of total glucose R_a. The percent suppression of basal endogenous glucose R_a during the final hour (300–360 min) by insulin was used as an index of the sensitivity of endogenous glucose production to insulin (percent suppression of endogenous R_a).

Other analytical procedures. Plasma glucose concentration was measured in duplicate with the glucose oxidase method (24) using a Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Serum free insulin concentrations were determined after precipitation with polyethylene glycol (25) using the Phadeseph insulin radioimmunoassay (RIA) kit (Pharmacia, Uppsala, Sweden). Serum total insulin concentrations were measured with the same RIA kit without polyethylene glycol precipitation. Serum FFAs were measured by a fluorometric method (26). C-peptide concentration was determined by RIA (27). Total cholesterol, HDL cholesterol, and triglycerides were measured as previously described (28). HbA_{1c} was measured by high-performance liquid chromatography (29) using the fully automated Glycosylated Hemoglobin Analyzer System (Bio-Rad, Richmond, CA). Insulin antibodies were determined after incubation of serum with [^{125}I]-insulin at room temperature overnight by measuring total radioactivity and that remaining in the supernatant after precipitation with polyethylene glycol (25,30).

Statistical analyses. All correlation analyses were performed using Spearman's nonparametric rank correlation coefficient. Areas above basal in the insulin absorption study were calculated using the trapezoid rule. Goodness of fit of individual nonlinear equations to mean data in the insulin absorption test was analyzed using the runs test and R^2. Goodness of fit of different equations was compared by calculating the significance of *F* ratio—i.e., whether, when using a complicated versus simple model, the relative increase in sum of squares was greater than the relative increase in degrees of freedom. All calculations were performed using GraphPad Prism version 2.01 (GraphPad, San Diego, CA). All data are shown as mean ± SE.

RESULTS

Variation in insulin absorption and action as causes of variation in insulin requirements

Absorption and action of subcutaneous insulin. Figure 2 depicts the mean ± SE for serum free and total insulin, plasma glucose, and FFA concentrations and the glucose infusion rate during the 480-min period after the subcutaneous insulin injection. Half-maximal increases in serum free and total insulin concentrations and the glucose infusion rate were observed at 14, 5, and 93 min. The half-time for suppression of FFA concentrations was 46 min. The amount of free insulin absorbed after the subcutaneous injection, calculated as the area above basal, area above basal divided by FFM, and area above basal divided by kilogram of body weight, varied 9.2-, 10.6-, and 10.9-fold, respectively. The corresponding values for fold variation of total insulin were 34, 37, and 39.

The action of absorbed insulin was assessed by 2 parameters, suppression of serum FFAs (area under the FFA curve) and the amount of glucose infused to maintain isoglycemia between 0 and 480 min (*M* value). The *M* value varied 11.5-fold. The ability of subcutaneous insulin to suppress FFAs (area under the FFA curve between 0 and 480 min) varied 7.5-fold. The amount of free insulin absorbed (area above basal over the 480-min period) was significantly correlated with both the area under the FFA curve during the 480-min period ($r = –0.63$, $P < 0.005$) and the *M* value ($r = 0.74$, $P < 0.001$) (Fig. 3). The corresponding correlations for total insulin were $r = –0.48$, $P < 0.05$, and $r = 0.62$, $P < 0.001$ (Fig. 3).

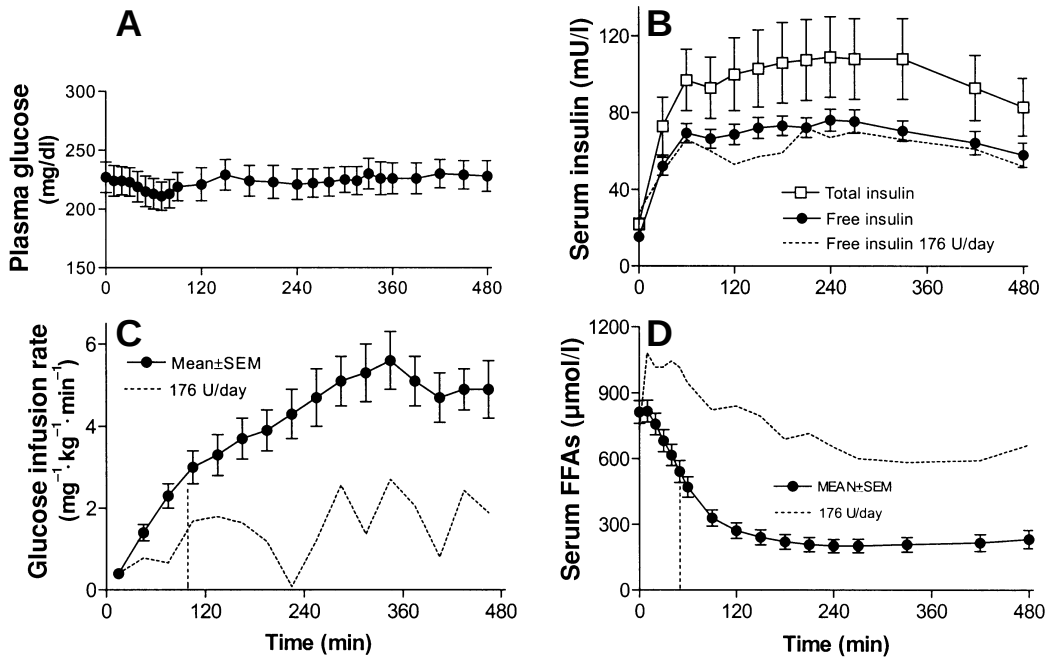

FIG. 2. Absorption and action of subcutaneous insulin study. Plasma glucose concentrations (*A*), serum insulin concentrations (*B*), glucose infusion rate (*C*), and serum FFA concentrations (*D*) during the 480-min period after subcutaneous injection of a fixed dose of regular insulin. ●, Mean values; ○, patient with an exceptionally high bedtime insulin dose (176 U).

The patient requiring a bedtime insulin dose of 176 U is shown separately in Fig. 2 as an extreme example of individual variation. The free insulin concentrations were close to the mean value of all patients, but the glucose infusion rate was the lowest and the FFA concentrations the highest of the entire group.

Action of intravenous insulin. The time course for plasma glucose, serum free insulin, and FFA concentrations and the glucose infusion rate for the intravenous insulin action study are shown in Fig. 4. The intravenous insulin infusion increased serum free insulin concentrations from 13 ± 1 (basal) to 30 ± 1 mU/l during hyperinsulinemia (30–360

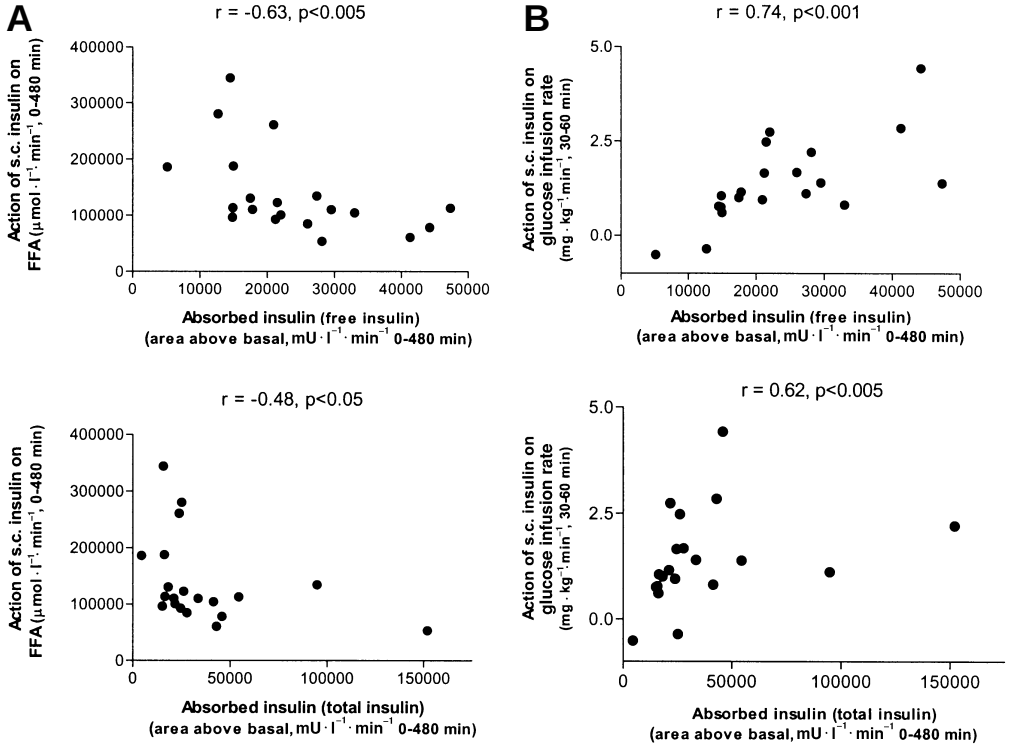

FIG. 3. The relationship between the amount of free (upper panels) and total (lower panels) insulin absorbed after subcutaneous (s.c.) injections (area above basal) and the action of subcutaneous insulin on FFAs (*A*) and *M* values (*B*).

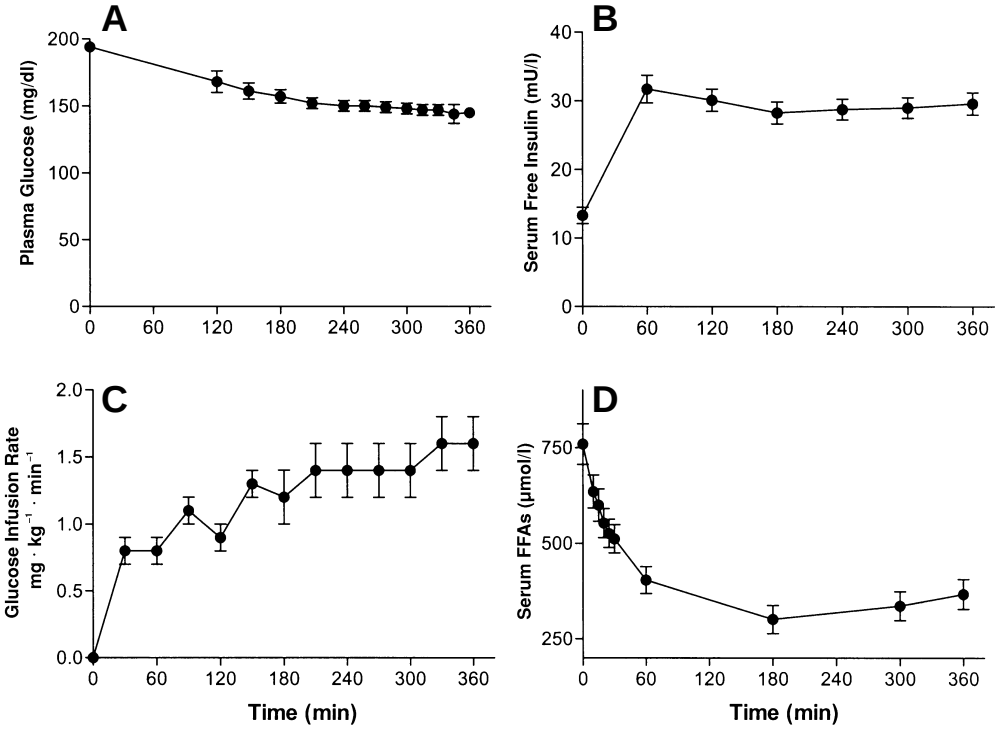

FIG. 4. Plasma glucose (*A*), glucose infusion rate (*B*), serum free insulin (*C*), and FFAs (*D*) during the isoglycemic insulin clamp study. Data are means ± SE.

min). Plasma glucose concentrations were similar in all subjects between 240 and 360 min and averaged 8.2 ± 0.2 mmol/l. The *M* value (0–360 min) varied 10.8-fold. During the final 2 h, glucose R_d averaged 2.75 ± 0.20 mg · kg^{-1} · min^{-1} and endogenous R_a 1.17 ± 0.23 mg · kg^{-1} · min^{-1}, which represented a 67 ± 7% suppression below basal. FFAs averaged 759 ± 53 basally and decreased to 352 ± 38 µmol/l between 240 and 360 min. Action of intravenous insulin to suppress serum FFAs (area under the FFA concentration curve between 0 and 360 min) varied by 4.6-fold.

Insulin antibodies. The titer of insulin antibodies averaged 9.8 ± 1.6% (range 3.6–27.3). The correlation between the titer of insulin antibodies and the daily insulin dose did not reach statistical significance ($r = 0.37$, $P = 0.11$).

Relationships between insulin absorption and action, insulin antibodies, and the daily insulin dose. The amount of insulin absorbed, measured either as free ($r = 0.38$, NS) or total ($r = –0.27$, NS) insulin, during any 30-min time period between 0 and 480 min did not correlate significantly with the insulin dose, whereas the ability of subcutaneous insulin to suppress serum FFAs ($r = 0.70$, $P < 0.001$) and the *M* value ($r = –0.61$, $P < 0.005$) were significantly correlated with the daily insulin dose (Fig. 5). The correlation between action of absorbed insulin on *M* value between 30 and 60 min ($r = 0.61$, $P < 0.005$) was better than at any other time point (data not shown), which is why this measure of action of subcutaneous insulin was used in all analyses. The relationship between the action of intravenous insulin to suppress serum FFAs ($r = 0.46$, $P < 0.05$, FFA area under curve at 0–360 min) and *M* value ($r = –0.46$, $P < 0.05$, 0–360 min) and the daily insulin dose were significant, albeit weaker than the relationships between the actions of subcutaneous insulin and the insulin dose (Fig. 5).

To examine the combined effects of insulin absorption, action, and antibodies on daily insulin requirements, multiple linear regression analysis was used. The patient with an exceptionally high insulin dose (176 U) was excluded to ensure normal distribution of the variables. The greatest *F* ratio (13.4, $P < 0.001$ for model) and R^2 (61.3%) were found when the daily insulin dose (units per day) was the dependent variable and the actions of subcutaneous insulin to suppress FFAs ($P < 0.001$) and insulin antibodies ($P = 0.05$) were the independent variables. The *F* ratio was 10.2 ($P < 0.002$ for model), and R^2 was 54.7 when the actions of intravenous insulin to suppress FFAs ($P < 0.002$) and insulin antibodies ($P = 0.05$) were included as dependent variables. Inclusion of the amount of absorbed insulin, measured as either free or total insulin, did not improve the model. Use of the *M* value or intravenous insulin action on R_d or endogenous R_a instead of insulin suppression of serum FFAs as a measure of insulin action also did not improve the model (data not shown).

Contribution of variation in insulin absorption to variation in insulin action. The abilities of subcutaneous and intravenous insulin to suppress FFAs ($r = 0.82$, $P < 0.001$, R^2 67%) and the *M* values ($r = 0.64$, $P < 0.005$, R^2 41%) were significantly interrelated. These data imply that variation in insulin absorption, day-to-day variation in methods to assess insulin action (variation in glucose and FFA concentrations), and other factors together explained 33 and 59% of variation in insulin action on suppression of FFAs and the *M* value.

Causes of variation in insulin absorption and action

Absorption and action of subcutaneous insulin. The area above basal under the insulin absorption curve, measured as free or total insulin, during the 480-min period was calculated for each individual to obtain an index of the amount of insulin absorbed. This index for free insulin, expressed as mil-

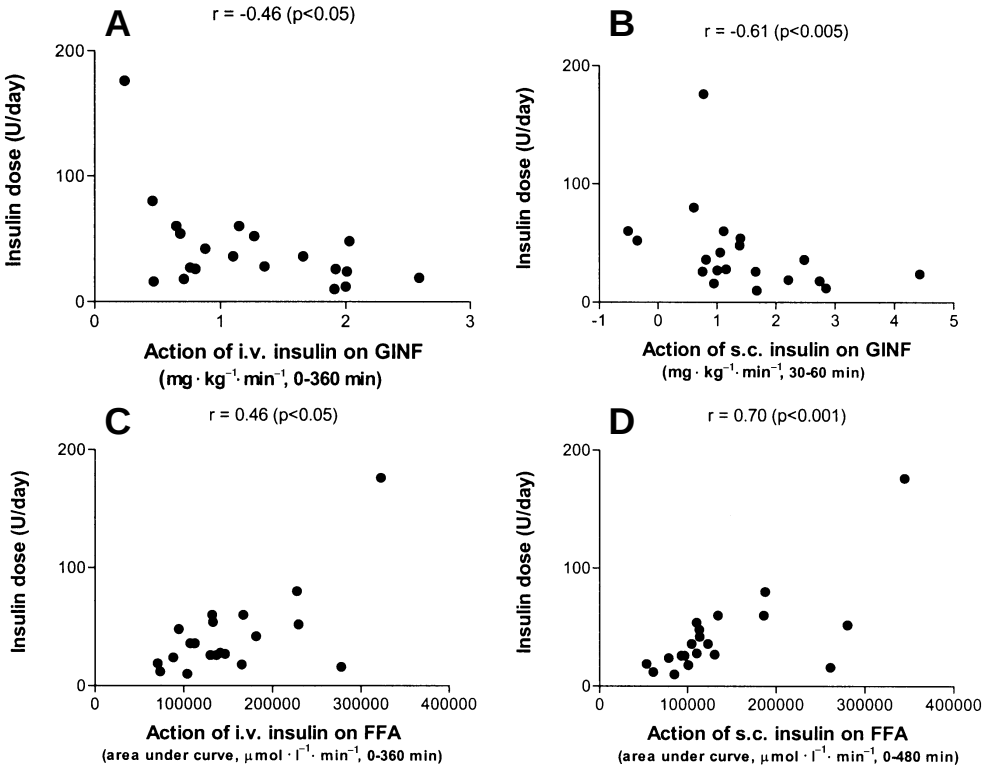

FIG. 5. The relationship between actions of intravenous (i.v.) (*A* and *C*) and subcutaneous (s.c.) (*B* and *D*) insulin on glucose infusion rate (*A* and *B*) and antilipolysis (*C* and *D*) and insulin dose. GINF, glucose infusion rate.

liunits per liter times minutes, was closely correlated to that expressed as milliunits per liter times minutes per kilogram of body weight ($r = 0.93$, $P < 0.0001$) or milliunits per liter times minutes per kilogram of FFM ($r = 0.92$, $P < 0.0001$). The correlation between the amount of insulin absorbed, measured as the area under the serum total insulin curve (milliunits per liter times minutes) and that expressed as milliunits per liter times minutes per kilogram body weight was $r = 0.95$, $P < 0.0001$. The correlation between the amount of insulin absorbed and that expressed as milliunits per liter times minutes per kilogram of FFM was $r = 0.95$, $P < 0.0001$. We then analyzed the relationship between parameters thought to influence insulin absorption and the indexes of insulin absorption (Table 1). The best correlates for both free and total insulin were visceral fat volume, subcutaneous fat volume, and BMI. The thickness of subcutaneous fat, measured with ultrasound, varied between 1.6 and 3.8 cm but was not significantly correlated with insulin absorption (Table 1).

Action of intravenous insulin. To analyze how various measures of body fat content and distribution are associated with insulin sensitivity of endogenous R_a, the relationships between such parameters and the percent suppression of

TABLE 1
Relationships (Spearman's *r*) between measures of overall adiposity and body fat distribution and the amount of absorbed insulin during the 480-min period in patients with type 2 diabetes

	Insulin area above basal (mU · l^{-1} · min^{-1})				Insulin area above basal (mU · l^{-1} · min^{-1} · kg^{-1} FFM)			
	Free		Total		Free		Total	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Measures of overall adiposity								
Visceral fat volume (ml)	–0.73	0.0004	–0.55	0.015	–0.77	0.0001	–0.55	0.014
Subcutaneous fat volume (ml)	–0.60	0.0072	–0.57	0.010	–0.53	0.02	–0.59	0.0072
Subcutaneous fat (cm)	–0.33	NS	–0.29	NS	–0.29	NS	–0.30	NS
BMI (kg/m^2)	–0.59	0.006	–0.57	0.0085	–0.72	0.0004	–0.65	0.0018
Fat mass (kg)	–0.41	NS	–0.41	NS	–0.46	0.04	–0.48	0.032
Body fat (%)	–0.38	NS	–0.44	0.05	–0.25	NS	–0.43	NS
Weight (kg)	–0.28	NS	–0.24	NS	–0.55	0.01	–0.40	NS
Measures of fat distribution								
WHR	–0.34	NS	–0.25	NS	–0.51	0.023	–0.24	NS
Visceral/subcutaneous fat ratio	–0.22	NS	–0.01	NS	–0.33	NS	–0.10	NS

TABLE 2
Relationships (Spearman's *r*) between measures of overall adiposity and body fat distribution and the sensitivity of endogenous R_a to insulin in patients with type 2 diabetes

	Percent suppression 300–360 min			
	Range	Fold variation	*r*	*P*
Liver fat (%)	2–28	14	0.72	0.0013
Measures of overall adiposity				
Visceral fat volume (ml)	179–2,053	11.5	0.56	0.0297
Subcutaneous fat volume (ml)	526–1,765	3.4	0.67	0.0065
Subcutaneous fat (cm)	1.6–3.8	2.3	0.32	NS
BMI (kg/m^2)	23.3–36.4	1.6	0.54	0.026
Fat mass (kg)	15–39	2.6	0.60	0.01
Body fat (%)	22–39	1.8	0.51	0.037
Body weight (kg)	67–127	1.9	0.44	NS
Measures of fat distribution				
WHR	0.92–1.17	1.3	0.58	0.01
Visceral/subcutaneous fat ratio	0.34–1.37	4.0	–0.02	NS

endogenous R_a during the final hour of the intravenous insulin infusion were calculated (Table 2). The percent of hepatic fat was the parameter most closely associated with percent suppression of hepatic glucose production by insulin (Fig. 6).

Hepatic fat content and its relationship to measures of total adiposity and fat distribution. The percent fat in the liver varied 14-fold, from 2 to 28%. To establish whether the percent fat in the liver was related to measures of total adiposity or fat distribution, Spearman's rank correlation coefficients were calculated. In univariate analysis, several measures of adiposity (BMI, $r = 0.63$, $P = 0.006$; percent body fat, $r = 0.60$, $P = 0.011$; subcutaneous fat volume, $r = 0.61$, $P = 0.016$) but especially total fat mass were significantly correlated with percent of liver fat (Fig. 6). Of the two measures of fat distribution, neither waist-to-hip ratio (WHR) ($r = 0.40$, NS) nor the ratio between visceral and subcutaneous fat volumes ($r = 0.02$, NS) was correlated with percent fat in the liver.

Measures of adiposity as predictors of insulin requirements. We also analyzed whether and how the various measures of adiposity were related to the daily insulin dose. Percent hepatic fat was most closely correlated with insulin dose (Fig. 6; Table 3).

DISCUSSION

The present study is the first to examine the contribution of insulin absorption and action to variation in insulin require-

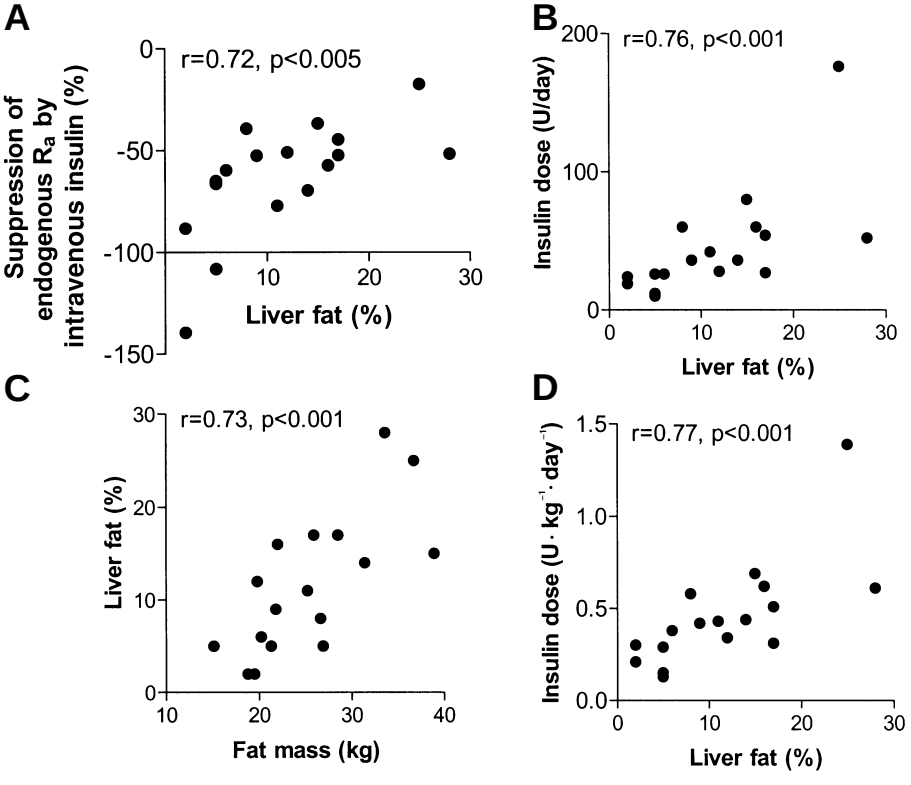

FIG. 6. The relationship between fat mass and percent liver fat (*C*) and between percent liver fat and suppression of endogenous R_a by intravenous insulin (*A*), insulin dose (U/day [*B*] and U · kg^{-1} · day^{-1} [*D*]).

TABLE 3
Relationships (Spearman's *r*) between measures of overall adiposity and body fat distribution and the daily insulin dose expressed as units per day and units per kilogram per day in patients with type 2 diabetes

	Insulin dose (U/day)		Insulin dose ($U \cdot kg^{-1} \cdot day^{-1}$)	
	r	*P*	*r*	*P*
Liver fat (%)	0.76	0.0004	0.77	0.0003
Measures of overall adiposity				
Visceral fat volume (ml)	0.46	0.048	0.42	0.07
Subcutaneous fat volume (ml)	0.47	0.041	0.50	0.03
Subcutaneous fat (cm)	0.04	NS	0.03	NS
BMI (kg/m^2)	0.68	0.0009	0.61	0.004
Fat mass (kg)	0.60	0.005	0.58	0.007
Body fat (%)	0.31	NS	0.37	0.11
Body weight (kg)	0.63	0.003	0.50	0.02
Measures of fat distribution				
WHR	0.69	0.0008	0.61	0.004
Visceral/subcutaneous fat ratio	0.01	NS	–0.05	NS

ments in patients with type 2 diabetes. Furthermore, to our knowledge this is the first study to determine whether hepatic fat content is correlated with the sensitivity of endogenous glucose production to insulin and to the daily insulin dose. The main findings can be summarized as follows. First, the amount of insulin absorbed per se was not significantly correlated with the insulin dose but did contribute to variation in insulin action (Fig. 3). Subcutaneous insulin action was better correlated with insulin requirements (Fig. 5*B* and *D*) than intravenous insulin action (Fig. 5*A* and *C*). The latter remained true also in multiple linear regression analysis, where the daily insulin dose was the dependent variable and subcutaneous insulin action and insulin antibodies, or intravenous insulin action and insulin antibodies, were the independent variables. Second, we found that percent liver fat was more closely correlated than other measures of adiposity with both insulin dose (Table 3) and the sensitivity of endogenous glucose production to insulin (Table 2).

The mean insulin dose of the patients using bedtime NPH insulin and metformin was 36 U/day (not counting the 1 patient requiring 176 U/day). To mimic the mean dose, to enable ranking of patients with respect to their insulin sensitivity, and to make the absorption study feasible to perform, 36 U of regular insulin was injected subcutaneously, and the increase in free and total insulin concentrations and glucose requirements for the ensuing 8-h period were followed. This period was not long enough to allow the entire absorption and action profile to be determined (Fig. 2). The amount of absorbed insulin therefore only provides an index of insulin absorption. Even in normal subjects, absorption of regular insulin is markedly slow. In the study by Ziel et al. (14), the maximal concentration of regular insulin in nondiabetic nonobese subjects after a 10-U subcutaneous injection was observed at 112 min, and the glucose infusion rate was maximal at 256 min. Even after subcutaneous injection of a short-acting insulin analog (21 U to nonobese nondiabetic subjects), the glucose infusion rate remains increased until 8 h (31). Consistent with these observations, the possibly slower insulin absorption in obese type 2 diabetic patients than in type 1 diabetic patients (15) and a decrease in the rate of insulin absorption at increasing insulin doses, the glucose infusion rate did not reach maximum until ~360 min after the subcutaneous injection in the present study (Fig. 2). Although the insulin concentrations, the action of subcutaneous insulin on serum FFA concentrations, and the glucose infusion rate did not return to baseline within the 8-h period, the absorption study nevertheless provided some useful information about absorption and action of subcutaneous insulin. First, the significant correlations between the amount of absorbed insulin and its action on both FFAs and glucose metabolism (Fig. 3) suggest that variation in insulin absorption had biologically significant consequences. Second, the significant correlations between the actions of absorbed insulin and the insulin dose on one hand (Fig. 5) and the better correlations between subcutaneous than intravenous insulin action and the insulin dose on the other support the idea that measurement of insulin absorption provided physiologically meaningful information.

In analyses searching for factors associated with insulin requirements, the correlation between the ability of insulin to suppress serum FFAs and the insulin dose was better than that between the *M* value and the insulin dose (Fig. 5). Also, the correlation between actions of subcutaneous and intravenous insulin was clearly better for action on FFAs than on glucose metabolism. The superiority of FFA suppression versus the *M* value as a measure of insulin action is likely to be technical because the glucose concentrations were variable during the insulin absorption study. Such variation in glucose concentrations will induce some variation in *M* values because of the mass action effect of hyperglycemia (32). Because antilipolysis is not regulated by glucose in humans (33), use of the area under the FFA curve during insulin infusion or injection is subject to less variability than the *M* value under conditions of varying glycemia.

Regarding causes of variation in insulin absorption, we found several measures of body size (visceral and subcutaneous fat volumes, BMI, weight, and fat mass) rather than subcutaneous thickness at the injection site to be associated with the amount of insulin absorbed, regardless of whether the area under the absorption curve or the area divided by FFM was used as the measure of the amount of insulin absorbed. Previous data in type 2 diabetic patients are sparse. In the study of Clauson and Linde in type 2 diabetic patients (15), insulin absorption was followed using injection of 5 U regular [^{125}I]-

insulin. In that study, which included obese and nonobese patients, no correlation was observed between depth of the fat layer and residual radioactivity at the 3 injection sites examined (15). On the other hand, consistent with the present data, studies performed in type 1 diabetic patients have demonstrated significantly slower insulin absorption, measured as residual radioactivity at the injection site, in obese than in nonobese patients (11,12). As in the present study, however, subcutaneous fat thickness did not explain variation in insulin requirements in these studies (11,12).

Because inhibition of endogenous glucose production represents the major target for insulin therapy (34,35), we were particularly interested in searching for parameters that might explain interindividual variation in hepatic insulin sensitivity. Percent fat in the liver was found to be most closely correlated with suppression of endogenous glucose production by insulin within each patient (Table 2). It was also correlated with the insulin dose, with an r value of 0.77 (Fig. 6). These results suggest that 60% of the variation in the daily insulin dose was attributable to variation in hepatic fat content, possibly via effects of hepatic adiposity on the sensitivity of endogenous glucose production to insulin (Fig. 6). These relationships may seem surprisingly strong considering that daily insulin requirements should also be influenced by interindividual differences in diet composition and exercise habits. When body weight is stable and physical activity habits are constant, however, insulin requirements also should stabilize. Because physical activity and body weight are key determinants of insulin sensitivity (36), and inhibition of endogenous glucose production is the primary target of insulin therapy (37), the correlations between liver fat content, insulin sensitivity, and insulin requirements are physiologically feasible and expected.

The relationship between liver fat percentage and hepatic insulin sensitivity supports recent evidence that has led to the classification of nonalcoholic steatohepatitis (NASH) as a disease of affluence and part of the insulin resistance syndrome (38). In the Third National Health and Nutrition Examination Survey, 2.6% of the U.S. population had raised values of serum alanine aminotransferase (ALT) for which no potential cause of chronic liver disease could be found (38). The raised ALT concentration was significantly and independently associated with indexes of insulin resistance and with HbA_{1c} concentrations. The risk of steatosis increases exponentially with each addition of a component of the insulin resistance syndrome, such as impaired glucose tolerance, hypertension, and dyslipidemia (39). Our patients had normal transaminases, but percent liver fat varied 14-fold, from 2 to 28%, suggesting that noninvasive measurement of liver fat content is a more sensitive index of steatosis than elevation of ALT, although the diagnosis of NASH still rests on histopathological features (38). The mechanisms linking insulin resistance and fatty liver are unclear. It has been suggested, but not confirmed because of the inaccessibility of the portal vein for blood sampling in humans, that fatty liver is a consequence of fatty acid mobilization from visceral fat depots to the liver (40). In the present study, visceral fat volume, WHR, and the visceral/subcutaneous fat ratio were not significantly correlated with percent liver fat, whereas measures of overweight (total and subcutaneous fat mass, percent body fat, and BMI) were. No causal conclusions can be made based on these correlation analyses. They do not exclude the possibility that hyperinsulinemia, which could result from obesity-induced primary peripheral insulin resistance in skeletal muscles (41) and from exogenous insulin injections, itself might increase hepatic fat content (42).

The present study has clinical implications in that it helps to make verified guesses of individual insulin requirements in patients with type 2 diabetes. Of the simple parameters measured, BMI was one that correlated with the amount of absorbed insulin (r = –0.72), the percent fat in the liver (r = 0.63), the sensitivity of endogenous glucose production to insulin (r = 0.54), and the insulin dose (r = 0.68 for U per day and r = 0.61 for U/kg per day). Thus in obese patients, insulin is more slowly absorbed and acts less efficiently to suppress hepatic glucose production and FFA levels than in nonobese patients. These factors increase not only the absolute insulin dose but also the dose needed per kilogram of body weight. Regarding the relative importance of insulin absorption versus insulin action on insulin requirements, there was no correlation between the amount of insulin absorbed and insulin dose, but there was a highly significant correlation between subcutaneous insulin action and insulin dose. The better correlation between subcutaneous insulin action and insulin dose than between intravenous insulin action and insulin dose (Fig. 5) suggests that the amount of absorbed insulin does influence insulin requirements because it influences insulin action. From the relationship between actions of subcutaneous and intravenous insulin on FFA metabolism (R^2 = 67%), it can be calculated that maximally 33% of the variation in insulin action can be explained by interindividual variation in insulin absorption.

The present data support the clinical impression of great heterogeneity among patients with type 2 diabetes during insulin therapy. Although easily measured parameters such as BMI significantly predict insulin requirements, BMI is not accurate enough to be useful on an individual basis, making it difficult for health care professionals to safely adjust the insulin dose within a reasonable time frame to achieve good glycemic control. The best option is to teach patients to adjust insulin dose themselves, based on home glucose monitoring results such as fasting glucose measurements during combination therapy with bedtime insulin. We have recently shown that such a simple approach results in sustained good glycemic control (3).

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