Insulin-Like Growth Factor Binding Protein 1 as a Novel Specific Marker of Hepatic Insulin Sensitivity

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Background and Aims: The liver is the main source and insulin the main regulator of IGF binding protein 1 (IGFBP-1) in humans. Here we examined how serum IGFBP-1 concentrations are related to directly measured hepatic insulin sensitivity and liver fat content in humans.

Methods: We measured fasting serum (fS) IGFBP-1 concentrations and liver fat content by proton magnetic resonance spectroscopy in 113 nondiabetic subjects. In addition, hepatic insulin sensitivity was measured using the euglycemic hyperinsulinemic clamp (insulin 0.3 mU/kg·min) technique in combination with the infusion of [3-(3)H]glucose in 78 subjects.

Results: fS-IGFBP-1 concentrations were inversely related to liver fat content ($r = -0.38$, $P < 0.0001$). Of circulating parameters, fS-IGFBP-1 was better correlated to hepatic insulin sensitivity ($r = 0.48$, $P < 0.0001$) than fS-insulin ($r = -0.42$, $P = 0.0001$), fS-C-peptide ($r = -0.41$, $P = 0.0002$), fS-triglyceride ($r = -0.33$, $P = 0.003$), or fS-high-density lipoprotein cholesterol ($r = 0.30$, $P = 0.007$). In multiple linear regression analyses, body mass index ($P < 0.0001$) and fS-IGFBP-1 ($P = 0.008$), but neither age nor gender, were independently associated with hepatic insulin sensitivity ($P < 0.0001$ for ANOVA). Neither fS-insulin nor fS-C-peptide were independent determinants of hepatic insulin sensitivity after adjusting for age, gender, and body mass index.

Conclusions: fS-IGFBP-1 is inversely correlated with liver fat and is an obesity-independent and liver-specific circulating marker of hepatic insulin sensitivity. (***J Clin Endocrinol Metab*** **93: 4867–4872, 2008)**

It is well established that nonalcoholic fatty liver disease (NAFLD) is an independent predictor of type 2 diabetes, the metabolic syndrome, cardiovascular disease, and advanced liver disease (1). Hepatic fat accumulation is tightly linked to hepatic insulin resistance and characterized by decreased ability of insulin to suppress hepatic glucose and triglyceride-rich very low-density lipoprotein particle production in the liver (1). This in turn leads to hyperglycemia, hypertriglyceridemia, and a low high-density lipoprotein (HDL) cholesterol concentration. Hepatic insulin resistance is thus likely to be a core component of the metabolic syndrome (2).

When estimated using proton magnetic resonance spectroscopy (^{1}H-MRS), every third adult has a fatty liver (3). There is a need to identify novel specific markers of hepatic fat because ^{1}H-MRS is not available in clinical practice, and current markers of hepatic steatosis, such as serum (S)-alanine aminotransferase (ALT), S-aspartate aminotransferase (AST), fasting serum (fS)-insulin, and fS-C-peptide, are both insensitive and unspecific (4). Assessment of hepatic insulin sensitivity by state-of-the-art methodology requires prolonged insulin and glucose infusions in combination with glucose tracer. Because these measurements are complicated and costly, they also cannot be used in routine clinical practice. fS-insulin concentrations are often used as a marker of hepatic insulin sensitivity, but they are influenced by insulin resistance in tissues other than the liver, as well as by insulin clearance and insulin secretion.

IGF binding protein 1 (IGFBP-1) belongs to a six-member family of IGFBPs that modulate the bioavailability of IGF-I (5).

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Abbreviations: ALT, Alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FFA, free fatty acid; fS, fasting serum; HbA_{1c}, glycosylated hemoglobin 1c; HDL, high-density lipoprotein; ^{1}H-MRS, proton magnetic resonance spectroscopy; HOMA-IR, homeostatic model assessment of insulin resistance; IGFBP-1, IGF binding protein 1; NAFLD, nonalcoholic fatty liver disease; NS, not significant; R_a, rate of appearance; S, serum.

We have previously shown that insulin decreases IGFBP-1 concentrations acutely *in vivo* (6). The liver has been shown to be the main site of production of IGFBP-1 in humans (7). Previous studies have shown that serum IGFBP-1 concentrations are negatively related to indirect estimates of insulin resistance, such as homeostatic model assessment of insulin resistance (HOMA-IR) (8–10), and positively related to whole-body insulin sensitivity measured using the euglycemic hyperinsulinemic clamp technique (11–13). The associations between serum IGFBP-1 concentrations, liver fat, and directly measured hepatic insulin sensitivity have not been examined previously.

In the present study, we measured liver fat using ^{1}H-MRS, serum IGFBP-1 concentrations, and other features of insulin resistance in 113 nondiabetic subjects. Hepatic insulin sensitivity was measured directly by use of a prolonged low-dose (0.3 mU/kg·min) insulin infusion in combination with [3-^{3}H]glucose.

Subjects and Methods

Subjects and study design

A total of 113 nondiabetic subjects were recruited using the following inclusion criteria: 1) age 18 to 60 yr; 2) no known acute or chronic disease based on history, physical examination, and standard laboratory tests (blood counts, serum creatinine, TSH, electrolyte concentrations and electrocardiogram); 3) alcohol consumption less that 20 g/d; and 4) no evidence of hepatitis A, B, or C, autoimmune hepatitis, clinical signs or symptoms of inborn errors of metabolism, or history of use of toxins or drugs known to induce hepatitis. The study protocol was approved by the ethics committee of the Helsinki University Central Hospital. Baseline characteristics of these subjects were included in a recent large analysis of liver fat in subjects with and without the metabolic syndrome (4).

Insulin action on glucose rate of appearance (R_a; hepatic insulin sensitivity) was measured directly in 78 subjects using a prolonged low-dose euglycemic hyperinsulinemic clamp to optimize conditions for measuring hepatic insulin sensitivity (14). Characteristics of these subjects have been reported previously (15).

Insulin action on glucose R_a

Hepatic insulin sensitivity was measured using a 6-h euglycemic hyperinsulinemic clamp. At 0800 h, after an overnight fast, two indwelling catheters were placed in an antecubital vein and retrogradely in a heated hand vein for infusion of glucose, insulin, and [3-^{3}H]glucose and for sampling of arterialized venous blood. To determine R_a under basal and hyperinsulinemic conditions, [3-^{3}H]glucose was infused in a primed (20 μCi), continuous (0.2 μCi/min) fashion for a total of 360 min. Baseline blood samples were taken for measurement of fasting serum insulin and glucose concentrations and for biochemical measurements [fS-IGFBP-1, fS-insulin, fS-C-peptide, fasting plasma glucose, glycosylated hemoglobin 1c (HbA_{1c}), S-ALT, S-AST, fS-triglycerides, fS-HDL and fS-low-density lipoprotein (LDL) cholesterol, and fS-free fatty acids (FFAs)]. After 120 min, insulin was infused in a primed continuous (0.3 mU/kg·min) fashion. Plasma glucose was maintained at 5 mmol/liter (90 mg/dl) until 360 min using a variable rate infusion of 20% glucose (16). Blood samples for measurement of glucose specific activity and FFA concentrations were taken at 90, 100, 110, and 120 min in the basal state and at 15- to 30-min intervals during the last 2 h of the insulin infusion. Serum free insulin concentrations were measured at 0, 120, 150, 180, 240, and 360 min. S-IGFBP-1 concentrations during the insulin infusion were measured at 360 min. Glucose specific activity was determined as

TABLE 1. Characteristics of study subjects

	Women	Men	*P* value
n	49	64	
Age (yr)	43 ± 1	41 ± 1	NS
Body composition			
BMI (kg/m^2)	30.2 ± 0.5	26.6 ± 0.5	<0.0001
% Fat	35.2 ± 0.4	20.8 ± 0.7	<0.0001
Waist (cm)	101 ± 2	96 ± 2	0.042
Hip (cm)	109 ± 1	100 ± 1	<0.0001
Waist-to-hip ratio	0.93 ± 0.01	0.96 ± 0.01	0.008
Subcutaneous fat (cm^3)	5100 ± 220	2630 ± 150	<0.0001
Intraabdominal fat (cm^3)	1340 (1060–1790)	1320 (790–2150)	NS
Liver fat (%)	5.0 (3.0–11.0)	3.5 (2.0–14.5)	NS
Serum insulin and IGFBP-1			
fS-insulin (mU/liter)	9.0 (6.0–14.0)	7.0 (5.0–10.0)	NS
fS-C-peptide (nmol/liter)	0.86 (0.61–1.05)	0.66 (0.43–0.92)	NS
fS-IGFBP-1 (μg/liter)	18 (10–27)	16 (12–23)	NS
Glycemic parameters			
fP-glucose (mmol/liter)	5.8 ± 0.1	5.6 ± 0.1	NS
HbA_{1c} (%)	5.6 ± 0.1	5.5 ± 0.1	NS
Serum lipids			
fS-triglycerides (mmol/liter)	1.55 ± 0.11	1.75 ± 0.23	NS
fS-HDL cholesterol (mmol/liter)	1.31 ± 0.05	1.33 ± 0.04	NS
fS-LDL cholesterol (mmol/liter)	3.23 ± 0.11	3.15 ± 0.12	NS
fS-FFA (μmol/liter)	708 ± 27	627 ± 27	0.040
Blood pressure			
Systolic (mm Hg)	124 (113–132)	132 (120–144)	NS
Diastolic (mm Hg)	78 (70–80)	83 (78–94)	<0.0001
Liver enzymes			
S-ALT (U/liter)	26 (19–40)	32 (21–35)	NS
S-AST (U/liter)	25 (22–36)	29 (24–38)	NS

Data are shown as mean ± SEM or, for nonnormally distributed data, as median (25th-75th percentiles). FP, Fasting plasma; LDL, low-density lipoprotein.

previously described (16). Glucose R_a was calculated using the Steele equation, assuming a pool fraction of 0.65 for glucose and a distribution volume of 200 ml/kg for glucose. Glucose R_a during the last 2 h of the insulin infusion was used to determine the percentage and absolute suppressions of endogenous glucose production by insulin.

Liver fat content (^{1}H-MR spectroscopy)

Localized single voxel ($2\times2\times2$ cm^3) proton spectra were acquired using a 1.5-T whole-body system (Siemens Magnetom Vision, Erlangen, Germany), which consisted of a combination of whole-body and loop surface coils for radio frequency transmitting and signal receiving. T1-weighted high-resolution magnetic resonance imaging scans were used for localization of the voxel of interest within the right lobe of the liver. ^{1}H-MRS measurements of the liver fat were performed in the middle of the right lobe of the liver at a location that was individually determined for each subject; vascular structures and sc fat tissue were avoided when selecting the voxel. Subjects lay on their stomachs on the surface coil, which was embedded in a mattress to minimize abdominal movement due to breathing. The single voxel spectra were recorded using the stimulated-echo acquisition mode sequence, with an echo time of 20 msec, a repetition time of 3000 msec, a mixing time of 30 msec, and 1024 data points over 1000 kHz spectral width with 32 averages. Water-suppressed spectra with 128 averages were also recorded to detect weak lipid signals. A short echo time and long repetition time were chosen to ensure a fully relaxed water signal, which was used as an internal standard. Chemical shifts were measured relative to water at 4.80 ppm. The methylene signal, which represents intracellular triglyceride, was measured at 1.4 ppm. Signal intensities were quantified by using an analysis program, VAPRO-MRUI (http://www.mrui.uab.es/mrui/). Spectroscopic intracellular triglyceride content (liver fat) was expressed as a ratio of the area under the methylene peak to that under the methylene and water peaks ($\times100$ = liver fat percentage). This measurement has been validated against histologically determined lipid content (15, 17). When measured by proton spectroscopy, normal liver fat is approximately 5% (3). All spectra were analyzed by physicists who were unaware of any of the clinical data. The reproducibility of repeated measurements of liver fat in nondiabetic subjects studied on two separate occasions by the same reader in our laboratory is 11% (4).

Measurements of body composition

Intraabdominal and sc fat content were determined by magnetic resonance imaging as previously described (4). The percentage body fat was determined using bioelectrical impedance analysis (BioElectrical Impedance Analyzer System, model number BIA-101A; RJL Systems, Detroit, MI). Waist circumference was measured midway between spina iliaca superior and the lower rib margin, and hip circumference was measured at the level of the greater trochanters.

Analytical procedures, calculations, and other measurements

Serum IGFBP-1 concentrations in the fasting state and during the last hour of insulin infusion were determined by an in-house RIA using a polyclonal antibody and human IGFBP-1 as a standard (18). The intra- and interassay coefficients of variance are 3 and 10%, respectively. Plasma glucose concentrations were measured in duplicate with the glucose oxidase method using Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). HbA_{1c} was measured by HPLC using the fully automated Glycosylated Hemoglobin Analyzer System (Bio-Rad, Richmond, CA). Serum free insulin concentrations were measured with the Auto-DELFIA kit (Wallac, Turku, Finland), and C-peptide concentrations by RIA. fS-HDL cholesterol and fS-triglyceride concentrations were measured with enzymatic kits from Roche Diagnostics using an autoanalyzer (Roche Diagnostics Hitachi, Hitachi Ltd., Tokyo, Japan). S-ALT and S-AST activities were determined as recommended by the European Committee for Clinical Laboratory Standards. S-FFA concentrations were measured as previously described (15). The HOMA-IR index was calculated using the following formula: HOMA-IR = fasting insulin (milliunits per liter) $\times$ fasting glucose (millimoles per liter)/22.5 (19).

Statistical analyses

Nonnormally distributed data were used after logarithmic transformation. The unpaired Student's *t* test was used to compare mean values between women and men. Analysis of covariance was used to adjust for age, gender, and BMI. Correlation analyses were performed using Pearson's rank correlation coefficient. Analysis of covariance was used to compare slopes of regression lines and intercepts between women and men. Multiple linear regression analyses were used to determine sources of variation in fS-IGFBP-1, fS-insulin, and fS-C-peptide concentrations. Data are shown as mean ± SEM or, for nonnormally distributed data, as median followed by the 25th and 75th percentiles. Calculations were made using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA) and SPSS 15.0 for Windows (SPSS, Chicago, IL). A *P* value of less than 0.05 was considered statistically significant.

Results

Subject characteristics (Table 1)

The men and women were comparable with respect to age. Women had higher BMI, waist circumference, waist-to-hip ratio, percentage whole-body fat, and sc fat volume than men, but intraabdominal and liver fat were comparable between the groups. fS-triglyceride, HDL and LDL cholesterol, HbA_{1c}, and

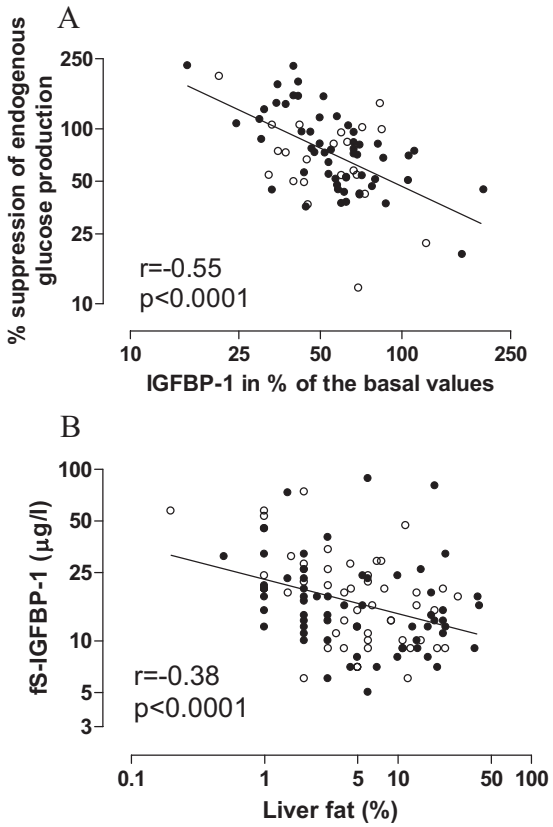

FIG. 1. The relationship between the percentage suppression of hepatic glucose and serum IGFBP-1 concentrations by insulin (A) and between liver fat content and fS-IGFBP-1 (B). *Open circles* denote women, and *filled circles* denote men.

liver enzyme concentrations were comparable between women and men, as were fS-insulin and fS-IGFBP-1 concentrations.

Circulating markers of the suppression of hepatic glucose production by insulin

The percentage suppression of endogenous glucose production by insulin was closely correlated with the percentage S-IGFBP-1 concentrations of the basal levels during the insulin infusion ($P = -0.55$, $P < 0.0001$, Fig. 1). The percentage suppression of endogenous glucose production by insulin was also significantly correlated with fS-insulin ($r = -0.42$, $P = 0.0001$, Fig. 2), HOMA-IR ($r = -0.43$, $P < 0.0001$), fS-C-peptide ($r = -0.41$, $P = 0.0002$, Fig. 2), fS-triglyceride ($r = -0.33$, $P = 0.003$, Fig. 2), fS-HDL cholesterol ($r = 0.30$, $P = 0.007$), fS-ALT ($r = -0.32$, $P = 0.004$, Fig. 2), and fS-FFA ($r = -0.24$, $P = 0.032$, Fig. 2) concentrations. Of circulating parameters measured in fasting serum, fS-IGFBP-1 was the best correlate of hepatic insulin sensitivity ($r = 0.48$, $P < 0.0001$, Fig. 2).

The relationships between liver fat, fS-IGFBP-1, the suppression of S-IGFBP-1 by insulin, and fS-insulin and fS-C-peptide concentrations

fS-IGFBP-1 concentrations were inversely related to liver fat content ($r = -0.38$, $P < 0.0001$, Fig. 1). The percentage

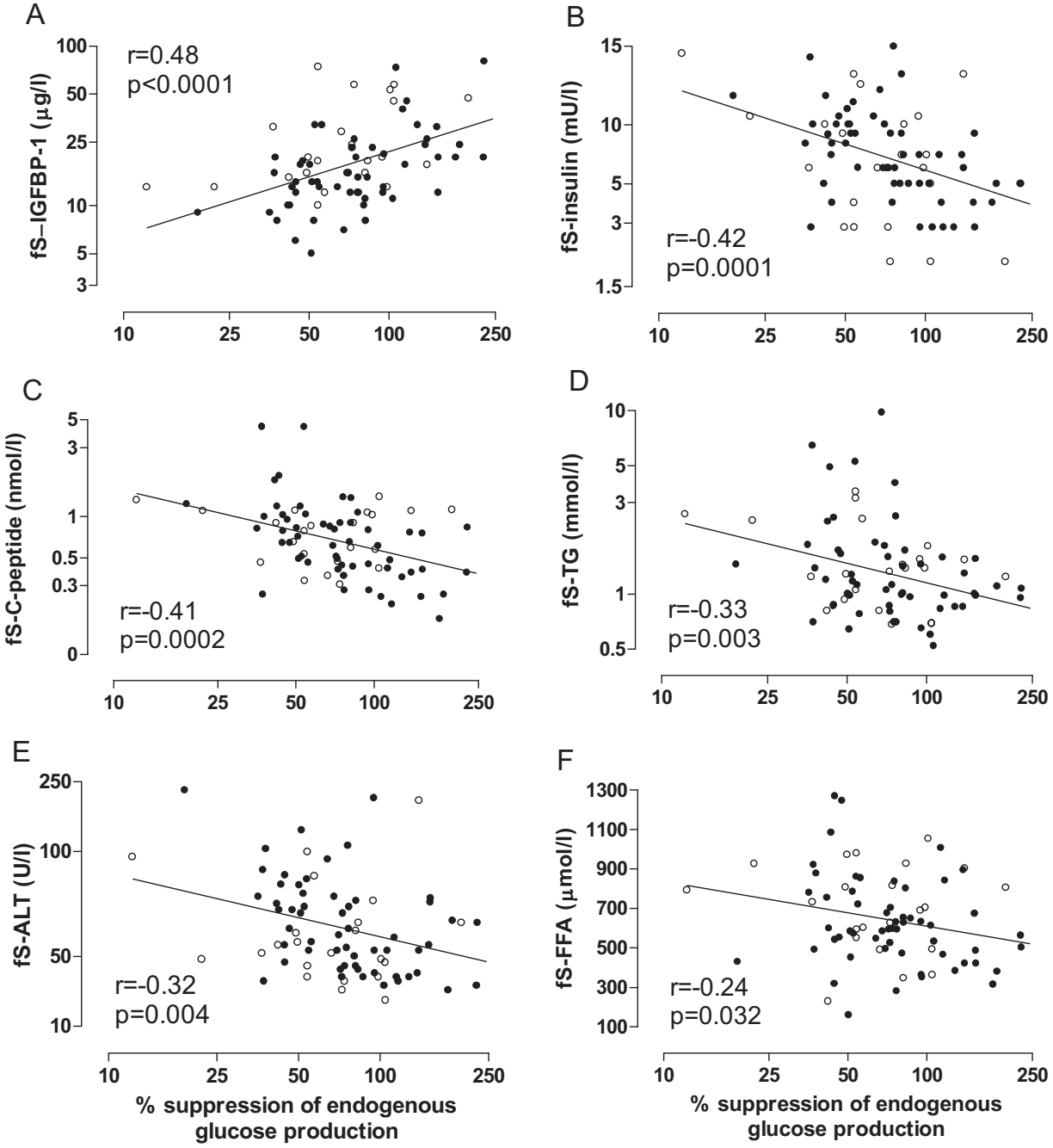

FIG. 2. The relationships between hepatic insulin sensitivity and fS-IGFBP-1 (A), fS-insulin (B), fS-C-peptide (C), fS-triglyceride (D), fS-ALT (E), and fS-FFA (F) concentrations. TG, Triglyceride. *Open circles* denote women, and *filled circles* denote men.

S-IGFBP-1 of the basal levels during the insulin infusion was positively related to liver fat content (r = 0.36, $P = 0.001$). This relationship did not persist statistically significant after adjusting for age, gender, and BMI [r = −0.17, not significant (NS)]. fS-IGFBP-1 concentrations below 16 μg/liter corresponded to a fatty liver (liver fat > 5.6%) (3). The relationship between liver fat and other circulating markers were as follows: fS-insulin (r = 0.57, $P < 0.0001$), fS-C-peptide ($P = 0.57$, $P < 0.0001$), fS-triglycerides (r = 0.41, $P < 0.0001$), fS-HDL cholesterol (r = −0.37, $P < 0.0001$), fS-ALT (r = 0.58, $P < 0.0001$), and fS-FFA (r = 0.13, NS).

Hepatic insulin sensitivity and IGFBP-1: effects of age, gender, and BMI or waist

To examine further the impact of age, gender, and BMI on the relationships between hepatic insulin sensitivity and fS-IGFBP-1, fS-insulin, and fS-C-peptide, multiple linear regression analyses were employed. fS-IGFBP-1 but neither fS-insulin nor fS-C-peptide concentrations were independently associated with hepatic insulin sensitivity (Table 2). Of other variables, BMI was an independent correlate of hepatic insulin sensitivity in all models. The results remained essentially unchanged if BMI was replaced by waist (Table 2). In a model including waist, fS-insulin, fS-ALT, and fS-IGFBP-1 concentrations as independent variables, only fS-IGFBP-1 concentrations were independent determinants of hepatic insulin sensitivity (Table 2). Use of liver fat percentage instead of fS-ALT did not change the results.

TABLE 2. The multivariate regression model searching for the independent determinants of hepatic insulin sensitivity

R^2 (*P* value for the model)	Independent variable	*P*
42% ($P < 0.0001$)	Age	NS
	Gender	NS
	BMI	<0.0001
	fS-IGFBP-1	0.008
36% ($P < 0.0001$)	Age	NS
	Gender	NS
	Waist	0.041
	fS-IGFBP-1	<0.0001
37% ($P < 0.0001$)	Age	NS
	Gender	NS
	BMI	<0.0001
	fS-Insulin	NS
22% ($P < 0.0001$)	Age	NS
	Gender	NS
	Waist	0.027
	fS-Insulin	NS
36% ($P < 0.0001$)	Age	NS
	Gender	NS
	BMI	<0.0001
	fS-C-peptide	NS
20% ($P < 0.0001$)	Age	NS
	Gender	NS
	Waist	0.032
	fS-C-peptide	NS
28% ($P < 0.0001$)	Waist	NS
	fS-insulin	NS
	fS-ALT	NS
	fS-IGFBP-1	0.004

Dependent variable: hepatic insulin sensitivity.

Discussion

In the present study, we examined whether serum concentrations of IGFBP-1, which is produced mainly by the liver in humans (7), are related to liver fat content and hepatic insulin sensitivity. Liver fat and hepatic insulin sensitivity were measured using state-of-the-art methodology in a relatively large number of nondiabetic subjects. The data show that fS-IGFBP-1 concentrations are inversely related to liver fat content and that fS-IGFBP-1 concentrations are closely and independently related to hepatic insulin sensitivity. In addition, IGFBP-1 was a better correlate of hepatic insulin sensitivity than fS-insulin or fS-C-peptide concentrations, or any other circulating parameter measured. However, even the best model explained only 42% of the variation in hepatic insulin sensitivity.

IGFBP-1 has been shown to modify the short-term effects of IGFs (20). Insulin in turn acutely suppresses hepatic production of serum IGFBP-1 (6, 21), which increases bioavailability of IGFs (22). The exact function of IGFBP-1 is unknown. Tissue-specific overexpression in the liver is associated with abnormalities in brain development, reduced body weight gain, reduced fecundity, and kidney damage (23). In human studies, low circulating IGFBP-1 concentrations have been linked with increased risk of macrovascular disease in several studies, but there is no evidence for a cause-and-effect relationship (24). Thus, although IGFBP-1 is unique among known markers of hepatic insulin resistance in that it is regulated by insulin and predicts worsening of glucose tolerance (25), it has not yet been established as a cause of either abnormal glucose metabolism or cardiovascular disease.

Previous studies have suggested that fS-IGFBP-1 concentrations are related to insulin sensitivity measured using HOMA-IR (8–10) or whole-body insulin sensitivity measured using the euglycemic hyperinsulinemic clamp and an insulin infusion rate of 1 mU/kg·min (11–13). The latter reflects mainly peripheral insulin sensitivity (14). Because glucose tracers were not used (11–13), it was not possible to distinguish between hepatic and peripheral insulin sensitivity. The associations between fS-IGFBP-1 and hepatic insulin sensitivity have not previously been studied. Use of a prolonged low-dose insulin infusion enables accurate assessment of interindividual variation in hepatic insulin sensitivity (14). Here we show that fasting IGFBP-1 is a marker of hepatic insulin sensitivity in nondiabetic subjects independent of age, gender, BMI, and fS-insulin concentrations.

Paradoxically, serum concentrations of IGFBP-1 have been reported to be increased in patients with hepatocellular carcinoma and liver cirrhosis (26, 27). Liver fat decreases once cirrhosis develops (28). In addition, insulin resistance in patients with cirrhosis is peripheral rather than hepatic (29). The relationships between IFGBP-1 and other liver diseases have not been previously examined in humans. In the present study, we investigated how IGFBP-1 concentrations change in NAFLD, the most prevalent liver abnormality in the general population (3). Currently available markers of NAFLD, such as S-ALT and S-AST, are poor correlates of liver fat content (4). Thus, novel liver-specific markers of hepatic fat accumulation are needed. In the present study, liver fat content was quantitated, using the most accurate method available to date, and related to serum

IGFBP-1 concentrations. The results suggest that IGFBP-1 is inversely related to liver fat content in nondiabetic subjects.

We conclude that fS-IGFBP-1 is a novel marker of hepatic insulin sensitivity independent of age, gender, and BMI in this population of nondiabetic subjects. fS-IGFBP-1 concentrations reflected hepatic insulin sensitivity better than fS-insulin or C-peptide concentrations. In addition, we found that liver fat content is inversely related to fS-IGFBP-1. The data thus suggest that measurement of IGFBP-1 could provide a simple and reliable estimate of hepatic insulin sensitivity in humans.

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