The IGF system is increasingly implicated in the development of cardiovascular disease. The effects of circulating IGFs on the vasculature are largely modulated by IGFBPs, which control their access to cell-surface IGF receptors. IGFBP-1 has been proposed as the acute regulator of IGF bioavailability because of its metabolic regulation by glucoregulatory hormones. Posttranslational phosphorylation of IGFBP-1 significantly increases its affinity for IGF-I and therefore represents a further mechanism for controlling IGF bioavailability. We have therefore examined the IGF system and IGFBP-1 phosphorylation status, using specifically developed immunoassays, in a cohort of 160 extensively characterized type 2 diabetic subjects on two occasions 12 months apart. Total IGFBP-1 (tIGFBP-1), which is predominantly highly phosphorylated, was significantly lower in subjects with known macrovascular disease (geometric mean [95% CI], 48.7 μg/l [33.7–63.6]; P = 0.01) than in patients with no vascular pathology (80.0 μg/l [52.2–107]; P = 5.4, P = 0.01). A similar relationship was found for highly phosphorylated IGFBP-1 (hpIGFBP-1) concentration (known macrovascular disease, 45.1 μg/l [35.1–55.2]; no macrovascular disease, 75.8 μg/l [56.2–95.3]; F = 4.8, P = 0.01). Logistic regression showed that for every decrease of 2.73 μg/l in IGFBP-1 concentration, there was a 43% increase in the odds of a subject having macrovascular disease (odds ratio 0.57 [95% CI 0.40–0.83]; P = 0.001). hpIGFBP-1 correlated negatively with systolic blood pressure (P = 0.30, P < 0.01), diastolic blood pressure (P = 0.45, P < 0.001), and mean arterial pressure (MAP) (P = 0.41, P < 0.001). Linear regression modeling showed that 40% of the variance in tIGFBP-1 was accounted for by MAP, triglycerides, and nonesterified fatty acids. In contrast, levels of nonphosphorylated and lesser-phosphorylated IGFBP-1 (lpIGFBP-1) were unrelated to macrovascular disease or hypertension but did correlate positively with fasting glucose concentration (P = 0.350, P < 0.01). tIGFBP-1 concentrations were higher in subjects treated with insulin alone (n = 29) than for any other group. This effect persisted after adjustment of tIGFBP-1 levels for BMI, C-peptide, age, and sex (F = 6.5, P < 0.001, P = −0.46). Such an effect was not apparent for lpIGFBP-1. We conclude that low circulating levels of hpIGFBP-1 are closely correlated with macrovascular disease and hypertension in type 2 diabetes, whereas lpIGFBP-1 isoforms are associated with glycemic control, suggesting a dual role for IGFBP-1 in the regulation of IGF actions in type 2 diabetes. Our data suggest that high circulating concentrations of highly phosphorylated IGFBP-1 may protect against the development of hypertension and cardiovascular disease by reducing the mitogenic potential of IGFs on the vasculature.

Type 2 diabetes is associated with a three- to fivefold increased risk of macrovascular disease that cannot be completely explained by known risk factors. IGFs and IGFBPs are increasingly being recognized as contributing to cardiovascular risk (1–4). In addition, there are well-established links between IGFs and other surrogate markers for cardiovascular risk. IGF-I has been shown to correlate with LDL cholesterol in normal women (5), patients with hypothyroidism (6), and the elderly (6) and with apolipoprotein B and cardiovascular risk in normal subjects (7). There is also evidence that the IGF system is involved in the underlying pathophysiological processes associated with cardiovascular disease. In vivo animal studies suggest a role for IGF-I as a mediator of the hypertrophic responses of vascular smooth muscle cells (VSMCs) in hypertension (8) and implicate IGF-I as a mediator of cardiac hypertrophy in congestive cardiac failure (9), whereas in vitro, IGF-I receptor availability is closely linked with VSMC growth (10).

IGF binding proteins predominantly control the access of IGFs to tissues and cell-surface IGF receptors. Of these, IGFBP-1 is the most likely candidate for acute regulation of IGF actions because of its acute downregulation by...
insulin and upregulation by other gluconeoregulatory hormones and cytokines and in catabolic states (11–13). Furthermore, transendothelial movement of IGFBP-1 into the tissues is known to occur and to be enhanced by insulin (14). IGFBP-1 thus provides an important link between intermediary metabolism and regulation of IGF actions.

We and others have previously shown that IGFBP-1 is negatively related with insulin resistance, blood pressure, BMI, waist-to-hip ratio, and triglyceride levels, as found previously in a population-based sample and in type 2 diabetes (2,4,15). With the development of type 2 diabetes (15), however, IGFBP-1 concentrations rise again despite persisting hyperinsulinemia, suggesting a complex interaction between pancreatic insulin output and hepatic insulin sensitivity. IGFBP-1 can exist in several phosphorylated isoforms. Phosphorylation of IGFBP-1 significantly alters its affinity for IGF-I (16,17). Under normal circumstances, circulating IGFBP-1 exists primarily in a highly phosphorylated form, which has the highest affinity for IGF-I (17). Changes in the phosphorylation state of IGFBP-1, therefore, could profoundly influence its ability to modulate IGF actions such that lesser-phosphorylated and nonphosphorylated variants would potentially increase, and highly phosphorylated forms reduce, IGF bioavailability. We have previously shown in diabetic pregnancy that increased levels of highly phosphorylated IGFBP-1 are associated with reduced fetal growth, whereas lesser-phosphorylated IGFBP-1 species are more closely associated with maternal weight gain, suggesting that phosphorylation of IGFBP-1 can differentially affect the metabolic and mitogenic actions of IGFs in different tissues (18). It therefore seems likely that in type 2 diabetes, the known dysregulation of the IGF/IGFBP-1 system would result in changes in IGF bioavailability that subsequently could predispose an affected individual to develop premature cardiovascular disease.

In this study, we have examined the relationship between IGFBP-1 phosphorylation status and the IGF system in type 2 diabetic subjects with and without evidence of macrovascular disease.

RESEARCH DESIGN AND METHOD

Subjects and study design. One hundred sixty subjects were enrolled from two diabetes clinics (Hope Hospital and Manchester Royal Infirmary) in Manchester, U.K. The mean age of the participants was 58.1 ± 9.1 years, and subjects were matched for duration of diabetes (mean 8.2 ± 1.2 years). Previously, individuals had been randomly assigned to type of treatment according to U.K. Prospective Diabetes Study (UKPDS) protocols. Their current treatments were diet alone (n = 20), sulfonylurea (chlorpropamide or glipizide) treatment (n = 71), insulin alone (n = 29), and insulin (once or twice a day) plus sulfonylurea treatment (n = 40). They belonged to three different ethnic groups: European (n = 121), Pakistani (n = 23), and African-Caribbean (n = 16). There was no significant difference between the ethnic groups for age or duration of diabetes. All subjects gave informed consent, and the protocol was approved by local ethics committees.

All blood samples were obtained after an overnight fast, and the patients’ usual medications were withheld until after venesection. Plasma was separated from the remaining blood by centrifugation at 4°C. Plasma samples were stored at −70°C until analysis. Blood samples were taken at baseline and 12 months later.

Blood pressure was the mean of three readings taken with the subject in the supine position. A survey of medical records was carried out to obtain a detailed medical history.

Macrovascular disease was defined as a history of any of the following: ischemic heart disease including previous myocardial infarction and congestive heart failure, intermittent claudication, cerebrovascular accident, transient ischemic attack, peripheral vascular disease (defined by unilateral or bilateral absence of foot pulses), or systolic blood pressure >170 mmHg.

Assays. Levels of IGF-I (19), IGFBP-1 (tIGFBP-1) (using monoclonal antibody [mAb] Ab303, which detects all IGFBP-1 phosphoforms) (21), and lesser-phosphorylated/nonphosphorylated IGFBP-1 (lpgIGFBP-1) (using mAb Ab305, which detects only the lesser- and nonphosphorylated forms of IGFBP-1) (21) were determined by previously reported antibody-based assays. The assays have respective detection limits of 28 ng/ml, 3 μg/l, and 3 μg/l and within- and between-assay CVs of <10%. Where necessary, highly phosphorylated IGFBP-1 (hpgIGFBP-1) was calculated by subtraction of lpgIGFBP-1 from tIGFBP-1 for each subject.

Insulin and insulin C-peptide were measured using the Mercodia (Uppsala, Sweden) enzyme-linked immunosorbent assay (ELISA) for intact insulin and the Dako (Ely, U.K.) ELISA for intact insulin C-peptide, with respective detection limits of 0.15 mEq/l and within- and between-assay CVs of <3%. Cross-reactivity of the insulin assay for insulin C-peptide and the C-peptide assay for insulin is <0.1%.

Fasting cholesterol and triglycerides were measured by enzymatic methods using the Cobasintegra autoanalyzer (Roche Diagnostics, Welwyn Garden City, U.K.), with HDL cholesterol measured by a direct method (Cobasintegra autoanalyzer). LDL cholesterol was calculated according to the Friedewald formula (22).

Fasting nonesterified fatty acid (NEFA) concentrations were measured using the Wako (Neuss, Germany) ELISA for total NEFAs. Sensitivity of the assay is 0.05 mEq/l and within- and between-assay CVs <10%. Glucose was assayed using the hexokinase method (Cobasintegra autoanalyzer). HBA1c was measured using the Cobasmara autoanalyzer (Roche Diagnostics).

Nondenaturing PAGE. To confirm the validity of antibody-based measurements of IGFBP-1 phosphoforms, we also separated and visualized the IGFBP-1 phosphoforms using nondenaturing PAGE (23). Briefly, for a subset of subjects with no manifest macrovascular disease, IGFBP-1 in 250 μl plasma was immunoprecipitated with mAb Ab303 (1:1,000; volume 250 μl) (provided by Medix Biochemica, Kauniainen, Finland), which immunoprecipitates all phosphoforms of IGFBP-1 (21), and incubated overnight at 4°C. IGFBP-1 in 500 μl plasma from subjects with known macrovascular disease was required for immunoprecipitation to be able to detect lower concentrations. Two controls were used, HepG2 conditioned medium containing all the phosphoforms of IGFBP-1 and amniotic fluid, which contains all the lesser-phosphorylated isoforms but no hpgIGFBP-1. After incubation, the antigen-antibody complexes were immunoprecipitated using Saccel rabbit anti-mouse antibody (IDS, Tyne & Wear, U.K.). Immunoprecipitated IGFBP-1 was applied to a 10% native polyacrylamide gel (23). After overnight transfer to nitrocellulose membranes, nonspecific binding was blocked by incubation with 1% BSA in 0.15 mol/l sodium chloride. The proteins were revealed by incubation with 150,000 cpm/ml [125I]IGF-I for 3 hr at 25°C in accordance with the method of Hossenlopp et al. (24), followed by phosphor-image analysis (Fuji bio-imaging analyzer) and autoradiography.

Statistics. Multiple logistic regression was used to assess the risk of macrovascular disease in relation to changes in IGFBP-1 concentration. Spearman correlations, both simple and partial, were calculated to investigate the relation between IGFBP-1 and other metabolic variables, BMI, and blood pressure.

 Differences in metabolic variables between treatment groups and ethnic groups were assessed using ANCOVA. Non–normally distributed data were logarithmically transformed before analysis. Post hoc testing used Scheffe’s test. Significant predictors for IGFBP-1 were examined using multiple regression analysis. Comparison of IGFBP-1 at initial assessment and follow-up was carried out using linear regression analysis. All analyses were carried out using the statistical package SPSS, version 9.0.

RESULTS

Relationship of IGFBP-1 with macrovascular disease. Relationship of IGFBP-1 with macrovascular disease, tIGFBP-1 was markedly reduced in subjects (n = 40) with known macrovascular disease (48.7 μg/l [33.7–63.6], geometric mean [95% CI]) compared with patients (n = 120) with no manifest macrovascular pathology (80.0 μg/l [52.2–107]; F = 5.4, P = 0.01) (Table 1). A similar relationship was also found for hpgIGFBP-1 concentration (known macrovascular disease, 45.1 μg/l [35.1–55.2]); no macrovascular disease, 75.8 μg/l [56.2–95.3]; F = 4.8, P = 0.01) (Table 1). Logistic regression showed that for every unit decrease in the log tIGFBP-1 (increase of 2.73 μg/l in the tIGFBP-1 concentration), there was a 43% increase in the
odds of the subject having macrovascular disease (odds ratio 0.57 [95% CI 0.40–0.83]; \(P = 0.001\)) (Fig. 1).

There was no difference in lpIGFBP-1 by macrovascular disease status. For the whole group, lpIGFBP-1 (12.2 μg/l [10.9–13.5]) was significantly lower than tIGFBP-1 (76.1 μg/l [56.8–95.5]); \(P < 0.001\).

To confirm the assay results, native PAGE was performed on a subset of subjects with high \((n = 11)\) versus low \((n = 12)\) tIGFBP-1. Representative results are shown in Fig. 2. The concentration of hpIGFBP-1 was less in those with macrovascular disease than in those without manifest macrovascular pathology. There was no corresponding difference in lpIGFBP-1 by macrovascular disease status.

Table 1: IGFBP-1 by macrovascular disease status

<table>
<thead>
<tr>
<th>Macrovascular disease</th>
<th>No macrovascular disease</th>
<th>(F)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGFBP-1 (μg/l)</td>
<td>48.7 (33.7–63.6)</td>
<td>80.0 (52.2–107)</td>
<td>5.4</td>
</tr>
<tr>
<td>hpIGFBP-1 (μg/l)</td>
<td>45.1 (35.1–55.2)</td>
<td>75.8 (56.2–95.3)</td>
<td>4.8</td>
</tr>
<tr>
<td>lpIGFBP-1 (μg/l)</td>
<td>12.6 (10.7–14.6)</td>
<td>11.3 (9.9–12.8)</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Data are arithmetic means (95% CI).

The relationship between tIGFBP-1 and hypertension was robust. Using multiple regression modeling, 40% of the variance in tIGFBP-1 was accounted for by three variables, MAP (unstandardized coefficient \(B = -3.4 \pm 0.8; P < 0.001\)), triglycerides \((B = -23.1 \pm 7.4, P = 0.003)\), and NEFAs \((B = -0.6 \pm 0.03, P = 0.04)\) (Figs. 3A–C) in a model that included IGF-I, IGF-II, insulin C-peptide, cholesterol, triglycerides, NEFAs, fasting glucose, Hba1c, age, sex, BMI, and MAP. When this analysis was repeated by treatment group, the relationship with MAP was greatest in the group treated with insulin alone, where 69% of the variance in IGFBP-1 was accounted for by MAP alone \((P < 0.001)\) (Fig. 3D).

**IGFBP-1 and glycemic control.** As anticipated, there was a strong negative correlation of tIGFBP-1 concentration with insulin C-peptide concentration and also with peripheral insulin concentration and triglyceride level (Table 2). However, there was no relationship between tIGFBP-1 and fasting glucose or Hba1c. This result was in complete contrast to lpIGFBP-1, which correlated positively with fasting glucose \((\rho = 0.350, P < 0.01)\).

**Effect of different treatments on IGFBP-1 and macrovascular risk.** In agreement with our previously reported findings (25), tIGFBP-1 concentration was higher in subjects treated with insulin alone than in any other group (Table 3; Fig. 4). This difference persisted even when tIGFBP-1 was corrected for BMI and C-peptide in addition to age and sex \((F = 6.5, P < 0.001)\). In contrast, there was no variation in lpIGFBP-1 concentration by treatment group (Table 3). Peripheral insulin levels were higher in those treated with insulin (with or without additional sulfonylureas) than in subjects not receiving insulin, whereas C-peptide levels were lower in subjects treated with insulin (with or without additional sulfonylureas). There was no significant difference in fasting glucose level or Hba1c by treatment group (Table 3).

**Relationships of IGF-I and IGF-II with macrovascular disease and type of treatment.** There were no differences in IGF-I or IGF-II levels between subjects with and without evidence of macrovascular disease. IGF-I correlated positively with IGF-II and negatively with NEFAs. Interestingly, IGF-II concentration was strongly positively correlated negatively with mean arterial pressure (MAP) (Spearman’s \(\rho = -0.26, P = 0.002\)). The latter relationship persisted even when corrected for age, sex, BMI, and insulin C-peptide concentration (partial \(\rho = -0.30, P < 0.001\)). hpIGFBP-1 correlated negatively with systolic blood pressure \((\rho = -0.45, P < 0.001)\), and MAP \((\rho = -0.41, P < 0.001)\). In contrast to tIGFBP-1 and hpIGFBP-1, no consistent relationships were found with lpIGFBP-1 and macrovascular disease, hypertension, or cardiovascular risk markers.
correlated with elevated total, LDL, and HDL cholesterol levels (Table 2).

IGF-I concentration was not affected by treatment category, but IGF-II levels were lower in subjects treated with insulin alone than in any other group (Table 3). There was a trend for total cholesterol to be lower in subjects treated with insulin alone ($F = 2.2, P = 0.06$), but otherwise there were no detectable differences in lipid profile by treatment group.

Robustness of IGFBP-1 over a 12-month period. Because hepatic IGFBP-1 synthesis is acutely regulated by glucoregulatory hormones, we wished to establish whether assessment of fasting IGFBP-1 levels could robustly predict association with macrovascular disease even after a gap of 12 months. Therefore, tIGFBP-1 concentration was assessed at initial visits and 12-month follow-ups as shown in Fig. 5. Linear regression analysis demonstrated that tIGFBP-1 on follow-up was closely related to tIGFBP-1 at initial visit by the equation $t\text{IGFBP-1}_{\text{follow-up}} = 3.2 + (t\text{IGFBP-1}_{\text{initial visit}}) 	imes 0.92$ ($r^2 = 0.90, P < 0.001$). This suggests that fasting tIGFBP-1 concentration is a consistent measure with little variation over a 12-month period.

![Figure 2: Native PAGE for IGFBP-1 by macrovascular disease status. Controls used HepG2 conditioned medium containing all the phosphorylated forms of IGFBP-1 (lane A) and amniotic fluid containing the less-phosphorylated forms but not highly phosphorylated IGFBP-1 (lane B). 1, the position of hIGFBP-1; 2, the position of lIGFBP-1.](image1)

![Figure 3: Scatter plots of MAP versus IGFBP-1 concentrations (A), triglyceride versus IGFBP-1 concentrations (B), and NEFA versus IGFBP-1 concentrations (C) for all patients in the study and MAP versus IGFBP-1 concentrations for the group treated with insulin alone (D).](image2)
TABLE 3
Effects of ethnicity on the IGF system. There were no differences in IGFBP-1 levels between the ethnic groups (Table 4). IGF-I concentration was higher in African-Caribbeans than in Europeans or Pakistanis, and IGF-II was higher in Europeans than in African-Caribbeans or Pakistanis. The IGF-I results are in accord with previous findings in non-diabetic subjects (26) and indicate that the onset of type 2 diabetes does not mask underlying differences in circulating IGF concentrations related to ethnicity. Also in keeping with previous studies (2), we found a close correlation between IGF-II and total, LDL, and HDL cholesterol. The significance of the IGF-II/cholesterol relationship is unknown, although similarities in clearance pathways between the β Very low density lipoprotein (β-VLDL) receptor and the mannose-6-phosphate receptor may suggest a possible mechanism (27). Insulin levels were similar in all three ethnic groups, with C-peptide being lower in African-Caribbeans than in Europeans or Pakistanis. There was no significant difference in total cholesterol, LDL cholesterol, or triglyceride levels by ethnic group. There was no difference in age, duration of diabetes, BMI, or fasting glucose between the three ethnic groups.

DISCUSSION
This is the first study to demonstrate close links between fasting lpIGFBP-1 and the presence of macrovascular disease and hypertension in type 2 diabetes. In addition to the negative correlation between IGFBP-1 and macrovascular disease, we found significant negative relationships between tIGFBP-1 and other known cardiovascular risk factors, namely BMI, triglyceride concentrations, circulating insulin C-peptide, and insulin levels. These relationships were robust and persisted over a 12-month period.

IGFBPs have been clearly shown to modulate the actions of IGFs. IGFBP-1 in particular is accepted as an acute modulator of IGF bioavailability (13). We and others have shown a close relationship between low IGFBP-1 levels and surrogate markers of cardiovascular risk in a nondiabetic population (3,15). We showed that low circulating IGFBP-1 concentrations significantly correlated with impaired glucose tolerance (IGT). Because the subjects in our previous study (15) did not have any evident macrovascular pathology, and because IGT precedes the development of type 2 diabetes, it is probable that a low circulating IGFBP-1 concentration predates the development of macrovascular disease. As proposed previously (15), we believe that the development of peripheral insulin resistance, with maintenance of hepatic insulin sensitivity, explains the decrease in circulating IGFBP-1 (and thus the increase in IGF bioavailability) long before the development of type 2 diabetes. Our current data suggest that this pattern persists in a type 2 diabetic population and further supports the hypothesis that IGFBPs may be early indicators of significant macrovascular disease.

TABLE 2
Universal correlation matrix for metabolic variables

<table>
<thead>
<tr>
<th></th>
<th>IGFBP-I</th>
<th>lpIGFBP-I</th>
<th>IGF-I</th>
<th>IGF-II</th>
<th>C-peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGFBP-1</td>
<td>X</td>
<td>0.48*</td>
<td>−0.02</td>
<td>−0.16‡</td>
<td>−0.46‡</td>
</tr>
<tr>
<td>lpIGFBP-1</td>
<td>0.48*</td>
<td>X</td>
<td>−0.12</td>
<td>−0.06</td>
<td>−0.17</td>
</tr>
<tr>
<td>IGF-I</td>
<td>−0.02</td>
<td>−0.12</td>
<td>X</td>
<td>0.51‡</td>
<td>−0.07</td>
</tr>
<tr>
<td>IGF-II</td>
<td>−0.05</td>
<td>0.06</td>
<td>0.51‡</td>
<td>X</td>
<td>0.01</td>
</tr>
<tr>
<td>C-peptide</td>
<td>−0.46‡</td>
<td>−0.17</td>
<td>−0.19†</td>
<td>0.04</td>
<td>0.19†</td>
</tr>
<tr>
<td>Insulin</td>
<td>−0.23†</td>
<td>−0.07</td>
<td>−0.08</td>
<td>−0.12</td>
<td>0.01</td>
</tr>
<tr>
<td>NEFAs</td>
<td>0.03</td>
<td>0.17</td>
<td>0.11</td>
<td>0.23*</td>
<td>0.08</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>−0.13</td>
<td>−0.08</td>
<td>0.05</td>
<td>0.32‡</td>
<td>0.12</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>−0.10</td>
<td>−0.07</td>
<td>0.11</td>
<td>0.23*</td>
<td>0.08</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.06</td>
<td>−0.01</td>
<td>0.09</td>
<td>0.19†</td>
<td>−0.24*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>−0.23</td>
<td>−0.14</td>
<td>−0.13</td>
<td>0.12</td>
<td>0.30‡</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.08</td>
<td>0.35*</td>
<td>0.01</td>
<td>0.01</td>
<td>0.09</td>
</tr>
<tr>
<td>HbA1c</td>
<td>−0.02</td>
<td>0.17</td>
<td>−0.01</td>
<td>−0.01</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Data are Spearman correlations (p). *P < 0.01; †P < 0.05; ‡P < 0.001.

TABLE 3
Metabolic variables by treatment group adjusted for age and sex

<table>
<thead>
<tr>
<th>n</th>
<th>Diet only</th>
<th>SU</th>
<th>Insulin treatment</th>
<th>Insulin + SU</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>55.4 (31.9–78.8)</td>
<td>52 (40.9–63.1)</td>
<td>102.1 (60.3–143.8)*</td>
<td>51.4 (36.6–66.2)</td>
<td>12.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>71</td>
<td>13.6 (8.3–18.9)</td>
<td>11.4 (10.1–12.7)</td>
<td>13.7 (10.3–17.1)</td>
<td>10.3 (8.8–11.8)</td>
<td>1.2</td>
<td>NS</td>
</tr>
<tr>
<td>29</td>
<td>60.6 (28.3–92.9)</td>
<td>61.7 (48.7–74.7)</td>
<td>148.1 (92.2–204)*</td>
<td>138.4 (95.3–181.4)*</td>
<td>5.0</td>
<td>0.003</td>
</tr>
<tr>
<td>40</td>
<td>980 (795–1,165)</td>
<td>959 (871–1,047)</td>
<td>721 (480–961)*</td>
<td>706 (608–864)*</td>
<td>2.5</td>
<td>0.03</td>
</tr>
<tr>
<td>188</td>
<td>188 (153–224)</td>
<td>199 (172–225)</td>
<td>200 (164–237)</td>
<td>202 (169–237)</td>
<td>1.3</td>
<td>NS</td>
</tr>
<tr>
<td>667</td>
<td>667 (553–781)</td>
<td>628 (572–683)</td>
<td>576 (494–659)*</td>
<td>625 (541–799)</td>
<td>3.8</td>
<td>0.003</td>
</tr>
<tr>
<td>5.5 (5.2–6.1)</td>
<td>5.7 (5.4–5.9)</td>
<td>5.5 (5.1–5.8)</td>
<td>5.7 (5.4–6.0)</td>
<td>2.2</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>3.5 (3.2–3.9)</td>
<td>3.6 (3.4–3.9)</td>
<td>3.4 (3.1–3.8)</td>
<td>3.6 (3.3–4.0)</td>
<td>1.3</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>2.1 (1.7–2.5)</td>
<td>2.1 (1.8–2.3)</td>
<td>1.9 (1.6–2.2)</td>
<td>2.1 (1.8–2.3)</td>
<td>1.4</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>9.2 (8.0–10.5)</td>
<td>10.8 (9.9–11.7)</td>
<td>9.8 (8.1–11.5)</td>
<td>10.2 (9.0–11.4)</td>
<td>1.0</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>4.9 (3.9–6.1)</td>
<td>6.3 (5.8–6.8)</td>
<td>5.8 (5.1–6.3)</td>
<td>6.4 (5.7–7.1)</td>
<td>2.4</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>28.1 (26.3–29.9)</td>
<td>30.4 (29.0–31.9)</td>
<td>29.7 (28.2–31.3)</td>
<td>32.5 (30.8–34.1)</td>
<td>3.8</td>
<td>0.003</td>
<td></td>
</tr>
</tbody>
</table>

Data are arithmetic means (95% CI). *Significantly different by post-hoc testing (Scheffe), P < 0.05. SU, sulfonylureas.
predisposes affected individuals to premature macrovascular disease.

The majority of circulating IGFBP-1 is in the highly phosphorylated state, implying that it is this isoform of IGFBP-1 that is most relevant to the development of hypertension and macrovascular disease (2). hpIGFBP-1 has the highest affinity for IGF-I relative to other isoforms (16,17), and its affinity for ligand is significantly greater than that of the type 1 IGF receptor (28). Thus hpIGFBP-1 has the greatest potential to inhibit IGF-I/IGF receptor interactions.

In nondiabetic hypertensive humans, circulating IGF-I levels independently predict 16% of the variability in left ventricular mass (29). In another study, a reduction in serum IGF-I level in relation to bezafibrate treatment in post–myocardial infarction patients was independently associated with angiographic measures of coronary artery disease regression (30). Furthermore, in acromegaly, a human model of IGF-I excess (where World Health Organization criteria indicate that 37.5% patients have hypertension), blood pressure positively correlates with IGF-I concentration (31). These findings strongly support an endocrine effect of IGF-I in vivo. It is important, however, to recognize that IGFBP-1 is capable of inhibiting endocrine, paracrine, and autocrine IGF effects because of its ability to access the extracellular space and to associate with specific cell-surface integrins (32). Thus a high circulating level of hpIGFBP-1 will inhibit IGF effects whatever the cellular source of the IGF-I.

An elevated concentration of hpIGFBP-1 would therefore serve to protect vascular tissues from the mitogenic potential of circulating IGFs, whereas a consistently reduced level—induced by the diabetic state per se, hyperinsulinemia, or both—would increase IGF availability to susceptible tissues. This situation could be further worsened in type 2 diabetes, where reports of increased IGFBP-3 proteolysis (33) would further contribute to the exposure of the vascular compartment to the mitogenic actions of IGFs. It is interesting in this respect to note that African-Caribbean subjects in this and other studies tend to have higher IGF-I levels and are known to be more susceptible to resistant hypertension than other ethnic groups (34).

There are no studies examining the ability of liver to differentially phosphorylate IGFBP-1 in humans. Peripheral dephosphorylation seems a more likely explanation for the presence of hpIGFBP-1. Evidence for the ability of ectophosphatases (present in many cell types) to dephosphorylate IGFBP-1 has been reported (35). Cell-surface dephosphorylation would significantly affect the bioavailability of IGF-I at the cellular level. Thus IGFBP-1 in the highly phosphorylated state would significantly reduce interaction of IGF-I with its receptors, but with subsequent dephosphorylation could enhance local IGF bioavailability. Thus changes in tissue phosphatase/protease activity would further influence IGFBP-1/IGF-I binding kinetics and so IGF bioavailability at the tissue level (35). Further
studies will be required to determine how the regulation of these factors influences cell-specific IGF effects.

In this study, we found that lesser-/nonphosphorylated IGFBP-1 correlated positively with fasting glucose. This finding was unexpected and has not been described previously. It implies that IGFBP-1 phosphorylation status may play an important part in mediating the effects of IGFBP-1 on glucose homeostasis. The finding of a positive correlation between fasting glucose and IgPFBP-1 may suggest a mechanism to increase insulin-like activity at times of high ambient glucose concentration and, given the lower affinity of IgPFBP-1 for IGF-I, parallels reports of increased IGFBP-3 proteolysis associated with hyperglycemia in type 2 diabetes (35).

This study confirms our earlier findings of higher IGFBP-1 levels in subjects with type 2 diabetes who were treated with insulin alone (25). A simple explanation for this finding would be that a reduced portal insulin concentration in these subjects would remove the tonic inhibition of hepatic IGFBP-1 synthesis, leading to increased circulating levels of IGFBP-1. To address this issue in this study, we measured C-peptide levels, which undergo little hepatic extraction and therefore better represent hepatic insulin exposure via the portal system. There were no significant differences in insulin and insulin C-peptide concentrations between the groups treated with insulin alone or insulin plus sulfonylureas, although IGFBP-1 levels were significantly different, suggesting that it is less likely to be pancreatic β-cell insulin output and more likely to be hepatic insulin resistance that determines the differences in tIGFBP-1 level between treatment groups. This result is in keeping with the multiple linear regression analysis showing that factors other than insulin were important in determining tIGFBP-1 concentration, particularly MAP, NEFAs, and triglycerides.

The close relationship between fasting tIGFBP-1 levels at inception of the study and 1-year follow-up confirms the robustness of fasting tIGFBP-1 measurement over time and points to the utility of tIGFBP-1 as a predictive biomarker for macrovascular disease in type 2 diabetes.

In summary, these studies demonstrate the novel and important finding of close links between low circulating levels of the highly phosphorylated isoform of IGFBP-1 and the presence of macrovascular disease and hypertension in type 2 diabetes. In addition, we have shown that lesser-phosphorylated IGFBP-1 levels are associated with ambient glucose concentration, suggesting that the phosphorylation status of IGFBP-1 may be important in glucose homeostasis. Because IGFBPs largely determine the mitogenic and metabolic potential of IGFs, our findings suggest an etiopathogenic role for IGFBP-1 in modulating the development of large vessel disease in type 2 diabetes.

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