Vascular endothelial function and blood pressure homeostasis in mice overexpressing IGF binding protein-1.

Vascular Endothelial Function and Blood Pressure Homeostasis in Mice Overexpressing IGF Binding Protein-1

Stephen B. Wheatcroft,[1] Mark T. Kearney,[1] Ajay M. Shah,[1] David J. Grieve,[1] Ian L. Williams,[1] John P. Miell,[2,3] and Paul A. Crossey[1]

IGFs and their binding proteins (IGFBPs) play a significant role in metabolic regulation, and there is growing evidence that they also exert important vascular effects. IGFBP-1 contributes to glucose counterregulation, and observational studies demonstrate an inverse association between circulating IGFBP-1 levels and cardiovascular risk factors. Furthermore, IGFBP-1 levels are lower in subjects with overt macrovascular disease. We therefore hypothesized that IGFBP-1 exerts potentially beneficial effects, either directly or indirectly, on blood pressure regulation and vascular function. We tested this hypothesis using a unique transgenic mouse, which overexpresses human IGFBP-1, and explored the effect of this protein on metabolic, blood pressure, and vascular homeostasis. IGFBP-1–overexpressing mice exhibited postprandial hyperinsulinemia with preservation of glucocompetence and insulin sensitivity. Blood pressure was unchanged in the fasting state but was significantly lower in transgenic mice after a carbohydrate load. Aortic rings from IGFBP-1–overexpressing mice were hypocontractile in response to vasoconstrictors, and relaxation responses were unimpaired. Basal nitric oxide production was increased and endothelial nitric oxide synthase mRNA expression upregulated in aortae of these mice. Our data suggest that IGFBP-1 plays an important and potentially beneficial role in regulating metabolic and vascular homeostasis. *Diabetes* 52: 2075–2082, 2003

It is now established that a key early event in the development of atherosclerosis is vascular endothelial cell dysfunction (1). A characteristic feature of endothelial dysfunction is a reduction in the bioactivity of the antiatherosclerotic molecule nitric oxide (NO) (2). The evidence is compelling that abnormalities of metabolism may lead to a reduction in NO bioavailability and hence play an important role in the pathophysiology of cardiovascular atherosclerosis (3,4).

Insulin is a key peptide in metabolic homeostasis, but IGF-I may also play an important complementary role in the maintenance of normoglycemia (5). Recently, it has been demonstrated that insulin and IGF-I can increase endothelial cell production of NO (6). Resistance to insulin's glucoregulatory actions is associated with conditions characterized by accelerated atherosclerosis, including type 2 diabetes, hypertension, and obesity (7). Consonant with this, robust evidence has established insulin resistance and hyperinsulinemia as independent risk factors for the development of coronary artery disease (8,9), although a potential contribution of IGF-I and its regulatory proteins was not assessed in these studies.

Only a small fraction of IGF-I is present in the circulation in the active free form. A substantial proportion of circulating IGF-I is bound to a family of six binding proteins (IGFBPs), which have a number of physiological roles, including modulation of IGF-I bioavailability at target cells (10,11). One of these binding proteins, IGFBP-1, has been proposed as a short-term regulator of IGF-I bioavailability and is thought likely to play an important role in metabolic homeostasis (5,12). Unlike the other members of the binding protein family, IGFBP-1 possesses a short plasma half-life and exhibits dynamic variation in plasma levels (13). Plasma IGFBP-1 levels are regulated acutely by nutritional status, showing an increment after fasting and a rapid decline after a high-carbohydrate meal. These effects are mediated, at least in part, by an inhibitory action of insulin on hepatic IGFBP-1 production (14,15).

Interest in a potential role for IGFBP-1 in vascular disease derives from a series of recently published cross-sectional observational studies that demonstrated an inverse correlation between IGFBP-1 levels and the presence of risk factors for cardiovascular disease, including elevated blood pressure, low HDL cholesterol, and measures of obesity (16–20). Furthermore, two recent studies have shown that low circulating levels of IGFBP-1 are closely associated with the presence of macrovascular disease in subjects with type 2 diabetes (21,22). These findings led us to propose the hypothesis that IGFBP-1 favorably influences blood pressure and vascular function, either directly or perhaps by modulating the actions of IGF-I or insulin on the vasculature.

We explored this hypothesis using a novel transgenic mouse, generated in our laboratory, which overexpresses

IGFBP-1 (23). The transgene employed in this mouse is a cosmid clone encompassing the entire human IGFBP-1 structural gene and its regulatory sequences, allowing the expression of IGFBP-1 to remain responsive to normal hormonal regulation. This is unique when compared with other reported models of IGFBP-1 overexpression, in which heterologous promoters were used (24–26). In our mice, total fasting IGFBP-1 protein levels in the serum of transgenic animals are fivefold higher than those of controls (23). As the human protein is fully compatible with murine IGF-I, our model allows us to study the effects of chronic exposure to increased concentrations of IGFBP-1 on vascular function and blood pressure regulation.

RESEARCH DESIGN AND METHODS

Animals. Transgenic mice were generated as previously described (23). Mice were bred on a CBA background and allowed free access to water and standard laboratory food. Genotyping was carried out by PCR of tail-tip lysates using primers specific for human IGFBP-1 (23). Male transgenic mice were studied between 4 and 6 months of age and compared with littermate wild-type controls. All experiments were performed in accordance with U.K. Home Office regulations on the use of animals and were approved by the institution's ethics committee.

Assessment of metabolic regulation and plasma lipids. Glucose or insulin tolerance tests were performed in conscious animals by repeated blood sampling following intraperitoneal injection of glucose (1 mg/g) or human insulin (0.75 units/kg), respectively. Blood glucose was measured using a portable device (Hemocue, Sheffield, U.K.), and insulin levels were assessed by a specific hypersensitive rat insulin radioimmunoassay (Linco Research, St. Charles, MO). Homeostasis model assessment scores of insulin resistance (HOMA-IR) were calculated from fasting glucose and insulin levels using the formula HOMA-IR = insulin (mU/l) × glucose (mmol/l)/22.5 (27). HDL, VLDL, total cholesterol, and triglycerides were measured in whole blood (Cholestech LDX, Hayward, CA). Free fatty acids in plasma were measured by a colorimetric enzyme assay (Roche).

Blood pressure measurement. Systolic blood pressure was measured by tail-cuff plethysmography in conscious mice prewarmed for 10 min in a thermostatically controlled restrainer (XBP1000; Kent Scientific). Three training sessions were performed during the week before measurement. Animals underwent blood pressure assessment after fasting for 4 h and 30 min after an intraperitoneal injection of glucose (1 mg/g). The mean of at least six separate recordings was taken to represent systolic blood pressure on each occasion.

Vascular function. After overnight fasting, the thoracic aorta was excised, cleaned of adherent connective tissue, and cut into rings 3 mm long. Rings were suspended between a fixed support and a force transducer in an organ bath containing 10 ml Krebs Henseleit solution (containing, in mmol/l, NaCl 119, KCl 4.7, KH_2PO_4 1.18, $NaHCO_3$ 25, $MgSO_4$ 1.19, $CaCl_2$ 2.5, and glucose 11) at 37°C and bubbled with 95% O_2/5% CO_2. After 45 min of equilibration at a resting tension of 3 g, which was found to be optimal in preliminary experiments, the maximal contractile response to 40 mmol KCl was assessed. After washout and re-equilibration, a cumulative dose-response curve to phenylephrine was measured. Rings were then constricted to 70% of their maximal phenylephrine-induced tension, and relaxation responses to cumulative addition of acetylcholine (1 nmol/l to 10 μmol/l) or sodium nitroprusside (10 pmol/l to 1 μmol/l) were assessed. Relaxation responses to acetylcholine were repeated in some animals in the presence of the cyclooxygenase inhibitor indomethacin (0.1 mmol/l) or the nonselective NO synthase inhibitor L-N-monomethyl-arginine (L-NMMA) (0.1 mmol/l). Each ring was exposed to only one vasodilator. Relaxation responses were expressed as percentage decrement in the preconstricted tension. Basal production of NO was assessed in rings maximally preconstricted with phenylephrine by recording the increase in tension elicited by incubation with L-NMMA (0.1 mmol/l) for 30 min.

Vascular endothelial NO synthase mRNA expression. Relative expression of endothelial NO synthase (eNOS) (NOS 3) mRNA was assessed in aortae of transgenic and wild-type mice by RT-PCR. Total RNA was extracted from three aortic samples per group (each comprising pooled tissue from three to four animals) using a commercial kit (Roche). Equal amounts of RNA from each sample were reverse transcribed (SuperScript II; Gibco) and subjected to separate PCR amplifications, performed in triplicate, using primers specific for murine eNOS (forward: 5′-GGCTCCCTCCTTCCGGCTG-3′, reverse: 5′-TCCCGCAGCACGCCGAT-3′) and 18sRNA (forward: 5′-GTAACCCGTTGAACCC

CATT-3′; reverse: 5′-CCATCCAATCGGTAGTAGCG-3′). 18sRNA was chosen as an internal control in preference to GAPDH because expression of the latter may be regulated by insulin. PCR products were separated by agarose gel electrophoresis, and band densities were quantified by densitometry (Syn gene, Cambridge, U.K.). Mean densitometry scores for eNOS, normalized against 18sRNA, were compared between transgenic and wild-type mice. The amplification profiles comprised 30 cycles (for eNOS) or 24 cycles (for 18s RNA) of denaturation for 1 min at 96°C, annealing for 1 min at 60°C, and extension for 1 min at 72°C. These were shown in preliminary studies to result in amplification within the linear range.

Statistical methods. Data are expressed as means ± SE. Metabolic and blood pressure data were compared using Student's t test. Dose-response relationships and concentrations at half-maximal stimulation (EC_{50}) for aortic ring studies were assessed by the Origin software program (OriginLab, Northampton, MA) and compared using two-way repeated measures ANOVA. $P < 0.05$ was considered significant.

RESULTS

There were no gross morphological or developmental changes in mice overexpressing IGFBP-1 (28). Body weight of transgenic mice was similar to controls (42.1 ± 0.6 vs. 41.4 ± 1 g at 4–6 months), and there was no significant difference in the mass of aortic rings (0.99 ± 0.19 vs. 1.09 ± 0.15 mg).

Metabolic data. We reported previously that total serum IGFBP-1 levels (murine plus human) in fasting transgenic mice were approximately fivefold higher than in control mice when assessed by Western ligand blot analysis (23). Circulating levels of human IGFBP-1 in transgenic mice fluctuate according to nutritional status, as they do in humans, with a fivefold decrease in fasting levels after refeeding (23). In keeping with this, we reported a 12-fold reduction in hepatic expression of human IGFBP-1 in these mice after refeeding and found that the normal inverse relationship between serum insulin and IGFBP-1 levels was maintained (23). Serum IGF-I levels were not significantly different between transgenic and wild-type animals in the fasting state (517 ± 38 vs. 549 ± 64 μg/l) or after a glucose challenge (507 ± 20 vs. 559 ± 62 μg/l). There was no significant difference in the expression profile of IGF binding proteins (other than IGFBP-1) between transgenic and wild-type mice when assessed by ligand blot analysis of serum proteins using I^{125}-labeled IGF-I (not shown).

In the current study, there were no significant differences in fasting blood glucose (8.9 ± 0.5 vs. 8.5 ± 0.5 mmol/l) or insulin (0.24 ± 0.04 vs. 0.25 ± 0.04 μg/l) between transgenic and wild-type mice. Transgenic mice exhibited a significantly greater hyperinsulinemic response to a glucose challenge than wild-type mice (Fig. 1A). Intraperitoneal glucose and insulin tolerance tests did not differ between groups (Fig. 1B and C). HOMA-IR scores were similar in transgenic and wild-type mice (2.2 ± 0.4 vs. 1.7 ± 0.4; $P = 0.3$).

Lipid data. Fasting plasma lipoprotein, triglyceride, and free fatty acid levels are shown in Table 1. There were no significant differences between groups.

Hemodynamic data. Systolic blood pressure was not significantly different between transgenic and wild-type animals in the fasted state (Fig. 2A). Transgenic mice, however, experienced a significant fall in systolic blood pressure 30 min after an intraperitoneal glucose challenge (Fig. 2B). This hypotensive response was absent in wild-type animals.
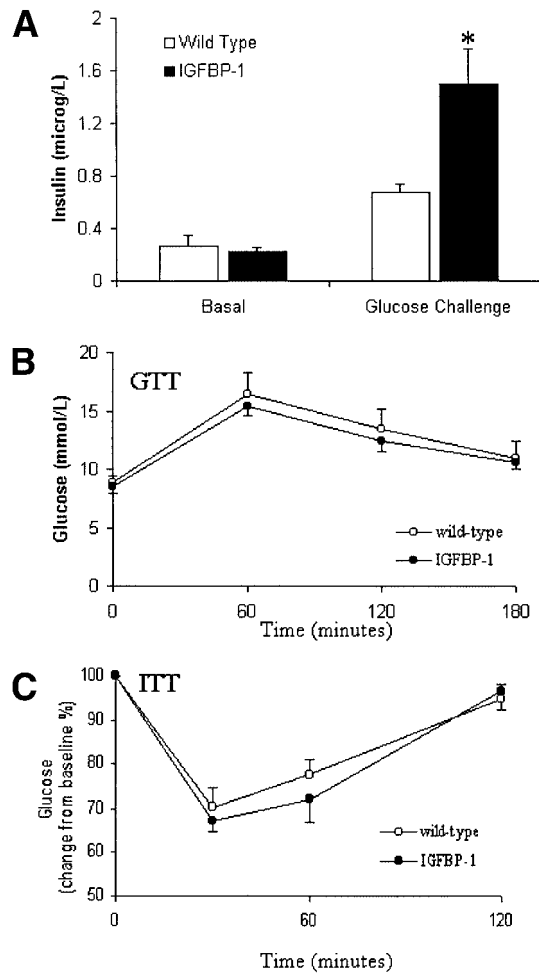
Vasomotor responses. Vasoconstriction of aortic rings

FIG. 1. Metabolic data. *A*: Insulin levels were measured after an overnight fast and 30 min after an intraperitoneal glucose injection (1 mg/g). *$P < 0.05$ for change in IGFBP-1–overexpressing mice vs. wild-type. In intraperitoneal glucose and insulin tolerance tests (GTT and ITT, respectively), blood glucose was measured at intervals following injection of glucose (1 mg/g) (*B*) or insulin (0.75 units/kg) (*C*). There were no significant differences in responses between transgenic and wild-type mice. All data are expressed as means ± SE ($n = 6$–8 per group).
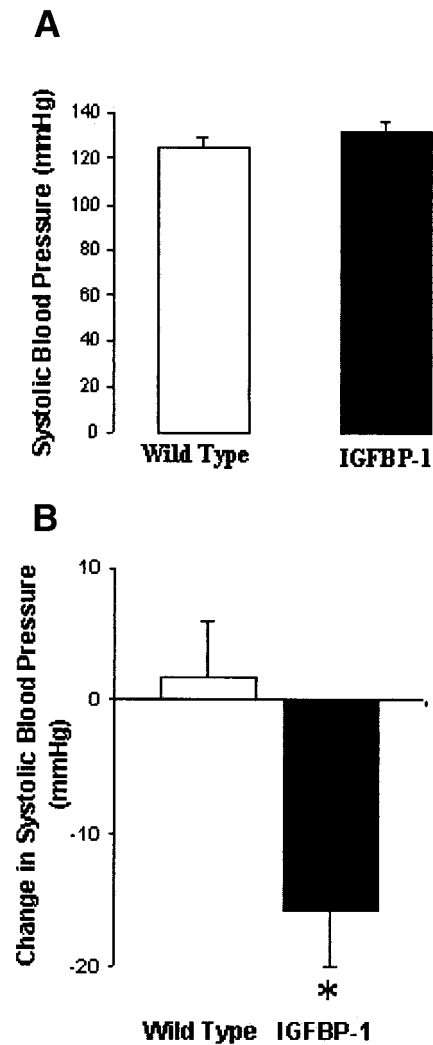

FIG. 2. Hemodynamic data. *A*: Systolic blood pressure measured by tail cuff plethysmography in the fasting state. There was no significant difference between transgenic and wild-type animals (means ± SE; $n = 12$ per group). *B*: Effect of intraperitoneal glucose injection on systolic blood pressure (means ± SE, $n = 12$ per group). *$P < 0.05$ for change in IGFBP-1–overexpressing mice vs. wild-type.

was assessed by cumulative exposure to increasing concentrations of phenylephrine (Fig. 3*A*). Although the sensitivity to phenylephrine did not differ between transgenic and wild-type animals (pEC_{50} 6.85 ± 0.04 vs. 6.83 ± 0.07), the magnitude of the response (E_{max}) was significantly attenuated in transgenic mice (E_{max} 1.13 ± 0.08 vs. 1.55 ± 0.07 g/mg; $P < 0.05$). Vasoconstriction to potassium chloride (40 mmol/l) was also reduced in transgenic mice (0.7 ± 0.1 vs. 1.1 ± 0.1 g/mg; $P < 0.05$).

When preconstricted rings were incubated for 30 min

TABLE 1
Plasma lipids

	Wild-type	IGFBP-1	P
Cholesterol (mmol/l)	3.0 ± 0.1	3.0 ± 0.1	0.2
HDL cholesterol (mmol/l)	2.4 ± 0.2	2.3 ± 0.1	0.5
VLDL cholesterol (mmol/l)	0.5 ± 0.1	0.5 ± 0.1	0.5
Triglycerides (mmol/l)	1.0 ± 0.2	1.0 ± 0.1	0.9
Free fatty acids (mmol/l)	0.8 ± 0.2	1.0 ± 0.2	0.3

Data are means ± SE ($n = 8$–10 per group).

with the nonselective NO synthase inhibitor L-NMMA, there was a greater percentage increment in tension in rings from transgenic than wild-type mice (Fig. 3*B*). The maximum tension achieved was similar in transgenic and wild-type mice (1.98 ± 0.09 vs. 1.85 ± 0.09 g/mg).

Relaxation responses were assessed following preconstriction of each ring to 70% of the maximal tension achieved by prior exposure to phenylephrine. Cumulative addition of the endothelium-dependent vasodilator acetylcholine and the endothelium-independent vasodilator sodium nitroprusside to preconstricted rings resulted in similar degrees of relaxation in transgenic and wild-type mice (Fig. 3*C* and *D*). There were no significant differences in EC_{50} or E_{max} values for these agents between the two groups. Relaxation to acetylcholine was unchanged in the presence of indomethacin in both transgenic and wild-type animals and was totally blocked in the presence of L-NMMA ($n = 4$ per group; data not shown).

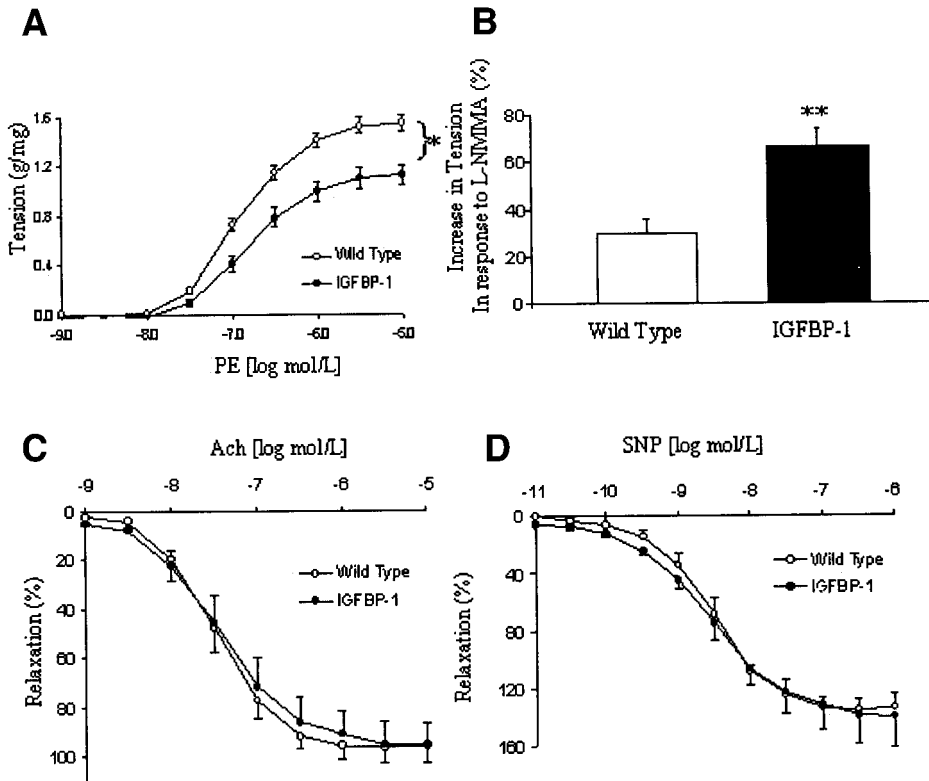**Vascular eNOS expression.** Aortic expression of eNOS mRNA was significantly increased in transgenic mice (Fig.

FIG. 3. Vasomotor responses of aortic rings ex vivo. *A*: Dose-response curve for constriction of aortic rings to phenylephrine. Tensions are normalized to mass of ring. *$P < 0.02$; $n = 12$ per group. *B*: Response of preconstricted rings to the NO synthase inhibitor L-NMMA (0.1 mmol/l), expressed as percent increase in preconstricted tension. **$P < 0.05$; $n = 6$ per group. *C* and *D*: Relaxation responses to acetylcholine (Ach) and sodium nitroprusside (SNP), expressed as percent reversal of phenylephrine-induced contraction ($n = 6$ per group). All data are means ± SE.

4). Analysis of data from nine separate reactions revealed threefold greater eNOS mRNA levels in transgenic aorta than in wild-type.

DISCUSSION

The demonstration of an inverse correlation between IGFBP-1 levels and cardiovascular risk factors (16–20), and the recent findings of lower IGFBP-1 levels in subjects

FIG. 4. Vascular eNOS expression. Total RNA of aortic segments from wild-type and IGFBP-1–overexpressing mice was isolated, RT was performed, and relative expression of eNOS mRNA (normalized for 18sRNA) was assessed by PCR. *A*: Representative agarose gels of amplified DNA fragments. *B*: Densitometric analysis of amplified eNOS PCR fragments, normalized to corresponding 18sRNA PCR signals. Data are means ± SE. *$P < 0.05$ for IGFBP-1–overexpressing mice vs. wild-type.

with diabetes and macrovascular disease (21,22), led us to hypothesize that IGFBP-1 may protect the vasculature against the development of atherosclerosis. The present study was designed to examine the cardiovascular phenotype of transgenic mice that overexpress the human IGFBP-1 gene.

The principal new findings of the present report are that *1*) animals overexpressing IGFBP-1 demonstrate significant hyperinsulinemia after a carbohydrate load; despite this, transgenic animals have preserved glucocompetence and insulin sensitivity; *2*) transgenic animals are normotensive in the fasting state but display a significant fall in systolic blood pressure after a carbohydrate load; *3*) the ability of aortic rings from transgenic animals to vasoconstrict to pressor agents is blunted; and *4*) transgenic animals have functional evidence of increased basal production of NO and demonstrated increased expression of eNOS mRNA in the aorta. These data suggest that IGFBP-1 may have beneficial effects on the vasculature by reducing blood pressure and increasing basal production of NO.

IGF-I and IGF binding proteins in cardiovascular risk. A potential role for the IGF-I/IGFBP system in the pathophysiology of a number of cardiovascular disorders has been hypothesized. The association of higher IGFBP-1 levels (after correcting for insulin) with a more favorable cardiovascular risk factor profile (16–21) raises the possibility that IGFBP-1 may, directly or indirectly, exert beneficial effects on the blood vessel wall.

Whereas the effect of insulin and resistance to its actions on vascular risk has received much attention (29), the effect of IGFBPs on vascular function remains poorly understood and incompletely explored. The possibility of a vasoprotective role for increased levels of IGFBP-1 is clinically relevant, especially as IGFBP-1 may be increased
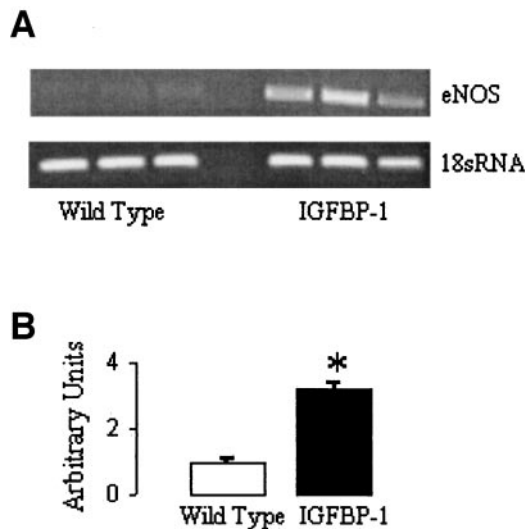
by simple lifestyle measures such as changes in diet and exercise (30).

Metabolic sequelae of increased IGFBP-1. We have previously shown that IGFBP-1 overexpressing mice are hyperinsulinemic after a glucose challenge (23). Despite this postprandial hyperinsulinemia, transgenic animals in this study were normoglycemic in the fasting state and appeared to be glucocompetent when glucoregulation was assessed by an intraperitoneal glucose tolerance test. Although hyperinsulinemia in the context of a normal glucose response after a carbohydrate challenge is a feature of some insulin-resistant states, the transgenic mice in our study did not appear to be insulin resistant. Insulin sensitivity was demonstrated to be preserved at this age (4–6 months) on the basis of a normal response to exogenous insulin in intraperitoneal insulin tolerance tests and by HOMA-IR scores, which were not different from control animals. We propose, therefore, that IGFBP-1–overexpressing mice exhibit hyperinsulinemia in the absence of overt insulin resistance. We postulate that this hyperinsulinemia results from the overexpression of IGFBP-1 leading to reduced bioavailability of IGF-I, which, in transgenic animals, leads to a compensatory rise in insulin levels to maintain normoglycemia after carbohydrate challenge. This concept is consistent with data in rats, where injection of recombinant IGFBP-1 causes hyperinsulinemia and blunts the hypoglycemic response to exogenous IGF-1 (31). Although other binding proteins also have the potential to modulate IGF-I bioavailability, we detected no change in the abundance of other IGFBPs in IGFBP-1–overexpressing mice as assessed by ligand blot analysis of serum proteins.

Lipid homeostasis. Cross-sectional studies in humans have shown a positive correlation between IGFBP-1 levels and HDL cholesterol (16,19,20) and an inverse association between IGFBP-1 and triglycerides (16,18). The association with HDL cholesterol persists after correcting for insulin levels (16,20), and it has been postulated that IGFBP-1 may play a role, independent of insulin, on HDL metabolism (16). In our study, there was no difference in total cholesterol, HDL, triglyceride, or free fatty acid levels between IGFBP-1–overexpressing mice and controls. These data do not support a role for IGFBP-1 in influencing murine lipid metabolism.

IGFBP-1 and vascular function. Human studies have shown an inverse relationship between IGFBP-1 and systolic blood pressure, which appears to be independent of insulin levels (19). We found no significant difference in systolic blood pressure between IGFBP-1–overexpressing mice and controls in the fasted state. Interestingly, however, there was a divergence in the systolic blood pressure response to a carbohydrate load. In IGFBP-1–overexpressing mice, systolic blood pressure fell by a mean of 16 mmHg after a glucose challenge, an effect not seen in control mice. Although it is possible that IGFBP-1 plays a direct role in blood pressure homeostasis, we speculate that this postprandial fall in blood pressure may be accounted for by vasodilation mediated by insulin, the levels of which increase to a significantly greater degree in transgenic animals than in controls. Furthermore, the difference in blood pressure was apparent 30 min following intraperitoneal glucose injection, a time at which

insulin levels increase substantially but before IGFBP-1 levels begin to decline.

There are compelling data to suggest that insulin acts as a vasoregulatory peptide and may play an important role in blood pressure regulation. It has been demonstrated using a number of techniques that insulin leads to vasorelaxation in vivo and ex vivo (32–34). Furthermore, insulin-mediated vasodilation is inhibited by pharmacological blockade of NO production in humans (32,35) or by deletion of the eNOS gene in mice (36), demonstrating that the vasorelaxant properties of insulin are largely NO dependent. As insulin levels rise substantially following carbohydrate ingestion, it may be predicted that hyperinsulinemia may be an important determinant of vascular tone and hence blood pressure in the postprandial state. The influence of hyperinsulinemia on blood pressure depends on the balance between insulin-mediated vasodilation and stimulation of the sympathetic nervous system (37). In keeping with the present dataset, we and others have demonstrated that the vasodepressor actions of insulin predominate and contribute significantly to postprandial hypotension in humans (38–40).

In support of a favorable role for IGFBP-1 on the vascular wall, we demonstrated a reduction in the maximal vasoconstrictor response to phenylephrine in aorta of IGFBP-1–overexpressing mice. We also showed that incubation of preconstricted aortic rings with the nonselective NO synthase inhibitor L-NMMA caused a greater increment in tension in IGFBP-1–overexpressing mice, consistent with an increase in basal production of NO, a potent vasodilator with antiatherosclerotic properties. The maximum tension achieved after cumulative addition of phenylephrine and L-NMMA was similar in transgenic and control mice, suggesting that increased basal production of NO is responsible for reducing the vasoconstrictor responsiveness of transgenic mice. Consistent with the physiological data demonstrating increased basal NO production, we found that eNOS mRNA levels were threefold higher in aorta of IGFBP-1–overexpressing mice. As there are no published data to support a direct role for IGFBP-1 in the regulation of eNOS expression, we postulate that the upregulation of eNOS in our model may have been mediated indirectly, for example, through the effects of hyperinsulinemia. Exposure to increasing physiological concentrations of insulin leads to dose-dependent increases in eNOS mRNA and protein levels in cultured bovine aortic endothelial cells (41) and human coronary endothelial cells (42). It is likely, therefore, that long-term exposure to hyperinsulinemia in the IGFBP-1–overexpressing mouse accounts for the upregulation of eNOS demonstrated in our study. In support of this hypothesis, rats subject to hyperinsulinemia by repetitive injection of insulin or implantation of a subcutaneous insulin-releasing pellet exhibit upregulation of eNOS mRNA levels to a degree similar to that seen in the present dataset (43,44). Both insulin and IGF-I increase NO production in endothelial cells, but insulin has been shown to be twice as effective in this regard as IGF-I (6). We speculate that in our model, hyperinsulinemia arises in IGFBP-1–overexpressing animals to compensate for reduced bioavailaibity of IGF-I. It is likely, therefore, that the increased NO production in transgenic animals is mediated by a stimu-

latory effect of hyperinsulinemia on eNOS expression and activity that outweighs the potential effects of reduced IGF-I bioavailability.

It is also possible that mechanisms other than hyperinsulinemia and increased NO production contribute to the reduced vasoconstrictor responsiveness to phenylephrine in IGFBP-1–overexpressing mice. However, other explanations such as downregulation of α-adrenergic receptors are unlikely, as the response of non–receptor-dependent vasoconstriction to potassium chloride was also blunted in these animals. Likewise, the mass of aortic rings was unchanged in transgenic mice compared with controls, suggesting that there were no gross structural changes of the vessel wall induced by altered IGF-I bioavailability.

Although a reduction in IGF-I bioavailability (and an accompanying reciprocal increase in insulin levels) is the most attractive explanation for the influence of IGFBP-1 overexpression on the metabolic and vascular phenotypes of transgenic animals, we cannot discount the possibility that other mechanisms may be involved. One intriguing possibility is that IGFBP-1 per se exerts direct effects on metabolically active tissues and the vasculature. Ligand-independent cellular actions have been described for several of the binding proteins (45). IGFBP-1 possesses an $\alpha_5\beta_1$ integrin binding site and is able to independently stimulate cell migration through this mechanism (46). Whether similar IGF-independent effects are important in other actions of IGFBP-1 remains to be studied.

Despite evidence of increased basal NO production and eNOS mRNA upregulation in this study, overexpression of IGFBP-1 had no effect on acetylcholine-induced vasorelaxation. This may be due to differential regulation of basal and agonist-stimulated eNOS activity, but may also reflect limitations of aortic vasomotor studies in demonstrating an improvement in endothelial function when baseline function is already maximal. In support of this, it is clear that increased eNOS abundance may have divergent effects on vasomotor function depending on the experimental model. Gene transfer of recombinant eNOS to rabbit carotid arteries, for example, slightly improves the sensitivity to acetylcholine, but does not change the maximal response (47), whereas overexpression of eNOS in mice counterintuitively leads to a significant deterioration in acetylcholine-mediated dilatation, perhaps due to the development of nitrate tolerance (48).

The demonstration of increased NO production, blunted vasoconstrictor responsiveness, and preserved vasodilatory capacity in our model of IGFBP-1 overexpression is relevant to human studies, in which lower IGFBP-1 levels are associated with an increased prevalence of cardiovascular risk factors and overt macrovascular disease (16–22). Although IGFBP-1 levels are reduced in subjects with type 2 diabetes and macrovascular disease (18,22), speculation on the extent to which loss of the potential beneficial effects of IGFBP-1 contribute to impaired vascular function in this condition is complicated by confounding effects of insulin resistance, hyperinsulinemia, and hyperglycemia.

Insulin and endothelial function. The preservation of endothelium-dependent vasomotor function in our model provides further insight into the role of hyperinsulinemia in endothelial dysfunction. Data from animal and human studies show a clear association between insulin resistance and impaired endothelium-dependent vasorelaxation, although a causal role of hyperinsulinemia per se remains contentious. Infusion of exogenous insulin at pathophysiological levels has been shown to exert a detrimental effect on endothelial function in humans (49). Furthermore, mice rendered hyperinsulinemic by targeted deletion of IRS-1, a key protein in the cellular insulin signaling cascade, have endothelial dysfunction (50). Conversely, others have shown that insulin exerts desirable effects on the vasculature by increasing eNOS gene expression and NO bioavailability (41–44). In support of this, in our model of IGFBP-1, overexpression of endothelial function is preserved despite the presence of hyperinsulinemia. Our data, therefore, do not support a deleterious effect of repetitive postprandial hyperinsulinemia on endothelial function, at least when insulin signaling pathways remain intact.

Study limitations. The present study assessed mice at 4–6 months of age, so the effect of IGFBP-1 overexpression in older mice is unclear. We and others have shown that the compensatory hyperinsulinemia seen in mice overexpressing IGFBP-1 eventually leads to pancreatic secretory dysfunction and glucose intolerance (23). The changes in vascular function in older mice warrant attention, although interpretation of findings may be confounded by the coexisting glucose intolerance. Second, we acknowledge that although the aorta is widely employed as a vessel in which to study vasomotor function, tone in smaller resistance arteries is a major determinant of systemic vascular resistance, and hence blood pressure, in vivo. Finally, our data do not allow us to draw conclusions regarding a therapeutic effect of IGFBP-1 in already insulin-resistant animals prone to atherosclerosis; this also warrants further study.

In conclusion, we have demonstrated that overexpression of IGFBP-1 in mice modulates the insulin/IGF-I axis and has potentially favorable effects on cardiovascular homeostasis. These data identify the IGF-I/IGFBP-1 axis as a novel and useful target to address mechanisms of vascular disease and raise the intriguing possibility that IGFBP-1 may be a useful peptide in protecting the vascular wall from developing early atherosclerosis by reducing blood pressure and increasing basal production of NO.

REFERENCES

1. Wever R, Luscher T, Consentino F, Rabelink TJ: Atherosclerosis and the two faces of endothelial nitric oxide synthase. *Circulation* 97:108–112, 1998
2. Harrison DG: Cellular and molecular mechanisms of endothelial cell dysfunction. *J Clin Invest* 100:2153–2157, 1997
3. Grundy SM: Obesity, metabolic syndrome, and coronary atherosclerosis. *Circulation* 105:2696–2698, 2002
4. Wheatcroft SB, Williams IL, Shah AM, Kearney MT: Pathophysiological

implications of insulin resistance on vascular endothelial function. *Diabet Med.* 20:255–268, 2003

5. Lewitt MS, Baxter RC: Insulin-like growth factor-binding protein-1: a role in glucose counterregulation? *Mol Cell Endocrinol* 79:C147–C152, 1991

6. Zeng G, Quon MJ: Insulin-stimulated production of nitric oxide is inhibited by wortmannin: direct measurement in vascular endothelial cells. *J Clin Invest* 98:894–898, 1996

7. Cefalu WT: Insulin resistance: cellular and clinical concepts. *Exp Biol Med (Maywood)* 226:13–26, 2001

8. Despres JP, Lamarche B, Mauriege P, Cantin B, Dagenais GR, Moorjani S, Lupien PJ: Hyperinsulinemia as an independent risk factor for ischemic heart disease. *N Engl J Med* 334:952–957, 1996

9. Pyorala M, Miettinen H, Laakso M, Pyorala K: Hyperinsulinemia predicts coronary heart disease risk in healthy middle-aged men: the 22-year follow-up results of the Helsinki Policemen Study. *Circulation* 98:398–404, 1998

10. Baxter RC: Insulin-like growth factor binding proteins in the human circulation: a review. *Horm Res* 42:140–144, 1994

11. Kelley KM, Oh Y, Gargosky SE, Gucev Z, Matsumoto T, Hwa V, Ng L, Simpson DM, Rosenfeld RG: Insulin-like growth factor-binding proteins (IGFBPs) and their regulatory dynamics. *Int J Biochem Cell Biol* 28:619–637, 1996

12. Lee PD, Giudice LC, Conover CA, Powell DR: Insulin-like growth factor binding protein-1: recent findings and new directions. *Proc Soc Exp Biol Med* 216:319–357, 1997

13. Lee PD, Conover CA, Powell DR: Regulation and function of insulin-like growth factor-binding protein-1. *Proc Soc Exp Biol Med* 204:4–29, 1993

14. Ketelslegers JM, Maiter D, Maes M, Underwood LE, Thissen JP: Nutritional regulation of the growth hormone and insulin-like growth factor-binding proteins. *Horm Res* 45:252–257, 1996

15. Brismar K, Fernqvist-Forbes E, Wahren J, Hall K: Effect of insulin on the hepatic production of insulin-like growth factor-binding protein-1 (IGFBP-1), IGFBP-3, and IGF-I in insulin-dependent diabetes. *J Clin Endocrinol Metab* 79:872–878, 1994

16. Mohamed-Ali V, Pinkney JH, Panahloo A, Cwyfan-Hughes S, Holly JM, Yudkin JS: Insulin-like growth factor binding protein-1 in NIDDM: relationship with the insulin resistance syndrome. *Clin Endocrinol (Oxf)* 50:221–228, 1999

17. Harrela M, Koistinen R, Tuomilehto J, Nissinen A, Seppala M: Low serum insulin-like growth factor-binding protein-1 is associated with an unfavourable cardiovascular risk profile in elderly men. *Ann Med* 32:424–428, 2000

18. Heald AH, Cruickshank JK, Riste LK, Cade JE, Anderson S, Greenhalgh A, Sampayo J, Taylor W, Fraser W, White A, Gibson JM: Close relation of fasting insulin-like growth factor binding protein-1 (IGFBP-1) with glucose tolerance and cardiovascular risk in two populations. *Diabetologia* 44:333–339, 2001

19. Gibson JM, Westwood M, Young RJ, White A: Reduced insulin-like growth factor binding protein-1 (IGFBP-1) levels correlate with increased cardiovascular risk in non-insulin dependent diabetes mellitus (NIDDM). *J Clin Endocrinol Metab* 81:860–863, 1996

20. Janssen JA, Stolk RP, Pols HA, Grobbee DE, Lamberts SW: Serum total IGF-I, free IGF-I, and IGFB-1 levels in an elderly population: relation to cardiovascular risk factors and disease. *Arterioscler Thromb Vasc Biol* 18:277–282, 1998

21. Heald AH, Siddals KW, Fraser W, Taylor W, Kaushal K, Morris J, Young RJ, White A, Gibson JM: Low circulating levels of insulin-like growth factor binding protein-1 (IGFBP-1) are closely associated with the presence of macrovascular disease and hypertension in type 2 diabetes. *Diabetes* 51:2629–2636, 2002

22. Leinonen ES, Salonen JT, Salonen RM, Koistinen RA, Leinonen PJ, Sarna SS, Taskinen MR: Reduced IGFBP-1 is associated with thickening of the carotid wall in type 2 diabetes. *Diabetes Care* 25:1807–1812, 2002

23. Crossey PA, Jones JS, Miell JP: Dysregulation of the insulin/IGF binding protein-1 axis in transgenic mice is associated with hyperinsulinemia and glucose intolerance. *Diabetes* 49:457–465, 2000

24. D'Ercole AJ, Dai Z, Xing Y, Boney C, Wilkie MB, Lauder JM, Han VK, Clemmons DR: Brain growth retardation due to the expression of human insulin like growth factor binding protein-1 in transgenic mice: an in vivo model for the analysis of IGF function in the brain. *Brain Res Dev Brain Res* 82:213–222, 1994

25. Rajkumar K, Barron D, Lewitt MS, Murphy LJ: Growth retardation and hyperglycemia in insulin-like growth factor binding protein-1 transgenic mice. *Endocrinology* 136:4029–4034, 1995

26. Gay E, Seurin D, Babajko S, Doublier S, Cazillis M, Binoux M: Liver-specific expression of human insulin-like growth factor binding protein-1 in transgenic mice: repercussions on reproduction, ante- and perinatal mortality and postnatal growth. *Endocrinology* 138:2937–2947, 1997

27. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985

28. Crossey PA, Pillai CC, Miell JP: Altered placental development and intrauterine growth restriction in IGF binding protein-1 transgenic mice. *J Clin Invest* 110:411–418, 2002

29. Ginsberg HN: Insulin resistance and cardiovascular disease. *J Clin Invest* 106:453–458, 2000

30. Hellenius ML, Brismar KE, Berglund BH, de Faire UH: Effects on glucose tolerance, insulin secretion, insulin-like growth factor 1 and its binding protein, IGFBP-1, in a randomized controlled diet and exercise study in healthy, middle-aged men. *J Intern Med* 238:121–130, 1995

31. Mortensen DL, Won WB, Siu J, Reifsnyder D, Gironella M, Etcheverry T, Clark RG: Insulin-like growth factor binding protein-1 induces insulin release in the rat. *Endocrinology* 138:2073–2080, 1997

32. Chen YL, Messina EJ: Dilation of isolated skeletal muscle arterioles by insulin is endothelium dependent and nitric oxide mediated. *Am J Physiol* 270:H2120–H2124, 1996

33. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD: Obesity/insulin resistance is associated with endothelial dysfunction: implications for the syndrome of insulin resistance. *J Clin Invest* 97:2601–2610, 1996

34. Laight DW, Kaw AV, Carrier MJ, Anggard EE: Pharmacological modulation of endothelial function by insulin in the rat aorta. *J Pharm Pharmacol* 50:1117–1120, 1998

35. Baron AD: Hemodynamic actions of insulin. *Am J Physiol* 267:E187–E202, 1994

36. Duplain H, Burcelin R, Sartori C, Cook S, Egli M, Lepori M, Vollenweider P, Pedrazzini T, Nicod P, Thorens B, Scherrer U: Insulin resistance, hyperlipidemia, and hypertension in mice lacking endothelial nitric oxide synthase. *Circulation* 104:342–345, 2001

37. Anderson EA, Hoffman RP, Balon TW, Sinkey CA, Mark AL: Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. *J Clin Invest* 87:2246–2252, 1991

38. Kearney MT, Cowley AJ, Stubbs TA, Evans A, Macdonald IA: Depressor action of insulin on skeletal muscle vasculature: a novel mechanism for postprandial hypotension in the elderly. *J Am Coll Cardiol* 31:209–216, 1998

39. Kearney MT, Cowley AJ, Stubbs TA, Macdonald IA: Effect of a physiological insulin infusion on the cardiovascular responses to a high fat meal: evidence supporting a role for insulin in modulating postprandial cardiovascular homoeostasis in man. *Clin Sci (Colch)* 91:415–423, 1996

40. Masuo K, Mikami H, Ogihara T, Tuck ML: Mechanisms mediating postprandial blood pressure reduction in young and elderly subjects. *Am J Hypertens* 9:536–544, 1996

41. Kuboki K, Jiang ZY, Takahara N, Ha SW, Igarashi M, Yamauchi T, Feener EP, Herbert TP, Rhodes CJ, King GL: Regulation of endothelial constitutive nitric oxide synthase gene expression in endothelial cells and in vivo: a specific vascular action of insulin. *Circulation* 101:676–681, 2000

42. Ding YVN, Coulson R, Kananna VS, Roh DD: Effects of simulated hyperglycaemia, insulin, and glucagon on endothelial nitric oxide synthase expression. *Am J Physiol Endocrinol Metab* 279:E11–E17, 2000

43. Kobayashi T, Kamata K: Effect of chronic insulin treatment on NO production and endothelium-dependent relaxation in aortae from established STZ-induced diabetic rats. *Atherosclerosis* 155:313–320, 2001

44. Shinozaki K, Kashiwagi A, Nishio Y, Okamura T, Yoshida Y, Masada M, Toda N, Kikkawa R: Abnormal biopterin metabolism is a major cause of impaired endothelium-dependent relaxation through nitric oxide/O_2^- imbalance in insulin-resistant rat aorta. *Diabetes* 48:2437–2445, 1999

45. Firth SM, Baxter RC: Cellular actions of the insulin-like growth factor binding proteins. *Endocr Rev* 23:824–854, 2002

46. Jones JI, Gockerman A, Busby WH Jr, Wright G, Clemmons DR: Insulin-like growth factor binding protein 1 stimulates cell migration and binds to the alpha 5 beta 1 integrin by means of its Arg-Gly-Asp sequence. *Proc Natl Acad Sci U S A* 90:10553–10557, 1993

47. Kullo IJ, Mozes G, Schwartz RS, Gloviczki P, Tsutsui M, Katusic ZS, O'Brien T: Enhanced endothelium-dependent relaxations after gene transfer of recombinant endothelial nitric oxide synthase to rabbit carotid arteries. *Hypertension* 30:314–320, 1997

48. Ohashi Y, Kawashima S, Hirata K, Yamashita T, Ishida T, Inoue N, Sakoda T, Kurihara H, Yazaki Y, Yokoyama M: Hypotension and reduced nitric oxide-elicited vasorelaxation in transgenic mice overexpressing endothelial nitric oxide synthase. *J Clin Invest* 102:2061–2071, 1998

49. Arcaro G, Cretti A, Balzano S, Lechi A, Muggeo M, Bonora E, Bonadonna RC: Insulin causes endothelial dysfunction in humans: sites and mechanisms. *Circulation* 105:576–582, 2002

50. Abe H, Yamada N, Kamata K, Kuwaki T, Shimada M, Osuga J, Shionoiri F, Yahagi N, Kadowaki T, Tamemoto H, Ishibashi S, Yazaki Y, Makuuchi M: Hypertension, hypertriglyceridemia, and impaired endothelium-dependent vascular relaxation in mice lacking insulin receptor substrate-1. *J Clin Invest* 101:1784–1788, 1998