Dysregulation of the Insulin/IGF Binding Protein-1 Axis in Transgenic Mice Is Associated With Hyperinsulinemia and Glucose Intolerance

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The insulin/IGF binding protein-1 (IGFBP-1) axis is important in coordinating insulin- and IGF-mediated regulation of glucose metabolism and glycemia. Dysregulation of the axis may play a role in the pathophysiology of disorders of insulin deficiency and resistance. We have investigated this hypothesis by generating transgenic mice that overexpress hIGFBP-1. To study the axis in its true physiological context, we used a human (h) IGFBP-1 cosmid clone so that transgene expression is responsive to normal hormonal stimuli. hIGFBP-1 mRNA is expressed in a tissue-specific fashion, and measurement of serum protein levels by specific immunobssay indicates normal physiological regulation in response to fasting/feeding and appropriate post-translational modification as indicated by the detection of phosphorylated and nonphosphorylated isoforms of the protein. The hypoglycemic response to exogenous IGF-I is attenuated in transgenic mice. Transgenic mice exhibit an enhanced insulin secretory response to a glucose challenge, although basal and stimulated blood glucose levels are similar to controls. There is a sexual dimorphism in phenotypic expression: male transgenic had higher stimulated glucose and insulin levels than did females. Transgenic mice exhibit fasting hyperglycemia and hyperinsulinemia and glucose intolerance in later life, indicating an age-related decline in glucocompetence. These findings demonstrate the importance of the normal inverse relationship between serum insulin and IGFBP-1 levels in glucoregulation and that sustained dysregulation of the insulin/IGF-I/IGFBP-1 axis is associated with impaired glucose tolerance and abnormalities of insulin action. Diabetes 49:457–465, 2000

The IGFs (IGF-I and -II) have insulin-like actions on glucose metabolism in vivo, stimulating peripheral glucose uptake and utilization and reducing hepatic glucose production (1). These observations have led to speculation that the IGFs play a role in regulating carbohydrate metabolism and glycemia. The actions of the IGFs are modulated by the IGF binding proteins (IGFBPs), a family of high-affinity binding proteins that determine IGF bioavailability (2). One of the major functions of serum IGFBPs is regulation of the hypoglycemic potential of circulating IGFs, which are ~1,000-fold greater than insulin levels (3). IGFBP-1 is believed to play an important role in the acute regulation of serum free IGFs.

Insulin has multiple reciprocal interactions with the IGFs and their binding proteins. Insulin is a positive regulator of IGF-I synthesis in vivo and in vitro, acting at the level of hepatic gene transcription (4,5). In vivo studies of IGF-I administration have consistently shown an inhibitory effect on insulin secretion (6,7). Furthermore, IGF-I increases tissue sensitivity to insulin in patients with type 1 or type 2 diabetes (8,9). Serum IGFBP-1 levels fluctuate in response to nutritional status, largely as a result of negative regulation of hepatic IGFBP-1 gene expression by insulin (10,11). Epinephrine, glucagon, and corticosteroids are positive physiological regulators of IGFBP-1, although their effects are secondary to the inhibitory action of insulin. The hormonal regulation of IGFBP-1 synthesis is characteristic of counterregulatory hormones, leading to suggestions of a possible role in glucose counterregulation (12).

Dysregulation of the insulin/IGFBP-1 axis is a consistent feature of the human disease states of insulin deficiency and resistance. IGFBP-1 levels are elevated in type 1 diabetic patients and in some forms of acquired insulin resistance, such as type 2 diabetes (13,14). The elevated IGFBP-1 levels in these disease states reflect the reduced inhibitory effect of insulin on IGFBP-1 synthesis. The increase in IGFBP-1 levels may not be merely a consequence of insulin deficiency/resistance but might play a role in the pathophysiology of these conditions by virtue of its inhibitory effects on IGF activity. Some of the metabolic derangements in type 1 diabetic patients may be due to attenuation of the insulin-like actions of the IGFs (15). Furthermore, it has been suggested that increased levels of IGF inhibitory IGFBPs may play a role in growth impairment in prepubertal children with poorly con-
trolled type 1 diabetes and also in late-onset complications of type 1 diabetes, such as retinopathy and nephropathy (16). To explore the role of the insulin/IGFBP-1 axis, we have experimentally manipulated the axis so that the normal inverse relationship between insulin and IGFBP-1 is disturbed. This has been achieved by overexpressing human (h) IGFBP-1 in transgenic mice. A transgenic model of IGFBP-1 overexpression has been previously generated, but the use of a heterologous promoter to control transgene expression means that its usefulness in elucidating the physiological function of the insulin/IGFBP-1 axis is limited (17). We have improved on this model by developing transgenic mice using a cosmold clone encompassing the entire hIGFBP-1 structural gene and its regulatory sequences that is responsive to normal hormonal stimuli, thereby allowing us to study the axis in its true physiological context.

RESEARCH DESIGN AND METHODS

Generation of transgenic mice. A cosmold clone, chBP1:2, encompassing the hIGFBP-1 structural gene, has been reported previously (18). A 35-kb Not fragment of chBP1:2 was gel purified, quantitated, and then diluted to give a final concentration of 0.5 µg/ml. The DNA was microinjected into the male pronucleus of fertilized C57Bl/6J/CBA oocytes. Transgenic mice were initially identified by Southern blot analysis of DNA isolated from tail biopsies taken at weaning. EcoRI digests of mouse DNA were transferred to Hybond N+ hybridization membrane by standard procedures (19) and probed with a (α-32P)5dCTP-labeled hIGFBP-1 cDNA (20). Founder animals positive for the transgene were mated with CBA/Ca mice to produce heterozygous transgenic F1 animals. F2 transgenic mice were initially identified by Southern blot analysis of DNA from tail biopsies. Transgenic males and females positive for the transgene were mated to produce homozygous transgenic F2 animals. F4 transgenic animals in each group were pooled, and 2.5 µg of it was reverse transcribed using Superscript II and random hexamers following the manufacturer’s guidelines. cDNA was amplified by PCR with the primer pair HBP1 F and HBP1 R. PCR products were analyzed by PCR with the primer pair HBP1 F and HBP1 R. PCR products were analyzed by agarose gel electrophoresis. The amplified product distinguished from the target by agarose gel electrophoresis. The amplified product and the mean ratio of the intensity of the target (A+) to competitor (A-) was calculated for each amount of the internal standard added.

Construction of the internal cRNA standard for competitive RT-PCR. The internal cRNA standard for quantitative RT-PCR was prepared by amplifying human liver cDNA with the primer pair CPT THBP1 F (5'-CGGGATCCGGATCCAT CGGAGCAGAGAAGACCTGTCGAGCGCTTACC-3') and CPHBP1 R (5'-AGGGATCTCTTCCATCCATCAAGGGTACGTTGAGTTGGC AG-3'). The internal competitor is designed to be amplified by the same primer pair as the target but it generates a product that is 52 bp smaller that can be distinguished from the target by agarose gel electrophoresis. The amplified product was transcribed in vitro using T7 polymerase (Stratagene, Cambridge, U.K.), and the transcript was run on a 15% denaturing formaldehyde gel to confirm its size and integrity and quantitated by spectrophotometry. cDNA synthesis and PCR amplification. Total liver RNA was isolated from fasted and ad libitum-fed transgenic mice. An equal mass of RNA from individual transgenic animals in each group was pooled, and 2.5 µg of it was reverse transcribed in the presence of twofold dilutions of the internal standard cRNA. cDNA was amplified using the primer pair HBP1 F and HBP1 R. PCR products were analyzed by electrophoresis on 2% agarose gels. The band intensity was determined by densitometry (GDS 7000 and Gel Pro Analyser software; UVP, Cambridge, U.K.), and the mean ratio of the intensity of the target (A+) to competitor (A-) was calculated for each amount of the internal standard added.

Western ligand blot analysis. Samples of sera from transgenic and wild-type animals containing an equal mass of protein were diluted with sample buffer and denatured at 100°C for 5 min before electrophoresis on a 12% SDS-polyacrylamide gel. The gel was electroblotted to nitrocellulose (Hybond C; Amersham, Amersham, U.K.). After incubation with [32P]-labeled IGF-I and –II, the filter was washed to remove unbound ligand, and the IGF BP's were visualized by autoradiography.

Assays for hIGFBP-1, insulin, and IGFI-1. Total and nonphosphorylated hIGFBP-1 in sera of transgenic mice was measured by immunoradiometric assay (IRMA) (Diagnostic Systems Laboratories, Webster, TX) (21). Both assays show absolute specificity for hIGFBP-1. Murine insulin and IGF-I were measured by radioimmunoassay (Biogenesis, Poole, U.K.). For comparison of fasting and nonfasting sera, samples were collected from overnight fasted mice. These mice were then allowed access to food for 4 h, after which a second sample was taken.

Intraperitoneal glucose tolerance tests. Mice were fasted overnight before testing. Blood samples were collected before and 30 min after administration of an intraperitoneal injection of glucose (1 mg/g body weight in sterile saline). Samples for measurement of hIGFBP-1, insulin, and IGF-I were centrifuged for 10 min at 2,500 rpm, and the plasma was stored at −20°C. The time course of glucose uptake was determined by measurement of whole blood glucose before and 30, 60, and 120 min after a similar glucose challenge. Whole blood glucose was determined in duplicate at each time point using a portable blood glucose analyzer (Hemocon, Sheffield, U.K.).

IGF-1 administration. Recombinant human IGF-1 (0.2 µg/kg body weight) was administered subcutaneously to overnight fasted mice, and whole blood glucose was measured as described above.

Statistical analysis. Results are presented as means ± SE. Comparisons were made by analysis of variance or Student’s t test after log normalization of nonparametric data. Where P < 0.05, analysis of variance was performed using Fisher’s least significant difference test. Correlations were made by simple regression analysis.

RESULTS

Generation of hIGFBP-1 transgenic mice. Three of seven mice (designated c1:2-1, c1:2-2, and c1:2-11) born after microinjection of the Not fragment of chBP1:2 were positive for the transgene. Southern blot analysis of tail DNA from all three founders indicates that the cosmold integrated without any gross rearrangements and was present in multiple copies (Fig. 1B). The copy number was estimated to range from 8 to 20 by slot blot analysis in the three lines (data not shown).

FIG. 1. Identification of hIGFBP-1 transgenic mice by Southern blot analysis. A: Schematic diagram of the hIGFBP-1 cosmold clone chBP1:2. Exons (1-4) are represented by solid bars and the positions of EcoRI (E) sites are indicated. B: Southern blot analysis of EcoRI digested DNA from two founder mice showing the characteristic 5.2- and 4-kb cross-hybridizing bands of the hIGFBP-1 gene (lanes 1 and 2). Lanes 3–8 contain DNA from nontransgenic animals. Wild-type mouse (wt) and human liver DNA (hl) were loaded as controls.
Analysis of hIGFBP-1 expression. The tissue distribution and transcript size of hIGFBP-1 mRNA in transgenic mice were determined by Northern blotting and RT-PCR analysis (Fig. 2). hIGFBP-1 mRNA was most abundant in the liver, followed by the kidney and uterus, with much lower levels in heart, small intestine, spleen, and brain (Fig. 2A). The sites of expression and relative abundance of hIGFBP-1 mRNA in tissues of transgenic mice are very similar to those of the endogenous murine gene in nontransgenic mice (Fig. 2B). The liver transcript size was estimated to be 1.6 kb by Northern blot analysis, which is identical in size to the transcript size in human liver (Fig. 2C; data shown only for line c1:2-2).

Serum IGFBP-1 levels in transgenic mice. Total IGFBP-1 levels (i.e., murine plus hIGFBP-1) in sera of transgenic mice were assessed by Western ligand blot analysis. Figure 3 shows a Western ligand blot of fasting sera from transgenic mice and wild-type controls. In sera of transgenic mice, there is a marked increase in the abundance of a 30-kDa IGFBP corresponding to IGFBP-1.

Estimation of serum hIGFBP-1. Total and nonphosphorylated hIGFBP-1 were quantitated in sera of transgenic mice using specific IRMAs. The mean fasting total hIGFBP-1 in sera of transgenic mice was 174.1 ± 29.7 µg/l (mean nonphosphorylated hIGFBP-1 was 99.1 ± 32 µg/l). hIGFBP-1 was undetectable in sera of wild-type controls, confirming the specificity of both assays.

Physiological regulation of hIGFBP-1. To determine whether hIGFBP-1 is responsive to normal physiological stimuli in transgenic mice, we compared hepatic gene expression and circulating levels of hIGFBP-1 in relation to nutritional status. Matched fasting and nonfasting sera from transgenic mice were assayed for total hIGFBP-1, nonphosphorylated hIGFBP-1, and insulin (Table 1 and Fig. 4). The nonfasting hIGFBP-1 level was reduced by 81% compared with fasting levels. The normal inverse relationship between insulin and IGFBP-1 is maintained in transgenic mice (Fig. 4B). Figure 5 shows the results of competitive RT-PCR analysis of liver RNA from fasted and fed mice. There is a 12-fold reduction in expression levels in the nonfasting state, accounting for the decrease in the serum level of the protein.

Response to IGF-I administration. The hypoglycemic response to exogenous IGF-I is attenuated in transgenic mice.
Blood glucose levels in transgenic mice were higher at all time points after the glucose challenge compared with controls, although this was only significant at 60 and 120 min. There is a marked sexual dimorphism in the response to IGF-I. In wild-type males, there is an increase in blood glucose levels at 30 min and then a rapid decline. This initial increase in blood glucose is abolished in male transgenic mice. In contrast, both wild-type and transgenic females show a rapid fall in blood glucose levels, the fall being more pronounced in wild-type animals.

**Glucoregulation in transgenic mice.** Comparison of fasting and nonfasting blood glucose and insulin levels in transgenic and wild-type littermates at age 16–20 weeks indicated no significant differences between groups. However, there was a marked difference in the response to an intraperitoneal glucose challenge in transgenic mice (Table 2 and Fig. 7). The 30-min insulin levels were 2.5-fold greater in transgenic males (37% increase in females), although blood glucose levels were similar to controls. Fasting insulin and glucose levels in transgenic males did not differ significantly from sex-matched controls. The glucose/insulin ratio is significantly lower in transgenic males after a glucose challenge. Transgenic males had significantly higher 30-min glucose and insulin levels compared with females. Analysis of data from control mice indicates no similar differences.

Although the fasting and 30-min hIGFBP-1 levels were similar, there was a significant increase in the relative proportion of the nonphosphorylated isoform in sera of transgenic mice (Table 2). IGF-I levels did not differ between groups in either basal or stimulated sera (Fig. 7B). Transgenic mice have normal glucose tolerance based on blood glucose levels 30, 60, and 120 min after a glucose challenge (data not shown).

**Age-related changes in glucoregulation.** We measured fasting blood glucose, total hIGFBP-1, and insulin and performed intraperitoneal glucose tolerance tests at age 40–44 weeks to determine if sustained overexpression of hIGFBP-1 in transgenic mice is associated with more profound abnormalities of glucoregulation. There was a marked deterioration in glucocompetence with age, as demonstrated by increased fasting blood glucose and insulin levels in transgenic male mice (Fig. 8A). Fasting hIGFBP-1 levels were reduced, reflecting the relative hyperinsulinemia. Intraperitoneal glucose tolerance tests indicated a marked impairment in the ability to dispose of a glucose load compared with age- and sex-matched controls (Fig. 8B). Fasting blood glucose and insulin levels were moderately elevated in female transgenic mice compared with sex-matched controls, but the data did not reach statistical significance. This was also true of glucose

<table>
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<th>Glucose (mmol/l)</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
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<tr>
<td>Glucose/insulin</td>
<td>45.4±2.6</td>
<td>39.8±9.9</td>
<td>19.5±5.9</td>
<td>11.3±2.6</td>
</tr>
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<td>Total hIGFBP-1 (µg/l)</td>
<td>191.2±56.1</td>
<td>160±29.9</td>
<td>35±6.5</td>
<td>31.7±5.2</td>
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<tr>
<td>Nonphosphorylated hIGFBP-1 (µg/l)</td>
<td>130.6±60.5</td>
<td>73.3±31.5</td>
<td>ND</td>
<td>ND</td>
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</table>

Data are means ± SE for the indicated number of animals. ND, not determined.
tolerance, which showed a mild but statistically insignificant impairment.

**Growth of hIGFBP-1 transgenic mice.** The body weights of transgenic and control mice were measured to examine possible effects of hIGFBP-1 overexpression on postnatal growth (Fig. 9). Transgenic mice were ~10% lighter than wild-type littermates at weaning, but by 8 weeks of age this deficit had narrowed such that body weights were essentially identical. The parity in body weight was maintained until the last recorded measurements at 12–16 weeks. A significant inverse correlation was observed between fasting serum hIGFBP-1 and body weight and IGF-I levels in adult transgenic mice (Fig. 9B). Measurements of serum IGF-I levels correlate well with body weights and sex in both groups, but no significant differences were observed between transgenic and control animals.

**DISCUSSION**

This study was undertaken to investigate the physiological significance of the inverse relationship between insulin and IGFBP-1 and its role in regulating glucose metabolism and glycemia. The role of IGFBP-1 in the acute regulation of serum free IGFs and the manner of its regulation by insulin and contra-insulin hormones, as well as the reciprocal relationship between insulin and IGF-I, are consistent with a model whereby the insulin/IGF-I/IGFBP-1 axis coordinates the metabolic and hypoglycemic activities of insulin and the IGFs in relation to fuel supply.

We have developed transgenic mice that overexpress hIGFBP-1 to investigate this hypothesis. The transgene is a human cosmid clone encompassing the entire structural gene and all the cis-acting sequences necessary to direct appropriate tissue-specific and regulated gene expression. This approach avoids many of the problems associated with the use of heterologous promoters, such as atypical expression sites and low levels of expression. More importantly, the use of a constitutively active promoter would not provide insights into the changing nature of the axis in response to nutritional status. An important advantage of using the human gene is that although the human protein is fully compatible with the rodent IGF/IGFBP system, it is sufficiently distinct to allow us to distinguish it from the murine homologue at the mRNA and protein level. The availability of reagents for the detection of specific isoforms of hIGFBP-1 allows analysis of the phosphorylation status of the protein.

The tissue distribution of hIGFBP-1 mRNA in transgenic mice is more similar to the human than the murine pattern. In transgenic mice, renal hIGFBP-1 expression is low compared with the liver; but in wild-type mice, endogenous IGFBP-1 mRNA is equally abundant in the liver and the kidney. Serum levels of hIGFBP-1 in transgenic mice are in the pathophysiological range. Fasting IGFBP-1 levels in nor-

**FIG. 5.** Competitive RT-PCR analysis of hepatic hIGFBP-1 gene expression in transgenic mice. A representative ethidium bromide-stained gel shows amplification of liver RNA from fasting and non-fasting transgenic mice in the presence of twofold dilutions of the competitor RNA. Amplification of the target generates a band of 445 bp compared with 393 bp for the competitor using the same primer pair and amplification profile indicated in the legend to Fig. 2A. The log of the ratio of the absorbance of target to competitor (A_t/A_c) against the log of the competitor dilution was plotted. Extrapolation from A_t/A_c = 0 (i.e., equal amplification of target and competitor) indicates a 12-fold induction of target mRNA in the fasting group (P < 0.01). Data were derived from three separate amplifications of RNA pooled from six animals per group.

**FIG. 6.** Hypoglycemic effects of exogenous IGF-I. Recombinant human IGF-I (0.2 µg/g body weight) was administered subcutaneously to overnight fasted mice. Blood glucose was measured at the indicated intervals. Data are means ± SE for 25 wild-type (△) and 26 transgenic animals (▲) (A). In B, the data are presented to demonstrate sex differences in the response to IGF-I. The groups are denoted as follows: ○, wild-type male (n = 8); ●, transgenic male (n = 14); □, wild-type female (n = 17); ■, transgenic female (n = 12). *P < 0.02 for transgenic vs. wild-type; **P < 0.05 for sex-matched transgenic vs. wild-type; #P < 0.05 for transgenic males vs. females.
Dysregulation of Insulin/IGFBP-1 Axis

Table 2
Glucose tolerance tests: basal and stimulated serum chemistry in male hIGFBP-1 transgenic mice and sex-matched controls

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<th></th>
<th>Basal</th>
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<th>Stimulated</th>
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<tr>
<td></td>
<td>Transgenic</td>
<td>Wild type</td>
<td>Transgenic</td>
<td>Wild type</td>
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<tr>
<td>Glucose (mmol/l)</td>
<td>7.55 ± 0.4</td>
<td>7.3 ± 0.5</td>
<td>15.4 ± 0.7</td>
<td>13.7 ± 1.4</td>
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<tr>
<td>Insulin (µg/l)</td>
<td>0.24 ± 0.04</td>
<td>0.25 ± 0.1</td>
<td>1.7 ± 0.32</td>
<td>0.69 ± 0.08*</td>
</tr>
<tr>
<td>Glucose/insulin</td>
<td>42.3 ± 7.8</td>
<td>43.6 ± 8.4</td>
<td>10.8 ± 1.1</td>
<td>22.6 ± 5.6†</td>
</tr>
<tr>
<td>Total hIGFBP-1 (µg/l)</td>
<td>75.9 ± 18</td>
<td>—</td>
<td>74 ± 27.3</td>
<td>—</td>
</tr>
<tr>
<td>Nonphosphorylated hIGFBP-1 (µg/l)</td>
<td>26.6 ± 10.6</td>
<td>—</td>
<td>41.5 ± 20.3</td>
<td>—</td>
</tr>
<tr>
<td>Nonphosphorylated/total hIGFBP-1</td>
<td>0.28 ± 0.06</td>
<td>—</td>
<td>0.49 ± 0.1*</td>
<td>—</td>
</tr>
<tr>
<td>IGF-I (µg/l)</td>
<td>517.5 ± 37.6</td>
<td>548.8 ± 64.2</td>
<td>507.4 ± 20.3</td>
<td>559.3 ± 62.4</td>
</tr>
<tr>
<td>Animals per group</td>
<td>8-11</td>
<td>6-10</td>
<td>8-11</td>
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Data are means ± SE for the indicated number of animals. Matched serum samples were collected before and 30 min after an intraperitoneal glucose challenge. Statistically significant differences between transgenic and age-matched control animals in stimulated sera and between the nonphosphorylated/total hIGFBP-1 ratio in basal and stimulated sera of transgenic mice are shown. *P < 0.05; †P < 0.02.

**FIG. 7. Glucose tolerance tests in transgenic mice.** Transgenic and age-matched controls (16-20 weeks) were fasted overnight and then given glucose intraperitoneally. Matched basal and stimulated samples were assayed for insulin and glucose (A) and total IGF-I (B). Data are presented as means ± SE for the number of animals shown in Table 2. Groups are as follows: □, transgenic male; ◻, wild-type male; ○, transgenic female; ●, wild-type female. *P < 0.01; **P < 0.05.

Mal human serum are in the range of 50–80 ng/ml, falling to <10 ng/ml in the nonfasting state.

hIGFBP-1 levels in sera of transgenic mice vary in response to nutritional status, and the normal inverse relationship between hIGFBP-1 and insulin levels is evident. Hepatic hIGFBP-1 mRNA levels are reduced in the nonfasting state consistent with the inhibitory effect of insulin on hepatic IGF-1 gene transcription. However, the relationship between insulin and hIGFBP-1 levels is not absolute. It can be seen that even in the nonfasting state when insulin levels are maximal, hIGFBP-1 levels in the circulation, although reduced compared with fasting levels, are considerably higher than would be expected. Therefore, regulated overexpression of hIGFBP-1 in transgenic mice is a model of reduced sensitivity of IGFBP-1 to insulin regulation. This is the only transgenic model of IGFBP-1 overexpression to show appropriate post-translational modification of the protein. IGFBP-1 has both inhibitory and stimulatory effects on IGF.
activity in vitro (23,24). This differential action appears to be
due to the phosphorylation status of the protein, which can vary
depending on its origin. Phosphorylated IGFBP-1 has a three-
to fivefold greater IGF binding affinity than the nonphospho-
rylated protein. In vivo regulation of IGFBP-1 phosphorylation
has not been convincingly demonstrated, and its physiological
significance remains unclear. However, the striking differences
in the phosphorylation status of IGFBP-1 in different fet-
maternal compartments argues in favor of it having an in vivo
role in modulating IGFBP-1 function, at least in fetal growth and
development (25).

IGFBP-1 in normal human serum is present predominantly
as a single, highly phosphorylated species (>95%). The pro-
portion of phosphorylated hIGFBP-1 in sera of transgenic
mice is significantly lower (60–70%). This may be due to the
fact that murine species lack hepatic protein kinases with the
appropriate substrate specificity to phosphorylate hIGFBP-1
on the serine residues (serine 98 and 169) that are not con-
served in murine IGFBP-1. Alternatively, the decrease in
hIGFBP-1 phosphorylation may represent an adaptive
response to increased IGF BP-1 synthesis, increasing the pro-
portion of the nonphosphorylated (i.e., less inhibitory) form
of the protein. The increase in the proportion of nonphos-
phosphorylated hIGFBP-1 after a glucose challenge is intriguing, particularly when total hIGFBP-1 levels remain unchanged. In physiological terms, there is a compelling rationale for such a phenomenon. Nonphosphorylated IGFBP-1 would be less inhibitory to the insulin-like actions of the IGFs at a time when this activity needs to be maximized, i.e., in response to a glucose challenge. Insulin itself, or perhaps glucose, may stimulate hepatocytes to alter the pattern of protein phosphorylation. Hormonal regulation of IGFBP-1 phosphorylation has been reported in humans, but the physiological significance and mechanisms involved require clarification (26).

We have demonstrated specific abnormalities of glucose-regulation in hIGFBP-1 mice. At age 16–20 weeks, hIGFBP-1 transgenic mice have normal fasting glucose and insulin levels. Even after a glucose challenge, blood glucose levels in transgenic mice are similar to controls, albeit at the expense of a twofold increase in insulin secretion. The coordinated actions of insulin on glucose transport and IGFBP-1 expression enhance the ability to respond to a glucose challenge when insulin levels are high, and the insulin-like activity of the IGFs can be expressed because of the insulin-mediated suppression of IGFBP-1. The finding of normal fasting blood glucose in transgenic mice and the observation that insulin secretion is only perturbed following a glucose challenge are consistent with this analysis.

Our hypothesis is that the insulin/IGFBP-1 axis is important in coordinating the metabolic and hypoglycemic activities of insulin and the IGFs and that dysregulation of the axis may give rise to abnormalities of insulin action, leading to insulin resistance and ultimately glucose intolerance. The rationale is that if insulin-mediated reduction of IGFBP-1 levels in the non-fasting state is not absolute, then serum IGFBP-1 has the potential to attenuate the hypoglycemic actions of the IGFs. In our view, this would precipitate nonfasting hyperinsulinemia by a number of mechanisms acting in synergy and which are related to a reduction in IGF bioavailability. Attenuation of the hypoglycemic actions of the IGFs by IGFBP-1 may elicit a compensatory increase in insulin secretion to maintain normal blood glucose levels. The reduced inhibitory action of the IGFs on insulin secretion would have a similar outcome. Because IGF-1 is reported to increase insulin sensitivity in humans and rodents, the reduction in IGF bioactivity could lead to desensitization of insulin-responsive tissues, namely skeletal muscle. The increase in insulin secretion after a glucose challenge in hIGFBP-1 mice is of the order of 2.5-fold greater than in controls. The cumulative effect of this level of insulin hypersecretion over a long period of time would inevitably lead to a decrease in tissue sensitivity to insulin, stimulating yet further insulin secretion. This prediction is borne out in hIGFBP-1 mice with the onset of fasting hyperglycemia and hyperinsulinemia and glucose intolerance with advancing age. The progression from normoglycemia and hyperinsulinemia to overt fasting hyperglycemia, hyperinsulinemia, and glucose intolerance in hIGFBP-1 is similar to some animal models of type 2 diabetes.

Abnormalities of hepatic insulin sensitivity offer the most plausible scenario by which the insulin/IGFBP-1 axis may be perturbed. Defects in insulin action at the receptor or postreceptor level could attenuate the inhibitory action of insulin on IGFBP-1 gene transcription. Liver-enriched transcription factors such as the hepatocyte nuclear factor (HNF) family (HNF-1 to -4) are important in regulating the expression of genes such as IGFBP-1 that are highly expressed in the liver. Recently, mutations in the HNF-1β and HNF-4α genes have been reported in patients with maturity-onset diabetes of the young (MODY), a rare form of type 2 diabetes (27,28). The role of the three known MODY genes in susceptibility to the more common late-onset form of type 2 diabetes remains uncertain. Finally, the phenomenon of “metabolic programming” is thought to underlie the epidemiological association between low birth weight and the incidence of type 2 diabetes in adult life (29). It has been suggested that abnormalities of the insulin/IGFBP-1 axis may be programmed in utero in response to fetal undernutrition (30). Circulating levels of IGFBP-1 and insulin are increased in fetuses with intrauterine growth restriction (31), and several studies have shown that children who were small at birth have impaired glucose tolerance and insulin resistance in adult life (32,33).

Our findings are distinct from those reported in IGFBP-1 transgenic mice in which gene expression is regulated by the mouse phosphoglycerate kinase (PGK) promoter (PGK-rIGFBP-1). PGK-rIGFBP-1 mice exhibit basal and stimulated hyperglycemia and hyperinsulinemia and have impaired glucose tolerance from an early age (34). At a comparable age, hIGFBP-1 mice have normal fasting and nonfasting blood glucose and insulin but exhibit hyperinsulinemia after a glucose challenge. The increase in insulin secretion in response to a glucose challenge in PGK-rIGFBP-1 mice is less than that of wild-type controls (1.6- vs. 3.99-fold increase over basal levels). The glucose/insulin ratio falls in hIGFBP-1 mice but increases in PGK-rIGFBP-1 mice after a glucose load. Another divergence in the phenotypes is the age-related decline in serum insulin levels in PGK-rIGFBP-1 mice, in contrast to the increase in insulin secretion in hIGFBP-1 mice between the ages of 4 and 10 months.

hIGFBP-1 transgenic mice have a moderate degree of growth restriction in early postnatal life before weaning, but there appears to be “catch-up growth,” as the weight difference is no longer evident by 8 weeks of age. In contrast, the growth restriction in PGK-IGFBP-1 transgenic mice follows a different pattern. The growth trajectories of transgenic and wild-type mice diverge early in postnatal life and continue into adult life with no evidence of catch-up growth.

Given the similarity in the weights of adult hIGFBP-1 transgenic and wild-type littersmates, the negative correlation between hIGFBP-1 levels and IGF-1 levels and body weight in adult transgenic mice is surprising. Gross body weight is a crude measurement of growth, because it does not distinguish between linear and nonlinear growth, i.e., increased adiposity. Therefore, it is possible that a reduction in linear growth may be counterbalanced by an increase in adiposity with no net change in body weight. We have demonstrated specific defects in glucose regulation in hIGFBP-1 mice that are consistent with insulin resistance. The conventional view that obesity predisposes to the development of insulin resistance is well established, but an alternative, though not mutually exclusive hypothesis, is that resistance to insulin-mediated glucose disposal in muscle can result in an increase in adiposity (35). Altered nutrient partitioning, leading to an increase in triglyceride synthesis and storage in adipose tissue, may be a consequence of diminished muscle glucose utilization. In addition, the hyperinsulinemia associated with insulin resistance may have direct anabolic actions on adipose tissue. Further investigation of lean body mass and adiposity
in hIGFBP-1 transgenic mice will be necessary to determine if this applies. The observation of early postnatal growth restriction suggests that its origins may be in utero life. Increased hepatic expression and circulating IGFBP-1 levels are a consistent finding in animal models of intrauterine growth restriction and in human studies of small-for-gestational-age fetuses. We have recently demonstrated hepatic transgene expression in hIGFBP-1 transgenic conceptuses (P.A.C., C.C. Pillai, J.P.M., unpublished observations).

In conclusion, we have generated transgenic mice that overexpress hIGFBP-1 as a model system to study the regulation and function of the insulin/IGF-I/IGFBP-1 axis in an in vivo physiological context. The analyses performed here confirm the fundamental importance of the normal inverse relationship between insulin and IGFBP-1 in glucoregulation. We present evidence that sustained dysregulation of the axis leads to profound abnormalities of glucose homeostasis that have some similarities to the pathophysiology of type 2 diabetes.

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REFERENCES