Transgenic mice overexpressing growth hormone (GH) have been extensively used to study the chronic effects of elevated serum levels of GH. GH is known to have many acute effects in the liver, but little is known about the chronic effects of GH overexpression on hepatic gene expression. Therefore, we used DNA microarray to compare gene expression in livers from bovine GH (bGH)-transgenic mice and littermates. Hepatic expression of peroxisome proliferator-activated receptor-α (PPARα) and genes involved in fatty acid activation, peroxisomal and mitochondrial β-oxidation, and production of ketone bodies was decreased. In line with this expression profile, bGH-transgenic mice had a reduced ability to form ketone bodies in both the fed and fasted states. Although the bGH mice were hyperinsulinemic, the expression of sterol regulatory element-binding protein (SREBP)-1 and most lipogenic enzymes regulated by SREBP-1 was reduced, indicating that these mice are different from other insulin-resistant models with respect to expression of SREBP-1 and its downstream genes. This study also provides several candidate genes for the well-known association between elevated GH levels and cardiovascular disease, e.g., decreased expression of scavenger receptor class B type I, hepatic lipase, and serum paraoxonase and increased expression of serum amyloid A-3 protein. We conclude that bGH-transgenic mice display marked changes in hepatic genes coding for metabolic enzymes and suggest that GH directly or indirectly regulates many of these hepatic genes via decreased expression of PPARα and SREBP-1.

MANY DIFFERENT TRANSGENIC MODELS with chronic elevation of growth hormone (GH) levels have been used to study the long-term effects of GH. Mice transgenic for GH have increased size due to increased lean body mass, linear growth, and organomegaly (13, 67). The GH-transgenic mice have decreased fat mass (20, 45) and changed serum levels of several hormones other than GH. The bovine GH (bGH)-transgenic mice used in this study have elevated serum levels of corticosterone (4), triiodothyronine (T₃) (4), insulin (20), and IGF-I (20) but decreased levels of thyroxine (T₄) (4) compared with littermate controls. Moreover, hyperinsulinemia and insulin resistance, but normal or nearly normal glucose tolerance, have also been described in GH-transgenic mice (29). Furthermore, these bGH-transgenic mice have impaired cardiac function (5) and increased locomotor activity (60). Regarding the lipid and lipoprotein metabolism in GH-transgenic models, both GH-releasing factor- and bGH-transgenic mice revealed elevated serum cholesterol levels and normal or decreased serum triglyceride levels (6, 20, 46). We have recently shown (20) that bGH-transgenic mice have increased HDL-cholesterol and LDL-apolipoprotein B
Bovine growth hormone-transgenic mice have major alterations in hepatic expression of metabolic genes | American Journal of Physiology - Endocrinology and Metabolism

(apoB) levels but decreased VLDL-apoB and triglyceride levels. Moreover, these mice showed decreased hepatic triglyceride secretion and increased lipoprotein lipase activity in adipose tissue, heart, and skeletal muscle but unchanged LDL receptor activity (20). Patients with acromegaly have many features that are similar to those of GH-transgenic mice, including organomegaly, decreased body fat (3), insulin resistance (19), and disturbed lipoprotein metabolism. However, these patients have elevated serum triglycerides and, in some individuals, increased hepatic triglyceride secretion (39, 44).

The influence of GH on hepatic metabolism has also been investigated in GH-deficient states, such as in GH-deficient adults and hypophysectomized rats. In terms of lipoprotein metabolism, GH treatment of hypophysectomized rats increases HDL- but decreases LDL-cholesterol levels (41), increases hepatic VLDL secretion (14, 58), and increases LDL receptor expression (2). Lipoprotein lipase activity was increased in skeletal muscle and heart but was unchanged in adipose tissue by GH treatment of hypophysectomized rats (43). On the other hand, GH treatment of GH-deficient adults decreased adipose tissue lipoprotein lipase activity (42). Thus the effect of GH treatment is different depending on the model of investigation and may also depend on the time course of treatment.

Several hepatic genes that are influenced by GH on a transcriptional level have been identified, e.g., the GH receptor (GHR), IGF-I, signal transducer and activator of transcription 5, members of the CYP family, major urinary proteins, serine proteinase inhibitor 2.1 (SPI2.1), and the prolactin receptor (26, 27, 34, 38, 47). Also, the short-term effect of GH on the expression of several hepatic genes in the normal and aging rat and in hypophysectomized rats has recently been described (16, 62-64). However, the long-term effect of GH on hepatic metabolic genes has not previously been described. Therefore, in this study, we investigated hepatic gene expression by DNA microarray in mice with chronic elevation of GH. We conclude that the livers of bGH-transgenic mice display marked changes in the expression of genes coding for metabolic enzymes and suggest that many of these changes are mediated via peroxisome proliferator-activated receptor-α (PPARα) and sterol regulatory element-binding protein (SREBP)-1. Moreover, the reduced expression of SREBP-1, despite hyperinsulinemia, shows that these mice are different from other insulin-resistant models with respect to expression of SREBP-1 and its downstream genes.

MATERIAL AND METHODS

Animals. In this study, we used 6-mo-old male bGH-transgenic mice and littermates. The bGH-transgenic mice have been described previously (20, 50). The mice were housed with a light cycle of 14 h of light (0500-1900) and 10 h dark (1900-0500) and had free access to mouse standard chow and tap water (Rat/Mouse Standard Diet; B&K Universal, Sollentuna, Sweden). The mice were anesthetized with ketamine hydrochloride (77 mg/kg Ketalar; Parke-Davis, Detroit, MI) and xylazine (9 mg/kg Rompun; Bayer, Lever-Kusen, Germany) and killed by heart puncture. The livers
used for RNA preparations were excised and immediately frozen in liquid nitrogen and stored at -135°C. The study was performed after approval from the regional ethics committee for animal experimentation.

**RNA preparation.** Total RNA from livers of four male bGH-transgenic mice and four male littermate controls were extracted by Tri Reagent (Sigma Diagnostics, St. Louis, MO) and further purified using an RNeasy Total RNA Isolation Kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions. The integrity of RNA was examined by agarose gel electrophoresis and ethidium bromide staining. The total RNA from the four mice in each group was pooled using equal amounts of RNA from each mouse. Reverse transcription was performed with Superscript Choice System (GIBCO-BRL, Rockville, MD), followed by an in vitro transcription using BioArray HighYield Transcript Labeling Kit (Enzo Diagnostics, Farmingdale, NY) according to the Affymetrix (Affymetrix, Santa Clara, CA) user manual. After this procedure, the integrity of the samples was examined by agarose gel electrophoresis and ethidium bromide staining. The hybridization and washing procedures were performed using an Affymetrix hybridization oven and fluidics station according to the Affymetrix user manual. The duplicate microarrays started from the same livers, but two separate RNA preparations and cDNA syntheses and in vitro transcriptions were performed. The microarrays were scanned with a Hewlett-Packard confocal laser scanner and visualized using Affymetrix Genechip 3.1 software (Affymetrix).

**Calculations.** The mouse 11ka and 11kb (Affymetrix microarrays) contain 11,000 genes and expressed sequence tags (ESTs) together. To study all well-known genes, we decided to use both chips, since the well-known genes were divided onto both chips. To allow cross-comparisons between different samples, the mean target signal on each microarray was scaled to an average intensity of 500. Both the mouse 11ka and the mouse 11kb microarrays were run in duplicates for each group (bGH and control). Each bGH microarray was compared with each control microarray for either 11ka or 11kb, creating four comparison files. The differences were selected solely on the difference call (DC) parameter, determined by an algorithm based on signal intensity and quality. Only the genes that differed in three or four of the comparisons (DC 3-4) were set as a significant change. The fold change given was calculated as the average fold change of all comparisons. EST accessions were identified using the TIGR Mouse Gene Index database and compared with GenBank with NCBI BLAST.

**Real-time PCR.** Livers from four transgenic males and four littermate controls were dissected. Total RNA was extracted with Tri Reagent (Sigma). First-strand cDNA was synthesized from total RNA using the Superscript preamplification system (Life Technologies). Real-time (Rt)-PCR analysis was performed in individual mice \((n = 6)\) with an ABI Prism 7900 Sequence Detection System (Perkin Elmer Applied Biosystems) using 6-carboxyfluorescein- and 6-carboxy-tetramethylrhodamine-labeled fluorogenic probes. The expression data were normalized against mouse acidic ribosomal phosphoprotein P0 (M36B4). The SREBP-1 total (including 1a and 1c isoforms), SREBP-1a,
SREBP-2, PPARα, fatty acid synthetase, hepatic lipase, and M36B4-specific primers amplified nucleotides 600-648 (GenBank accession no. U09103), 1053-1120 (acc. no. U09103), 1037-1106 (acc. no. X57638), 1053-1120 (acc. no. X13135), 1053-1120 (acc. no. X58426), and 986-1059 (acc. no. X15267), respectively (Table 1). The relative expression levels were calculated according to the formula 2^-ΔCT, where ΔCT is the difference in threshold cycle (CT) values between the target and the M36B4 internal control (User Bulletin no. 2, Perkin Elmer).

Table 1. Primer and probe sequence for real-time PCR

| Primer and probe sequence for real-time PCR | Serum analysis. Plasma β-hydroxybutyrate (β-HB) and free fatty acid (FFA) levels were determined before and after a 16-h fast using a β-HB kit (Sigma) and NEFA C kit (Wako Chemicals, Neuss, Germany) according to the manufacturer's instructions. Plasma insulin was determined radioimmunochemically with the use of a guinea pig anti-rat insulin antibody, ^125^I-labeled human insulin as tracer, and rat insulin as standard (Linco Research, St. Charles, MO). Plasma glucose was determined with the glucose oxidase method. |

Statistics. Values are given as means ± SE. Comparisons between independent groups were performed with the Mann-Whitney U-test, and comparisons of repeated measurements within groups were performed with the Wilcoxon signed rank pair test. P values <0.05 were considered significant.

RESULTS

Microarray. GH is a potent regulator of metabolism and has been shown to influence hepatic lipid, cholesterol, and carbohydrate metabolism (2, 7, 10, 12, 17, 27, 46, 49, 56). However, few molecular targets for the effect of GH on metabolism in the liver have been identified. To study the effect of chronic elevation of GH on genes involved in hepatic metabolism, we performed microarray analysis on pooled RNA from livers of bGH-transgenic mice and littermate controls. To validate the use of DNA microarray to detect differences between bGH-transgenic mice and littermates, we first investigated the expression of well-known GH-regulated genes. In agreement with previous observations, the elevated GH levels in bGH-transgenic mice increased the hepatic gene expression of the GHR, IGF-I, and SPI2.1 (35, 40, 70) (Table 2).

Several regulated metabolic genes were identified and classified into regulators of transcription, fatty acid synthesis and oxidation, scavenger receptors, lipoprotein metabolism, cholesterol metabolism, and carbohydrate and amino acid metabolism (Tables 3 and 4). The most striking findings were that the expression of genes involved in all

https://www.physiology.org/doi/full/10.1152/ajpendo.00444.2002
aspects of fatty acid metabolism were decreased, e.g., fatty acid activation, β-oxidation, ketone body formation, and fatty acid synthesis and esterification, with the exception of diacylglycerol acyltransferase (DGAT), which was increased. Also, SREBP-1 and PPARα, two important regulators of transcription of genes involved in fatty acid metabolism, were decreased. Furthermore, genes involved in amino acid and HDL metabolism showed decreased gene expression.

Table 3. Difference in expression of genes in lipid metabolism between bGH-transgenic mice and littermate controls
Expand table

Table 4. Difference in expression of genes in lipoprotein, carbohydrate, amino acid, and cholesterol metabolism between bGH-transgenic mice and littermate controls
Expand table

Rt-PCR. To verify the results found in the microarray experiment, we performed Rt-PCR on five key genes in six bGH-transgenic mice and six littermate controls. PPARα was decreased 2.1 times on the microarray in the bGH-transgenic mice and 1.6 times in the Rt-PCR (Fig. 1). SREBP-1 exists in two different isoforms, SREBP-1a and -1c, which are transcribed from a single gene by the use of alternate promoters (24, 54). SREBP-1a is a potent activator of the cholesterol and lipogenic pathways, whereas SREBP-1c activates only the lipogenic pathway (24). SREBP-1 was decreased 2.6 times on the microarray, and the Rt-PCR showed a twofold decrease by use of primers directed to the common part of the SREBP-1 detecting both SREBP-1a and -1c (P < 0.05; Fig. 1). However, specific primers for the SREBP-1a isoform showed no difference between the bGH-transgenic mice and littermate controls (Fig. 1). Hence, the decrease in SREBP-1 is due to the decreased expression of SREBP-1c. SREBP-2 showed no difference in expression level between the bGH-transgenic mice and littermate controls. Fatty acid synthase and hepatic lipase were decreased 2.5 and 22.2 times, respectively, on the microarray, and Rt-PCR revealed a decrease of 2.4 times for fatty acid synthase and a decrease of 25.3 times for hepatic lipase (Fig. 1).
Real-time PCR verification in individual mice of some of the genes found differently expressed by microarray. Relative expression is shown in an arbitrary scale. bGH, bovine growth hormone-transgenic mice; SREBP, sterol regulatory element-binding protein; PPAR, peroxisome proliferator-activated receptor-α; FAS, fatty acid synthetase; HL, hepatic lipase. Values are expressed as means ± SE (n = 6). Comparisons between independent groups were performed with a Mann-Whitney U-test (*significant change). P < 0.05 was considered significant.

Download figureDownload PowerPoint

Serum analysis. Because the gene expression of enzymes involved in β-oxidation and ketone body formation was decreased, we conducted an experiment to study the plasma and serum levels of ketone bodies and fatty acids in response to fasting (Fig. 2, A and B). In the fed state, the levels of β-HB were undetectable in five of the seven bGH-transgenic mice. Fasting induced a rise in plasma β-HB levels in the bGH mice but to a markedly lower level than in littermate controls (Fig. 2A), indicating that the hepatic fatty acid β-oxidation and subsequent ketone body formation were impaired in bGH-transgenic mice. FFA levels were lower in the bGH-transgenic mice in the fed state, but they were not different from the littermate controls in the fasted state (Fig. 2B). The bGH-transgenic mice were clearly hyperinsulinemic (Fig. 2C) but showed normal blood glucose levels (Fig. 2D). Furthermore, bGH-transgenic mice as used in this study (same strain) had previously been shown to have elevated levels of IGF-I (bGH 526 μg/l vs. controls 262 μg/l) (20), T3 (bGH 7.2 pmol/l vs. controls 5.4 pmol/l) (4), and corticosterone (bGH 745 μg/l vs. controls 199 μg/l) (4) but decreased levels of T4 (bGH 9.3 μg/l vs. controls 13.7 μg/l) (4).
DISCUSSION

In this study, we used DNA microarray to investigate the effect of chronic elevation of GH on hepatic gene expression in bGH-transgenic mice. Gene expression of lipogenic enzymes was decreased. We also noted a decrease in the expression of SREBP-1, which is a potent regulator of lipogenic enzymes, indicating that decreased expression of SREBP-1 may be responsible for the decreased gene expression of these enzymes. The expression of genes involved in fatty acid oxidation and ketone body formation was also decreased in bGH-transgenic mice. In line with these findings, bGH-transgenic mice had a reduced ability to form ketone bodies in both the fed and fasted states.

https://www.physiology.org/doi/full/10.1152/ajpendo.00444.2002
This decrease coincided with decreased gene expression of PPARα. This finding suggests that decreased PPARα expression mediates the downregulation of the genes involved in fatty acid oxidation and ketone body formation. An alternative explanation is that enzymes involved in fatty acid oxidation and ketone body formation are downregulated in bGH-transgenic mice by an increased action of insulin on the liver. In line with this possibility, the liver seems to be less affected by the diabetogenic action of GH than is muscle tissue in GH-transgenic mice (11, 12).

Concerning the validation of our animal model, the gene expressions of GHR, IGF-I, and SPI2.1 were increased on the microarray, which is in line with previous reports (35, 40, 70). The decreased gene expression of enzymes involved in the catabolism of amino acids observed in the bGH-transgenic mice most likely reflects the known anabolic effects of GH. Thus, apart from a decreased supply of amino acids for gluconeogenesis and ketogenesis by the anabolic action of GH in skeletal muscle, GH also decreases the hepatic expression of genes involved in the catabolism of amino acids.

The expression of SREBP-1 was decreased in bGH-transgenic livers. In addition, we noted a decreased expression of genes regulated by SREBP-1, i.e., the HDL receptor scavenger receptor class B type I (SR-BI) (31), fatty acid synthetase (32,57), ATP-citrate lyase (51), glycerol-3-phosphate acyltransferase (GPAT) (15), stearoyl-CoA desaturase (SCD1) (57), and spot 14 (33). These findings indicate that SREBP-1 may be an important mediator of GH's long-term effect on the gene expression of enzymes involved in fatty acid metabolism.

Recent studies have shown that SREBP-1 is an important mediator of insulin action, since SREBP-1 mRNA and protein were directly stimulated by insulin in rat hepatocytes (18) and introduction of a dominant negative form of SREBP-1 in hepatocytes abolished insulin's ability to induce transcription of glucokinase (17). Furthermore, introduction of a dominant positive SREBP-1 in hepatocytes induced glucokinase without insulin stimulation (17), and dominant positive SREBP-1-transgenic mice did not show a decrease in lipogenic enzymes when fasted (23). Moreover, in mice with streptozotocin-induced diabetes, hepatic SREBP-1c was undetectable but was rapidly induced after insulin administration (55). The stimulatory effect of insulin on SREBP-1c has been shown to be mediated by an increase in gene expression (17, 55). Our finding of decreased SREBP-1 mRNA expression indicates that SREBP-1 is resistant to the stimulatory effect of hyperinsulinemia in bGH-transgenic mice, in contrast to other hyperinsulinemic mouse models such as the ob/ob mouse (56). Thus the decreased expression of SREBP-1 may be a direct effect of chronic overexpression of GH in the liver or, alternatively, an indirect effect via other accompanying hormonal or metabolic alterations than hyperinsulinemia such as elevated levels of T₃ or corticosterone. It is less likely that SREBP-1 levels decreased via changed hepatic cholesterol levels, because they are unchanged in these mice (20). Our findings therefore indicate that the effect of chronic overexpression of GH is dominant over the effect of hyperinsulinemia in the regulation of SREBP-1c gene expression.
We observed a contradiction in the GH-mediated regulation of two key enzymes in the triglyceride biosynthesis; i.e., the gene expression of GPAT decreased whereas the expression of DGAT increased. A decreased triglyceride biosynthesis is suggested to occur in these mice by the observations of normal hepatic triglyceride content and a decreased hepatic triglyceride secretion rate in bGH-transgenic mice (20), indicating that the decreased DGAT gene expression is of less importance for the hepatic triglyceride synthesis in these mice. The physiological role of DGAT has been questioned, because the $DGAT^{-/-}$ mice showed normal triglyceride formation and amount of adipose tissue (59). Furthermore, other enzymes with DGAT activity have recently been identified and cloned (8).

Expression of several genes involved in lipid metabolism, including fatty acid activation, peroxisomal and mitochondrial β-oxidation, and production of ketone bodies, was decreased in bGH-transgenic mice. This finding coincided with decreased gene expression of PPARα. PPARα has been shown to direct the activity of β-oxidation and subsequent ketone body formation in the liver via changed expression of mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthetase (48), acyl-CoA oxidase (28, 66), long-chain acyl-CoA synthetase (52), and medium-chain acyl-CoA dehydrogenase (21). PPARα also regulates the expression of SCD1, which mainly converts palmitic acid and stearic acid to their monounsaturated forms (36). All of these known PPARα target genes showed reduced expression. Moreover, we showed that bGH-transgenic mice have a reduced ability to produce ketone bodies in both the fed and fasted states.

GH has previously been shown to decrease the expression of PPARα and the effect of peroxisome proliferators on peroxisomal β-oxidation activity in hepatocytes (7, 64, 68, 69). Furthermore, insulin has been shown to decrease peroxisomal β-oxidation (22), indicating that GH may decrease the expression of enzymes in peroxisomal β-oxidation via decreased PPARα expression and via elevated insulin levels. Glucocorticoids have a stimulatory effect on PPARα expression (30), and even though bGH-transgenic mice have a threefold increase in serum corticosterone levels (9), the expression of PPARα was reduced. This finding suggests that the levels of glucocorticoids are subordinate to the levels of GH in the regulation of PPARα in this mouse model. The decreased expression of genes involved in β-oxidation and formation of ketone bodies, together with a reduced ability to form ketone bodies in response to fasting where fatty acids are ample, suggests that the hepatic fatty acid β-oxidation and subsequent ketone body formation are impaired in bGH-transgenic mice.

Epidemiological studies of patients with chronic elevation of GH in humans (acromegaly) indicate that increased levels of GH result in an increased risk for cardiovascular disease (1). One explanation for this effect of GH may be major alterations in the lipoprotein metabolism induced by the hormone (20). There are several observations in the present study that connect chronic elevation of GH with risk factors for atherosclerosis and cardiovascular disease. Thus our data indicate that the expression of SR-BI is decreased in the bGH-transgenic mice. Recent studies in
mice show that SR-BI deficiency resulted in elevated HDL-cholesterol levels, reduced biliary cholesterol levels, and drastically accelerated onset of atherosclerosis (65). Epidemiological studies indicate that elevated HDL-cholesterol levels are beneficial. However, increased HDL levels have to be related to the presumed function of HDL. Thus the elevated HDL levels in bGH-transgenic male mice (20) may therefore not be associated with an increase in reverse cholesterol transport, the transport that has been suggested to be one reason for the antiatherogenic effects of HDL. Other factors associated with atherosclerosis and cardiovascular disease that connect chronic elevation of GH with the development of the diseases are the reduced gene expression of hepatic lipase (37, 71) and serum paraoxonase (25, 53) and the increased gene expression of serum amyloid A-3 protein (61).

We conclude that bGH-transgenic mice display marked changes in genes coding for metabolic enzymes and suggest that many of these hepatic effects by GH are mediated via PPARα and SREBP-1. The decreased expression of SREBP-1 and its target genes indicates that GH may impair this insulin-dependent pathway.

DISCLOSURES

This work was supported by grants from Swedish Cancer Foundation, AstraZeneca Research and Development, Swedish Medical Research Council 14291, the Sahlgrenska University Foundation, Novo Nordisk Foundation, Gustaf V's, Queen Victoria's Foundation, and the Swedish Heart and Lung Foundation.

FOOTNOTES

- The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Barbro Basta for excellent technical assistance.

AUTHOR NOTES

- Address for reprint requests and other correspondence: B. Olsson, Research Center, Endocrinology and Metabolism, Dept. of Internal Medicine, Göteborg Univ., Vita Straket 12, SE-405 30 Goteborg, Sweden (E-mail: bob.olsson@medic.gu.se).