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Consequences of Overexpression of Growth Hormone in Transgenic Mice on Liver Cytochrome P450 Enzymes

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ABSTRACT. The effect of growth hormone (GH) on cytochrome P450 (CYP) and P450-dependent monooxygenases was studied in 4-, 6-, 8-, and 10-month-old female bovine growth hormone (bGH) transgenic mice that overexpress GH. Nontransgenic female mice (C57/SJL) littermates were used for baseline determinations. The body weights of the bGH mice were approximately 35% greater than those of the controls. The liver weights were 2-fold higher than those of the controls, resulting in a 25–60% increase in liver/body weight ratio during the life span of the bGH mice when compared with the controls. Similar increases in heart and kidney weights were observed. Since the GH transgene was transcriptionally regulated by a metallothionein-I gene promoter, metallothionein concentrations in livers of transgenic and nontransgenic mice were measured. No significant differences were observed. In marked contrast to increases in liver weights, hepatic cytochrome P450 content, benzphetamine *N*-demethylase, and benzo [*a*] pyrene hydroxylase activities were decreased by 36, 42 and 75%, respectively. No age-related changes in the decrease of the monooxygenases were observed. Microsomal heme oxygenase (HO) in the liver was induced 44% above the control values. Immunoblot analysis also showed a marked increase in HO-1 in the bGH mice. These results indicate that GH suppresses the carcinogen-metabolizing enzyme benzo [*a*] pyrene hydroxylase and the drug-metabolizing enzyme benzphetamine *N*-demethylase. This suppression was accompanied by an induction of HO activity in bGH transgenic mice. The consequences of prolonged exposure to supraphysiological levels of this hormone cannot always be predicted from the known physiological actions of GH. BIOCHEM PHARMACOL **55**;9:1481–1487, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. transgenic mice; cytochrome P450; heme oxygenase; drug-metabolizing enzymes; metallothionein; growth hormone

The CYP¶ family of enzymes participates in the metabolism of a large number of xenobiotics and endogenous substrates, such as fatty acids, prostaglandins, leukotrienes, and steroids. These enzymes exhibit a pattern of expression that is dependent on development and tissue factors, as well as genetic, environmental, physiological, and pathological factors. A number of hormones have been implicated in the regulation of cytochrome P450 proteins, including testosterone, thyroid hormone, and GH. Pituitary GH secretory patterns and circulating thyroid hormone levels are the most important endocrine regulators.

The secretory pattern of GH in the rat is sex-dependent. In male rats, GH is secreted in pulses with a low basal level between pulses, whereas in females circulating GH is present continuously. These differences in circulating GH have been associated with the expression of male-specific CYP2C11 and female-specific CYP2C12, respectively [1, 2]. In hypophysectomized rats, the expression of xenobiotic-metabolizing CYP3A1 and 3A2 increases by about 2-fold in males and more than 10-fold in females, at both the protein and mRNA levels [3]. Treatment of the hypophysectomized rats with GH restored the normal CYP3A expression. These observations suggest that GH suppresses CYP3A expression in rat liver. In both male and female hypophysectomized rats, intermittent GH suppresses CYP2A1, whereas continuous GH infusion induces CYP2A1 [4, 5], demonstrating that the expression of CYP2A1 is also regulated by GH. In GH-deficient homozygous Little (*lit/lit*) mice, which have a genetic defect in GH production, CYP2A4 enzyme activity and mRNA levels are elevated compared with the heterozygotes. Intraperitoneal

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¶ *Abbreviations:* CYP, cytochrome P450; GH, growth hormone; bGH, bovine growth hormone; and HO, heme oxygenase.

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injection of GH in the homozygous mice dramatically reduces liver microsomal testosterone 15α-hydroxylase activity and liver CYP2A4 mRNA levels [6]. These results further suggest that, as in rats, GH suppresses CYP2A4 expression in mice. As is the case with other CYP2A enzymes, the role of rat CYP2A1 and mouse CYP2A4 in xenobiotic metabolism has not been investigated intensively. Expression levels of the drug-inducible forms of CYP also appear to be altered by plasma GH. The induction by phenobarbital of CYP2B1- and CYP2B2-dependent monooxygenase activities was significantly higher in the livers of hypophysectomized rats when compared with control rats [7, 8]. Similarly, the induction by pregnenolone-16α-carbonitrile of hepatic aryl hydrocarbon hydroxylase activities (CYP1A1-dependent monooxygenase activities) was greater in hypophysectomized rats when compared with control rats [9]. However, Burke *et al.* [10] reported that the induction of hepatic CYP1A1-dependent monooxygenase activities by 3-methylcholanthrene was diminished in hypophysectomized rats even though there was no decrease in basal activities due to hypophysectomy.

Transgenic animals overexpressing GH provide a unique system in which one can study long-term as well as short-term effects of GH on CYP enzymes. Production of GH in transgenic mice is ectopic, with most of the hormone present in the circulation being derived from the liver and kidney [11]. Enhanced growth is thought to result from the activation of hepatic GH receptors, which, in turn, stimulate the synthesis and secretion of insulin-like growth factor I, a hormone involved in the normal development of multiple organs. Animals transgenic for bGH under the control of a metallothionein promoter have rapid somatic growth that becomes apparent after the age of 3 weeks [12–14]. Long-term exposure to GH can have clinical relevance in those patients being treated with human recombinant GH, as it may affect the metabolism of drugs and endogenous substrates by the liver cytochrome P450 enzymes. The present studies demonstrated that in bGH mice CYP and dependent enzyme activities were decreased markedly, even though body and liver weights were increased markedly.

MATERIALS AND METHODS

Animals

Transgenic mice that contained the bGH gene using mouse metallothionein promoter were generated by the standard egg microinjection technique. The plasmid construct and generation of the transgenic mice are described by Chen *et al.* [15]. Female bGH transgenic (C57/SJL) mice and their non-transgenic littermates were obtained from the Edison Biotechnology Institute, Ohio University. All mice had free access to food and water.

Tissue Preparation

bGH mice and littermates were killed by decapitation at 4, 6, 8, and 10 months following birth. Body, liver, heart, and kidney weights were recorded. Livers of transgenic and nontransgenic mice were kept at −80° until they were assayed. At the time of the assays, the livers were thawed and homogenized in 4 vol. of 1.15% KCl solution. Homogenates were centrifuged at 9000 *g* for 20 min. Supernatants were decanted, and aliquots were used for benzo [*a*] pyrene hydroxylase assay and metallothionein content determinations. The 9,000 *g* supernatant was subsequently centrifuged at 100,000 *g* to obtain the microsomal pellets. The microsomal pellets were suspended in 0.1 M of potassium phosphate buffer (pH 7.4) and assayed for benzphetamine *N*-demethylase and HO activities. Aliquots of the microsomal suspension from control and transgenic mice were used for the immunoblot analysis of heme oxygenase 1 (HO-1).

Assays Procedures

Benzo [*a*] pyrene hydroxylase activity was determined using the postmitochondrial fraction as described previously [16], and the amount of phenolic metabolites was measured fluorometrically by the method of Nebert and Gelboin [17]. Four milligrams of tissue, wet weight, was used for the assay, and incubation times of 5 min were used to maintain linearity of reaction rates. Benzphetamine *N*-demethylase activity was determined using 250 mg of liver, wet weight, by the method of Alvares and Mannering [18]. CYP content was determined by the method of Omura and Sato [19]. Metallothionein levels were determined by the method of Eaton and Toal [20]. Results are expressed by using 6 mol cadmium bound/mol metallothionein and assuming a M_r of 6000 for metallothionein [21]. Microsomal HO activity was measured using a modification of the method of Maines and Kappas [22], as described by Eiseman and Alvares [23]. The protein contents of the cellular fractions were determined by the method of Lowry *et al.* [24], using crystalline bovine serum albumin as a standard.

Immunoblot Analysis

Rabbit polyclonal antibody to rat HO-1 was purchased from Stress Gen. Phosphatase-labeled affinity-purified goat anti-rabbit IgG was purchased from Kirkegaard & Perry Laboratories, Inc., and the immunoblot assay kit was purchased from Bio-Rad. Microsomal proteins (50 μg) were separated electrophoretically on 10% SDS-polyacrylamide gel according to Laemmli [25] and transferred electrophoretically to a nitrocellulose sheet [26]. The immunoblot analysis was carried out according to directions in the assay kit.

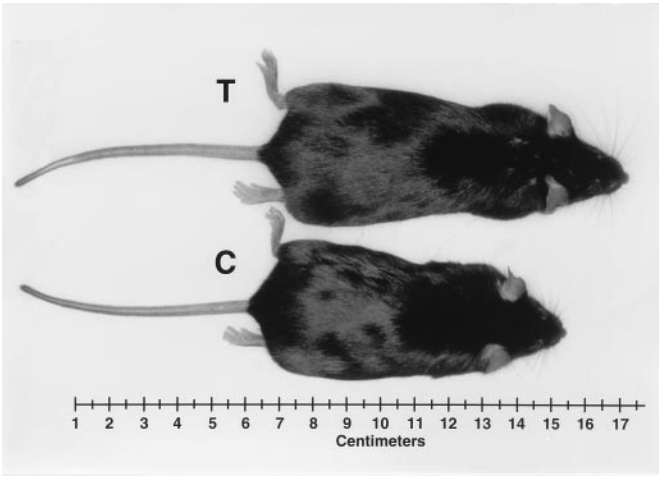

FIG. 1. bGH transgenic (T) and nontransgenic (C) C57/SJL mice at 10 months of age.

Statistical Analysis

Student's *t*-distribution was used as a test of the null hypothesis, using as a level of significance $P \leq 0.05$.

RESULTS

Transgenic mice overexpressing GH showed significantly greater body weights than their control littermates (Figs. 1 and 2). The body weights of the transgenic mice were approximately 35% greater than those of the controls. This increase was observed at 4, 6, 8, and 10 months, indicating that the mice were sensitive to the circulating GH prior to 4 months of age. The liver weights were also increased about 2-fold in the transgenic mice. The increment was proportionally greater than the body weight. The liver to body weight ratio increased 1.3-, 1.4-, 1.5-, and 1.6-fold in 4-, 6-, 8-, and 10-month-old transgenic mice, respectively, when compared with the ratio in nontransgenic littermates. In addition to liver weights, kidney and heart weights were also recorded at 4 and 10 months of age. As shown in Table 1, significantly higher organ weights were observed in the bGH mice with liver > kidneys > heart, when compared with their nontransgenic littermates. In addition to these organs, previous studies have also shown significant increases in lung, adrenal gland, spleen, intestinal tract, pancreas, and skin weights in bGH transgenic mice [27].

The effects of GH on total liver CYP and CYP-dependent monooxygenases are shown in Fig. 2 (right panel). CYP content of the liver microsomes was decreased 47, 28, 36, and 31% in transgenic mice at 4, 6, 8, and 10 months of age, when compared with their control littermates. Similar decreases were observed in benzphetamine *N*-demethylase by the liver preparations from transgenic mice. In contrast, the decrease in benzo [*a*] pyrene hydroxylase at all four ages was approximately 75% when compared with their control littermates. Thus, the two substrates were differentially affected by GH. The decreases in the specific activities of these enzymic activities were not related to differences in liver microsomal protein. The hepatic microsomal protein in control mice, 14.5 ± 0.7 mg/g of liver, was not significantly different from that in the transgenic mice (15.9 ± 0.9 mg/g of liver).

To determine the role of the heme catabolic enzyme in the decrease in CYP-dependent enzyme activities, hepatic microsomal HO activities of control and transgenic mice at 4 months of age were determined, and the data are shown in Fig. 3. A significant increase of 45% was observed in this enzymic activity in the transgenic mice when compared with their control littermates. Two forms of HO, HO-1 and HO-2, have been characterized in mammalian species. Heme has been shown to induce HO-1, but not HO-2 [28]. As shown in Fig. 3, the immunoblot analysis showed that antibody to HO-1 strongly reacted with HO-1 from transgenic and control mice. The 32 kDa polypeptide has been shown previously to be the M_r of HO-1 [29]. In data not shown, the marked increase in HO-1 staining and HO activity observed in the transgenic mice at 4 months of age was also observed at 6, 8, and 10 months.

Since the bGH gene is fused to the mouse metallothionein-I gene promoter, it was of interest to determine the metallothionein contents of the livers of the transgenic mice that stably incorporated fusion genes. The liver metallothionein contents of control and bGH transgenic mice were 5.4 ± 0.4 and 5.3 ± 0.4 mg of metallothionein/g of liver, demonstrating no significant differences between the two strains of mice.

DISCUSSION

Interest in understanding the possible metabolic effects of GH has increased as a consequence of the current therapeutic use of recombinant human GH to promote growth in children with short stature and also as a potential treatment for increasing bone and muscle mass in the elderly and in patients with HIV. Transgenic mice expressing the gene for bGH are a useful model to study the long-term effects of the hormone on the hepatic xenobiotic-metabolizing CYP. In studies carried out with hypophysectomized rats, GH appears to exert a major suppressive effect on CYP expression in the liver.

Previous studies have shown that by the age of 3 months, bGH transgenic mice are two to three times larger than their normal littermates [13, 14, 27]. However, in the present study, body weight of the female bGH mice was only increased about 1.4-fold by 4, 6, and 8 months and 1.2-fold by 10 months of age when compared with their littermates, indicating that the growth rate might be slowed down in the later stages of life in the transgenic mice. It has been shown that progressive glomerulosclerosis occurs in bGH mice [30], which could cause the decrease in growth rate in the 10-month-old mice. Heart and kidney weights were increased significantly in the transgenic mice. In addition, present studies also showed that the liver/body weight ratio was also increased, which resulted in a significantly larger growth rate of the liver when compared with

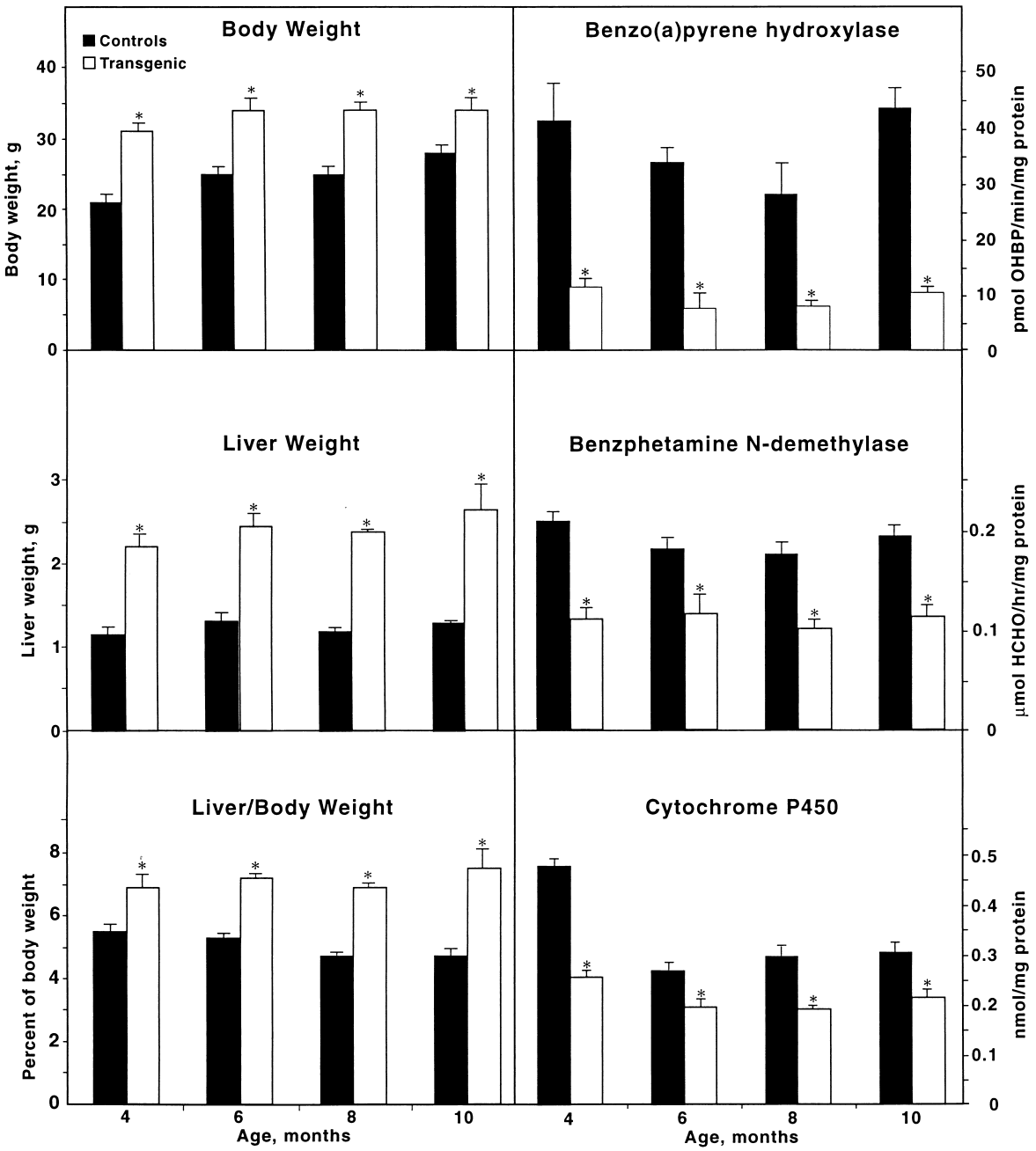

FIG. 2. Differences in physical parameters, CYP content, and CYP-dependent enzyme activities of bGH transgenic and control mice. Each bar represents the mean ± SEM for 3 mice. The liver/body weight ratio is expressed as g/100 g of body weight. Abbreviations: OHBP, hydroxy benzo [*a*] pyrene; and HCHO, formaldehyde. *Significantly different from the respective control value ($P < 0.05$).

whole body weight of the mice. GH is the primary regulator of insulin-like growth factor I (IGF-I) [31]. bGH transgenic mice are characterized by a 2-fold increased level of circulating IGF-I and display a marked overgrowth of their body as well as visceromegaly [32]. IGF-II does not affect body weight in the presence of elevated GH levels [27].

Changes in drug-metabolizing enzymatic activities are now recognized to be associated with an alteration in endocrine factors. GH plays an important role in the regulation of the male-specific CYP2C11 and the female-specific CYP2C12 in rats. In the present studies, the effects of elevated serum GH levels on liver CYP and on CYP-dependent benzphetamine N-demethylation and benzo [*a*] pyrene hydroxylase activities were determined. These substrates were selected because they are metabolized predominantly by the CYP3A and CYP1A subfamilies of cytochrome P450, respectively [33]. Hepatic cytochrome P450, benzphetamine *N*-demethylase, and benzo [*a*] pyrene hydroxylase activities were decreased by 36, 42, and 75%, respectively, in the transgenic mice. No age-related changes in the decrease of the monooxygenase activities were observed. The differential decrease in the metabolism

TABLE 1. Differences in organ weights between nontransgenic and bGH transgenic mice at 4 and 10 months of age

Mice	Age (months)	Kidney	Liver	Heart
		(mg/g body weight)		
Nontransgenic	4	5.74 ± 0.05	55.3 ± 2.30	4.41 ± 0.32
Transgenic	4	6.82 ± 0.23* (19%)†	70.3 ± 3.30* (27%)	4.99 ± 0.20 (13%)
Nontransgenic	10	6.02 ± 0.25	48.3 ± 1.90	3.95 ± 0.10
Transgenic	10	8.43 ± 0.42* (40%)	76.3 ± 4.70* (58%)	4.84 ± 0.22* (23%)

Each value is the mean ± SEM for 4 mice per group.
*Significantly different from the respective nontransgenic control ($P \leq 0.05$).
†Percent increase.

of these prototypic substrates has been reported previously [34, 35]. Induction of experimental malaria with the parasite *Plasmodium berghei* depressed the drug-metabolizing capability of a number of drugs whose metabolism is catalyzed by CYP, and the effect appears to be isoform-specific. However, the expression of individual CYP proteins was not investigated following the induction of malaria. The mechanism for the down-regulation of hepatic CYP is not known. Previous studies have reported plasma bGH levels of approximately 500 ng/mL in MT-bGH mice, and nontransgenic mice to have murine GH levels of approximately 20 ng/mL [36]. Overexpression of GH in transgenic mice is associated with a significant increase in the binding of ^{125}I-bGH to liver microsomes, and the increase in binding was associated with an increase in GH receptors [37]. Another possible mechanism is that GH can induce the microsomal HO involved in the degradation of heme. In the rat, endocrine hormones such as thyroid hormone and insulin have been reported to increase hepatic HO [38, 39]. In the present studies, overexpression of GH in the transgenic mice produced a significant increase in HO enzymic activity. Two forms of HO are present in liver microsomes, HO-1 and HO-2 [29]. HO-1 is inducible by chemicals and stress, whereas HO-2 is refractory to such stimuli. They also differ in their molecular weights, and antibody raised against one form does not cross-react with the other. In the present studies, western blot analysis showed that antibody raised against HO-1 showed a greater reactivity with HO-1 from transgenic mice than from controls. This increased reactivity was observed at all time points, which were characterized by decreased CYP activities. HO enzyme activity reflects the sum of HO-1 and HO-2, whereas the immunoblot analysis reflects the increase of HO-1 only in the bGH mice. Consequently, the increase in HO activity appears quantitatively lower than the staining for HO-1 (Fig. 3).

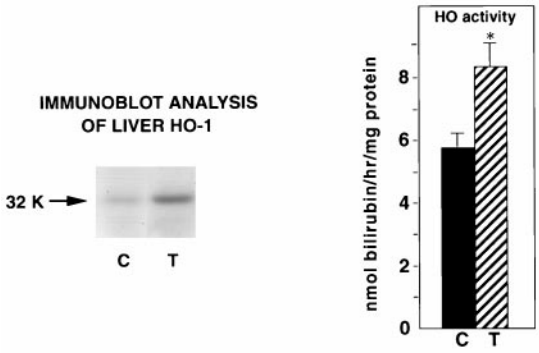

FIG. 3. Left panel: Immunoblot analysis of liver carried out on 50 μg of microsomal protein. Following separation by SDS–PAGE, the protein bands were probed with rabbit anti-rat HO-1 serum. Right panel: Liver HO activities of control (C) and transgenic (T) mice. Microsomal HO activity was determined in 4-month-old mice. Each bar represents the mean ± SEM for 3 mice. *Significantly different from the respective control value ($P < 0.05$).

Mice under stress from chemicals or infection have been shown previously to have elevated HO-1, while CYP content is reduced. This raises the possibility that the mice in the present studies are responding to a toxic substance and not specifically to bGH itself. In the present studies, the mice showed increased HO-1 levels and HO activities at 4, 6, 8, and 10 months, with a corresponding decrease in CYP-dependent enzymic activities. The only difference between the livers of the two groups of mice used in the present studies is the expression of the bGH transgene. They were housed in the same environment over the 10-month experimental period. Thus, the changes observed in heme metabolism are due specifically to the overexpression of bGH, although it may be indirect through IGF-I, and are not due to stress or environmental toxins. In addition, the transgenic mice used in these studies carried a metallothionein-I gene promoter, and because oxidative stress and certain hormones induce metallothionein [40], it was of interest to determine metallothionein levels in the livers of bGH transgenic mice. No significant differences were observed in metallothionein levels in transgenic mice when compared with their control littermates.

In summary, results of the present study indicate that in marked contrast to the increase in liver and body weights observed in bGH transgenic mice, there was a down-regulation of two subfamilies of CYP isozymes accompanied by a significant increase in one isoform of HO. In light of increasing use of GH as therapy for short stature in children and its use to increase milk production in cows, the present study merits further investigation in clinical and veterinary

medicine on the ability of these subjects to metabolize drugs, carcinogens, and other environmental toxins.

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