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The growth hormone (GH)-independent growth of the obese Zucker rat is not due to increased levels of GH receptor messenger RNA in the liver

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Abstract

Obese Zucker rats maintain normal rates of linear growth and circulating concentrations of insulin-like growth factor-I (IGF-I) and of IGF-binding protein-3 (IGFBP-3) in spite of low GH secretion. The mechanisms underlying this GH-independent growth in obesity are unknown. To assess whether the liver expression of the GH receptor (GHR) messenger RNA (mRNA) is increased and/or if the liver expression of IGFBP-3 mRNA is maintained in the obese, Zucker rats of both genders and phenotypes (four groups, $n = 6$/group) were studied at 12 weeks of age. By Northern analysis, mRNA levels for GHR and GHBP were not increased in obese rats compared to their sex-matched lean littermates; the expression of these two transcripts was sexually dimorphic and the changes in GHBP mRNA/GHR mRNA ratios associated with obesity were sex-specific. In both genders, IGFBP-1 and IGFBP-3 mRNAs were decreased in the obese. We conclude that the GH-independent growth of obese Zucker rats is not due to increased GHR mRNA or to maintained IGFBP-3 mRNA levels in the liver.

Keywords: Obesity (Zucker rat); Growth; Growth hormone; Growth hormone receptor; Insulin-like growth factor-I; Binding protein

1. Introduction

The first step in the action of growth hormone (GH) on peripheral tissues is its binding to a specific transmembrane protein, the GH receptor (GHR). Transcription of the rat GHR gene generates a messenger RNA (mRNA) for the GHR itself and an alternatively spliced mRNA which is translated into the circulating GH binding protein (GHBP) (Baumbach et al., 1989); both these mRNA species are most abundant in liver (Matthews et al., 1989; Tiong and Herington, 1991). Likewise, the liver contains abundant mRNA for insulin-like growth factor-I (IGF-I, the principal mediator of GH action) (Daughaday and Rotwein, 1989) and for the IGF binding proteins (IGFBPs) (Rechler and Brown, 1992). The IGFBPs constitute a family of six proteins (IGFBP-1–6) which are under hormonal and nutritional regulation and modulate the peripheral actions of IGF-I (Clemmons and Underwood, 1991). While IGFBP-3 is the major circulating IGFBP and is increased by both GH and insulin, IGFBP-1 is not influenced by GH, is acutely decreased by insulin, and likely regulates the amount of free IGF-I available to tissues (Lee et al., 1993).

The effects of malnutrition and fasting on the GH-IGF-I axis have been well studied in animals and in humans (reviewed by Thissen et al., 1991), but situations of overnutrition such as obesity have received relatively little attention. A decrease in GH secretion has been documented but data on the GH-IGF-I interaction and on IGFBPs are scanty (reviewed by

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Van Vliet and Deal, 1994). Snyder et al. (1988) have reported that diet-restricted obese humans maintain normal plasma IGF-I which increases to supraphysiological levels upon GH admistration, while non-obese subjects have decreased IGF-I and become GH resistant in such a situation (Thissen et al., 1994).

The genetically obese Zucker rat is an appropriate model for the study of the hormonal control of growth in obesity: as in children with exogenous obesity, linear growth and circulating IGF-I levels are normal or increased and IGFBP-3 levels are normal in spite of a diminished GH secretion; moreover, the increase in plasma IGF-I after GH administration is two-fold greater in obese animals than in their lean littermates (Nguyen-Yamamoto et al., 1994).

The mechanisms underlying the maintenance of normal linear growth and IGF-I levels in the face of low GH secretion in the obese state are unknown. Obese animals have been reported to have a 2–3-fold increased binding of GH to liver membranes (Postel-Vinay et al., 1990), which could be a reflection of increased GHR synthesis. An alternative explanation is that the primary effect of the hyperinsulinism characteristic of the obese state compensates for the low GH and maintains normal IGFBP-3 levels; because one of the main functions of IGFBP-3 is that of a reservoir for IGF-I (Jones and Clemmons, 1995), this would then lead to normal IGF-I levels.

The present work was undertaken to test the hypothesis that IGF-I levels remain normal in the face of low GH secretion in obesity through increased GHR mRNA and/or maintained IGFBP-3 mRNA expression in the liver. We measured steady-state mRNA levels for GHR, GHBP and for IGFBP-1–4 in obese and lean, male and female adult Zucker rats. Serum GHBP levels were also measured, as they may be correlated with GHR activity (Carmignac et al., 1993).

2. Materials and methods

2.1. Animals

Zucker rats of both genders and phenotypes (four groups, $n = 6$/group) were obtained at 9 weeks of age from Canadian Breeding Farms. They were housed under constant conditions of temperature (21°C) and light (on at 06:00 h, off at 18:00 h) with free access to pellet food and water. At 12 weeks of age, the animals were weighed and measured before being killed by decapitation starting at 10:00 h. Trunk blood was collected into glass tubes on ice. Samples were immediately centrifuged and the serum kept frozen at −70°C until assayed. The livers were removed, rapidly rinsed in saline and frozen in liquid nitrogen; they were maintained at −70°C until homogenization. The protocol was approved by our local animal research ethics committee.

2.2. Hormone assays

Serum insulin concentrations were measured by RIA according to a previously published method (Tannenbaum et al., 1990), courtesy of Dr. G.S. Tannenbaum, McGill University, Montreal, Quebec. Serum GHBP levels were determined by a sensitive heterologous RIA as previously described (Carmignac et al., 1992), courtesy of Dr. I.C.A.F. Robinson, National Institute for Medical Research, London, U.K. IGF-I concentrations were measured by RIA (Nguyen-Yamamoto et al., 1994) on pooled serum samples after acid chromatography (Donovan et al., 1989) and the results were expressed in ng of biosynthetic human IGF-I (Lot DDC BO4; Gibco, BRL, Burlington, Ontario) per ml of serum; insulin was unable to displace tracer even at high concentrations (1 μg/ml).

2.3. DNA preparation

Total RNA was isolated from liver tissue by the guanidinium isothiocyanate-lithium chloride (LiCl) precipitation method (Cathala et al., 1983) as modified by Näntö-Salonen et al. (1991), but with precipitation of the RNA in a 2 M LiCl solution. Poly(A)$^+$ RNA was further purified by affinity chromatography on oligo(dT)-cellulose minicolumns according to the method of Sambrook et al. (1989a). The amount of RNA was quantified by measuring optical density at 260 nm and the RNA samples were stored in TS buffer (20 mM Tris, pH 7.5, and 0.1% SDS) at −70°C until use.

2.4. cDNA probes

For GHR/GHBP, a 799-basepair (bp) *Eco*RI-*Ava*II fragment coding for the entire extracellular region common to both receptor and binding protein, cloned into pGEM3Z and cut out with *Eco*RI and *Pst*I was kindly provided by Dr. W.R. Baumbach (American Cyanamid Co., Princeton, NJ). For IGFBP-1–4, the following coding region probes were used: for IGFBP-1, a 1.0-kb *Eco*RI fragment of Hep G2 cells-IGFBP-1 (courtesy of Dr. D. Powell, Houston, TX); for IGFBP-2, a 1.295-kb *Eco*RI restriction fragment of 1.5-kb cDNA isolated from rat liver cDNA library (courtesy of Dr. J. Schwander, Basel, Switzerland); for IGFBP-3, a 507-bp cDNA coding for amino acids 72–240 and synthetized by RT-PCR of rat kidney RNA using the antisense primer CGACCGGCCGAGCAGTATCC and the sense primer GTCCACGCACCAGCAGAAGC; and for IGFBP-4, a 444-bp *Sma*I/*Hin*dIII fragment of the 1.2-kb cDNA from an osteosarcoma cell library (courtesy of Dr. M. Mohan, Loma Linda,

CA). cDNA probes for the 28S ribosomal RNA (1.4-kb fragment) and/or chicken β-actin (2.1-kb fragment) were hybridized as a control for lane loading with probes cloned into the plasmid pBR 322 and cut out with *Bam*HI (courtesy of Dr. A. Petit and of Dr. J. Tanner, respectively; University of Montreal, Montreal, Quebec).

The probes were labeled with [^{32}P]dATP by random oligo priming using a commercial kit (Random Primers DNA Labeling System, Gibco BRL, Burlington, Ontario) and purified on spin columns of Sephadex G-50 (Sambrook et al., 1989b) before use.

2.5. Northern blot analysis

Pooled (n = 6 animals/group) total RNA samples (20 μg/lane) and poly(A)$^+$-enriched RNA samples (5 μg/lane), as well as individual total RNA samples (20 μg/lane) were denatured and electrophoresed on 1.2% agarose-formaldehyde gels. Gels were soaked for 30 min in NH_4Ac^- 0.1 M and incubated for 10 min in 10× SSC (1× = 150 mM sodium chloride and 15 mM sodium citrate) and the RNA was transferred by capillarity onto nylon membranes (Hybond-N Nylon 0.45 μm, Amersham International, Amersham, UK). Post-transfer membranes were baked under a vacuum for 2 h at 80°C. The blots were then hybridized according to the method of Wahi et al. (1979) with addition of 0.1% SDS in the hybridization mixture. Labeled probes (specific activities of 0.7–2.2 × 10^9 dpm/μg) were denatured with 0.2 M NaOH and were used at a final concentration of approximately 1 × 10^6 counts/min per ml of hybridization solution.

After overnight hybridization at 42°C, the membranes were washed twice at 55°C for 10 min in 0.2× SSC–0.1% SDS and air dried. Autoradiography was performed by exposure to Fuji RX films (Fuji Photo Film Co., Japan) for 12 h to 8 days at −70°C with intensifying screens. Prior to rehybridization, probes were stripped from the blots by washing twice with 100°C water.

2.6. Densitometric analysis

Autoradiographs were scanned using a laser densitometer (LKB UltroScan XL, LKB Produkter AB, Bromma, Sweden). The relative densities of the mRNA bands detected by specific hybridization were deduced directly from the integrated area under the curve and expressed as arbitrary absorbance units (AU) × mm. For each mRNA, values were normalized for the corresponding amount of β-actin and the data were expressed as percent of AU × mm for β-actin mRNA.

2.7. Statistical analysis

Unpaired Student's t-tests were used for comparisons of body weight and naso-anal length between the groups. Paired Student's t-tests were used for comparisons between the liver expression of the GHBP mRNA and the GHR mRNA in the same animal. For all other densitometric analyses and for hormonal values, statistical differences between lean and obese animals were determined by the Mann-Whitney test. The level of significance was set at $P < 0.05$. The data are presented as mean ± SD or as median and range.

3. Results

3.1. Body weight, naso-anal length and serum hormone levels

The body weights and the naso-anal lengths of the animals are given in Table 1 by gender and phenotype.

Serum insulin and GHBP levels are also given in Table 1 for the four groups of animals. Insulin levels were markedly increased in obese males and females compared to their lean littermates ($P < 0.005$). Circulating GHBP levels were higher in obese animals of both genders; however, the difference between the two phenotypes was significant only for males ($P < 0.05$).

IGF-I concentrations on pooled acid-chromato-

Table 1
Animal weights, naso-anal lengths and trunk blood hormone concentrations

	Body weight(g)	Naso-anal length(cm)	Insulin(ng/ml)	GHBP(ng/ml)
Obese males (n = 6)	561 ± 26**	22.6 ± 0.3**	16.94** (6.96–25.49)	142.3* (112.8–203.5)
Lean males (n = 6)	377 ± 26	21.0 ± 1.0	0.32 (0.19–2.86)	99.5 (75.0–131.1)
Obese females (n = 6)	415 ± 27**	19.7 ± 0.8	7.34* (2.11–8.78)	186.6 (90.5–664.5)
Lean females (n = 6)	241 ± 16	19.0 ± 0.4	0.32 (0.32–1.02)	163.8 (125.9–190.1)

Body weight, naso-anal length (mean ± SD) and serum insulin and GHBP levels (median and range) of obese and lean, male and female Zucker rats (n = 6/group). Statistical comparisons were made between obese and lean animals of the same gender (unpaired Student's t test for body weight and naso-anal length; Mann-Whitney test for serum hormone levels): (*$P < 0.05$); (**$P < 0.005$).

graphed serum were as follows (mean of duplicate measurements at three different dilutions): in males, 1385 ng/ml for obese and 1135 ng/ml for lean; in females, 1100 ng/ml for obese and 765 ng/ml for lean.

3.2. Steady-state liver mRNA levels of GHR and GHBP

Fig. 1 shows a Northern blot of pooled poly(A)$^+$-enriched RNA samples from the four groups of rats hybridized with the probe recognizing GHR and GHBP mRNAs, as well as with the 28S ribosomal and β-actin probes. Densitometric analysis of the mRNA bands gave similar results when expressed as percent of β-actin mRNA or of 28S ribosontal RNA (not shown). Therefore, β-actin mRNA alone was used as control for lane loading in all the following Northern blot analyses. As the results for GHR mRNA and GHBP mRNA were similar by densitometric analysis with electrophoresis of total RNA samples or poly(A)$^+$-enriched RNA samples (not shown), we chose to use total RNA for studies of individual samples (Figs. 2 and 3).

The steady-state liver mRNA levels of GHR and GHBP are compared between obese and lean annals in Table 2 (males) and Table 3 (females), in which the results of densitometric analyses are given as arbitrary AU × mm as well as in percent of the values for β-actin. In males, there was no significant difference of GHR mRNA and GHBP mRNA levels between the obese animals and their lean littermates, whether absolute or β-actin-corrected amounts were considered. In females, the mRNA levels of GHBP were similar in the two phenotypes, whereas those of GHR were slightly decreased in the obese ($P < 0.05$) when corrected for β-actin.

In males, the expression of liver GHBP mRNA was

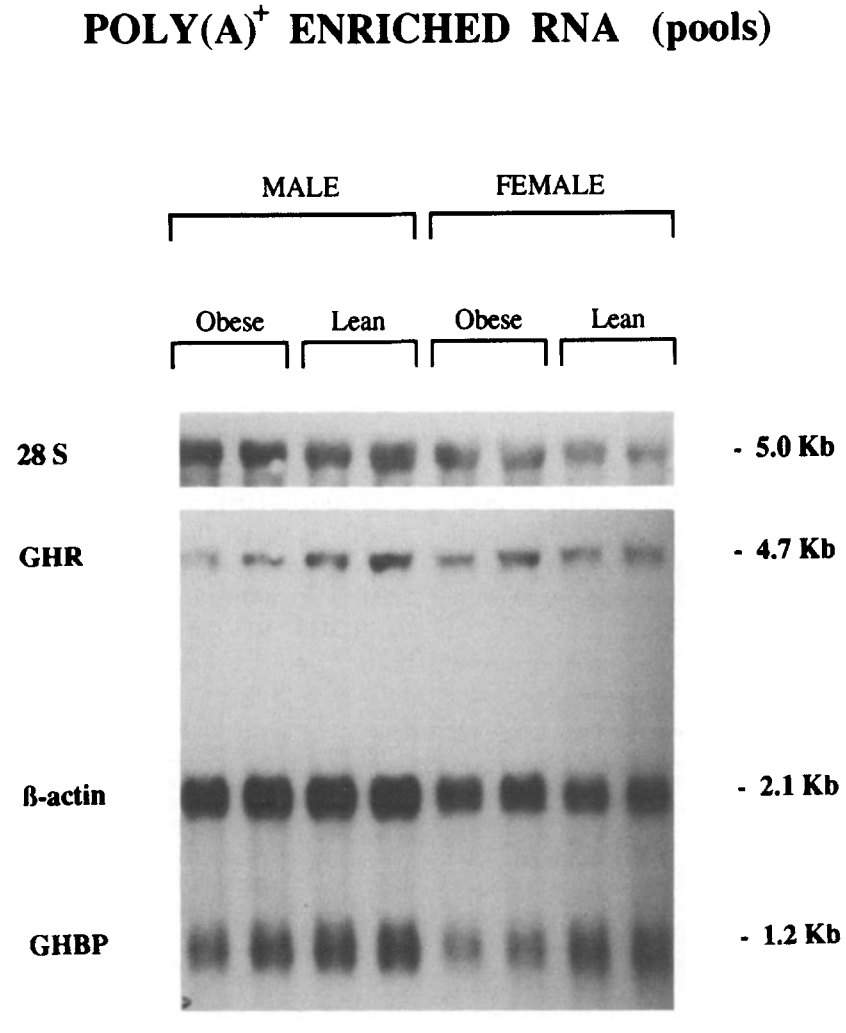

Fig. 1. Northern blot of pooled poly(A)$^+$-enriched liver RNA samples (5 μg/lane) from obese and lean Zucker rats of both genders (duplicate samples). A cDNA probe recognizing GHR mRNA and GHBP mRNA and the probe for β-actin mRNA were used in the same hybridization solution. 28S ribosomal RNA cDNA was used as a second control probe for lane loading, with similar results as with β-actin.

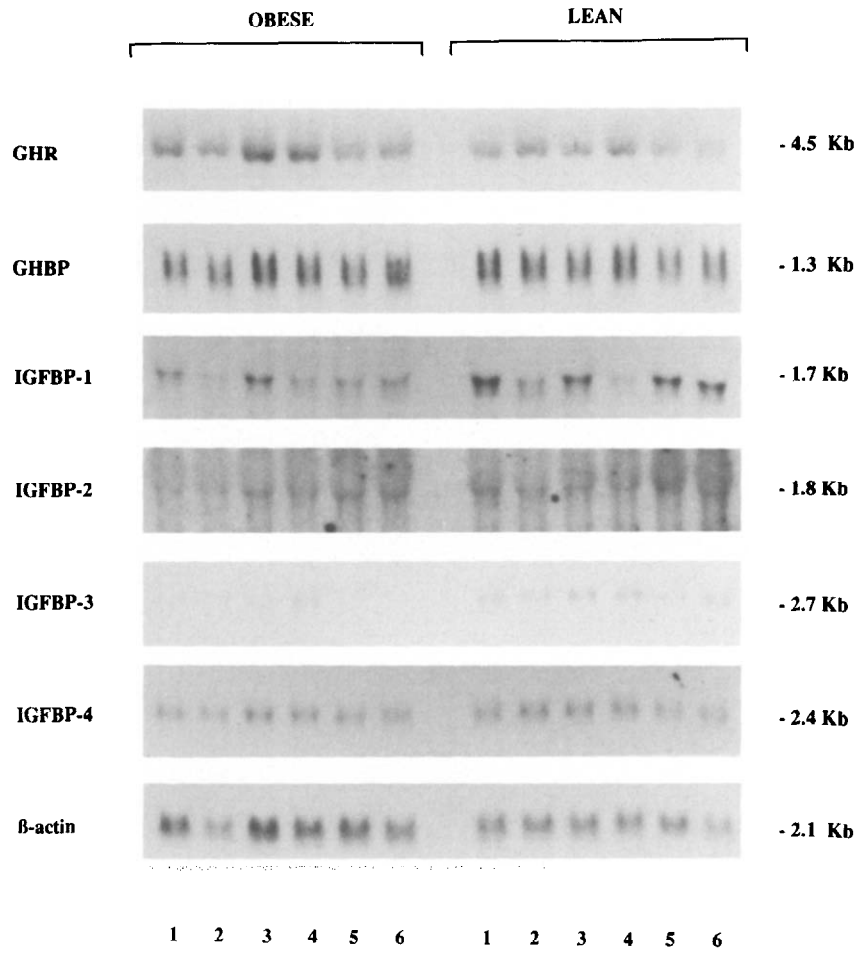

Fig. 2. Northern blot of individual total liver RNA samples (20 μg/lane) from obese and lean male Zucker rats. A cDNA probe recognizing GHR mRNA and GHBP mRNA was used, as well as probes for IGFBP-1–4 mRNAs. β-actin cDNA probe was used as control for lane loading. Densitometric analysis of this autoradiogram is shown in Table 2.

significantly greater than that of GHR mRNA, both in obese ($P < 0.005$) and in lean animals ($P < 0.0005$); the GHBP mRNA/GHR mRNA ratios were lower in the obese than in the lean (median of the ratios: 1.38 (range: 1.15–1.66) vs. 1.97 (range: 1.54–2.52), $P = 0.01$). Conversely, the liver GHBP mRNA expression was significantly less than that of GHR mRNA both in obese ($P < 0.01$) and in lean ($P < 0.025$) females; also in contrast to the males, the GHBP mRNA/GHR mRNA ratios were similar in the two phenotypes (median of the ratios: 0.74 (range: 0.60–0.91) in obese vs. 0.73 (0.51–1.02) in lean, P = NS).

3.3. Steady-state liver mRNA levels of IGFBP-1–4

Figs. 2 and 3 show Northern blotting of IGFBP-1–4 mRNAs after electrophoresis of individual total RNA samples from the four groups of rats and hybridization with the probes described in Materials and methods.

The relative densities of the mRNA bands for IGFBP-1–4 are given in Table 2 (males) and Table 3 (females) with comparisons between the obese animals and their lean littermates. In both genders, steady-state mRNA levels for IGFBP-1 and IGFBP-3 (corrected for β-actin) were decreased in the obese animals ($P < 0.05$ to $P < 0.005$); this was also true for IGFBP-2 and IGFBP-4 mRNA in females, but not in males.

4. Discussion

Our measurements of naso-anal length confirm our previous finding, i.e. that obese animals do not have a

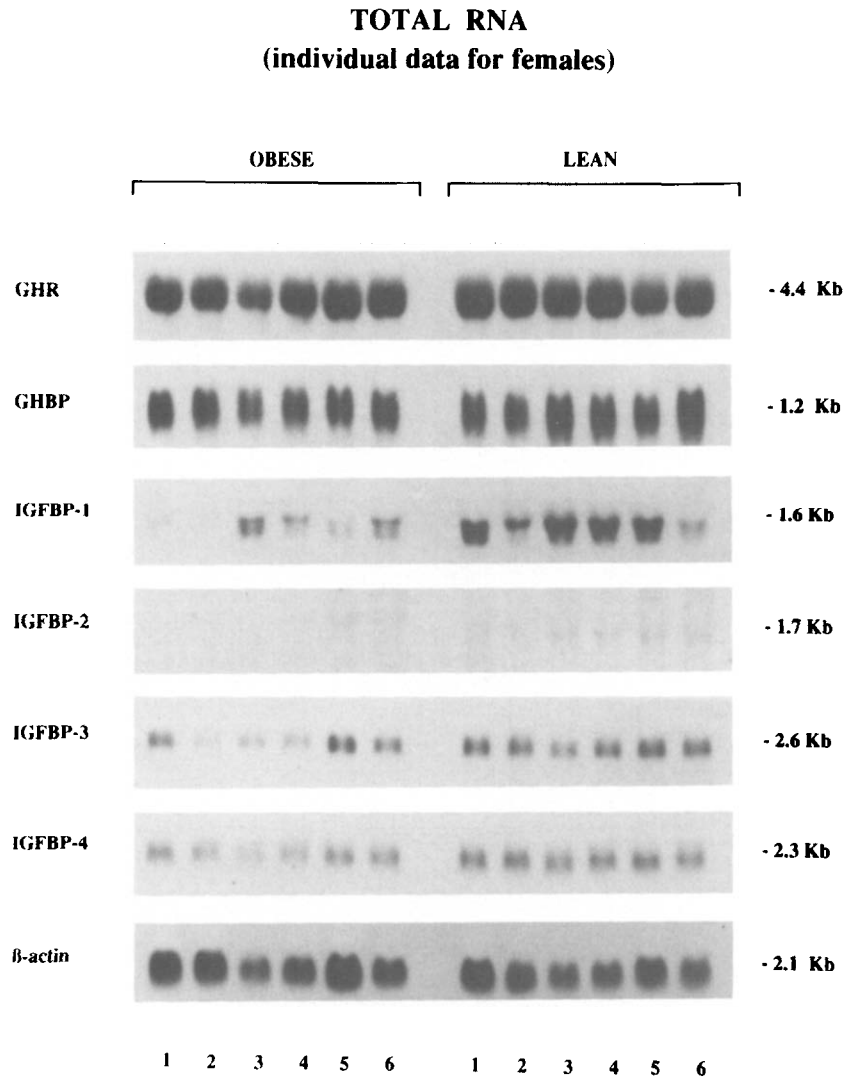

Fig. 3. Northern blot of individual total liver RNA samples (20 μg/lane) from obese and lean female Zucker rats. A cDNA probe recognizing GHR mRNA and GHBP mRNA was used, as well as probes for IGFBP-1–4 mRNAs. β-actin cDNA probe was used as control for lane loading. Densitometric analysis of this autoradiogram is shown in Table 3.

stunted linear growth in spite of the decrease in GH secretion that can be documented as early as 6 weeks of age in chronically cannulated animals (Tannenbaum et al., 1990; Finkelstein et al., 1986; Leidy et al., 1993). Trunk blood insulin levels are 20- (in females) to 50-fold (in males) higher in obese animals than in their lean littermates, a difference similar to that found in chronically cannulated animals by Leidy et al. (1993). Our measurements of serum after acid chromatography confirm our previous observations that IGF-I concentrations are slightly higher in the obese (male, 122% of lean; female, 144% of lean; compared to 115 and 153% in our previous study (Nguyen-Yamamoto et al., 1994)).

The maintenance of normal linear growth and IGF-I levels despite low GH secretion in the obese state could be due to a greater number of liver GHR protein as suggested by the increased GH binding reported by Postel-Vinay et al. (1990). In the present study, we found that steady-state liver GHR mRNA levels were not increased in the obese. Indeed, when corrected for β-actin, obese females even had a slight decrease of GHR mRNA compared to sex-matched lean littermates; discrepancies between absolute results and results corrected for β-actin have also been found by Maiter et al. (1992). Possible explanations for increased GHR binding activity in the face of unchanged steady-state GHR mRNA levels include regulation at either the post-transcriptional level (such as increased mRNA turnover or translation efficiency) or at the post-translational level (such as decreased GHR degradation). Given the massive hyperinsulinism of the obese Zucker rat (Table 1) and the findings of Tollet et al. (1990) that insulin in-

Table 2
Densitometric analysis of liver steady state mRNA levels in males

	β-actin	GHR	GHBP	IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4
Obese (n = 6)							
AU × mm	2.13 (1.38–2.63)	1.22 (0.98–1.87)	1.71 (1.53–2.43)	0.47 (0.22–0.73)	0.07 (0.02–0.09)	0.11 (0:09–0.19)	0.66 (0.60–0.88)
% β-actin	100.0	69.5 (50.8–73.5)	89.5 (67.3–112.4)	21.6 (15.0–31.1)	3.6 (1.0–5.3)	5.5 (4.6–9.1)	34.2 (29.8–44.7)
Lean (n = 6)							
0AU × mm	1.46 (1.05–1.48)	0.91 (0.59–1.18)	1.71 (1.21–2.00)	0.94 (0.24–1.36)	0.06 (0.05–0.11)	0.24 (0.15–0.28)	0.69 (0.40–0.81)
% β-actin	100.0	62.0 (41.3–79.8)	127.1 (83.4–141.1)	64.2 (16.7–92.8)	5.0 (3.1–7.4)	16.2 (10.3–25.4)	49.7 (27.3–56.5)
P*	NS	NS	NS	NS	NS	0.01	NS
P**	—	NS	NS	< 0.05	NS	< 0.005	NS

Results (median and range) are expressed in arbitrary absorbance units (AU × mm) and in percent of the β-actin densitometric values (% β-actin).

tatistical significance (Mann-Whitney test) of difference between obese and lean animals expressed in AU × mm.

** Statistical significance (Mann-Whitney test) of difference between obese and lean animals expressed in % of β-actin.

creases GH binding to cultured rat hepatocytes without increasing GHR mRNA, a translational or post-translational regulation is more likely.

Circulating GHBP is considered to reflect GH receptor activity (Massa et al., 1990; Hochberg et al., 1992; Carmignac et al., 1993): in our study, obese animals tended to have higher radioimmunoassayable GHBP in serum (Table 1) although the difference reached statistical significance only in males. Liver steady-state mRNA levels for this protein were not increased in either gender. Taken together, these data are compatible with a sex-specific post-transcriptional effect, possibly related to the more pronounced hyperinsulinism of the obese male.

The main function of the most abundant and most GH-dependent serum IGFBP (IGFBP-3) appears to be that of a reservoir of IGF-I leading to an increase in its half-life (Sommer et al., 1993). Therefore, aside from post-transcriptional changes in the GH receptor, another mechanism that may play a role in the maintenance of plasma IGF-I levels in the obese would be through the maintenance of liver IGFBP-3 mRNA

Table 3
Densitometric analysis of liver steady state mRNA levels in females

	β-actin	GHR	GHBP	IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4
Obese (n = 6)							
AU × mm	5.13 (3.04–7.50)	5.32 (2.76–6.12)	3.90 (2.38–4.07)	0.42 (0.13–1.08)	0.02 (0.00–0.10)	0.53 (0.29–1.18)	0.74 (0.47–0.86)
% β-actin	100.0	87.5 (78.4–128.7)	74.2 (50.6–86.5)	8.1 (2.4–35.4)	0.5 (0.04–2.0)	12.6 (5.1–15.7)	14.1 (10.5–17.8)
Lean (n = 6)							
AU × mm	3.88 (3.17–5.95)	5.98 (3.98–7.27)	3.86 (3.36–5.76)	2.63 (0.48–3.80)	0.09 (0.06–0.15)	0.83 (0.54–1.03)	0.98 (0.85–1.06)
% β-actin	100.0	163.1 (91.3–209.6)	101.2 (57.2–175.9)	52.4 (14.8–120.0)	2.8 (1.3–3.4)	20.5 (16.5–25.6)	25.7 (17.4–27.3)
P*	NS	NS	NS	0.02	NS	NS	0.005
P**	—	< 0.05	NS	0.02	< 0.05	< 0.005	0.005

Results (median and range) are expressed in arbitrary absorbance units (AU × mm) and in percent of the β-actin densitometric values (% β-actin).

* Statistical significance (Mann-Whitney test) of difference between obese and lean animals expressed in AU × mm.

** Statistical significance (Mann-Whitney test) of difference between obese and lean animals expressed in % of β-actin.

expression, as suggested by the normal IGFBP-3 levels that we previously found by Western ligand blot (Nguyen-Yamamoto et al., 1994). However, as shown in Figs. 2 and 3, the steady-state levels of IGFBP-3 mRNA were decreased in obese animals of both genders: again, this discrepancy between liver mRNA and serum protein levels could be due to post-transcriptional mechanisms. Moreover, our finding of a different pattern of migration of IGFBP-3 on Western ligand blotting in obese females (possibly a reflection of glycosylation differences) (Nguyen-Yamamoto et al., 1994) underlines that the complex hormonal changes associated with obesity influence post-translational processing of IGFBPs as well.

The decrease of IGFBP-1 mRNA parallels the decreased circulating concentrations of the protein as assessed by RIA (Lewitt et al., 1993) and supports the concept of an inverse relationship between insulin and IGFBP-1 synthesis and secretion (Lee et al., 1993). Furthermore, this agrees with the decreased serum IGFBP-1 reported in obese humans (Conover et al., 1992). Thus, in spite of insulin resistance at the level of glucose and amino acid uptake (Jacob et al., 1992), the expected effects of hyperinsulinism on hepatic IGFBP-1 mRNA levels is observed in the obese Zucker rat. As suggested by Conover et al. (1992), a decrease in this inhibitory binding protein would accentuate the growth-promoting effect of IGF-I and its negative feedback regulation of GH secretion. The regulation of IGFBP-4 being much less known, further studies will be needed to explain the fact that the liver mRNA levels for this protein are significantly decreased only in obese females (Table 3). Given the very low level of IGFBP-2 mRNA expression, the biological significance of its decrease in obese females is questionable.

Liver GHR and GHBP mRNA levels are known to be sexually dimorphic in normal rats (Carmignac et al., 1993). In the present study, there was approximately 2-fold more message for GHBP mRNA than for GHR mRNA in lean males, whereas lean females expressed less GHBP mRNA than GHR mRNA. Moreover, obesity was associated with a decreased GHBP mRNA/GHR mRNA ratio in males but not in females. Differential regulation of GHR and GHBP transcripts has been described in other situations (Tiong and Herington, 1991; Walker et al., 1992; Pacaud et al., 1994). It is also noteworthy that our studies of hypothalamic tissue of the same animals also reveal a decrease in GHRH mRNA expression in males while a reverse pattern is observed in females (Videau et al., 1994). The mechanisms responsible for the sexual dimorphism and for the sex-specific effect of obesity are unknown. Spontaneous GH secretory profiles are sexually dimorphic in lean Zucker rats (Leidy et al., 1993) and this may play a role in the sexually dimorphic expression of GHR and GHBP mRNAs. However, obesity in Zucker rats is associated with a decrease in GH secretion which is similar in both genders (with mean GH peak amplitude and mean 6-h GH concentrations decreased by 75–86% in the obese (Leidy et al., 1993)). It is therefore unlikely that the pattern of GH secretion is the only determinant of the differential expression of GHR and GHBP mRNAs. Studies of the interaction of sex steroids with GH on GHR mRNA and GHBP mRNA expression (Carmignac et al., 1993) would be of interest in Zucker rats.

In summary, we have shown that neither the liver levels of GHR mRNA nor that of GHBP mRNA are increased in obese Zucker rats, compared to their sex-matched lean littermates. Moreover, the steady-state liver mRNA levels of IGFBP-3 are decreased in obese animals of both genders. Thus, the maintenance of normal or even increased serum IGF-I levels in the face of low GH secretion in obese Zucker rats is not due to increased GHR mRNA or to maintained IGFBP-3 mRNA levels in the liver. Therefore, the increased binding of GH to liver membranes (Postel-Vinay et al., 1990) as well as the increased responsiveness of IGF-I to exogenous GH of the obese animals (Nguyen Yamamoto et al., 1994) are probably due to post-transcriptional or post-translational mechanisms. Intracellular events distal to the GHR may also be involved. On the other hand, adipose tissue possesses GH receptors (Vikman et al., 1991) and is capable of synthesizing and releasing IGF-I and IGFBPs (Gaskins et al., 1990; Peter et al., 1993). The markedly increased fat mass of the obese could therefore also contribute to the pool of circulating IGF-I and IGFBP-3. Finally, the sex-specific effect of obesity on the GHBP mRNA/GHR mRNA ratio requires further study.

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