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Hepatic reduction of insulin-like growth factor (IGF)-I and IGF binding protein-3 that results from fasting is not attenuated in genetically obese rats

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Abstract

Fasting or caloric restriction causes substantial reductions in serum IGF-I in normal weight humans and animals, and reductions of liver IGF-I and IGFBP-3 mRNAs in animals. Obese humans, however, have attenuated and delayed decrements in IGF-I in serum when subjected to caloric restriction. Obese Zucker rats show a clear tendency to preserve body protein during fasting. To determine whether obesity opposes the effects of fasting on IGF-I and IGFBP-3, and thereby contributes to preservation of lean tissue, we have examined the effect of 72 h of fasting on IGF-I and IGFBP-3 in lean and obese Zucker rats. We observe that between lean and obese animals, fasting for 72 h produces similar decrements in body weight, serum IGF-I, liver IGF-I mRNA, serum IGFBP-3 and liver IGFBP-3 mRNA. Our finding that the reduction of IGF-I and IGFBP-3 in liver that results from 72 h of fasting is not attenuated in obese Zucker rats raises the possibility that conservation of lean tissue in these animals during fasting is not related to the hepatic production of IGF-I and IGFBP-3.

Keywords: IGF-I; IGFBP-3; Fasting; Obesity

1. Introduction

Nutritional status is a key regulator of insulin-like growth factor-I (IGF-I) and its major carrier protein, IGF binding protein 3 (IGFBP-3) [1]. Fasting or protein restriction causes reductions in the serum concentrations of these peptides and corresponding reductions in their steady-state mRNAs in liver, a principal site of IGF-I and IGFBP-3 synthesis [2–5]. Obese humans, however, appear to have some protection from the effects of dietary restriction in terms of reduction of IGF-I in serum [6,7]. Genetically obese (fa/fa) Zucker rats have larger reserves of potential calories in their fat, and when fasted they lose less body protein and survive longer than their lean counterparts [8,9]. It is not known, however, whether these animals experience delayed or attenuated decrements in their serum IGF-I concentrations and hepatic IGF-I mRNA levels when fasted. Also, changes of IGFBP-3 in these animals during undernutrition have not been elucidated.

The metabolic responses to fasting change as fasting progresses. In obese rats, fasting can be divided into three phases: an early phase which lasts less than 48 h; a prolonged phase during which animals are conserving protein; and a terminal phase which parallels the increase in protein catabolism and exhaustion of lipid sources prior to death [9]. We used a 72 h fasting model of obese Zucker rats, because it permits us to investigate the correlation between protein conservation that occurs in the second phase of fasting (covering 72 h) and the production of IGF-I and IGFBP-3. Also, the reduction of hepatic IGF-I production in lean rats that results from 72 hr of fasting has been well studied [4,10]. The goals of the present study are to determine whether there are differences between lean and genetically obese rats in the regulation of IGF-I and IGFBP-3 that might provide an explanation for the ability of obese animals to conserve lean tissues during fasting.

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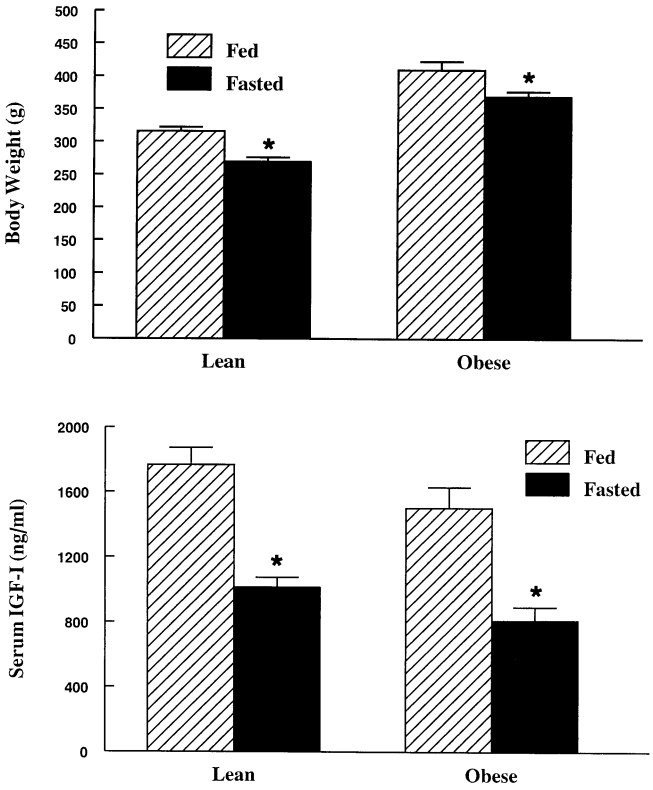

Fig. 1. Effect of fasting for 72 hr on body weight and serum IGF-I concentration of lean and obese rats. Fasting reduced mean body weight (top) and the serum IGF-I levels (bottom) of lean and obese rats (*, $p < 0.01$), but, the decrements in body weight and serum IGF-I level between fasted lean and fasted obese animals are not significantly different.

2. Materials and methods

2.1. Animals and experimental design

Ten-week-old male genetically obese (fa/fa, weighing 370 ± 26 g, n = 12) and lean (Fa/?, weighing 266 ± 18 g, n = 12) Zucker rats were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN), housed under controlled conditions (22°C; lights on from 0800–2000) and fed standard laboratory chow ad libitum for 7 days. They were then divided into four groups (6 each): obese, fasted for 72 hr; obese, fed; lean, fasted for 72 hr; and lean, fed. All animals were allowed free access to water during the fasting period, and their body weights were measured daily. Blood and liver tissue were collected from each animal under ether anesthesia. Serum was isolated and stored at −20°C until assayed for IGF-I and IGFBP-3. Livers were flash-frozen in liquid nitrogen and stored at −70°C. The protocol was approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill.

2.2. Measurements of serum IGF-I and IGFBP-3

Serum IGF-I concentrations were measured by RIA after removal of IGFBPs using C18 cartridge chromatography (Sep-Pak, Waters Associates, Milford, MA) [11]. 1988). Purified plasma-derived human IGF-I (PSIII) was used as a standard [12]. To measure serum IGFBP-3 by ligand blotting, serum proteins were size-separated on 12.5% discontinuous SDS polyacrylamide gels under denaturing, nonreducing conditions, electroblotted onto nitrocellulose membranes. Blots were incubated for 24 h in a ligand hybridization buffer containing 500,000 cpm/ml of recombinant [^{125}I] IGF-I, washed and subjected to autoradiography [13].

2.3. RNA extraction and northern blot analyses

Whole cellular RNA was extracted from frozen liver using the acidic guanidinium thyocynate-phenol-chloroform method [14], and quantified by UV spectrophotometry. Aliquots of 20 ug RNA were electrophoresed on 1% agarose gels containing formaldehyde, and transferred to nylon membranes. Blots were UV cross-linked, then vacuum baked at 80°C for 2 hr. For analysis of IGF-I mRNA, single stranded DNA probes were generated from a 358 bp human IGF-I cDNA by linear PCR using the antisense primer and ^{32}P-dCTP [15]. Membranes were hybridized with ^{32}P-labeled IGF-I probes overnight at 65°C and then washed under stringent conditions. Specific bands were detected by autoradiography. For analysis of IGFBP-3 mRNA, antisense RNA probes (426 bases) were synthesized from a linearized rat IGFBP-3 cDNA by in vitro transcription using T3 RNA polymerases, and labeled with DIG-11-UTP (Boehringer Mannheim, Indianapolis, IN). Membranes were hybridized with DIG-labeled IGFBP-3 riboprobes overnight at 68°C and then washed under stringent conditions. The detection protocol recommended by the vendor was followed. Content of the mRNA in each sample was normalized to 28S rRNA stained with methylene blue.

2.4. Data analyses

Blots were scanned and quantified using a computer-assisted image analysis system (Image–Pro, Media Cybernetics, Silver Springs, MD). Values are presented as mean ± SEM. Statistical significance was assessed by the Student's test (between obese-fed and obese-fasted, between lean-fed and lean-fasted) and the one-way analysis of variance (among the four experimental groups), with $p < 0.05$ considered significant.

3. Results

3.1. Effects of fasting on body weight, serum IGF-I and liver IGF-I mRNA

Fasting for 72 hr produced equivalent declines in mean body weights of fasted obese (decreased by 8.5% compared with the pre-fasting body weight) and fasted lean (decreased

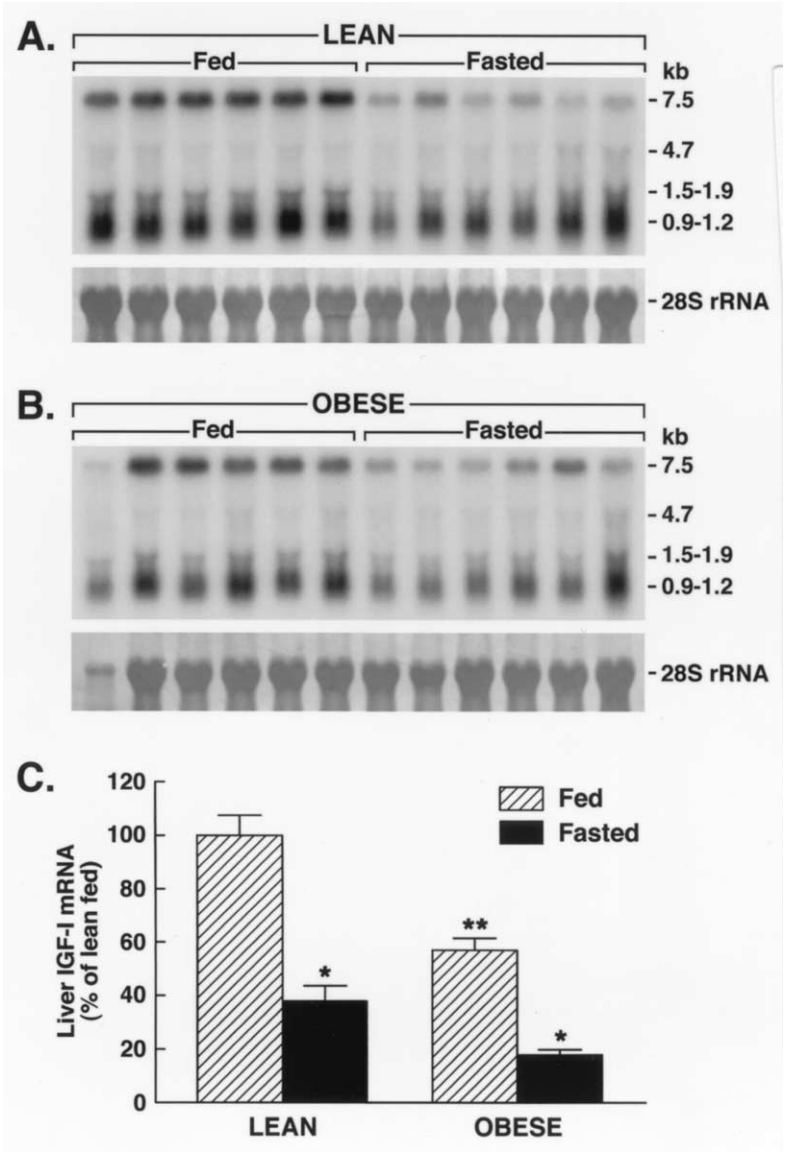

Fig. 2. Effect of fasting on liver IGF-I mRNA of lean and obese rats. Panel A: Northen blot comparing IGF-I mRNA of fed and fasted lean rats. Panel B: Northern blot comparing fed and fasted obese rats. Panel C: quantification of responses to fasting. Liver IGF-I mRNA abundance was significantly lower in obese fed rats than that in lean fed rats (**, $p < 0.01$). Compared with the corresponding fed animals, fasting caused a significant reduction of liver IGF-I mRNA in both lean and obese rats (*, $p < 0.01$). However, the reduction of IGF-I mRNA produced by fasting in lean and obese rats is similar.

by 6.9% compared with the pre-fasting body weight) animals ($p > 0.05$). In the same intervals, the body weights of the fed rats increased (obese, 7.0%; lean 5.6%, $p > 0.05$). At the end of dietary manipulation, the fasted animals weighed significantly less than the fed ($p < 0.01$; Fig. 1; top panel). The mean concentration of the IGF-I in serum of obese fed rats was not significantly different from that of lean fed rats (1503.4 ± 127.0 ng/ml vs 1766.5 ± 106.8 ng/ml, $p > 0.05$). With fasting, IGF-I serum values were decreased proportionally in the obese and the lean groups (by 46% for obese and by 43% for lean, $p > 0.05$; $p < 0.01$ compared with the respective fed group; Fig. 1; bottom panel). The serum IGF-I values of obese fasted and lean fasted were not significantly different after 72 h fasting (810.7 ± 82.9 ng/ml vs 1015.2 ± 59.4 ng/ml, $p > 0.05$).

Mean liver IGF-I mRNA abundance of obese fed rats was lower than that of the lean fed group (32.0 ± 3.2 D.U. vs 61.5 ± 5.2 D.U., $p < 0.01$; Fig. 2, Panels A and B). The magnitude of the decrease in liver IGF-I mRNA with fasting was similar among obese and lean fasted animals (68.1% vs 64.2%, Fig. 2, Panel C). In lean fasted rats, reduction of the 7.5 kb transcript of liver IGF-I mRNA was more pronounced than that of the 0.9–1.2 kb species (86.2% vs

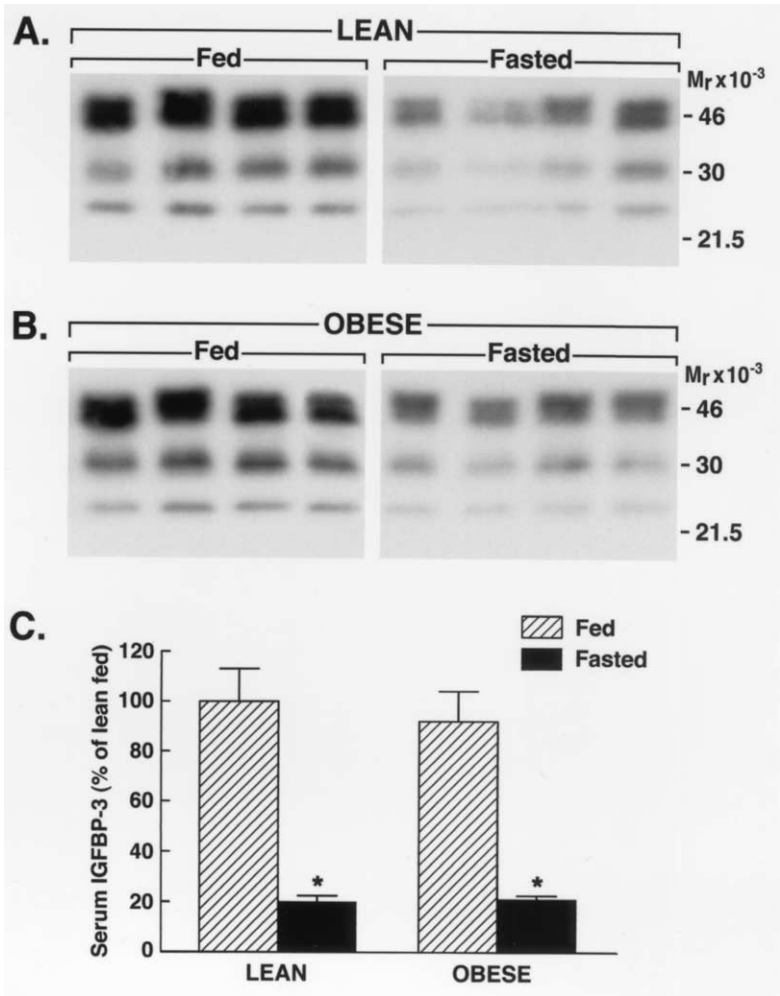

Fig. 3. Effect of fasting on serum IGFBP-3 in lean and obese rats. Panel A: ligand blots of IGFBPs from lean animals. Panel B: ligand blots from obese animals. IGFBP-3 is the band at 46K. Panel C: graphic display of changes with fasting showing comparable decrements in lean and obese animals.

55.5% reduction, $p < 0.05$). This difference, however, was not observed in obese fasted rats (76.8% vs 63.4% reduction, $p > 0.05$).

3.2. Effects of fasting on serum IGFBP-3 and liver IGFBP-3 mRNA

Serum values of IGFBP-3 in obese and lean fed rats were not different (10.9 ± 1.6 D.U. vs 11.8 ± 1.6 D.U., $p > 0.05$; Fig. 3, Panels A and B). Compared with lean and obese fed rats, fasting produced comparable decrements of serum IGFBP-3 (81.3% vs 78.9%, Fig. 3, Panel C). The other two bands detected in the ligand blots (30 kDal and 24 kDal) showed the same pattern of decline as IGFBP-3. The mean liver IGFBP-3 mRNA of obese fed rats was lower than that of the lean fed rats (17.6 ± 0.7 D.U. vs. 30.5 ± 3.0 D.U., $p < 0.01$; Fig. 4, Panels A and B), and the liver IGFBP-3 mRNA declined proportionately in the obese and lean fasted groups (64.9% vs 60.2%, Fig. 4, Panel C).

4. Discussion

Much is known about the stimulatory effects of growth hormone (GH) and the regulatory influence of nutrition on the IGF system [1]. Obesity is accompanied by attenuation of GH secretion and reduction of serum GH concentrations in humans [16,17] and lower animals [18]. The effects, however, of obesity and of change in body composition on IGF-I and IGFBP-3 are understood less well. Compared with normal weight individuals, concentrations of IGF-I in serum of obese humans are variously reported to be low, normal, or high [6,19,20]. Similar variability of IGF-I values are reported in obese and lean Zucker rats [21–25]. We found in this study that serum IGF-I of obese Zucker rats is lower though not statistically different from that of lean rats. We believe the affinity of different antibodies used for RIA measurement of IGF-I, and/or the efficiency of extraction of IGFBPs prior to assay of serum samples may account for some, if not all, of these differences.

Obese humans appear to be more resistant than non-

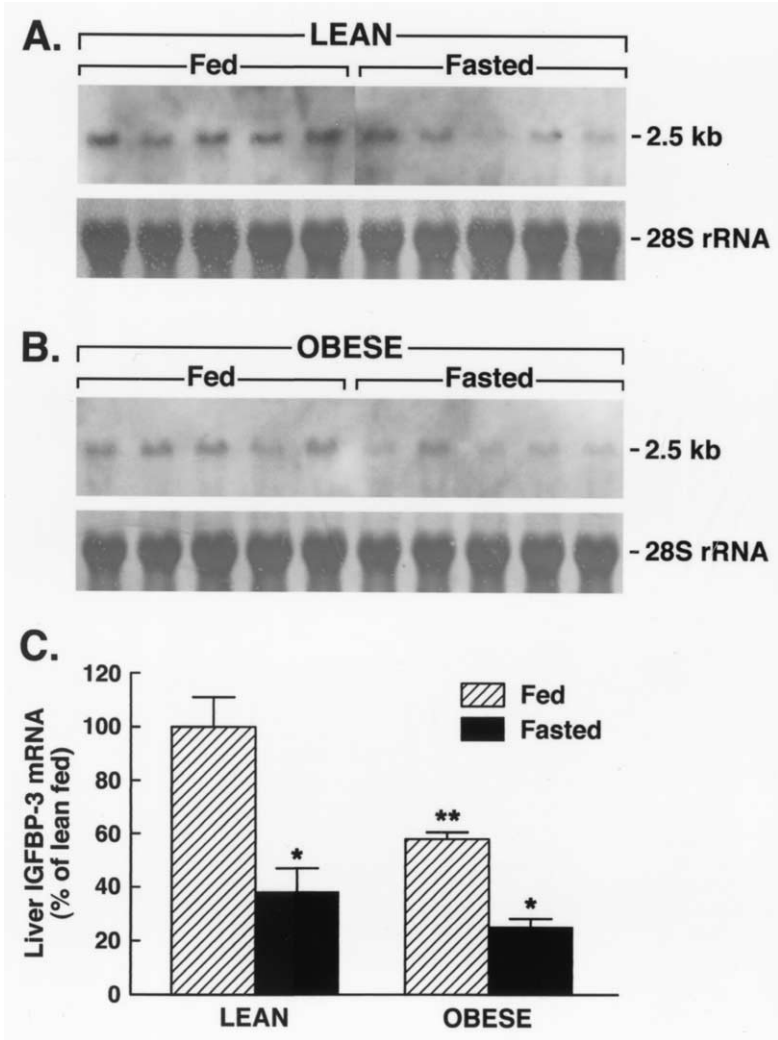

Fig. 4. Effects of fasting on liver IGFBP-3 mRNA. Panel A: Northern blot comparing IGFBP-3 mRNA of lean (fed and fasted) rats. Panel B: Northern blot of mRNA of obese animals. Panel C: graphic display showing proportional decline in IGFBP-3 mRNA of lean and obese during fasting. IGFBP-3 mRNA abundance was significantly lower in obese fed rats than that in lean fed rats (**, $p < 0.01$). Fasting produced a significant reduction of liver IGFBP-3 mRNA in both lean and obese rats compared with the respective fed groups (*, $p < 0.01$).

obese to undernutrition, in that they experience less marked decrements in serum concentrations of IGF-I and IGFBP-3 when deprived of calories [6,7]. Specifically, obese adults fed a low energy diet (445 kcal/day) relatively rich in protein (50 g/day) are reported not to experience a decrease in their serum IGF-I [6]. Bang et al [7] showed that the decline in IGF-I serum concentrations during 4 days of fasting in obese subjects was delayed and attenuated, compared with that occurring in non-obese subjects. Obese rats also are resistant to nutrient deprivation. While fasting of Zucker rats for 10 days produces comparable weight loss in obese and non-obese littermates, the obese rats lose nearly twice as much lipid, but only 1/5 as much protein as lean rats [8]. Similarly, smaller reductions in protein synthesis and smaller increases in protein degradation are produced when older, larger rats are fasted, compared with younger, smaller counterparts [26]. We speculated, therefore, that obese rats subjected to fasting would be able to use their reserves of fat as a source of energy, and would be better able to maintain their serum IGF-I and IGFBP-3 peptide and liver mRNAs for IGF-I and IGFBP-3. Success with these adaptive mechanisms might provide an explanation for the ability of obese animals to conserve lean tissue when subjected to undernutrition. Our results, however, indicate that obesity in Zucker rats does not attenuate the fasting-related diminution of IGF-I and IGFBP-3 peptides in serum, or their respective mRNAs in liver. Consistent with the knowledge that the liver is the principal source of serum IGF-I and IGFBP-3 [27,28], we observed that fasting for 72 h in obese and lean rats produced similar decrements in serum IGF-I and IGFBP-3 concentrations, accompanying parallel decrements in their mRNAs in liver. However, we note that a lack of protection by obesity against fasting-induced hepatic reduction in IGF-I and IGFBP-3 does not allow the conclusion that IGF-I does not protect against tissue protein conservation during fasting. Our finding simply suggests that

the serum concentrations and hepatic mRNA levels of IGF-I and IGFBP-3 in obese Zucker rats do not correlate with the protein conservation observed following 72 h fasting. Extrahepatic tissues, especially skeletal muscle and adipose tissue that produce IGF-I locally, might behave differently in the obese animals. Fasting or protein restriction in rats causes a decrease in IGF-I mRNA in skeletal muscle [3,29], a lean tissue that can be used to monitor protein sparing during fasting. Whether obesity attenuates or delays the reduction of IGF-I mRNA in skeletal muscle that results from fasting is not known. Also, the time-related changes in IGF-I and IGFBP-3 mRNAs in hepatic and extrahepatic tissues during the three phases of fasting have not been documented.

In keeping with the report of Melian et al [30], we observed that obese Zucker rats have lower basal-state liver IGF-I mRNA than their lean counterparts, but their serum IGF-I values are not significantly reduced. The lower IGF-I mRNA may be secondary to reduced GH, but another mechanism is needed to explain the discrepancy between reduced hepatic IGF-I mRNA and normal serum IGF-I concentrations. We speculate that the absence of lower IGF-I peptide could be secondary to absence of a lean-obese difference in IGFBP-3, the principal IGF carrier protein in serum. More problematic, however, is the explanation for the lack of difference in IGFBP-3 in serum of lean vs obese animals. The occurrence of reduced liver IGFBP-3 mRNA in obese-fed rats also might be secondary to reduced stimulation from GH. Another possible explanation, however, is that there is a more global adjustment in transcription control, which affects gene expression of both IGF-I and IGFBP-3.

Previous studies from our laboratory [31] indicate that reduction of IGF-I gene expression during fasting results from 2 events: a) defective splicing of heteronuclear IGF-I leading to the formation of mature mRNA, and b) decreased stability of the mature IGF-I mRNA. It is not known whether these mechanisms are also operative for IGFBP-3. The sharp decline in serum peptide and liver mRNA for IGF-I and IGFBP-3 during fasting in both the lean and obese animals raises the possibility that conservation of lean tissue in obese animals is not related to production or action of IGF-I. This supposition is further supported by the observation that the decrements in these indices measured during fasting were comparable between lean and obese animals.

While our results show that during 72 h fasting, obese Zucker rats are not able to maintain the hepatic production and serum concentrations of IGF-I and IGFBP-3 any better than their lean counterparts, we recognize that these observations might not be widely applicable to other forms of obesity, such as obese rats fed a high fat diet and obese human. Obese Zucker rats (fa/fa) bear a mutated leptin receptor gene, have elevated plasma concentrations of leptin, and are insensitive to leptin [32,33]. These changes do not afflict lean Zucker rats. Aside from obesity itself, altered leptin sensitivity might cause differences in the response to fasting between obese and lean rats. Weakening the notion that leptin sensitivity may be tied to reduction in IGF-I and IGFBP-3 gene expression in liver during fasting, is the observation that leptin secretion decreased rapidly during a 72 h fast and that the fasting-induced reduction of hepatic IGF-I gene expression was not corrected with leptin treatment in non-obese, leptin-sensitive rats [34].

In summary, the results of this study provide insight into some of the effects of fasting on the principal components of the IGF system of obese Zucker rats. Our findings raise the possibility that the ability of these animals to preserve body protein during fasting is mediated through mechanisms outside the IGF/IGFBP system.

Acknowledgments

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