Leptin alters the response of the growth hormone releasing factor–growth hormone–insulin-like growth factor-I axis to fasting

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Abstract

Proper nutritional status is critical for maintaining growth and metabolic function, playing an intimate role in neuroendocrine regulation. Leptin, the recently identified product of the obese gene, may very well be an integral signal which regulates neuroendocrine responses in times of food deprivation. The present study examines leptin's ability to regulate hormonal synthesis and secretion within the GRF–GH–IGF axis in the adult male rat during almost 3 days of fasting. Serum levels of GH and IGF-I were drastically suppressed by fasting. Daily leptin administration was able to fully prevent the fasting-induced fall in serum GH. Leptin failed to restore IGF-I to control levels, however, suggesting possible GH resistance. Fasting

caused an insignificant increase in GH mRNA, while leptin injections significantly increased steady-state levels of this message. The GRF receptor (GRFr) message was not altered with fasting or leptin treatment. Leptin also exhibited effects at the hypothalamic level. Fasting induced a sharp fall in GRF mRNA expression and leptin injections partially prevented this fall. However, there were no observed changes in the hypothalamic GRF content. These results provide evidence that leptin may function as a neuromodulator of the GRF–GH–IGF axis communicating to this hormonal system the nutritional status of the animal.

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Introduction

The proper regulation of the growth hormone (GH) axis is crucial for normal development, somatic growth and other immune and metabolic functions. Hypothalamic GH releasing factor (GRF) stimulates and hypothalamic somatostatin inhibits release of GH. Circulating GH then targets many tissues, including the liver, leading to the release of insulin-like growth factor-I (IGF-I), the essential mediator of most of GH's actions on growth. IGF-I also inhibits GH synthesis and secretion by direct effects at both the pituitary and the hypothalamus (Lumpkin *et al.* 1985, Yamashita & Melmed 1986, Ceda *et al.* 1987, Colonna *et al.* 1988, Le Roith 1991, Wood *et al.* 1991, Uchiyama *et al.* 1994).

Fasting has been shown to decrease serum GH levels in rats (Tannebaum 1983, Murphy *et al.* 1991, Ohashi *et al.* 1995, Carro *et al.* 1997). IGF-I is also depressed (Underwood *et al.* 1986, Vance *et al.* 1992, Ohashi *et al.* 1995) during fasting. It has been recently reported that impaired somatostatin distribution and release may also contribute to changes seen in the GH axis in nutritionally deprived animals (Aguila & McCann 1987, Sonntag *et al.* 1995).

The recent discovery of the obese (ob) gene product, leptin, and its close association with neuropeptide Y and food intake has suggested a possible role in other endocrine functions (Zhang *et al.* 1994, Bray & York 1997). Leptin is produced mainly in white adipocytes and is released into the general circulation (Masuzaki *et al.* 1995, Cinti *et al.* 1997). Leptin is capable of entering the brain where it presumably modulates food intake and body weight (Banks *et al.* 1996, Seeley *et al.* 1996). Rats fasted for 1–3 days exhibit a marked decrease in ob mRNA which can be reversed by refeeding (Frederich *et al.* 1995, Ahima *et al.* 1996). Many suggest that leptin's primary target is the hypothalamus since leptin administration decreases food intake (Schwartz *et al.* 1996). Since many of the hormones in the GH axis are disturbed during fasting states, we hypothesized that leptin can act as a mediator between food intake and normal hormonal regulation.

Materials and Methods

Animals

Male Sprague–Dawley rats, aged 65 days old at the start of the experimental treatment, were obtained from Harlan

Laboratories, Indianapolis, IN, USA. All rats were multi-housed and kept with a ratio of 12 h light:12 h darkness cycle at 22–24 °C. To minimize stress, the animals were handled and weighed daily. Two groups of eight rats each were fasted from 1600 h on day 1 until 1030 h on day 4 (approximately 66 h). Beginning on day 1 at 2030 h, rats were given intraperitoneal injections of either saline or leptin (1 µg/g body weight) at 0830 h and 2030 h. Leptin was purchased through PeproTech (Rocky Hills, NJ, USA). On day 4, the animals were killed by decapitation 2 h after the morning injection. The third group of eight rats was injected with saline as above and given rat chow *ad libitum* throughout the entire experiment. All animals were allowed free access to water throughout the experiment. Trunk blood was collected, centrifuged and serum taken. Anterior pituitaries and hypothalami were dissected and placed immediately on dry ice.

Radioimmunoassay (RIA) for GH, IGF-I and GRF

The GH RIA was conducted using material generously contributed by the NIDDK and the National Hormone and Pituitary Program through Dr A F Parlow. The procedure for the GH RIA was as previously described (Hojvat *et al.* 1982). The lower limits of detection of the assay were 0.98 ng/ml. The intra-assay and interassay coefficients of variation were 3.6 and 7.8% respectively.

The GRF RIA was done with antibody supplied by Dr W B Wehrenberg of the University of Wisconsin–Milwaukee. The methods for the GRF RIA were as previously described (Tentler *et al.* 1993). Assay sensitivity was 156 pg/ml. Interassay coefficient of variation was 8% and intra-assay coefficient of variation was 12%.

IGF-I levels were measured using an RIA assay kit from Amersham (Arlington Heights, IL, USA). Assay sensitivity was 260 ng/ml. Intra-assay coefficient of variation was 7.4%.

Hypothalamic GRF mRNA and pituitary GRFr mRNA determinations

Total RNA was extracted from individual rat hypothalami and pituitaries according to an established procedure (Chomczynski & Sacchi 1987). Gene expression for GRF and GRFr was analyzed by RT-PCR. The GRF mRNA protocol was followed according to a previously published procedure (Steiner *et al.* 1997). GRFr mRNA determination was also performed according to the above protocol with the following changes: only 1 µg total RNA was reverse transcribed and 32 cycles of PCR were used to amplify the gene. GRFr oligos were designed to the 3' end of the gene starting at bp 866–1285, resulting in a 420 bp product. The sequences are as follows:

5' CCTACTGGTGGATCATCAAAGG

3' TGAGCACCTTCACTCTCGATCG

Histone oligos were used to control for amplification and loading. Autoradiographic film was analyzed on a densitometer and relative amounts were compared in arbitrary densitometer units (ADU).

Pituitary GH mRNA determination

Total RNA was extracted from pituitaries and levels of GH mRNA determined using Northern blot hybridization analysis. A total of 10 µg RNA were loaded per lane and run on a 1.5% agarose–formaldehyde gel and fixed onto Nytran membrane using a Stratagene UV oven. Hybridization of the GH probe was accomplished under stringent conditions in 50% formamide in a Robbins hybridization oven at 42 °C and the filters washed in 0.2 × SSC, 0.5% SDS at 65 °C. The filters were then exposed to X-ray film and the autoradiographs were analyzed using a scanning densitometer. Histone 3.3 was used as a control for loading.

Statistics

Data was analyzed by one way analysis of variance (ANOVA). Tukey's studentized range test was performed as a follow-up statistical analysis. All data are expressed as means ± S.E.M.

Results

Chow fed rats allowed to feed *ad libitum* gained weight appropriately. In contrast, fasted/saline injected rats lost substantial weight and fasted/leptin injected animals lost the same amount of weight. A dramatic 90% fall in serum GH was caused by fasting and was completely prevented by leptin injection to fasted animals. While fasting led to a fall in serum GH, GH mRNA did not change, though there was a trend towards increased level of expression in fasted animals. Interestingly, the fasted/leptin treated animals had a marked elevation of steady-state levels of GH message compared with chow fed control and fasted/saline treated rats. Northern blot analysis is shown in Fig. 1.

Despite the clear-cut changes in serum GH with 66 h of fasting, GRFr mRNA levels did not change significantly, though there was a trend towards a rise in fasted/saline injected animals not seen in fasted/leptin treated animals.

Because GH secretion is under hypothalamic control, the effect of fasting and leptin treatments on GRF message and peptide content was examined. In fasted/saline treated animals, steady-state levels of GRF mRNA fell significantly compared with chow fed rats. Fasted/leptin treated animals exhibited intermediate GRF mRNA levels not significantly different from either fasted/saline injected or chow fed animals allowed to feed *ad libitum*. RT-PCR analysis is shown in Fig. 2. Total hypothalamic content of GRF did not change in any of the three groups.

Figure 1 Northern blot of GH mRNA (top panel) and histone (bottom panel). Lanes 1–4 represent chow fed; 5–8 represent fasted/saline; 9–12 represent fasted/leptin.

Because synthesis and secretion of IGF-I are, at least partly, under GH control, serum levels of IGF-I were determined. In fasted/saline rats, there was a sharp 69% fall in circulating IGF-I compared with animals allowed to feed *ad libitum* concordant with the fasting-induced fall in serum GH. Although leptin treatment prevented the fall in GH, serum levels of IGF-I fell even further in fasted/leptin treated rats. Data are shown in Table 1.

Discussion

Nutritional status is intimately interlinked with neuroendocrine and other hormonal regulation. Caloric deprivation has long been known to have damaging consequences on growth, reproduction and other metabolic functions mediated through effects on neuroendo-

Figure 2 RT-PCR analysis of GRF mRNA (top panel) and histone (bottom panel) using incorporating ^{32}P to detect PCR product levels by densitometer. Lanes 1–4 represent chow fed; 5–8 represent fasted/saline and 9–12 represent fasted/leptin.

crine hormones (Vance *et al.* 1992). Leptin, a 167 amino acid peptide made largely, though not exclusively, in adipose tissue has profound effects on food intake (Bray 1996). More recently it has been appreciated that leptin may also mediate, at least in part, some of the dramatic neuroendocrine consequences of nutritional deprivation (Ahima *et al.* 1996, Legradi *et al.* 1997, Yu *et al.* 1997). We report here the effects of leptin on the response of the GRF–GH–IGF axis to fasting. The model of fasting we have used has previously been demonstrated to be associated with marked reduction in circulating leptin levels and thus is a model of hypoleptinemia (Ahima *et al.* 1996). Leptin administration in doses similar to the ones used in this study have been reported to restore leptin to normal levels, i.e. those seen in animals allowed to feed *ad libitum*.

After almost 3 days of fasting, a sharp decrease in serum GH was observed, which concurs with the findings of others (Tannebaum 1983, Murphy *et al.* 1991, Ohashi *et al.* 1995, Carro *et al.* 1997). Leptin administration almost completely prevented this fasting-induced 90% decrease in serum GH, confirming the findings of Carro *et al.* (1997). In contrast to the fall in serum levels of GH, there was no concordant suppression of GH message with fasting. IGF-I reduces the level of expression of GH mRNA (Yamashita & Melmed 1986, Ceda *et al.* 1987, Wood *et al.* 1991). Therefore, in our fasted animals with very low levels of circulating IGF-I, a rise in GH mRNA would have been anticipated. Thus, the lack of change in GH mRNA probably represents a relative suppression. Our finding that leptin administration to fasted animals produced a 3-fold increase in GH mRNA probably indicates that leptin has important, previously unrecognized direct or indirect effects on GH gene transcription and/or stability, as well as on GH secretion.

Because pituitary GH synthesis and secretion are controlled in large part by the hypothalamus, with GRF acting as a stimulatory factor and somatostatin as an inhibitor, we examined the effects of fasting and leptin on the hypothalamic GRF system. Three days of fasting led to a reduction in hypothalamic GRF mRNA, confirming a previous report (Bruno *et al.* 1992). Leptin partially prevented this. This finding suggests that at least part of the dramatic effects of leptin on GH and GH mRNA may be mediated at the hypothalamic level. Neither fasting nor fasting and leptin administration altered the hypothalamic content of the GRF peptide. It is hazardous, however, to make inferences about secretory dynamics based on determinations of static tissue levels. Future studies utilizing either *in vitro* hypothalamic tissue slice preparations, and/or *in vivo* push–pull cannula technology, or hypothalamic–pituitary portal blood vessel sampling will be necessary to determine how the hypothalamic GRF system regulates leptin's effects on GH. GRF stimulates GH synthesis and secretion by attaching to specific receptors on pituitary somatotropes. Despite the rather large changes in serum GH, there was no effect caused either by

Table 1 Effects of leptin treatment after 3 days of fasting

	Weight (g)	Weight change (g)	Serum GH (ng/ml)	GH mRNA (ADU)	GRFr mRNA (ADU)	GRF mRNA (ADU)	GRF content (pg/mg tissue)	IGF-I (ng/ml)
Group 1 <i>Ad libitum</i> chow fed/saline	293 ± 6	+ 14 ± 1	159 ± 25	956 ± 57	1274 ± 636	5820 ± 1645	202 ± 85	473 ± 14
Group 2 Fasted/saline	246 ± 3 **	− 41 ± 2 **	16 ± 10 ***	3619 ± 2129	2253 ± 699	1503 ± 301 *	270 ± 12	145 ± 29 ***
Group 3 Fasted/leptin	248 ± 3 **	− 39 ± 1 **	125 ± 31 **	10 411 ± 198 **	1189 ± 703	2926 ± 444	261 ± 36	53 ± 7 ** ***
Significance	** <i>P</i> <0.01 compared with Gp 1	** <i>P</i> <0.01 compared with Gp 1	*** <i>P</i> <0.001 compared with Gp 1 ** <i>P</i> <0.01 compared with GP 2	** <i>P</i> <0.01 compared with Gp 1 and 2	no significance	* <i>P</i> <0.05 compared with Gp 1	no significance	*** <i>P</i> <0.001 compared with Gp 1 ** <i>P</i> <0.01 compared with Gp 2

Data are shown as means ± S.E.M.
ADU, arbitrary densitometer units; Gp, group.

fasting or by leptin on expression of the GRFr mRNA. Since the level of expression of the message and expression and/or activity of the translated protein do not always move in a coordinated fashion, it would be premature to conclude that GRF binding is actually unchanged in our paradigm. This will require future studies. Recently, Quintela *et al.* (1997) reported that leptin diminished somatostatin mRNA levels and decreased somatostatin secretion *in vitro*. This may also be part of the mechanism of leptin action on GH.

The 36-h fasting paradigm resulted in a more than two-thirds reduction in circulating levels of IGF-I, the molecule which mediates many of the growth promoting effects of GH. This finding confirms that of several other groups in both animals and man (Underwood *et al.* 1986, Vance *et al.* 1992, Thissen *et al.* 1994, Ohashi *et al.* 1995). Not only is secretion of IGF-I reduced with fasting, but also synthesis is reduced as evidenced by falls in IGF-I mRNA (Zhang *et al.* 1994, Kobayashi *et al.* 1995). In contrast to the ability of leptin to prevent the fall in GH, serum levels of IGF-I further declined in fasted/leptin treated animals compared with fasted/saline injected rats. It has been well recognized that while GH normally stimulates IGF-I, caloric restriction and fasting impose a state of GH resistance wherein the ability of GH to stimulate IGF-I secretion is impaired (Maes *et al.* 1991, Thissen *et al.* 1994). Leptin, at least in the doses used in this study, was unable to overcome this state of GH resistance. Indeed, leptin further decreased IGF-I concentration by another two-thirds compared with fasted/saline animals. Although the physiologic significance of this is unclear at this time, it suggests the possibility that leptin may act directly at liver and other sites of IGF-I synthesis.

Our data open new areas for possible study. These include the role of leptin in GRF secretion, GRF binding at the pituitary, regulation of GH and IGF-I gene expression, to name a few. These data also expand the exciting notion developed by others (Ahima *et al.* 1996, Barash *et al.* 1996, Chehab *et al.* 1996, Legradi *et al.* 1997, Yu *et al.* 1997) that the physiologic role of leptin stretches well beyond regulation of feeding and is an important molecule signaling other hormonal systems about the nutritional status of the organism.

References

Aguila MC & McCann SM 1987 Evidence that growth hormone-releasing factor stimulates somatostatin release *in vitro* via β -endorphin. *Endocrinology* **120** 341–344.
Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E & Flier JS 1996 Role of leptin in the neuroendocrine response to fasting. *Nature* **382** 250–252.
Banks WA, Kastin AJ, Huang W, Jaspan JB & Maness LM 1996 Leptin enters the brain by a saturable system independent of insulin. *Peptides* **17** 305–311.
Barash IA, Cheung CC, Weigle DS, Ren H, Kabigting EB, Kuijper JL, Clifton DK & Steiner RA 1996 Leptin is a metabolic signal to the reproductive system. *Endocrinology* **137** 3144–3147.
Bray GA 1996 Leptin and leptinomania. *Lancet* **348** 140–141.
Bray GA & York DA 1997 Leptin and clinical medicine: a new piece in the puzzle of obesity. *Journal of Clinical Endocrinology and Metabolism* **82** 2771–2776.
Bruno JF, Song JF & Berelowitz M 1992 Regulation of rat hypothalamic preprogrowth hormone-releasing factor messenger ribonucleic acid by dietary protein. *Endocrinology* **129** 1226–1232.
Carro E, Senaris R, Considine RV, Casanueva FF & Dieguez C 1997 Regulation of *in vivo* growth hormone secretion by leptin. *Endocrinology* **138** 2203–2206.
Ceda GP, Davis RG, Rosenfeld RG & Hoffman AR 1987 The growth hormone (GH)-releasing hormone (GHRH)-GH

- somatomedin axis: evidence for rapid inhibition of GHRH-elicited GH release by insulin-like growth factors I and II. *Endocrinology* **120** 1658–1662.
- Chehab FF, Lim ME & Lu R 1996 Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. *Nature Genetics* **12** 318–320.
- Chomczynski P & Sacchi N 1987 Single-step method of RNA isolation by guanidinium thiocyanate–phenol–chloroform extraction. *Analytical Biochemistry* **162** 156–159.
- Cinti S, Frederich RC, Zingaretti MC, De Matteis R, Flier JS & Lowell BB 1997 Immunohistochemical localization of leptin and uncoupling protein in white and brown adipose tissue. *Endocrinology* **138** 797–804.
- Colonna VDG, Cattaneo E, Cocchi D, Muller EE & Maggi A 1988 Growth hormone regulation of growth hormone-releasing hormone gene expression. *Peptides* **9** 985–988.
- Frederich RC, Lollmann B, Hamann A, Napolitano-Rosen A, Kahn BB, Lowell BB & Flier JS 1995 Expression of ob mRNA and its encoded protein in rodents: impact of nutrition and obesity. *Journal of Clinical Investigation* **96** 1658–1663.
- Hojvat S, Emanuele N, Baker G, Connick E, Kirsteins L & Lawrence AM 1982 Growth hormone (GH), thyroid-stimulating hormone (TSH), and luteinizing hormone (LH)-like peptides in the rodent brain: non-parallel ontogenetic development with pituitary counter-parts. *Developmental Brain Research* **4** 427–434.
- Kobayashi S, Nogami H & Ikeda T 1995 Growth hormone and nutrition interact to regulate expressions of kidney IGF-I and IGFBP mRNAs. *Kidney International* **48** 65–71.
- Legrati G, Emerson CH, Ahima RS, Flier JS & Lechan RM 1997 Leptin prevents fasting-induced suppression of prothyrotropin-releasing hormone messenger ribonucleic acid in neurons of the hypothalamic paraventricular nucleus. *Endocrinology* **138** 2569–2576.
- Le Roith D 1997 Insulin-like growth factors. *New England Journal of Medicine* **336** 633–640.
- Lumpkin MD, Samson WK & McCann SM 1985 Effects of intraventricular growth hormone-releasing factor on growth hormone release: further evidence for ultrashort loop feedback. *Endocrinology* **116** 2070–2074.
- Maes M, Maiter D, Thissen JP, Underwood LE & Ketelslegers JM 1991 Contributions of growth hormone receptor and postreceptor defects to growth hormone resistance in malnutrition. *Trends in Endocrinology and Metabolism* **2** 92–97.
- Masuzaki H, Ogawa Y, Isse N, Satoh N, Okazaki T, Shigemoto M, Mori K, Tamura N, Hosoda K, Yoshimasa Y, Jingami H, Kawada T & Nakao K 1995 Human obese gene expression. Adipocyte-specific expression and regional differences in the adipose tissue. *Diabetes* **44** 855–858.
- Murphy LJ, Seneviratne C, Moreira P & Reid RE 1991 Enhanced expression of insulin-like growth factor-binding protein I in the fasted rat: the effects of insulin and growth hormone administration. *Endocrinology* **128** 689–696.
- Ohashi S, Kaji H, Abe H & Chihara K 1995 Effect of fasting and growth hormone (GH) administration on GH receptor (GHR) messenger ribonucleic acid (mRNA) and GH-binding protein (GHBP) mRNA levels in male rats. *Life Sciences* **57** 1655–1666.
- Quintela M, Senaris R, Heiman ML, Casanueva FF & Dieguez C 1997 Leptin inhibits *in vitro* hypothalamic somatostatin secretion and somatostatin mRNA levels. *Endocrinology* **138** 5641–5644.
- Schwartz MW, Seeley RJ, Campfield LA, Burn P & Baskin DG 1996 Identification of targets of leptin action in rat hypothalamus. *Journal of Clinical Investigation* **98** 1101–1106.
- Seeley RJ, van Dijk G, Campfield LA, Smith FJ, Burn P, Nelligan JA, Bell SM, Baskin DG, Woods SC & Schwartz MW 1996 Intraventricular leptin reduces food intake and body weight of lean rats but not obese Zucker rats. *Hormone and Metabolic Research* **28** 664–668.
- Somnath WE, Xiaowei X, Ingram RL & D'Costa A 1995 Moderate caloric restriction alters the subcellular distribution of somatostatin mRNA and increases growth hormone pulse amplitude in aged animals. *Neuroendocrinology* **61** 601–608.
- Steiner JC, LaPaglia N, Hansen M, Emanuele NV & Emanuele MA 1997 Effect of chronic ethanol on reproductive hormones in the peripubertal male rat. *Journal of Endocrinology* **154** 363–370.
- Tannebaum GS 1983 Insulin-like growth factors: a role in growth hormone negative feedback and body weight regulation via brain. *Science* **220** 77–79.
- Tender JJ, Emanuele MA, Paloyan E, Hofmann E, Emanuele NV, Lawrence AM & Kelley MR 1993 Ethanol effects growth hormone releasing factor (GRF) synthesis *in vivo* but not *in vitro*. *Endocrine Journal* **1** 141–146.
- Thissen JP, Ketelslegers JM & Underwood LE 1994 Nutritional regulation of the insulin-like growth factors. *Endocrine Reviews* **15** 80–101.
- Uchiyama T, Kaji H, Abe H & Chihara K 1994 Negative regulation of hypothalamic growth hormone-releasing factor messenger ribonucleic acid by growth hormone and insulin-like growth factor. *Neuroendocrinology* **59** 441–450.
- Underwood LE, Clemmons DR, Maes MA, D'Ercole J & Ketelslegers JM 1986 Regulation of somatomedin-C/insulin-like growth factor I by nutrients. *Hormone Research* **24** 166–176.
- Vance ML, Hartman ML & Thorner MO 1992 Growth hormone and nutrition. *Hormone Research* **38** 85–88.
- Wood TL, Berelowitz M, Gelato MC, Roberts Jr CT, LeRoith D, Millard WJ & McKelvey JF 1991 Hormonal regulation of rat hypothalamic neuropeptide mRNAs: effect of hypophysectomy and hormone replacement on growth hormone releasing factor, somatostatin and the insulin-like growth factors. *Neuroendocrinology* **53** 298–305.
- Yamashita S & Melmed S 1986 Insulin-like growth factor I action on rat anterior pituitary cells: suppression of growth hormone secretion and messenger ribonucleic acid levels. *Endocrinology* **118** 176–182.
- Yu WH, Kimura M, Walczewska A, Karanth S & McCann SM 1997 Role of leptin in hypothalamic–pituitary function. *Proceedings of the National Academy of Sciences of the USA* **94** 1023–1028.
- Zhang Y, Proenca R, Maffei M, Barone M, Leopold L & Friedman J 1994 Positional cloning of the mouse obese gene and its human homologue. *Nature* **372** 425–432.

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