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Interaction between Leptin and Growth Hormone (GH)/IGF-I Axis

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Abstract. In order to identify the mutual interaction between GH and leptin, we studied the effect of GH on fatty Zucker rats. GH administration at a high dose (5.0 IU/kg) reduced % body fat after 7 days. The leptin mRNA level in subcutaneous fat tissue was not changed but that in epididymal fat tissue was decreased by an even lower dose of GH (1.5 IU/kg). IGF-I treatment (200 μg/kg/day) did not change the % body fat or leptin mRNA level. These observations suggest that GH directly interacts with visceral fat and reduces fat mass and leptin expression. We also measured serum leptin levels in patients. The levels in patients with acromegaly were significantly lower than those in normal subjects with the same amount of body fat, but serum IGF-I and urinary C peptide excretion rates were higher in the acromegalic. These observations also suggests that GH directly interacts with adipose tissue and reduces leptin expression. Next we investigated the direct action of leptin on GH release from the pituitary. Leptin pretreatment of pituitary cells in culture or rats in a fasted or fed condition did not change GRH induced GH secretion. As indicated also by other recent studies, leptin may increase GRH or decrease somatostatin secretion by the hypothalamus. Thus GH interacts with fat tissues and leptin may be a good marker of the interaction.

Key words: GH, Obese, *ob* gene, Leptin, Adipose tissue

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GROWTH hormone (GH) has been reported to have a number of metabolic actions on peripheral tissues in addition to the growth promoting effect mediated by IGF-I production [1]. As for adipose tissues, GH promotes lipolysis or reduces fat deposition. The direct actions of GH on adipocytes were already reported in the 1960s, including amino acid incorporation and lipolysis [2, 3]. In addition to these findings, GH-deficient patients are often associated with increase body fat and GH treatment in GH-deficient patients significantly reduces the total fat mass and intra-abdominal adipose tissue [4], so that GH possibly plays a role in the regulation of adiposity.

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One of the major advances related to adiposity is the identification of the gene for obesity. The obesity gene (*ob*-gene) was isolated by positional cloning, and its gene product, leptin, has been demonstrated [5]. Leptin is expressed exclusively in adipocytes, secreted into the blood stream and appears to send a signal to the hypothalamus through its specific receptor. It reduces appetite and stimulates energy expenditure. Thus it has as an endocrine function in body weight regulation by means of energy consumption and storage. Since leptin plays a pivotal role in the regulation of adiposity and energy homeostasis, the level of its expression is likely to fluctuate under nutritional or disease conditions. These observations suggest a role of GH in leptin expression in fat tissues, and understanding the interaction between GH and leptin may be very helpful in treating of obesity and in understanding of the mechanism of obesity

in patients with growth hormone deficiency (GHD).

The Zucker rat is a strain of obese rat and its recessive homozygous littermates (*fa/fa*) are characterized by obesity and hyperlipidemia. Serum GH levels in obese littermates are reduced and the rats are a model of obese patients with GH deficiency [6]. A recent study demonstrated a loss of the function mutation of the ob receptor gene and the leptin mRNA levels are increased in fat tissue in resistance to leptin [7].

We therefore investigated a possible role of GH in leptin gene expression and adiposity. We also studied the effect of leptin on GH secretion.

Materials and Methods

Male Zucker (*fa/fa*) rats and their lean littermates (*Fa/?*) were obtained from Kiwa Laboratory Animals Co., Ltd. (Wakayama, Japan). Body weight and % body fat was determined by the bioelectroimpedance method. Recombinant human (rh) GH was obtained from Pharmacia & Upjohn, Stockholm, Sweden and was dissolved in H_2O and injected s.c. once a day for 7 day (1.5 IU/kg or 5.0 IU/kg). Saline was injected into the control rats. Recombinant human IGF-I (Fujisawa Pharmaceutical Com. LTD., Osaka) in 0.1 M acetic acid was applied s.c. (200 μg/day) with miniosmotic pumps (Alzat Corp., Palo Alto, CA). On the day of sacrifice, the animals were anesthetized and epididymal and subcutaneous fat tissues were excised and immediately frozen in liquid nitrogen and stored at -80°C until RNA extraction.

Total RNA was extracted by the guanidine/CsCl method. Leptin mRNA levels were determined by reverse transcription-polymerase chain reaction (RT-PCR) according to the method previously reported [8]. RNA samples were reverse transcribed with oligo $(dT)_{15}$ or the reverse primer for the PCR. The primers for PCR were selected with a computer program, Primer 3, White Head Institute for Biomedical Research, MIT, Cambridge, MA. The rat leptin primers were from a sequence with the accession number D49653 [9]. The forward primer (118 to 138 nt) was 5'-ACA CCA AAA CCC TCA TCA AGA-3' and the reverse primer (301 to 283 nt) was 5'-GAA GGC AAG CTG GTG AGG A-3'. The expected size of the PCR product was 184 bp. PCR was carried out as previously reported [7]. The annealing temperature was 65 °C and the number of PCR cycles was 40 for quantitative PCR for leptin mRNA. The PCR products were run on 3% agarose gels and stained with ethedium bromide. After taking photographs, the amount of PCR product was determined with a computer program, Image, NIH, Bethesda, MD.

We also prepared dispersed pituitary cells from Wister rats with Disperse (Godo-Syuzo, Tokyo, Japan) and seeded in 98-well plates. The cells were cultured for 2 days in DMEM supplemented with 10% fetal calf serum. The cells were stimulated by the indicated concentrations of growth hormone releasing hormone (GRH1-44 NH_2) for 3 h. Some of the cells were pretreated with 10^{-8} M recombinant mouse leptin (Pepro Tech EC LTD., United Kingdom) for 30 min before the stimulation with leptin. We determined the amount of rat GH released from the cells by radioimmunoassay (RIA). We also administered leptin or saline to rats anesthetized by pentobarbiturate. After 30 min we injected 0.5 μg/kg GRH and determined the GH concentration in serum by RIA.

We also carried out a study on about 35 patients with active acromegaly (16 men and 19 women), 35 patients with GH deficiency (20 men and 15 women) and 87 normal subjects (50 men and 37 women). Body fat was calculated by the bioelectricimpedance method. Serum leptin was measured by RIA (Linco Research, St Charles, MO). IGF-I and urinary c-peptides were measured with commercial RIA kits (Kailon Co.Tokyo for IGF-I and Shionogi Pharmaceutical Co. Tokyo for c-peptide).

All data are expressed as the mean ± SD except as indicated. Significant difference ($P<0.05$) between experimental values was assessed by one-way ANOVA where appropriate. Correlation analysis was performed with Spearman's test. These calculations were carried out with a computer program, Stat View 4.5, Abacus Concepts, Inc., Berkeley, CA.

Results & Discussion

We first investigated leptin mRNA expression in hypophysectomized (hypox) rats in order to clarify the mechanism of obesity in patients with hypopituitarism. The leptin mRNA level in the epididymal fat of hypox rats was significantly lower than that in control rats and the body weight of the hypox rats was about the half that of the control

rats (data not shown). These data confirmed the results in our preliminary report [8]. Boni-Schnetzler *et al.* also reported a decrease in leptin mRNA levels in the fat tissues of hypox rats and they showed that the levels were dependent on body weight [11]. They also reported that GH treatment does not restore the reduced levels of leptin mRNA in hypox rats [11]. Therefore the reduced leptin mRNA in hypox rats is not related to GH deficiency. Glucocorticoid is one possible cause. As administration of glucocorticoid induces *ob* gene expression with a reduction in body weight and food intake in rats [12], glucocorticoid deficiency in hypox rats may decrease *ob* gene expression. Therefore hypox rats are not a good model for the study of GH action on leptin gene expression.

The molecular defect in obese Zucker rats has recently been shown to be similar to that in *db/db* mice and to involve the receptor for leptin [7]. Obese Zucker fatty rats are resistant to leptin treatment, which is similar to most obese humans. As for GH, the synthesis and secretion of GH in the animal decrease after the onset of obesity as seen in obese children [6]. The decrease in GH secretion is not a primary event but may play a role in the persistence of obesity. Zucker rats, therefore, can be more suitable for the study of the GH action on obesity.

First we treated the rats with 1.5 IU/kg GH for 7 days. GH at this dose did not change either body weight or % body fat (Fig. 1 left panel), but the leptin mRNA levels were decreased significantly in epididymal fat tissue but not in subcutaneous fat tissue by the GH treatment, as reported previously [8]. These data indicate that GH action may be specific for visceral fat tissue that is considered to be more important in the etiology of

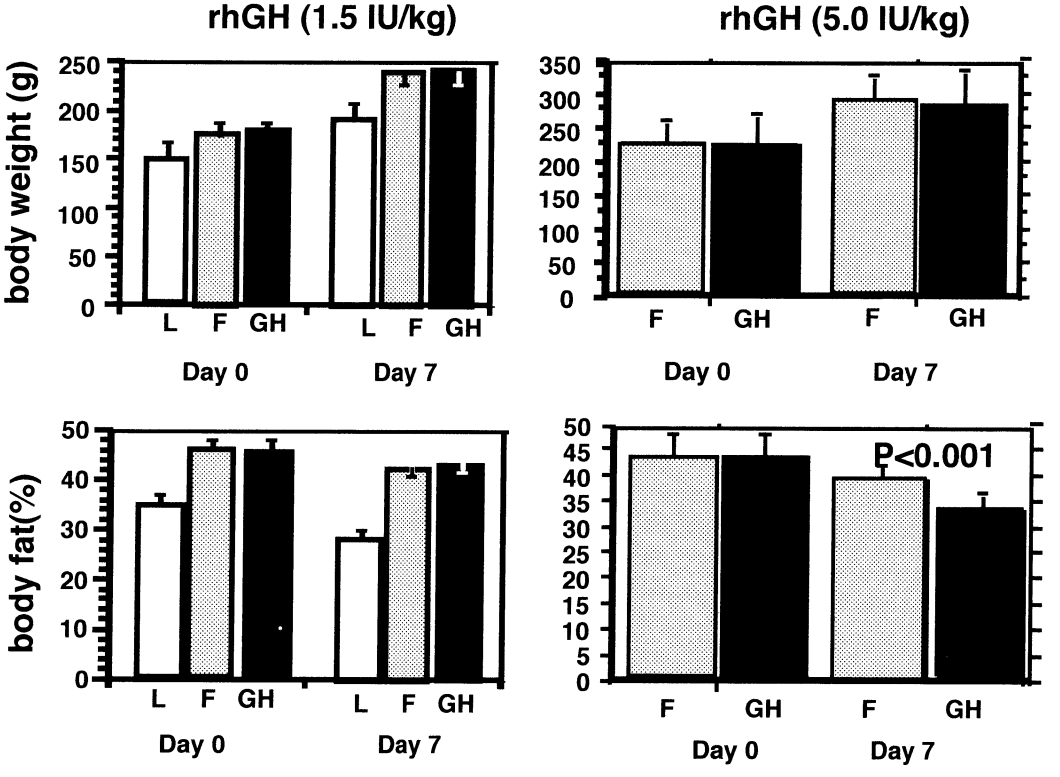

Fig. 1. Effect of growth hormone on body weight and % body fat mass of Zucker rats. The indicated doses of recombinant human GH were administered *i.c.* for 7 days and body weight and % body fat mass were determined as described in Materials and Methods. L indicates the control lean littermates (*Fa/?*) administered saline, F indicates the control fatty Zucker rats (*fa/fa*) given saline and GH indicates fatty Zucker rats given recombinant human GH at the doses indicated. As shown in the lower left panel, GH administration at 5.0 IU/kg decreased % body fat compared to the control fatty Zucker rats which received saline ($P<0.001$).

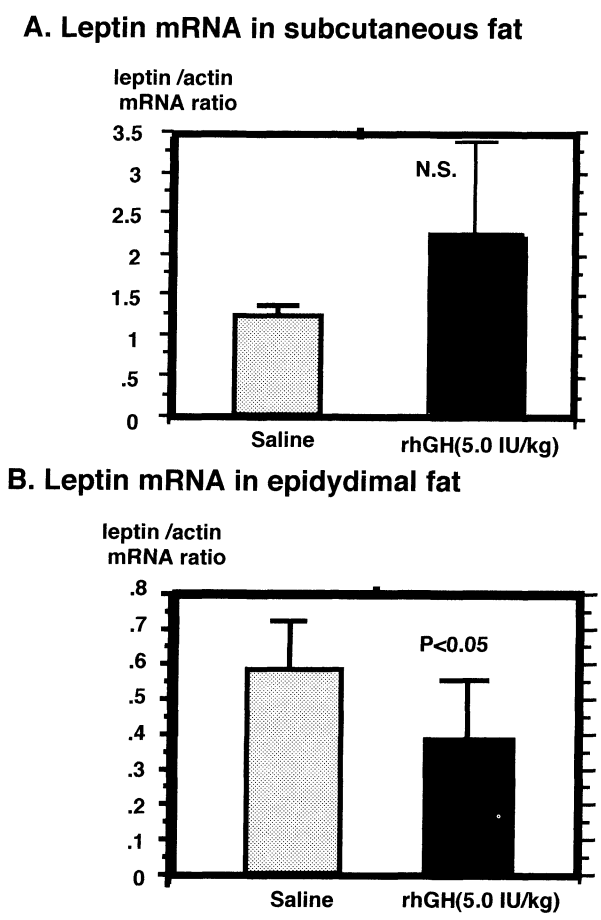

Fig. 2. Effect of growth hormone on leptin mRNA levels in subcutaneous and epididymal fat tissues of Zucker rats. As shown in the figure, administration of growth hormone (5.0 IU/kg) decreased the leptin/actin mRNA ratio in epididymal fat tissue in fatty Zucker rats (lower panel) but GH administration had no effect on the leptin mRNA level in subcutaneous fat tissue (upper panel).

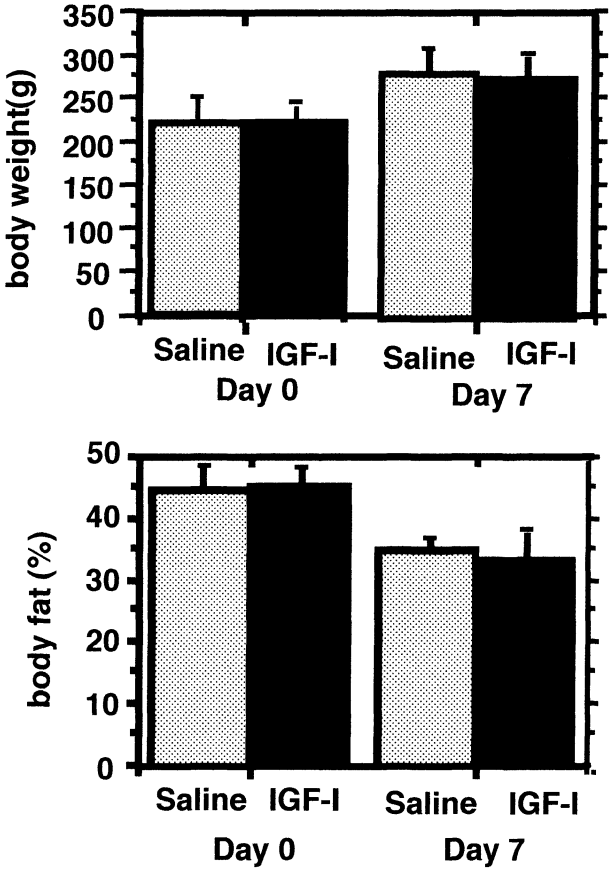

Fig. 3. Effect of recombinant human IGF-I on body weight and % body fat mass of Zucker rats. Recombinant human IGF-I was administered *i.c.* for 7 days (200 μg/day) with an osmotic minipump. The body weight and % body fat mass were determined before and after the experiments. As shown in the figure, IGF-I treatment did not change body weight or % body fat compared to the rats treated with saline.

various diseases. Similar responsiveness of visceral *vs.* peripheral fat tissue to metabolic or hormonal stimuli is reported in human adults as well as Zucker fatty rats [13].

Next we treated the fatty Zucker rats with a higher dose of GH (5.0 IU/kg). The body weight was not changed, as in the experiment with a lower dose of GH (1.5 IU/kg). In contrast in the lower dose experiment, GH treatment decreased % body fat significantly (Fig 1, left panel). GH was thus shown to reduce the fat mass in Zucker fatty rats. We also determined the leptin mRNA levels in these animals. GH at a high dose decreased leptin mRNA levels in epididymal fat tissue but not in subcutaneous fat tissue as GH did at a lower dose (Fig. 2). GH action on leptin gene expression was therefore specific for visceral fat even at a high-dose.

As the majority of the actions of GH are mediated by IGF-I production in peripheral tissues, we infused IGF-I in order to clarify the role of IGF-I in the GH action on fat cells. IGF-I infusion did not change the body weight or % body fat (Fig. 3). The leptin mRNA levels in epididymal and subcutaneous fat tissues were not changed (Fig. 4). Therefore IGF-I is not likely to mediate the action of GH on fat tissues and GH may interact with fat tissues by a direct mechanism and the decrease in the fat mass and leptin expression may be related to its lipolytic activity.

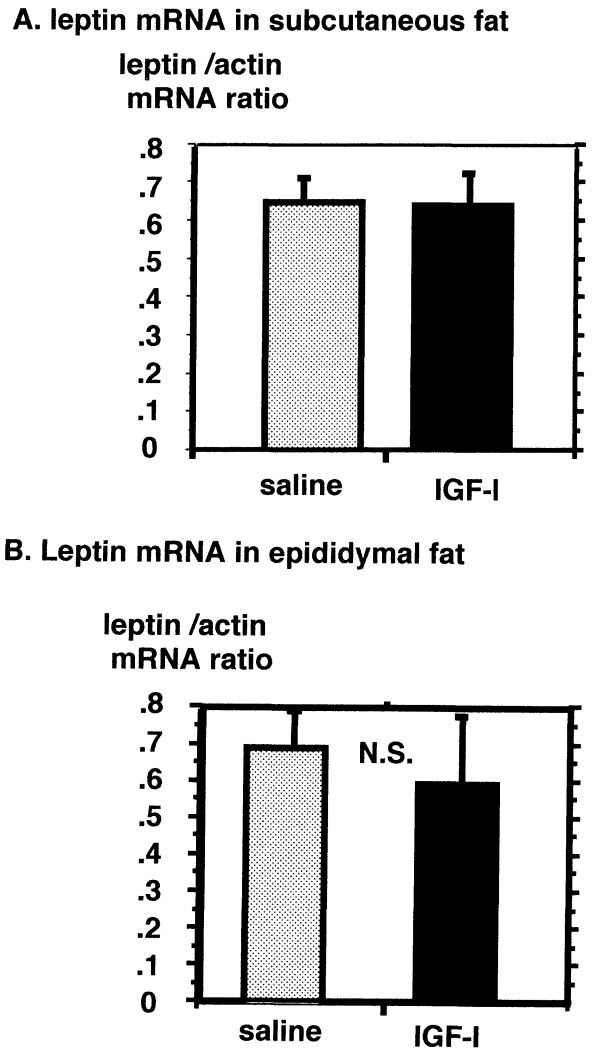

Fig. 4. Effect of IGF-I administration on leptin mRNA levels in fat tissues of Zucker fatty rats. IGF-I was administrated in the same manner as the previous experiments (Fig. 3) and the leptin mRNA levels in the epididymal and subcutaneous fat tissues were determined as described in Materials and Methods. As shown in the figure, leptin mRNA levels were not affected by IGF-I treatment compared the control rats treated with saline.

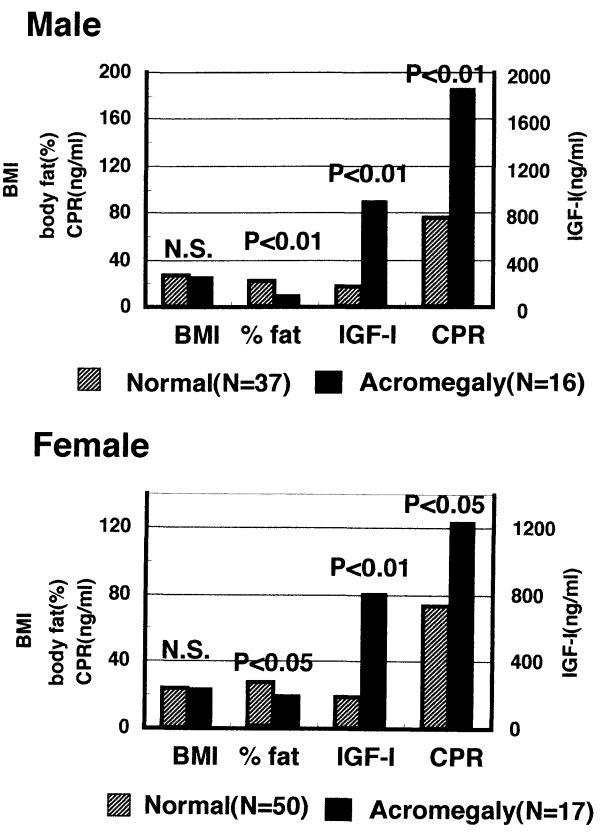

Fig. 5. Comparison of body mass index (BMI), % body fat mass(% fat), the serum IGF-I concentrations and urinary excretion rates of c-peptide (CPR) in patients with acromegaly and control subjects. We could not demonstrate any significant difference in BMI but % body fat was significantly decreased in patients of both genders with acromegaly. Serum IGF-I level and the rate of urinary c-peptide excretion were significantly increased in patients with acromegaly.

We then measured serum leptin concentrations in patients with acromegaly, those with growth hormone deficiency (GHD) and in normal subjects. We could not demonstrate any difference between the patients groups in the body mass index (BMI), but % body fat was significantly decreased in either gender in patients with acromegaly (Fig. 5). Leptin levels in the acromegalic patients were also significantly lower than in normal subjects (4.7 ± 2.7 ng/ml *vs.* 2.3 ± 2.3 ng/ml in males and 11.1 ± 9.1 ng/ml *vs.* 3.8 ± 2.8 ng/ml in females) as expected from the close relationships between fat mass and leptin production. In order to clarify the mechanism of the decrease in leptin levels in acromegalic patients, we selected normal subjects with the same degree of adiposity. As shown in Fig. 6, serum leptin levels were significantly lower in the patients with acromegaly but no statistical difference in % body fat was observed compared to the selected normal subjects in either gender. These data suggests that not the adiposity but the acromegaly itself contributes to the decrease in the leptin level in the patients.

Insulin is one of the positive regulators of leptin

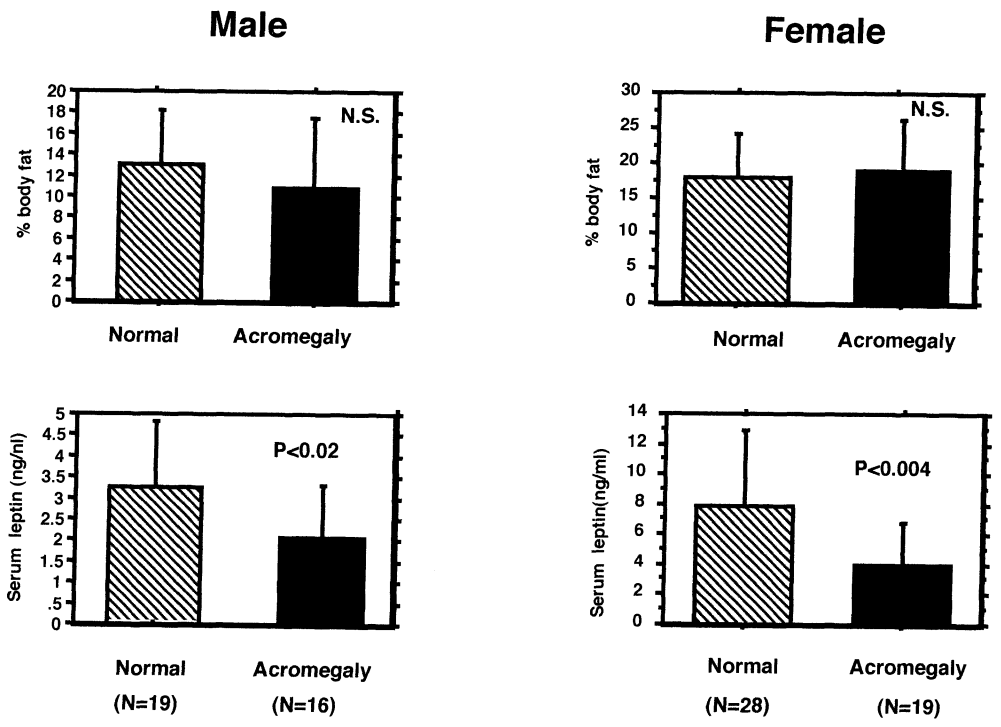

Fig. 6. Comparison of % body fat mass and serum leptin concentration in the acromegalic patients and selected patients with the same adiposity as the acromegalic patients. As shown in the figure, no statistically significant difference in % body fat was observed between the patients groups but the serum leptin concentration was significantly lower in the patients with acromegaly so that something other than adiposity decreases leptin levels in acromegalic subjects.

expression in fat cells [14]. Therefore the increase in the rate of urinary excretion of c-peptide in acromegalic patients may be related to insulin resistance induced by GH. IGF-I, which mediates the action of GH, was higher in the acromegalic patients, but it can increase leptin expression by its insulin-like activity [14]. Therefore the decrease in the leptin level in acromegalic patients is not explained by the IGF-I or insulin level. Considering the action of GH on the fat tissues of Zucker rats, GH itself is the most likely cause of the decrease in leptin production in fat cells because it is able to interact with adipocyte and induces lipolytic activity [1].

In contrast to the acromegalic patients, we could not demonstrate any difference between the serum leptin levels in control subjects and GHD patients (4.7 ± 2.7 *vs.* 5.1 ± 2.5 ng/m*l* in males, 11.1 ± 9.1 *vs.* 11.2 ± 11.2 ng/m*l* in females). This observation is inconsistent with a recent report stating that serum leptin levels are decreased in patients with GHD [15]. The serum leptin concentration is subject to fluctuation due to many factors. Age is one factor that influences the serum leptin concentration, and the serum GH concentration in elderly subjects is lower than in young subjects. As GHD patients as well as control subjects in our study (GHD: 37.1 ± 16.5 y in males and 48.5 ± 21.3 y in females, control: 48.7 ± 13.4 y in males and 44.0 ± 14.0 y in females) are older than those in the previous report, it is reasonable to detect no difference between GHD patients and normal patients in leptin levels in our study. In addition to age, replacement therapy including testosterone is also reported to lower the leptin level in GHD patients as testosterone is a potent inhibitor of leptin production in man [16]. In any case, together with the report that low-dose GH replacement decreases leptin and the fat store in adults with GHD and increases leptin [17], the serum leptin is a useful marker of the GH effect on these patients.

In order to investigate the direct actions of leptin on induced GH secretion, we prepared pituitary cells in culture and treated then with leptin. As

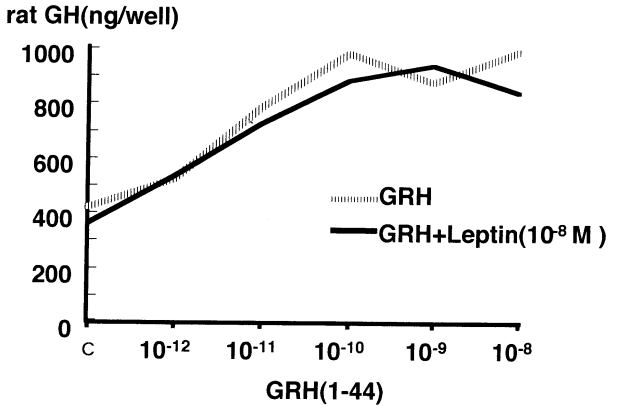

Fig. 7. Effect of leptin on GRH induced GH release from rat pituitary cells in culture. Rat pituitary cells in primary culture were prepared as described in Materials and Methods. The cells were pretreated with recombinant murine leptin (10^{-8} M) for 30 min and then stimulated with rat GRH (1-44NH_3) at the indicated concentrations for 3 h. The amount of GH released from the cells was determined by RIA. The values are the means of six determinations. As shown in the figure, the amount of GH released from the cells was not changed by pretreatment with leptin.

shown in Fig. 7 leptin had no effect on GRH induced GH secretion. Next we also carried out *in vivo* experiments and we found that leptin pretreatment does not change GRH induced GH secretion in fasted rats or fed rats (data not shown). In contrast to our data, secretion of GH in obese patients is often impaired [18] and GH secretion is disturbed in elderly subjects accompanied by a decrease in the leptin level. These observations suggest a role of leptin as a regulator of GH secretion [19]. Moreover, a recent report revealed that anti-leptin antibody abolished GH secretion during the daytime and that leptin administration increased GH secretion in fasted rats [20]. Therefore, leptin may regulate GRH or somatostatin secretion but not GH secretion.

GH therefore had direct effects on fat tissues and reduced leptin gene expression. Leptin may also increase GH secretion, so that understanding these mutual relationships should be important in treating endocrine disorders related to GH abnormalities and non-endocrine disorders such as obesity and hyperlipidemia.

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