Expression of leptin and β_3 -adrenergic receptors in rat adipose tissue in altered thyroid states

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The level of leptin [the *obese* (*ob*) gene product] mRNA is markedly elevated in hypothyroid male rats. The administration of tri-iodothyronine (T_3) to hypothyroid rats resulted in a 40% decrease in leptin mRNA at 8 h. This decrease in leptin mRNA was associated with a parallel decline in circulating leptin levels of about 50% at 24 h. Conversely, β_3 -adrenergic receptor mRNA levels were markedly decreased in epididymal adipose tissue from hypothyroid rats. T_3 administration resulted in a 147% increase at 12 h in β_3 -adrenergic receptor mRNA. There was a

corresponding increase due to T_3 in the lipolytic response to the specific β_3 -adrenergic agonist CL 316,243 that paralleled the increase in β_3 -adrenergic receptor mRNA. T_3 -mediated changes in leptin and β_3 -adrenergic receptor mRNAs were blocked by cycloheximide, suggesting the involvement of short-lived proteins in these effects. The present results indicate that T_3 has opposite effects to those of insulin on the white adipose tissue of rats with respect to leptin mRNA expression.

INTRODUCTION

Leptin is a 167-amino-acid secreted protein, expressed solely in adipose tissue, that is the product of a 4.5 kb mRNA encoded by the *ob* gene [1,2]. A mutation in this gene is responsible for one form of obesity (*ob/ob*) in mice, and another form of obesity in mice (*db/db*) is due to a defect in the receptor for leptin [3]. The expression of leptin mRNA in the adipose tissue of rodents is elevated by insulin [4,5], glucocorticoids [6] or obesity [7], and decreased by starvation [4,7–9]. In obese humans the level of leptin mRNA expression is actually elevated, and there is no evidence for mutations of the *ob* gene [10–13]. However, there is a defect in the β_3 -adrenergic receptor (Trp-64 → Arg) in some obese humans [14–16], and a pathogenic role for enhanced β_3 -adrenergic sensitivity has been postulated in human obesity [17].

The lipolytic response of white adipocytes to forskolin (a non-specific activator of all isoenzymes of adenylate cyclase), as well as that to noradrenaline (which stimulates β_1 -adrenergic receptors at low concentrations and β_3 -adrenergic receptors at higher concentrations), was depressed in adipocytes from hypothyroid rats [18]. The lipolytic sensitivity of adipocytes to a specific β_3 -adrenergic agonist, CL 316,243, is also depressed by hypothyroidism [19]. An increase in mRNA for the β_3 -adrenergic receptor was seen 24 h after the administration of tri-iodothyronine (T_3) to hypothyroid rats [19]. The present studies were designed to determine whether the expression and the plasma concentration of leptin are affected by thyroid status, and to examine the relationship between leptin and β_3 -adrenergic receptor expression.

MATERIALS AND METHODS

Animal treatment and preparation of rat adipose cells

Hypothyroidism was induced by feeding adult male rats for 3 weeks on a low-iodine diet with 6-*N*-propyl-2-thiouracil in the drinking water (62.5 mg/litre), as described by Mills et al. [18]. The iodine-deficient test diet was composed of 27% vitamin-free casein, 56% sucrose, 3% HMW (Hubbell, Mendel, Wakeman)

salt mix without iodine and 14% fat (Crisco), and was obtained in a pelleted form (Purina Mills). T_3 was injected intraperitoneally (25 μ g/100 g body weight) at the indicated times. In the studies shown in Figure 1, hypothyroid rats were compared with euthyroid rats of the same weight and age, which was accomplished by putting fairly large male rats (240–275 g) on the hypothyroidism-inducing diet for only 3 weeks.

The studies on lipolysis were carried out using retroperitoneal adipose tissue; RNA was extracted from epididymal adipose tissue, so that lipolysis and mRNA levels could be examined in the same animals. Retroperitoneal adipose tissue was removed from the dorsal region of the abdominal cavity, with care being taken to exclude any visible brown fat. Tavernier et al. [20] have shown that the lipolytic sensitivity of adipocytes from retroperitoneal adipose tissue to the specific β_3 -adrenergic agonist CL 316,243 is identical to that of cells from epididymal adipose tissue. The amount of hormone-sensitive triacylglycerol lipase activity is also the same in adipocytes from retroperitoneal adipose tissue as in those from epididymal adipose tissue [21].

Isolated adipocytes were prepared as described by Gokmen-Polar et al. [22] and incubated in buffer containing 4% albumin, 50 μ M ascorbic acid (to prevent oxidation of catecholamines) and 0.1 unit/ml adenosine deaminase to remove adenosine, thus preventing activation of G_i . Values for lipolysis in each experiment were based on duplicate samples analysed for glycerol release [22].

Analysis of RNA levels by Northern blotting

The leptin cDNA probe was a 209 bp fragment of mouse leptin cDNA that was cloned by reverse transcription and PCR. A 10 μ g sample of RNA from the fat pads of C57 mice was transcribed by reverse transcriptase and used as a template for amplification by PCR. The PCR primers used were: forward, 5'-TCATCAAGACCATTGTCACCAGG; reverse, 5'-ATTCTCCAGGTCATTGGCTATCTG. This resulted in a 209 bp fragment extending from +101 to +309 (relative to the initiator

Abbreviations used: T_3 , 3,3',5'-tri-iodo-L-thyronine; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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ATG) of mouse leptin cDNA [1]. The PCR product was subcloned into pGEM-T vector and sequenced. The rat β_3 -adrenergic receptor cDNA in pSVL [23] was a gift from Claire M. Frazer (TIGR Inc., MD, U.S.A.). The β_3 -adrenergic receptor probe was excised as a 1.5 kb *SacI*–*BamHI* fragment. The β_1 -adrenergic receptor probe was a 900 bp *PstI* fragment that was excised from the coding region of rat β_1 -adrenergic receptor cDNA [24]. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe was prepared from pTRI-GAPDH vector by religating the linearized plasmid (Ambion Inc.). The closed pTRI-GAPDH plasmid was digested with *SacI* and *BamHI* to generate a 318 bp fragment of rat GAPDH cDNA.

Total cellular RNA was extracted from epididymal fat pads by the guanidine isothiocyanate/ethanol precipitation method [25], and 5 or 25 μ g samples were subjected to electrophoresis on 1.2% agarose/3% formaldehyde gels [26]. The RNA was electroblotted from the gel to nylon membranes (Nytran; Schleicher and Schuell) overnight in 25 mM sodium phosphate, pH 6.5. The blot was prehybridized in 50% formamide, $5 \times$ SSC ($1 \times$ SSC is 150 mM NaCl, 15 mM sodium citrate, pH 7.0), 5% SDS, $2 \times$ Denhardt's solution and 250 μ g/ml sheared salmon sperm DNA at 42 °C for 6 h. After prehybridization, the blot was incubated with 2×10^6 c.p.m./ml radiolabelled probe for 16 h at 42 °C, then washed twice for 5 min in $2 \times$ SSC/0.5% SDS at 25 °C and twice for 15 min in $0.1 \times$ SSC/0.5% SDS at 54 °C. The washed blot was covered with Saran wrap, and subjected to direct counting of radioactivity using the Instant-image analyser (Packard) and then to autoradiography. To determine minor differences in RNA transfers between the lanes, the blot was then stripped of radioactivity as recommended by the manufacturer and rehybridized with a 32 P-radiolabelled rat GAPDH probe. The data for leptin and adrenergic receptor mRNAs were corrected for d.p.m. in the mRNA for GAPDH and expressed as a percentage of d.p.m. in RNA from hypothyroid animals.

The leptin content of 100 μ l aliquots of rat plasma was measured using radioimmunoassay kits with antibody raised against mouse leptin and mouse leptin standards from Linco Research, Inc. (St. Charles, MO, U.S.A.). A similar radioimmunoassay procedure using mouse leptin was used by Sliker et al. [27] to measure the release of leptin by rat adipocytes; these authors demonstrated a parallel dilution curve using serum from obese Zucker rats. The values obtained are in mouse equivalents, since rat leptin is not yet available to use as a standard.

RESULTS AND DISCUSSION

The average body weight of the euthyroid rats and hypothyroid rats was 336 ± 8 g and 328 ± 7 g respectively (means $\pm$ S.E.M. of 11 different groups of animals; 2 rats per group). The epididymal fat pad weight per rat was 3.7 ± 0.3 g for hypothyroid rats and 3.3 ± 0.2 g for euthyroid rats.

The data in Figure 1 demonstrate that expression of the mRNA for leptin was elevated in epididymal adipose tissue from hypothyroid rats compared with that euthyroid and hyperthyroid rats. In contrast, the level of β_3 -adrenergic receptor mRNA was lower in adipose tissue from hypothyroid rats than in that from controls (Figure 1).

The administration of T_3 to hypothyroid rats reversed the elevation in the level of leptin mRNA within 8 h (Figure 2). In a larger series of experiments ($n = 5$) we found that T_3 administration resulted in a 40% decrease in leptin at 8 h, and no greater decrease was seen at 12 or 24 h (Table 1). While T_3 administration to rats results in a more rapid induction of S14 mRNA in liver, there is no effect of T_3 on the expression of S14 mRNA in

Figure 1 Influence of altered thyroid states on leptin and β_3 -adrenergic receptor mRNA levels in rat adipose tissue

Hyperthyroidism was induced by the injection into normal male rats of 25 μ g of T_3 /100 g body weight 24 and 48 h before the rats were killed. In (A), 5 μ g of RNA was probed for leptin mRNA expression; in (B), 25 μ g of RNA was probed for β_3 -adrenergic receptor mRNA expression. The intensities of the bands were compared using a laser densitometer and the relative densities were as follows. β_3 -Adrenergic receptor density mRNA in epididymal adipose tissue was 50 units in hypothyroid, 155 units in euthyroid and 155 units in hyperthyroid rats. Leptin mRNA density in epididymal adipose tissue was 90 units in hypothyroid, 55 units in euthyroid and 15 units in hyperthyroid rats. The data are based on a representative gel prepared from RNA samples obtained by pooling the adipose tissue of 12 hypothyroid, seven euthyroid and eight hyperthyroid rats. The positions of 18 S and 25 S rRNA markers are indicated, and sizes (kb) of several standards are also shown.

epididymal adipose tissue at 4 h, but an increase can be seen at 8 h [28]. The present results indicate that the decrease in leptin mRNA is as rapid as the previously reported induction of S14 mRNA in adipose tissue [28].

The effects of T_3 on leptin mRNA in epididymal adipose tissue were accompanied by changes in serum leptin levels. The concentration of immunoreactive rat leptin (measured in mouse leptin units) was 2.06 ± 0.33 ng/ml in hypothyroid rats, and these levels had decreased by $\sim 50\%$ to 1.04 ± 0.18 at 24 h after T_3 administration (Figure 2C). These data indicate that the decrease of leptin mRNA levels due to T_3 was reflected in the circulating levels of leptin.

The administration of T_3 to hypothyroid rats also reversed the decrease in β_3 -adrenergic receptor mRNA. The increase in β_3 -adrenergic receptor mRNA in epididymal fat was not marked until 24 h after T_3 administration (Figures 3A and 3B). Pooled data from five pairs of animals revealed that T_3 increased β_3 -adrenergic receptor mRNA by 147% at 12 h and by 206% at 24 h (Table 1).

We measured cAMP and lipolysis in isolated adipocytes incubated with the β_3 -specific adrenergic agonist CL 316,243 (Figures 3C and 3D). Treatment with T_3 did not affect epididymal fat pad weight. In the experiment shown in Figure 3, the fat pad weight at 0, 8, 12, 48 and 72 h after injection of T_3 was 3.0, 3.5, 3.1, 3.6 and 3.2 g respectively, based on the average from two animals for each time point. There was an increase in cAMP and lipolysis at 24 h but not at 12 h after the administration of a single dose of T_3 to hypothyroid rats. No further increases were seen at 48 or 72 h (Figures 3C and 3D). In a larger series of experiments ($n = 5$), T_3 enhanced lipolysis in response to β_3 -

Figure 2 Time courses of the T_3 -induced decreases in adipose tissue leptin mRNA and plasma leptin levels

(A) RNA was isolated from the epididymal adipose tissue of hypothyroid rats at the indicated times after injection of the rats with T_3 . The blot was probed for leptin mRNA, and then stripped from the first probe and reprobed with a rat GAPDH probe. (B) An expanded time course for the effect of T_3 on leptin mRNA was generated as described for (A). The d.p.m. of ^{32}P incorporated into each band of leptin or GAPDH was obtained, and the ratio of leptin d.p.m. relative to GAPDH d.p.m. was determined for each lane. These ratios were divided by the leptin/GAPDH ratio in RNA from hypothyroid rats to calculate the percentage change in leptin mRNA after T_3 administration. (C) Plasma leptin in hypothyroid rats is shown in relative mouse units. The zero-time value is the mean $\pm$ S.E.M. for ten rats, the 12 h value that for seven rats and the 24 h value that for six rats; the 48 h value is the mean $\pm$ range for two rats.

adrenergic receptor activation by 10 nM CL 316,243 by 61% at 12 h and by 160% at 24 h (Table 1). The action of T_3 on β_3 -adrenergic receptors was specific, as there was no change in adipocyte GAPDH levels (Table 1) or β_1 -adrenergic receptor mRNA at 24 h after injection of T_3 (results not shown), which confirms the results from previous studies on the regulation of β_1 -adrenergic receptors by T_3 in adipocytes [29].

We examined the effect of blocking DNA transcription on the half-life of leptin mRNA and β_3 -adrenergic receptor mRNA in rats with altered thyroid states. We used 150 μ g of actinomycin D/100 g body weight, because our previous results have shown that this dose of actinomycin D is sufficient to down-regulate β_1 -

Table 1 Time course of the effects of T_3 on metabolism in adipocytes from hypothyroid rats

The values are percentage changes compared with uninjected hypothyroid controls, and are means $\pm$ S.E.M. from five experiments. Lipolysis is that due to β_3 -adrenergic receptor activation, and was measured by incubating isolated adipocytes for 20 min in the presence of 10 nM CL 316,243. The data for both leptin and β_3 -adrenergic receptor mRNAs are corrected for GAPDH mRNA, which was used as the internal recovery standard. Significant effects of T_3 are indicated by: * P < 0.05, ** P < 0.025; *** P < 0.01.

Time after T_3 (h)	Change due to T_3 (%)			
	β_3 -Adrenergic receptor mRNA	Leptin mRNA	GAPDH mRNA	Lipolysis
8	55 $\pm$ 39	-40 $\pm$ 10**	3 $\pm$ 9	1 $\pm$ 20
12	147 $\pm$ 40**	-45 $\pm$ 9***	-2 $\pm$ 6	61 $\pm$ 20*
24	206 $\pm$ 70*	-36 $\pm$ 9**	-1 $\pm$ 24	160 $\pm$ 55*

adrenergic receptor mRNA in rat salivary glands [30]. Moreover, Granneman and Lahners [31] reported that, in cultured 3T3-F442 adipocytes, exposure to 5 μ g/ml actinomycin D resulted in a loss of 25% of the β_3 -adrenergic receptor mRNA after 30 min and of 50% after 90 min. The down-regulation of β_3 -adrenergic receptor mRNA *in vivo* in rat fat pads was slow. We found only a 60% loss of mRNA for the β_3 -adrenergic receptor at 6 h in adipose tissue from euthyroid or hypothyroid rats (Table 2). At 4 h there was only an 18% decrease in mRNA in adipocytes from hypothyroid rats and a 30% loss in adipocytes from euthyroid rats. The reason for the slower loss of β_3 -adrenergic receptor mRNA in intact rats is unclear, but continuation of the experiments to longer periods of exposure to actinomycin D resulted in the death of the rats. Nevertheless, these data reveal that the rates of loss of β_3 -adrenergic receptor mRNA in hypothyroid and euthyroid rats are comparable, suggesting the involvement of a post-transcriptional mechanism in the effects of T_3 on β_3 -adrenergic receptor mRNA. The data in Table 2 also indicate that leptin mRNA levels in epididymal rat fat pads are insensitive to the administration of actinomycin D *in vivo*.

The possible involvement of short-lived proteins induced by T_3 in the regulation of leptin and β_3 -adrenergic receptor mRNA levels was examined using cycloheximide as an inhibitor of protein synthesis. Wong and Oppenheimer [32] demonstrated that the slow induction of S11 mRNA in the livers of hypothyroid rats by T_3 was blocked by the administration of cycloheximide (1 mg/100 g body weight). We used their experimental design, in which rats are first given T_3 , then injected with cycloheximide 6 h later and killed 8.5 and 14.5 h after the T_3 injection (Figure 4). There was no effect of cycloheximide on β_3 -adrenergic receptor mRNA if given at 6 h after T_3 with killing at 8.5 h. Our results were identical to those of Wong and Oppenheimer in that the up-regulation of β_3 -adrenergic receptor mRNA at 14.5 h was blocked by cycloheximide administered 6 h after T_3 injection (Figure 4B). Similar results were obtained with regard to leptin mRNA, in that the down-regulation of this message at 14.5 h was also blocked by administration of cycloheximide (Figure 4A). These data suggest that the effects of T_3 on both leptin mRNA and β_3 -adrenergic receptor mRNA *in vivo* involve antecedent regulatory proteins. Therefore, as for most other metabolic enzymes induced by thyroid hormones, leptin regulation probably involves short-lived protein(s) and is a post-transcriptional effect. The one complication is the possibility of *in vivo* effects of cycloheximide that are unrelated to the inhibition of protein synthesis.

Figure 3 Time courses of the T_3 -induced increase in β_3 -adrenergic receptor mRNA in adipose tissue and of β_3 -agonist-mediated cAMP accumulation and lipolysis in isolated adipocytes

(A) Changes in β_3 -adrenergic receptor (β_3 -AR) and GAPDH mRNAs in 25 μ g of RNA extracted from the epididymal fat pads of hypothyroid rats at the indicated times after injection of T_3 . (B) The change in the ratio of β_3 -adrenergic receptor mRNA relative to GAPDH mRNA for each time period was calculated. The data represent the percentage change in β_3 -adrenergic receptor mRNA relative to that in hypothyroid rats. (C) Rat adipocytes (170 000 per ml) from the same animals were suspended in 4% albumin and incubated for 20 min with 1 or 10 nM CL 316,243 (a specific β_3 -adrenergic agonist), and cAMP was determined at the end of the incubation. (D) Lipolysis is shown as glycerol release over a 20 min incubation in the presence of 1 or 10 nM CL 316,243.

Our data indicate that T_3 is a potent regulator of leptin and β_3 -adrenergic receptor mRNAs, but whether this is a direct or indirect effect is unclear from *in vivo* experiments. In hypothyroid rats the secretion of growth hormone is impaired [33], but no effects of growth hormone have yet been reported on leptin mRNA. The ability of insulin to stimulate adipocyte metabolism

Table 2 Effects of actinomycin D on levels of leptin and β_3 -adrenergic receptor mRNAs

Rats were injected with actinomycin D (150 μ g/100 g body weight), and killed 4 or 6 h later. The RNA extracted from the epididymal adipose tissue was fractionated on formaldehyde/agarose gels, and the mRNA content was analysed using labelled probes as described in the Materials and methods section.

Animals	Time after actinomycin D (h)	mRNA level (% of control)	
		Leptin mRNA	β_3 -Adrenergic receptor mRNA
Hypothyroid	4	94	82
Euthyroid	4	97	70
Hypothyroid	6	84	40
Euthyroid	6	91	40

is also depressed in hypothyroid rats [34]. However, enhancement of the insulin response by T_3 would have the opposite effect, since insulin elevates leptin mRNA in adipose tissue [4,5].

The administration of leptin to *ob/ob* mice corrects the observed hypometabolism, based on oxygen consumption and rectal temperature, in addition to causing a reduction in food intake [35]. There is a greater loss of weight (mostly fat) in *ob/ob* mice given leptin as compared with pair-fed controls, supporting the hypothesis that leptin increases the metabolic rate in addition to suppressing appetite [36]. The *ob/ob* mouse is hypothyroid [37,38], which is probably secondary to low levels of serum thyrotropin, and it also has low levels of serum prolactin, growth hormone, luteinizing hormone and follicle-stimulating hormone [39,40]. In contrast, the *ob/ob* mouse apparently has elevated levels of corticotropin ('ACTH'), as plasma corticosterone is markedly elevated and this parameter can be normalized by treatment with leptin [41]. These data indicate that, with the exception of corticotropin, there is a defective release of pituitary hormones which probably accounts for the hypometabolism and infertility of the *ob/ob* mouse. Possibly the increase in leptin formation in adipocytes in hypothyroid rats serves as an unrecognized stimulus in the hypothalamus for the release of thyrotropin-releasing hormone, which in turn enhances the secretion of thyrotropin by the anterior pituitary. However, in rats made hypothyroid by blocking the organification of iodine by the thyroid with propylthiouracil and feeding rats on a low-iodine diet, there is markedly impaired formation and release of thyroid hormones. When T_3 is administered there was a rapid correction (within 8 h) of the elevation in leptin mRNA in adipocytes (Table 1). Our data suggest that thyroid hormones should be added to the list of agents that affect leptin mRNA expression but, unlike insulin and glucocorticoids, T_3 inhibits rather than enhances leptin mRNA formation.

The choice of GAPDH as the 'housekeeping' mRNA may not have been ideal, since there is evidence for the regulation of this gene by T_3 in the liver [42]. However, examination of the data in Figures 2–4 and Table 1 indicates that there was little effect of T_3 on adipocyte GAPDH mRNA under the conditions of these experiments.

In mice, the *in vivo* administration of a selective β_3 -adrenergic agonist (CL 316,243) resulted in a marked decrease in serum leptin and adipose tissue leptin mRNA at 12 h but not at 6 h, and similar results were seen in mice deprived of food for the same period of time [43]. Trayhurn et al. [44] previously reported that

Figure 4 Inhibition by cycloheximide of the effects of T₃ administration on β_3 -adrenergic receptor mRNA and leptin mRNA

Hypothyroid rats were injected with T₃ (25 μ g/100 g body weight), and after 6 h cycloheximide (cyclo; 1 mg/100 g body weight) was also injected. The animals were killed 2.5 h or 8.5 h after the cycloheximide injection. (A) Northern blot analysis of the effect of cycloheximide on T₃-induced changes in leptin and β_3 -adrenergic receptor (β_3 -AR) mRNAs from one of the experiments. (B) The percentage change in leptin or β_3 -adrenergic receptor mRNA in treated rats relative to untreated hypothyroid rats is shown, corrected for GAPDH. The data are means $\pm$ range of two paired replications.

Table 3 Relationship between the effects of various conditions on leptin mRNA, lipolysis and β_3 -adrenergic receptor mRNA in rodent adipocytes

Condition	β_3 -Adrenergic receptor mRNA	Lipolysis	Leptin mRNA
Hypothyroidism	Down-regulated (Figure 1)	↓ [18,19,29]	Up-regulated (Figure 1)
Insulin	Down-regulated [46]	↓ [22]	Up-regulated [4,5,48]
Glucocorticoids	Down-regulated [31,47]	↑ [45]	Up-regulated [6,27,49]
Fasting	?	↑	Down-regulated [4,7,8]
Catecholamines	Down-regulated [31]	↑ [18,19,29]	Down-regulated [27,43,44]
Hyperthyroidism	Up-regulated (Figure 1)	↑ [18,19,29]	Down-regulated (Figure 1)

administration of isoprenaline to intact mice resulted in an almost complete disappearance of leptin mRNA from adipose tissue within 4.5 h. These results raise the question of whether the inhibition of leptin mRNA formation and leptin release by T₃ is mediated through enhanced release of catecholamines at nerve endings in adipose tissue. However, we have seen a 50 % increase in the release of leptin over a 24 h incubation by adipocytes from hypothyroid rats as compared with euthyroid rats, and a decrease of 30–50 % in the level of leptin mRNA after a 24 h incubation

of adipocytes from hypothyroid rats with 10 nM T₃ (J. N. Fain and S. W. Bahouth, unpublished work). Furthermore, the ability of T₃ to enhance the lipolytic sensitivity of adipocytes to adrenergic stimulation was not seen until 12 h after T₃ administration, while leptin mRNA in adipose tissue was reduced within 8 h of T₃ treatment (Table 1). In humans it has been reported that the leptin mRNA content of abdominal subcutaneous adipose tissue was not affected by 5 days of caloric restriction or by 3 h of insulin infusion, suggesting that leptin is not a tightly regulated factor in humans as it is in rodents [50].

A summary of the effects of various conditions on leptin mRNA, lipolysis and β_3 -adrenergic receptor mRNA is given in Table 3. The correlation between the down-regulation of β_3 -adrenergic receptor mRNA and the up-regulation of leptin mRNA seen in the hypothyroid state, as well as in the presence of insulin or glucocorticoids, is of interest.

It is unclear whether the down-regulation of leptin mRNA in fasting animals is secondary to the decrease in insulin, or whether it might involve increases in catecholamines. The down-regulation of leptin mRNA by thyroid hormones and the up-regulation by glucocorticoids may represent feedback effects of these hormones on adipocytes. In fasted mice the level of leptin is markedly depressed, as are the levels of thyroid hormones, while those of glucocorticoids are elevated [51]. The administration of leptin to fasted mice largely prevents these changes [51], which supports the results from the mice deficient in leptin, which have high levels of glucocorticoids and low levels of thyroid hormones. Ahima et al. [51] suggested that regulation of

the neuroendocrine system during starvation is the main physiological role of leptin in rodents. If leptin is a stimulus for thyroid hormone release, then the ability of thyroid hormones to inhibit leptin release suggests an inhibitory feedback role for these hormones. Conversely, a lack of leptin results in enhanced glucocorticoid secretion, which stimulates leptin release by adipocytes, resulting in a negative-feedback effect on corticotropin release.

The stimulation of leptin mRNA by glucocorticoids [6,27,49] is paradoxical, since glucocorticoids cause obesity in rodents [52] and enhance lipolysis in adipocytes. However, if leptin is more important as a neuroendocrine regulator than as an appetite suppressant, then the increase in leptin release by adipocytes might have a negative-feedback effect on corticotropin release.

This work was supported by National Institutes of Health grants RO1 HL-48169 and T32 HL07641.

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