Effect of tri-iodothyronine on leptin release and leptin mRNA accumulation in rat adipose tissue

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Leptin, the product of the *obese* gene, is produced by white adipocytes. The release of leptin, as well as leptin mRNA content, was enhanced in adipocytes isolated from hypothyroid rats. The administration of tri-iodothyronine (T_3) 8 h before death inhibited leptin release by adipocytes incubated for 6 or 24 h. Direct addition of T_3 to pieces of adipose tissue enhanced the loss of leptin mRNA seen over 24 h in the presence of dexamethasone plus the β_3-adrenergic agonist Cl 316,243. In contrast, if pieces of adipose tissue were incubated with dexamethasone plus insulin, enhanced the T_3 accumulation of leptin mRNA. These results indicate that T_3 enhances net adipocyte leptin mRNA accumulation in a condition that approximates the fed state (presence of insulin) but inhibits leptin mRNA accumulation in a condition that approximates the fasted state (absence of insulin).

INTRODUCTION

Major regulators of leptin mRNA accumulation as well as leptin release appear to be glucocorticoids, since dexamethasone (a synthetic glucocorticoid agonist) stimulates leptin release by rodent adipocytes in primary culture [1,2]. However, in fasted mice, plasma leptin decreases while plasma glucocorticoids as well as adrenocorticotropin are increased [3]. Leptin is apparently a negative regulator of adrenocorticotropin release while acting as a positive regulator of the release of other pituitary hormones. Ahima et al. [3] reported that the administration of leptin to fasted mice reversed the decreases in testosterone, luteinizing hormone, and thyroxine as well as the increases in plasma adrenocorticotropin and corticosterone.

Thyroid hormone status also influences leptin, since the levels of plasma leptin and adipose-tissue leptin mRNA are elevated in hypothyroid as compared with euthyroid rats [4]. The administration of tri-iodothyronine (T_3) to hypothyroid rats reduced adipose-tissue mRNA content by 40 % at 8 h without significantly affecting lipolysis [4]. However, Yoshida et al. [5] reported that T_3 enhanced the accumulation of leptin mRNA as well as leptin release by mouse 3T3-L1 cells. One problem is that the level of leptin expression in 3T3-L1 and 3T3-F442A cells is less than 1 % of that in intact adipose tissue [6,7]. The present studies were designed to determined whether enhanced leptin release and mRNA content is observed in adipocytes from hypothyroid rats in primary culture and whether T_3 directly inhibits leptin formation and release by rat adipocytes and adipose tissue.

MATERIALS AND METHODS

Preparation of rat adipocytes

Hypothyroidism was induced by feeding 240–275 g male Sprague–Dawley rats for 3 weeks on a low-iodine diet with 6-*N*-propyl-2-thiouracil in the drinking water (62.5 mg/l), as described by Fain et al. [4]. The body weight of the hypothyroid rats after 3 weeks on the diet was 335 ± 6 g (mean $\pm$ S.E.M. for 20 rats) which was comparable with that of age-matched euthyroid rats (330 ± 6 g; mean $\pm$ S.E.M. for 20 rats). Each experiment utilized pooled adipocytes or tissue from two rats.

Adipocytes were prepared by digestion of the epididymal adipose tissue from each rat in 5.5 ml of buffer containing 1 mg/ml bacterial collagenase (*Clostridium histolyticum* CLS1; 238 units/mg; from Worthington Biochemical, Freehold, NJ, U.S.A.). The BSA contained less than 0.05 mol of fatty acids/mol of albumin (Bovuminar; lot L59410; from Intergen, Purchase, NY, U.S.A.).

The buffer for digestion of adipose tissue was Dulbecco's modified Eagle's medium/Ham's F12 (1:1, Sigma, catalogue no. 2906) containing 17.5 mM glucose, 121 mM NaCl, 4 mM KCl, 1 mM $CaCl_2$, 25 mM Hepes, 2.4 mM $NaHCO_3$, 40 mg/ml albumin, 5 μg/ml ethanolamine, 0.1 ng/ml sodium selenite, 90 μg/ml penicillin G and 150 μg/ml streptomycin sulphate. Ascorbic acid (55 μM), leupeptin (10 μg/ml) and aprotinin (10 μg/ml) were added to the buffer for the 24 h incubation. Adipocytes were isolated and incubated under sterile conditions in 50 ml polypropylene tubes incubated on their side and shaken at 11 rev./min in a gyratory water bath. The incubation of the adipocytes (600000 to 1000000 per tube) was in 10 ml of medium for 24 h, adipocytes obtained from two rats being divided between 13 or 14 tubes. Measurement of lipolysis was based on analysis of glycerol release into the medium, and determined on 10 μl aliquots of the medium by the procedure of Boobis and Maughan [8]. The leptin content of 100 μl aliquots of incubation medium or rat serum was measured using RIA kits with antibody raised against rat leptin standards from Linco Research Inc. (St. Charles, MO, U.S.A.).

In studies using intact pieces of adipose tissue, the epididymal fat from two rats was cut into small pieces with scissors and divided between 13 tubes (400–600 mg/tube). Approx. 15–20 pieces of adipose tissue were present in each tube with 10 ml of medium, and the tubes were shaken on their side at 53 rev./min.

Measurement of leptin mRNA

Analysis of RNA levels was by Northern blot. The leptin cDNA probe was a 209 bp fragment of mouse leptin cDNA (nucleotides

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

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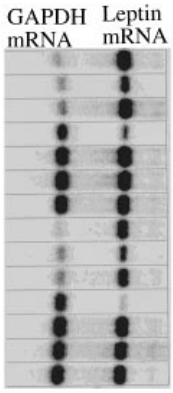

Condition	18S RNA	Leptin mRNA	GAPDH mRNA
#1 Zero time	100%	100%	100%
#2 6 h	93%	45%	109%
#3 6 h + DEX	113%	104%	113%
#4 24 h	104%	23%	233%
#5 24 h + DEX	173%	74%	228%
#6 24 h + DEX	161%	66%	262%
#7 24 h + DEX	164%	52%	223%
#8 Zero time	100%	100%	100%
#9 6 h	79%	56%	164%
#10 6 h + DEX	66%	129%	241%
#11 24h	92%	19%	358%
#12 24 h + DEX	109%	137%	453%
#13 24 h + DEX	89%	122%	453%
#14 24 h + DEX	94%	112%	470%

Figure 1 Concurrent Northern-blot analysis of leptin and GAPDH mRNAs

The data are from one of the five paired experiments illustrated in Figures 2 and 3. Lanes 1–7 are for adipocytes from control rats, and lanes 8–14 for adipocytes taken from T_3-treated rats. 18S RNA content was determined by analysis of ethidium bromide staining. Insulin at 10 nM was present in all tubes and dexamethasone (DEX) was present where indicated. The values for leptin and GAPDH are corrected for recovery of 18S RNA and expressed as percentage of the zero-time values.

+101 to +309) that was cloned by reverse transcription and PCR [4]. The rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe was prepared from pTRI-GAPDH vector by religating the linearized plasmid (Ambion Inc.) [4].

Total cellular RNA was extracted from adipocytes and adipose tissue by the procedure of Chomczynski and Sacchi [9] and dissolved respectively in 100 and 200 μl of water. A 20 μl portion of the RNA extract was subjected to electrophoresis on 1.2% agarose/3% formaldehyde gels [4]. The gels were washed extensively with water to remove the formaldehyde and then photographed under UV transillumination (Deltaimager). The digital images were stored as tagged image file format files and analysed by the NIH Image program, which calculates the relative intensities of 18S RNA bands. The gels were then transferred to Nytran membranes in buffer containing 3 M NaCl plus 0.3 M sodium citrate, pH 7.0, using a Turbo blotter (Schleicher and Schuell, Keene, NH, U.S.A.). After transfer, the blots were cross-linked by UV irradiation in a Stratalinker (Stratagene, LaJolla, CA, U.S.A.) and air-dried to fix the RNA to the Nytran membrane. The blots were prehybridized as previously described [4] and then incubated simultaneously with 2×10^6 c.p.m./ml radiolabelled leptin probe plus 2.5×10^5 c.p.m./ml GAPDH probe for 18–20 h at 42 °C.

The blots were analysed by electronic autoradiography for 8–40 h using a Packard Instantimager, which has 210240 microchannels combed in a 20 cm × 24 cm multilayer electron-avalanche amplification plate that detects, collimates and preamplifies ionizing radiation for read-out with an interpolating Multi-Wire Proportional Chamber. The proprietary X–Y signal-decoding, digitization and centroiding circuitry ensures a linear counting range of 1.2 million c.p.m. and high spatial read-out precision. The total counts under each peak were determined after subtraction of background counts. The direct quantification of the hybridized messages, the more consistent transfer of RNA to Nytran membranes using the Turbo blotter, and the fact that each experiment involving 13 or 14 experimental conditions was analysed on a single gel allowed a quantitative procedure. A digital image of a gel from a single experiment is shown in Figure 1 along with data on the recovery of 18S RNA in each lane plus the leptin and GAPDH mRNA content of each lane.

RESULTS

Increase in leptin release and leptin mRNA in adipocytes from hypothyroid rats

Serum leptin in hypothyroid rats was found to be 6.9 ± 0.6 ng/ml (mean ± S.E.M. for 12 experiments), whereas in euthyroid rats it was 3.8 ± 0.4 ($n = 6$). This effect of hypothyroid status on leptin levels was statistically significant ($P < 0.001$). An enhanced release of leptin was also seen in 11 experiments using isolated adipocytes from hypothyroid rats over a 4 h incubation. In these experiments, leptin release in ng/10^6 adipocytes was 45 ± 2 (mean ± S.E.M.) in cells from hypothyroid rats compared with 31 ± 3 in cells from euthyroid rats ($P < 0.001$).

The amount of leptin mRNA expressed as the ratio of counts in leptin mRNA divided by that in GAPDH mRNA in adipocytes from hypothyroid rats was 2.5-fold higher than in adipocytes from euthyroid rats at the end of a 24 h incubation (0.13 ± 0.03 in hypothyroid compared with 0.05 ± 0.01 in euthyroid rats; means ± S.E.M. for eight paired experiments). The change due to 25 nM dexamethasone (mean ± S.E.M. for the paired differences in these eight experiments) was $+665 \pm 270$% in adipocytes from hypothyroid rats and $+206 \pm 74$% in those from euthyroid rats at 24 h.

Effect of *in vivo* T_3 treatment on subsequent leptin release and leptin mRNA in isolated adipocytes

In the next series of experiments, we injected hypothyroid rats with 25 μg of T_3/100 g body weight 8 h before killing them. The administration of T_3 to hypothyroid rats resulted in a 32 ± 8% (mean ± S.E.M. for five paired experiments; $P < 0.025$) decrease in plasma leptin from 5.5 ± 0.7 ng/ml in untreated rats to 3.7 ± 0.8 ng/ml in T_3-treated animals. The adipocytes from control or T_3-treated hypothyroid rats were cultured in the presence of 10 nM insulin with and without dexamethasone (Figure 2). The basal rate of leptin release by adipocytes isolated from T_3-treated rats and incubated in the presence of 10 nM insulin (Figure 2) was reduced by 36 ± 8% at 6 h and 21 ± 6% at 24 h ($P < 0.025$ by paired comparisons). There was also a

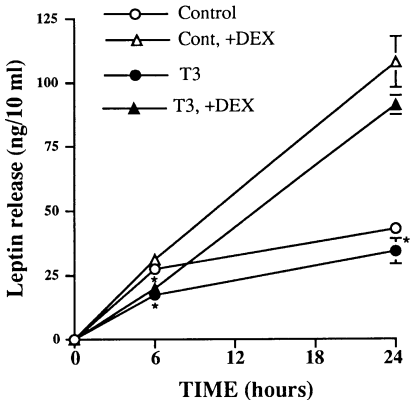

Figure 2 Effect of prior T_3 administration on leptin release by adipocytes

Rat adipocytes (750000 in control and 770000 in T_3-treated hypothyroid rats) were incubated for 6 or 24 h in primary culture with 10 nM insulin in the absence (○, ●) or presence (△, ▲) of 25 nM dexamethasone. Adipocytes from rats injected with 25 μg of T_3/100 g body weight 8 h before they were killed are indicated by filled symbols and those from uninjected hypothyroid controls by open symbols. The values are means ± S.E.M. for five paired experiments. Statistically significant ($P < 0.05$) decreases in leptin release caused by T_3 treatment based on paired comparisons are indicated by an asterisk.

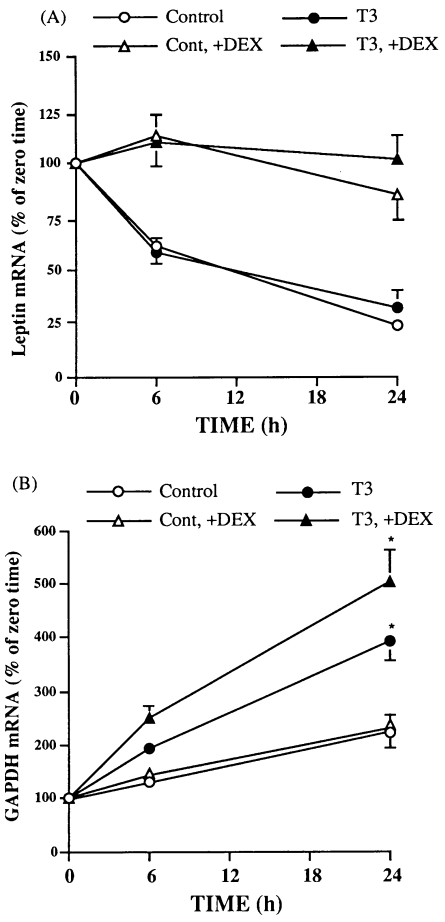

Figure 3 Effect of prior T_3 administration on accumulation of GAPDH mRNA (B) and leptin mRNA (A) in incubated adipocytes

The values for GAPDH and leptin mRNA are corrected for 18S RNA recovery and expressed as percentage of the zero-time value (mean ± S.E.M. from the five paired experiments shown in Figure 2). Statistically significant effects ($P < 0.05$) of T_3 treatment based on paired comparisons are indicated by an asterisk.

reduction in leptin release from adipocytes prepared from T_3-treated animals and exposed to dexamethasone in culture (Figure 2). The decrease in leptin release in the presence of dexamethasone was statistically significant only at 6 h ($P < 0.05$) in rats treated with T_3.

The level of leptin mRNA expressed as percentage of the amount present in adipocytes at the start of the incubation was markedly reduced after 24 h of culture, but this could be prevented in the presence of 25 nM dexamethasone (Figure 3A). However, the decrease in leptin release by adipocytes from hypothyroid rats previously injected with T_3 was not reflected in any change in the leptin mRNA content in either the absence or presence of dexamethasone (Figure 3B).

Initially we used GAPDH mRNA as a reference standard for recovery of mRNA and expressed the data in each experiment as the ratio of counts in leptin mRNA divided by those in GAPDH mRNA. However, the data in Figure 3(B) demonstrate that prior T_3 treatment enhanced the amount of GAPDH mRNA found in adipocytes after a 24 h incubation with insulin. Therefore we turned to the use of 18S RNA content as a reference standard. In Figure 3 as well as Tables 1 and 2 the values for both leptin mRNA and GAPDH mRNA are expressed as percentage of the zero-time value after correction for recovery of 18S RNA.

The digital image of the Northern-blot analysis for one of the five experiments summarized in Figures 2 and 3 is shown in Figure 1. All the samples from a single experiment were analysed on one gel. The counts under each peak were determined after subtraction of background counts then corrected for recovery of 18S RNA in order to measure only specific effects of hormones on leptin or GAPDH mRNAs. The counts were then normalized by expressing them as percentage of the counts present in the zero-time control (unincubated cells).

In subsequent studies, adipocytes or adipose tissue were incubated in primary culture for 24 h, since this enabled effects of agents on both leptin release and leptin mRNA to be examined. In adipocytes incubated for 6 h with dexamethasone, there was no increase in leptin release despite the marked effect on leptin mRNA (compare Figure 2 with Figure 3).

Effects of *in vitro* addition of T_3 to isolated adipocytes

The addition of T_3 to adipocytes incubated with dexamethasone and the lipolytic β_3-adrenergic receptor agonist Cl 316,243 was examined in studies shown in Table 1. T_3 significantly decreased leptin mRNA content at 24 h only in the presence of dexamethasone plus Cl 316,243, but no significant inhibition of leptin release was seen in adipocytes.

There was considerable loss of 18S RNA in adipocytes incubated for 24 h, and this was prevented by insulin (Table 1). We adjusted the leptin and GAPDH mRNA values for 18S RNA content, and, expressed in this way, there was no further effect of insulin on leptin mRNA or GAPDH mRNA content in the presence of dexamethasone. Insulin did block the enhanced loss of leptin mRNA caused by T_3 seen in the presence of dexamethasone plus Cl 316,243 (Table 1). There was a decrease in the leptin mRNA content due to Cl 316,243 in the presence of dexamethasone of about 50 %, and this inhibition was partially reversed in the presence of insulin (Table 1). There was also a 30 % decrease in leptin release due to Cl 316,243 in the presence of dexamethasone (Table 1). Leptin release was increased by insulin to about the same extent in the presence of dexamethasone plus Cl 316,243 as was seen in the absence of Cl 316,243 (Table 1).

T_3 inhibition of leptin mRNA accumulation by incubated pieces of adipose tissue is reversed to a stimulation in the presence of insulin

The loss of 18S RNA during incubation of adipocytes in the absence of insulin and the poor response of isolated adipocytes to T_3 suggested that intact pieces of adipose tissue might show a greater response to T_3. In cut pieces of adipose tissue incubated in the same culture medium, the 18S RNA content after 24 h of incubation was 158 ± 15 % of the initial value at the start of the incubation in the absence of insulin and 184 ± 124 % in the presence of 10 nM insulin (means ± S.E.M. for 12 paired experiments). In the same experiments, 10 nM insulin increased the accumulation of GAPDH mRNA from 195 to 330 % of the initial value.

In intact adipose tissue a small but significant inhibitory effect of T_3 (10 nM) was seen on leptin release in the presence of dexamethasone (Table 2). In contrast, there was a variable but statistically insignificant increase in leptin release due to T_3 over a 24 h incubation of intact adipose tissue in the presence of dexamethasone plus insulin (Table 2).

In the presence of dexamethasone plus insulin the addition of T_3 increased leptin mRNA accumulation at 24 h by 68 % (Table

Table 1 Effect of 10 nM T_3 on leptin release and accumulation of 18S RNA, leptin mRNA and GAPDH mRNA in adipocytes incubated in the presence of dexamethasone and 10 nM Cl 316,243

Rat adipocytes (720000) from hypothyroid rats were incubated for 24 h in the presence of 25 nM dexamethasone. Values are means ± S.E.M. for eight paired replications. Leptin mRNA and GAPDH mRNA are corrected for 18S RNA recovery.

Presence of 10 nM insulin	Leptin release (ng/24 h)			Change due to T_3 in the presence of Cl 316,243 (%)
	−Cl 316,243	+Cl 316,243	+Cl 316,243 +T_3	
−	76 ± 8	53 ± 5*	47 ± 4*	−11 ± 16
+	112 ± 6*	93 ± 5*	91 ± 5*	−2 ± 6
Presence of 10 nM insulin	**18S RNA (% of zero-time value)**			**Change due to T_3 in the presence of Cl 316,243 (%)**
	−Cl 316,243	+Cl 316,243	+Cl 316,243 +T_3	
−	47 ± 5	42 ± 3	41 ± 4	−2 ± 17
+	120 ± 5*	85 ± 5*	79 ± 5	−7 ± 6
Presence of 10 nM insulin	**Leptin mRNA (% of zero-time value)**			**Change due to T_3 in the presence of Cl 316,243 (%)**
	−Cl 316,243	+Cl 316,243	+Cl 316,243 +T_3	
−	62 ± 10	30 ± 3*	22 ± 2*	−27 ± 7†
+	46 ± 8	44 ± 5*	48 ± 7*	+9 ± 10
Presence of 10 nM insulin	**GAPDH mRNA (% of zero-time value)**			**Change due to T_3 in the presence of Cl 316,243 (%)**
	−Cl 316,243	+Cl 316,243	+Cl 316,243 +T_3	
−	130 ± 25	109 ± 21	100 ± 22	−8 ± 13
+	141 ± 20	162 ± 15	150 ± 14	−7 ± 5

* Statistically significant effects of insulin or Cl 316,243 based on paired comparisons ($P < 0.05$).

† Statistically significant effect of T_3 based on paired comparisons ($P < 0.05$).

Table 2 Effects of 10 nM T_3 on leptin release, leptin mRNA and GAPDH mRNA in intact adipose tissue

Pieces of epididymal adipose tissue (600 mg) from hypothyroid rats were incubated in 10 ml of medium for 24 h and all agents were added at the start of the incubation. Values are means ± S.E.M. for eight paired replications in the absence and 17 paired replications in the presence of 25 nM dexamethasone. Leptin mRNA and GAPDH mRNA are corrected for 18S RNA recovery.

Additions	Leptin release (ng/24 h)		Change due to T_3 (%)
	−T_3	+T_3	
None	67 ± 8	81 ± 6	+21 ± 9
+Cl 316,243 (10 nM)	59 ± 6	57 ± 9	−3 ± 9
+Insulin (10 nM)	101 ± 7*	110 ± 11*	+9 ± 11
+Dexamethasone (25 nM)	121 ± 6	106 ± 7	−12 ± 3†
+Dexamethasone and Cl 316,243	100 ± 5*	92 ± 6	−8 ± 4
+Dexamethasone and insulin	168 ± 10*	210 ± 17*	+25 ± 14
Additions	**Leptin mRNA (% of zero-time value)**		**Change due to T_3 (%)**
	−T_3	+T_3	
None	38 ± 8	42 ± 6	+10 ± 19
+Cl 316,243 (10 nM)	24 ± 4	23 ± 6	−4 ± 18
+Insulin (10 nM)	35 ± 7	36 ± 4	+2 ± 12
+Dexamethasone (25 nM)	95 ± 8	52 ± 7	−45 ± 10†
+Dexamethasone and Cl 316,243	60 ± 12*	41 ± 4	−31 ± 10†
+Dexamethasone and insulin	60 ± 8*	101 ± 9*	+68 ± 14†
Additions	**GAPDH mRNA (% of zero-time value)**		**Change due to T_3 (%)**
	−T_3	+T_3	
None	173 ± 24	170 ± 27	−2 ± 9
+Cl 316,243 (10 nM)	188 ± 30	180 ± 15	−4 ± 12
+Insulin (10 nM)	265 ± 45	260 ± 34	−2 ± 9
+Dexamethasone (25 nM)	160 ± 11	142 ± 13	−11 ± 7
+Dexamethasone and Cl 316,243	151 ± 12	152 ± 10	+1 ± 9
+Dexamethasone and insulin	344 ± 48*	330 ± 27*	−4 ± 8

* Statistically significant effects of insulin or Cl 316,243 based on paired comparisons ($P < 0.05$).

† Statistically significant effect of T_3 based on paired comparisons ($P < 0.05$).

2). However, there was an inhibitory effect due to T_3 on dexamethasone-stimulated leptin mRNA accumulation in intact adipose tissue in both the absence and presence of Cl 316,243 (Table 2). The GAPDH mRNA value at 24 h was 265% of the zero-time value and was insignificantly affected by 10 nM T_3 in the presence of insulin plus dexamethasone (Table 3). These data demonstrate that the increase in GAPDH mRNA seen in adipocytes after *in vivo* T_3 administration 8 h before death is an indirect effect that cannot be mimicked *in vitro*. Similarly the decline in leptin release by adipocytes from T_3-treated rats incubated in the presence of insulin is different from the *in vitro* effect of T_3 to increase leptin release in the presence of insulin and dexamethasone.

Stimulation of lipolysis by T_3

Adipocyte lipolysis is depressed by hypothyroidism and enhanced by thyroid hormones [10]. Direct effects of T_3 on lipolysis have been difficult to demonstrate in adipocytes and adipose tissue. Basal lipolysis was enhanced by 21% after a 24 h incubation of pieces of epididymal adipose tissue with 10 nM T_3 (Table 3). The effects of T_3 on leptin mRNA accumulation were quite different in tissue incubation with insulin from those seen in its absence (Table 2). However, the same was not the case for lipolysis, which was enhanced to the same extent by T_3 in the presence as in the absence of insulin (Table 3). Lipolysis was not affected by insulin alone but was markedly enhanced by 10 nM Cl 316,243. There was no further stimulation of lipolysis by the combination

Table 3 Stimulation of lipolysis by T_3

Pieces of epididymal adipose tissue (500–600 mg) from hypothyroid rats were incubated in 10 ml of medium containing 25 nM dexamethasone for 24 h and all agents were added at the start of the incubation. The effects of T_3 (10 nM) are shown as the percentage change (mean ± S.E.M. for the number of paired comparisons indicated in parentheses). Significant effects of T_3 are indicated by an asterisk based on paired comparisons ($P < 0.05$).

Additions	Lipolysis (μmol of glycerol/24 h)		Change due to T_3 (%)
	$-T_3$	$+T_3$	
None (15)	2.8 ± 0.2	3.4 ± 0.2	+21 ± 6*
Insulin, 10 nM (15)	3.4 ± 0.2	4.2 ± 0.3	+23 ± 5*
Cl 316,243, 10 nM (13)	12.6 ± 1.6	13.4 ± 1.3	+6 ± 6

of Cl 316,243 and T_3 as compared with Cl 316,243 alone (Table 3).

DISCUSSION

The present results indicate the complexity of the effects of T_3 on leptin release and leptin mRNA accumulation in adipocytes. We found that plasma leptin is increased in hypothyroid rats and that leptin release by adipocytes from hypothyroid rats is greater than that by adipocytes from euthyroid controls. Furthermore we observed a decrease in leptin release but not in leptin mRNA at 24 h in adipocytes from hypothyroid rats given T_3 8 h before they were killed.

Our results are in agreement with the finding that administration for 12–13 days by osmotic minipump of sufficient T_3 to increase plasma T_3 of thyroidectomized male rats from 1.5 to 3 nM reduced serum leptin concentration from 3.5 to 2 ng/ml [11]. In the presence of dexamethasone, direct inhibitory effects of T_3 on leptin release and leptin mRNA were seen in adipose tissue over a 24 h incubation period (Table 2). However, T_3 enhanced leptin release and leptin mRNA accumulation in pieces of adipose tissue incubated in the presence of insulin plus dexamethasone (Table 3 and Figure 3). These results indicate that whether T_3 inhibits or stimulates leptin release if added directly to adipose tissue depends on the hormonal milieu.

All our studies were carried out using adipocytes or tissue from fed rats incubated in the presence of 17.5 mM glucose. Under these conditions, the addition of insulin reproduces the fed situation with high levels of glucose and insulin. T_3 promotes leptin release and leptin mRNA accumulation. The presence of dexamethasone and Cl 316,243 reproduces to some extent the fasted state, and under these conditions there was an inhibitory effect of T_3 on leptin mRNA accumulation.

The present results suggest that thyroid hormone action on leptin release by adipocytes involves both direct and indirect effects. An indirect effect of T_3 is the marked increase in GAPDH mRNA seen in adipocytes from hypothyroid rats previously injected with T_3, which could not be reproduced *in vitro*. However, an inhibitory effect of T_3 on leptin mRNA content after 24 h could be seen in the presence of dexamethasone and absence of insulin.

The report that T_3 at a concentration of 10 nM increased the secretion of leptin as well as leptin mRNA content of murine 3T3-L1 adipocytes [5] is comparable with our finding in rat adipocytes incubated with dexamethasone plus insulin. However, MacDougald et al. [6] found that leptin mRNA expression was very low in 3T3-L1 adipocytes as compared with adipocytes. Furthermore, in order to obtain normal expression of leptin mRNA in 3T3-F4421 cells, they had to be implanted in athymic mice, where they developed into fat-pads with normal levels of leptin mRNA [7]. Leptin release per g of 3T3 L1 cells was 6 ng over 24 h [5], whereas we found that the basal release per g of intact tissue was 110 ng over 24 h (Table 2). It is unclear whether effects of ligands on leptin mRNA and leptin release in 3T3 cells differentiated into cells containing lipid have much relevance to what occurs in adipocytes because of the low level of leptin release and leptin mRNA [6,7].

The complexity of hormone effects on leptin is emphasized by the finding that treatment of young men with low plasma leptin values (3.2 ng/ml after an overnight fast) for 7 days with enough T_3 to increase plasma T_3 threefold did not affect plasma leptin [12]. In another study, hypothyroid humans actually had lower plasma leptin levels than euthyroid controls with a similar body mass index (4.7 and 8.6 ng/ml respectively) [13]. In hyperthyroid patients, plasma leptin levels were unaffected by treatment for 12–28 weeks with methimazole [13]. These data indicate that, in humans, the plasma leptin status in individuals with relatively low plasma leptin levels is little affected by thyroid status. Plasma leptin levels in humans are also unaffected by insulin levels or insulin sensitivity [14]. Furthermore, short-term insulinaemia (2 h or less) in humans has little effect on plasma leptin [15–20]. Glucocorticoid hormones increase adipose-tissue leptin mRNA accumulation in humans, but this does not explain the consistent findings that plasma leptin levels correlate with body mass index rather than the plasma levels of any known hormone [14–20].

Since all our studies were carried out with adipocytes or adipose tissue from hypothyroid rats, the conditions were optimal for demonstration of permissive effects of T_3 that might not be seen in tissue from euthyroid animals. In adipose tissue incubated for 24 h with dexamethasone, the addition of insulin decreased the accumulation of leptin mRNA in the absence of T_3 but enhanced that in the presence of T_3 (Table 2). Similarly the increase in leptin release due to insulin was doubled in the presence of T_3, whereas the increase in GAPDH mRNA due to insulin was the same in the absence as in the presence of T_3. The difference in thyroid hormone status may explain some of the contradictory effects of insulin that have been reported with respect to leptin mRNA content of adipose tissue. Insulin was found to inhibit [22,23], have no effect [19,20,24] or stimulate leptin mRNA accumulation in adipose tissue [25–27].

We conclude that T_3 can either inhibit or stimulate the net leptin mRNA content of white adipose tissue. In conditions that approximate the fed state (high glucose plus glucocorticoids and either insulin or growth hormone), the concurrent presence of T_3 stimulates leptin mRNA accumulation. In contrast, in conditions mimicking the fasted state (glucocorticoid alone or in the presence of a β_3-adrenergic agonist) the addition of T_3 enhances the loss of leptin mRNA.

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