Thyrotropin Decreses Leptin Production in Rat Adipocytes

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Leptin, which is secreted from adipocytes, has a role in the regulation of appetite and energy expenditure. The thyrotropin receptor (TSH-R) was recently found in adipocytes. We examined the effects of TSH on leptin production and lipolysis in rat epididymal adipocytes. TSH decreased the concentration of leptin in the medium time (~24 hours)- and dose ($\sim 10^{-7}$ mol/L)-dependently (half-maximal inhibition [IC_{50}] $\approx 10^{-9}$ mol/L). TSH also decreased the *ob* mRNA level approximately 55% in adipocytes. We confirmed the presence of TSH-R mRNA in the adipocytes by reverse transcription–polymerase chain reaction (RT-PCR). TSH stimulated glycerol release dose-dependently ($IC_{50} \approx 10^{-8}$ mol/L) in adipocytes. This TSH-induced glycerol release was further enhanced by adenosine deaminase (ADA). In summary, TSH reduced leptin production and stimulated lipolysis in rat epididymal adipocytes. Although the pathophysiological relevance of the regulation of leptin production and lipolysis by TSH is unknown, we speculate that TSH may affect the regulation of appetite and energy expenditure in pathophysiological states.

WHITE ADIPOSE TISSUE is the primary site of energy storage and lipolysis. Adipocytes secrete a satiety factor, leptin, and play a regulatory role in energy homeostasis. An extrathyroidal high-affinity binding of thyrotropin (TSH) has been reported in rat adipocytes,[1] although TSH was previously thought to act solely in the control of thyroid function.

TSH receptor (TSH-R) is a G-protein–coupled glycoprotein hormone receptor with a large extracellular domain fused to a canonical seven-membrane–spanning segment.[2] In thyroid follicular cells, TSH activates cell growth and thyroid hormone synthesis by binding to TSH-R and elevating the cyclic adenosine monophosphate (cAMP) level. β-Adrenergic receptor agonists have been reported to reduce *ob* mRNA levels in rat,[3,4] 3T3-L1,[5] and mouse[6,7] adipocytes. β-Adrenergic receptor agonists also induce lipolysis by elevating the cAMP level. It is thus conceivable that leptin production and lipolysis are modulated by TSH through cAMP-dependent mechanisms.

In the present study to investigate the functional roles of TSH-R in adipocytes, we examined the effects of TSH on leptin production and lipolysis in rat epididymal adipocytes.

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle's medium (DMEM) was obtained from GIBCO-BRL (Gaithersburg, MD). Purified bovine TSH and all chemicals were obtained from Sigma (St Louis, MO) unless otherwise noted.

Preparation of Isolated Rat Adipocytes

Isolated rat epididymal adipocytes were prepared from 12-week-old male Sprague-Dawley rats (200 g; Shimizu Breeding Laboratories, Kyoto, Japan) by collagenase (type I; Cooper Biochemical, Freehold, NJ) digestion as previously described.[8] The cells were washed three times with Krebs-Ringer solution buffered with 30 mmol/L HEPES at pH 7.4 (KRBH) containing 1% bovine serum albumin (BSA) (fraction V; Intergen, Purchase, NY) and 200 nmol/L adenosine. They were then washed twice with DMEM, pH 7.4 (containing 2 mmol/L glutamine, 200 nmol/L adenosine, gentamicin 50 μg/mL, 5% BSA, and 25 mmol/L HEPES). The cells were resuspended in DMEM at a cytocrit of approximately 20% and cultured at 37°C in an atmosphere of 5% CO_2 for 24 hours.

Radioimmunoassay of Leptin in Culture Medium

The leptin concentration in the culture medium was measured by a radioimmunoassay (RIA) for rat leptin as previously described.[9] The minimum detection limit of the RIA was 1 ng/mL and the 50% binding intercept was 10 ng/mL.

Total RNA Extraction and Reverse Transcription–Polymerase Chain Reaction of TSH-R

Total RNA from epididymal adipocytes was extracted as previously described.[10,11] Reverse transcription (RT) was performed on 2 μg RNA using the Superscript Preamplification System (GIBCO-BRL) according to the manufacturer's instructions. The cDNA was amplified by polymerase chain reaction (PCR) using two pairs of primers to amplify TSH-R fragments as follows (nucleotide positions according to Akamizu et al[2]): extracellular domain, 5′-CCTTTGGAGGAGTATACA-3′ (residues 659 to 676, sense strand) and 5′-GTGTAGTCATAGTGGC-TGT-3′ (residues 1245 to 1263, antisense strand); and domain from the transmembrane to the intracellular portion, 5′-GTATGCCAT-CACCTTCGC-3′ (residues 1560 to 1577, sense strand) and 5′-GAGACTC-TGCCTTGTGTC-3′ (residues 2179 to 2196, antisense strand).

Northern Blot Analysis

Total RNA (20 μg) was prepared and Northern blot analysis was performed using ^{32}P-labeled rat *ob*[10,11] and human lipoprotein lipase (LPL)[12] cDNA fragments as probes. The mRNA levels were normalized to the 28S ribosomal RNA level in the cells to correct for differences in the amount of RNA applied. The mRNA level (arbitrary units) is expressed in relation to the values for the control cells.

Glycerol Determination

A 50-μL aliquot of the adipocyte suspension was added to 450 μL KRBH containing 1% BSA and 200 nmol/L adenosine. Then, TSH or isoproterenol, with or without adenosine deaminase (ADA), was added. The incubations were performed in polypropylene tubes for 15 minutes at 37°C and terminated by the addition of 40 μL 60% perchloric acid. The samples were then neutralized with KOH and centrifuged. The glycerol content of the supernatant was determined by a glycerol F-kit (Boehringer, Mannheim, Germany).

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Statistical Analysis

Results are expressed as the mean ± SEM. Statistical significance was tested with a one-way ANOVA followed by Duncan's multiple-range test and the paired Student's t test as appropriate. A P level less than .05 was considered statistically significant.

RESULTS

Effect of TSH on Leptin Concentration in Adipocytes

To examine the effect of TSH on leptin production in adipocytes, we measured the leptin concentration in the culture medium. Figure 1A shows the time course (~24 hours) of the leptin concentration in the medium with or without TSH (10^{-7} mol/L). At 24 hours, TSH decreased leptin to 70% of the control level (control v TSH, 98 ± 5 v 68 ± 7 ng/mL, $P < .01$). The dose-response effect of TSH on the leptin concentration is shown in Fig 1B. TSH decreased the leptin level in a dose-dependent manner (0 to 10^{-7} mol/L). We further examined the additive effect of ADA on the TSH-induced decrease of leptin. ADA alone did not affect the leptin concentration (101 ± 6 ng/mL), but ADA enhanced the TSH-induced decrease (34 ± 7 ng/mL, $P < .01$; Fig 1C).

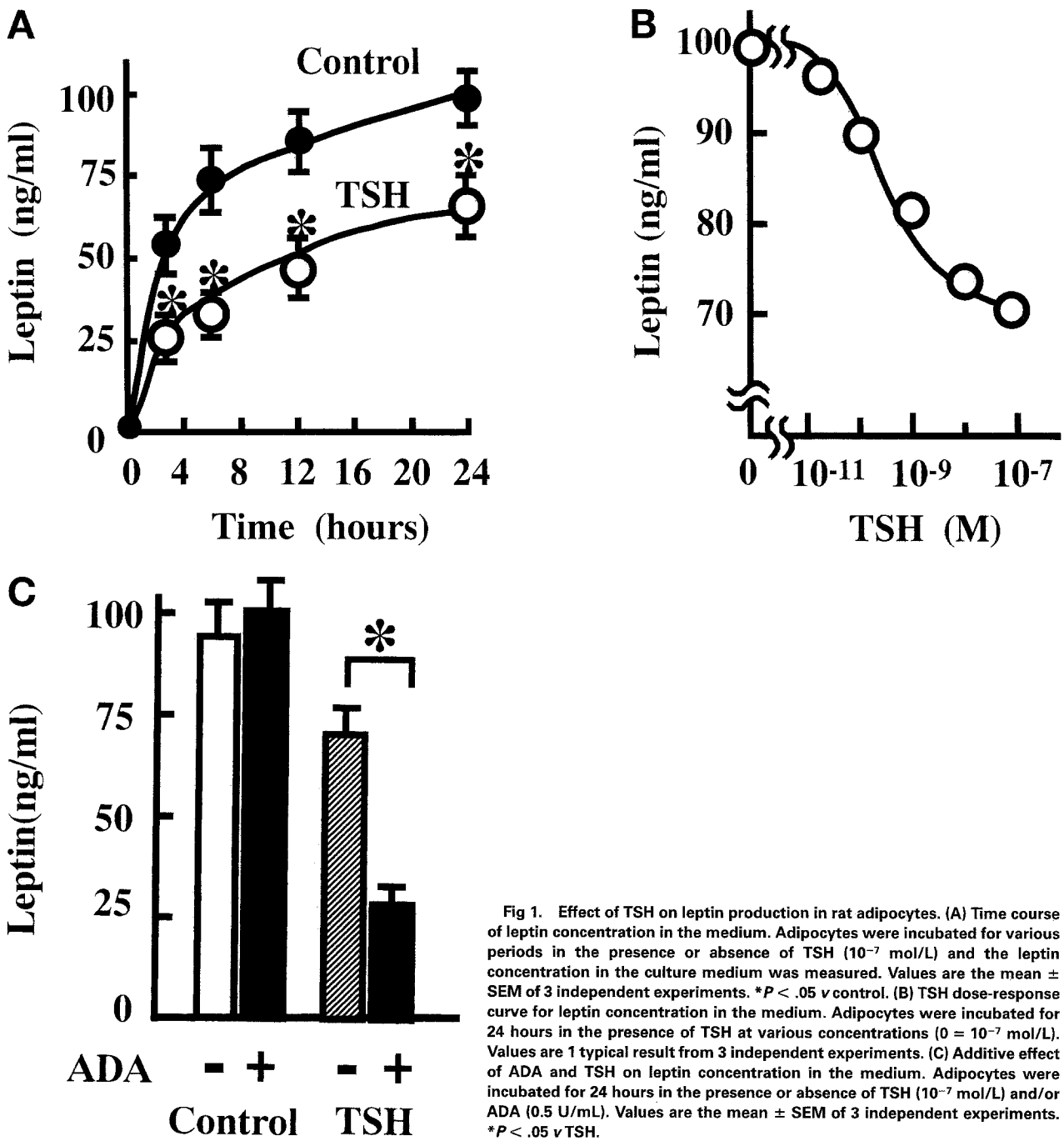

Fig 1. Effect of TSH on leptin production in rat adipocytes. (A) Time course of leptin concentration in the medium. Adipocytes were incubated for various periods in the presence or absence of TSH (10^{-7} mol/L) and the leptin concentration in the culture medium was measured. Values are the mean ± SEM of 3 independent experiments. *$P < .05$ v control. (B) TSH dose-response curve for leptin concentration in the medium. Adipocytes were incubated for 24 hours in the presence of TSH at various concentrations (0 = 10^{-7} mol/L). Values are 1 typical result from 3 independent experiments. (C) Additive effect of ADA and TSH on leptin concentration in the medium. Adipocytes were incubated for 24 hours in the presence or absence of TSH (10^{-7} mol/L) and/or ADA (0.5 U/mL). Values are the mean ± SEM of 3 independent experiments. *$P < .05$ v TSH.

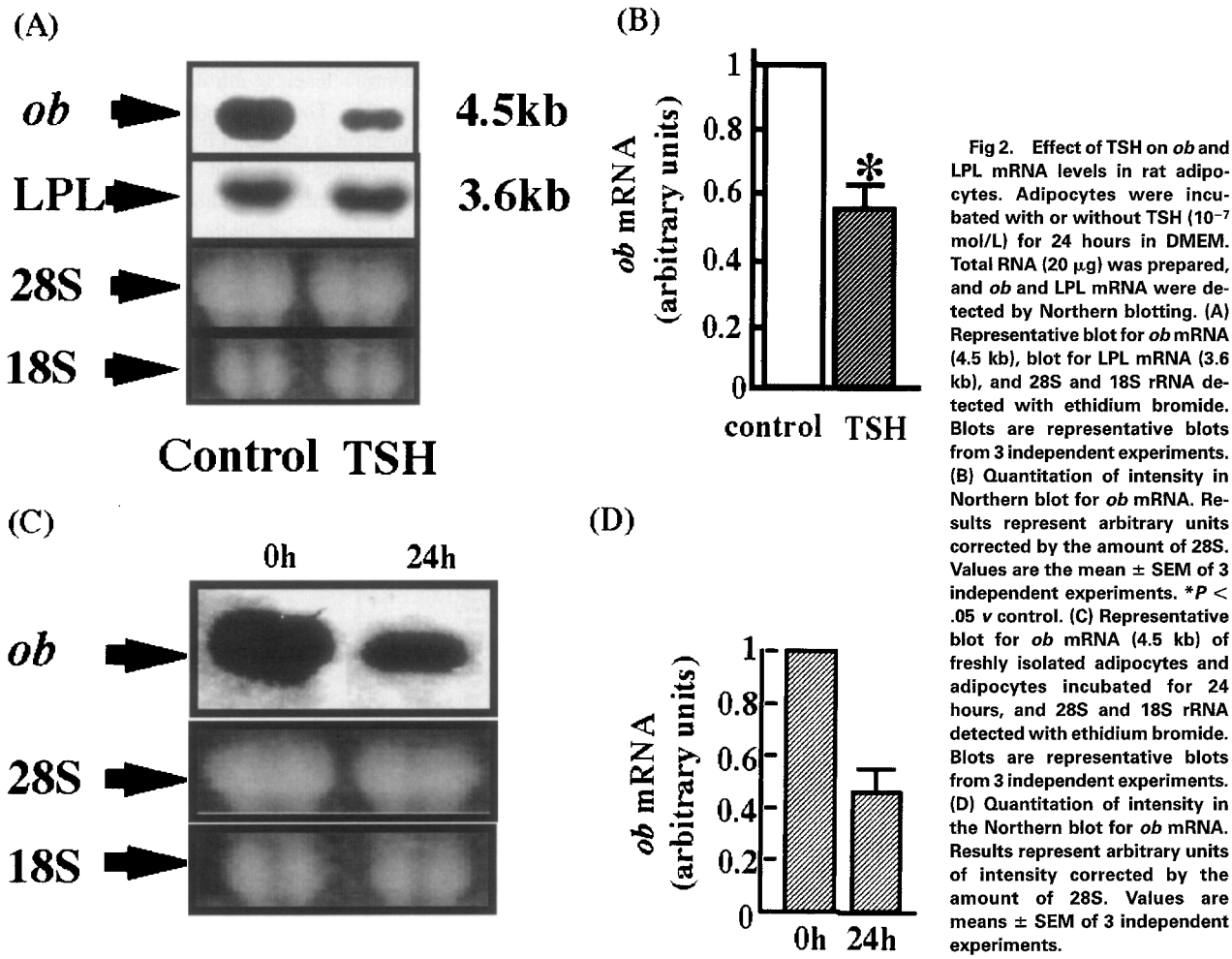

Fig 2. Effect of TSH on *ob* and LPL mRNA levels in rat adipocytes. Adipocytes were incubated with or without TSH (10^{-7} mol/L) for 24 hours in DMEM. Total RNA (20 μg) was prepared, and *ob* and LPL mRNA were detected by Northern blotting. (A) Representative blot for *ob* mRNA (4.5 kb), blot for LPL mRNA (3.6 kb), and 28S and 18S rRNA detected with ethidium bromide. Blots are representative blots from 3 independent experiments. (B) Quantitation of intensity in Northern blot for *ob* mRNA. Results represent arbitrary units corrected by the amount of 28S. Values are the mean ± SEM of 3 independent experiments. *$P <$ .05 *v* control. (C) Representative blot for *ob* mRNA (4.5 kb) of freshly isolated adipocytes and adipocytes incubated for 24 hours, and 28S and 18S rRNA detected with ethidium bromide. Blots are representative blots from 3 independent experiments. (D) Quantitation of intensity in the Northern blot for *ob* mRNA. Results represent arbitrary units of intensity corrected by the amount of 28S. Values are means ± SEM of 3 independent experiments.

Effect of TSH on ob and LPL mRNA Levels in Adipocytes

We investigated the effects of TSH on *ob* mRNA expression in adipocytes by Northern blot analysis (Fig 2A). TSH reduced the *ob* mRNA level to 55 ± 8% of the control ($P < .05$; Fig 2B). At the same time, we detected LPL mRNA as a typical adipocyte marker to clarify whether the decrease in *ob* mRNA was specific to TSH. LPL mRNA was not significantly affected by TSH (98 ± 7% of the control). The *ob* mRNA level was decreased to approximately 50% of the 0-hour level after 24 hours (Fig 2C and D). This decrease of *ob* mRNA was compatible with the decrease in the rate of leptin accumulation in the medium (Fig 1A).

Detection of TSH-R mRNA in Adipocytes

Figure 3 shows results of the RT-PCR using two pairs of primers designed to amplify the sequences of TSH-R cDNA. One of these primer pairs yielded the 605–base pair (bp) product of the extracellular domain. The other yielded the 640-bp product from the transmembrane to intracellular domain of the TSH-R. Both of the PCR products corresponding to TSH-R cDNA were present in the adipocytes, whereas no bands were observed in the control reactions where the RT was omitted.

Effect of TSH on Lipolysis in Adipocytes

We next examined the lipolytic activity of TSH in the adipocytes. TSH (10^{-7} mol/L) increased the glycerol concentration in the medium time-dependently and linearly up to 15 minutes (data not shown). In subsequent experiments, we measured the glycerol concentration at 15 minutes to calculate the initial velocity of glycerol release. Figure 4 shows the dose-response relationship between TSH and glycerol release with and without ADA (0.5 U/mL). TSH significantly increased glycerol release at concentrations above 10^{-8} mol/L ($P < .01$). ADA alone significantly increased glycerol release (control *v* ADA, 5.6 ± 2.2 *v* 15.9 ± 1.4 μmol/min/mL cells, $P < .01$) and enhanced TSH-induced glycerol release additively.

DISCUSSION

The present study demonstrates for the first time that TSH decreases the leptin concentration in the medium and *ob* mRNA expression in rat epididymal adipocytes. Leptin production is reportedly regulated by a variety of hormones and agents under both in vivo and in vitro conditions. Leptin is upregulated by insulin and glucocorticoids[3,14-16] and downregulated by β-adrenergic agonists, free fatty acids, and thiazolidinedione deriva-

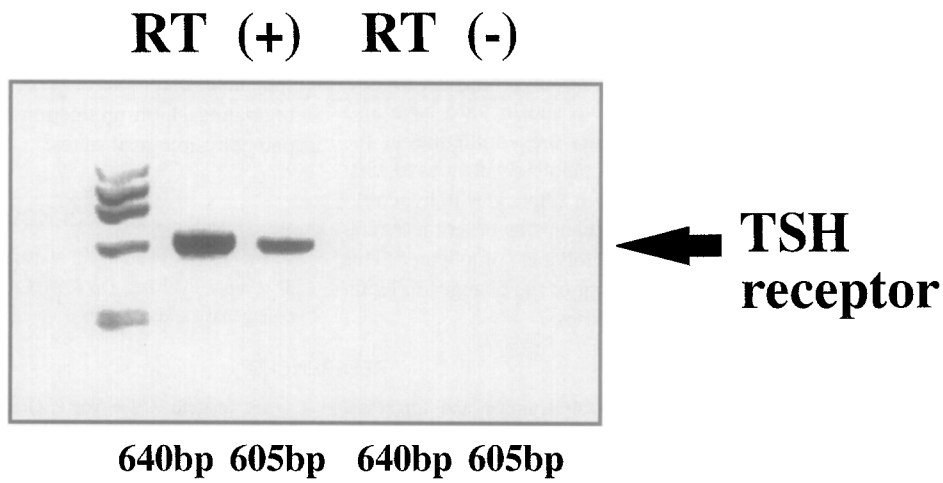

Fig 3. Detection of TSH-R in rat adipocytes by RT-PCR. RT-PCR analysis was performed using 2 pairs of primers for TSH-R (640 and 605 bp). The 2 right lanes show negative controls that did not contain RT [RT(−)]. Molecular size markers are 1,353, 1,078, 872, 603, and 310 bp.

tives.[3,4,6,7,17-19] Herein, we add TSH to the list of substances that reduce leptin and *ob* mRNA levels in adipocytes.

We confirmed the presence of TSH-R in rat adipocytes by RT-PCR and TSH-induced lipolysis in rat epididymal adipocytes as reported by Endo et al.[1] The binding activity of TSH and the responsiveness of cAMP to TSH are almost indistinguishable in adipocytes versus the thyroid gland.[1] β-Adrenergic receptor agonists have been reported to reduce *ob* mRNA levels by increasing cAMP in primary cultures of rat adipocytes[3,4] and 3T3-L1 adipocytes.[5] In the present study, TSH reduced leptin production and this decrease was further enhanced by the elimination of adenosine by ADA (Fig 1C). It has also been reported that a chemical analog of cAMP decreases leptin release and increases glycerol release in rat adipocytes incubated for 2 hours.[4] TSH might reduce leptin production by a cAMP-dependent mechanism. Further, the TSH-R has been reported to activate phosphatidylinositol turnover, diacylglycerol accumulation, and protein kinase C (PKC) activation.[20] This pathway may also be involved in the regulation of leptin production in adipocytes, although a role for PKC in leptin production has not been reported.

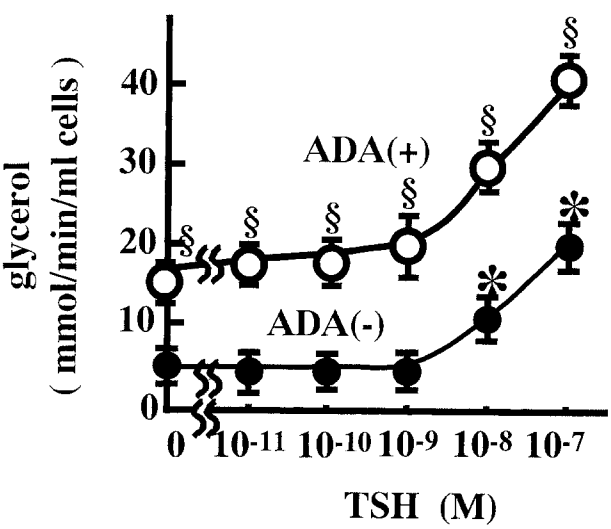

Fig 4. Dose-response relationship between TSH and glycerol release with or without ADA. Rat epididymal adipocytes were incubated with TSH (0-10^{-7} mol/L) for 15 minutes with or without ADA (0.5 U/mL) in KRBH buffer. Glycerol was measured in triplicate using the glycerol F-kit. Values are the mean ± SEM of 3 independent experiments. *$P < .01$ *v* control; §$P < .01$ *v* without ADA.

It is unlikely that the results of the present study are due to unexpected cytotoxic effects or cell damage by TSH. To eliminate these possibilities, we measured LPL mRNA as a typical adipocyte marker, which has been reported to be unchanged by an elevation of cAMP in rat adipocytes.[21] The level of LPL mRNA was not changed by TSH in the present study. Furthermore, the loss of cells as estimated by the cytocrit and lipid weight was less than 10%. Therefore, we consider that the reduction in leptin production was induced specifically by TSH.

Basal leptin accumulation amounted to almost 500 ng/g of packed adipocytes over 24 hours in this study. In contrast, Fain and Bahouth[13] found basal leptin release of approximately 30 ng/g by adipocytes over 24 hours in the absence of dexamethasone. Although the cause of this discrepancy is not clear, one possibility is that unknown factors in BSA in the medium may contribute to the high level of leptin. It has been reported that fetal calf serum has the ability to mimic the stimulation of leptin release by insulin.[13] Alternatively, the discrepancy may be due to the difference between our RIA and theirs.

Regarding the clinical relevance of these results, we suspect that the high level of thyroid-stimulating antibody (TSAb) may affect leptin production and lipolysis in adipocytes, in addition to thyroid hormones. We showed that a high level of TSH is necessary to reduce leptin production (~10^{-9} mol/L; Fig 1B) and to induce lipolysis (~10^{-8} mol/L; Fig 4). In hyperthyroid patients, the highest biological activity of TSAb could be equivalent to 10^{-8} to 10^{-7} mol/L of TSH.[22] It has also been demonstrated that immunoglobulin from patients with Graves' disease stimulates cAMP formation in nonthyroid cells expressing recombinant TSH-R.[23] Furthermore, the roles of TSH and TSAb have been described as lipolytic in infant adipocytes.[24] Thus, it is conceivable that a high level of TSAb could affect leptin production and lipolysis in hyperthyroid patients who have high levels of TSAb activity. In contrast, in patients with overt symptomatic primary hypothyroidism, serum TSH is usually elevated to 10^{-9} to 10^{-10} mol/L.[25] This concentration of

TSH can also affect leptin production (Fig 1B) in adipocytes without affecting lipolysis (Fig 4).

The effects of thyroid hormone (triiodothyronine and thyroxine) on leptin production both in vivo and in vitro have also been reported.[26-29] However, the data are conflicting in the human, cell culture, and rat. It is not completely clear as to what primarily regulates leptin production in adipocytes in hyperthyroidism and hypothyroidism. The mechanisms of leptin regulation in in vivo thyroid disorders are probably complicated. This study contributes to our understanding of the changes in leptin production that accompany such diseases.

The present study is the first report that TSH reduces leptin production and the *ob* mRNA level in rat adipocytes. We speculate that TSH has roles in adipocyte metabolism by regulating leptin production and/or inducing lipolysis in some pathophysiological states.

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