Regulation of adipose tissue lipoprotein lipase gene expression by thyroid hormone in rats

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Abstract Lipoprotein lipase (LPL) is an important enzyme in lipid metabolism, and adipose LPL activity is increased in rats that are deficient in thyroid hormone. To examine the mechanism of thyroid hormone's effect on LPL, LPL gene expression was assessed in the epididymal fat pads of hypothyroid rats. When compared to control rats, LPL activity, mass, and synthetic rate in hypothyroid rats were increased; heparin-releasable LPL activity and mass were increased to 448% and 300% of control, respectively, and [^{35}S]methionine incorporation into LPL was increased to 250% of control. The increases in LPL activity and mass were reversed by treatment of hypothyroid rats with triiodothyronine (T$_3$). However, there was no change in the level of LPL mRNA when compared to the level of gamma-actin mRNA and no effect on LPL transcription using run-off assays. Isolated adipocytes were prepared from normal rats and exposed to 2 nM T$_3$ in vitro for 24 h. The addition of T$_3$ to cultures of adipocytes resulted in a decrease in LPL activity, mass, and [^{35}S]methionine incorporation, but still no change in LPL mRNA level. To determine whether thyroid hormone regulated catecholamine responsiveness, adipocytes were prepared from hypothyroid and control rats, and the responses to epinephrine were compared. Although epinephrine inhibited [^{35}S]methionine incorporation into LPL in control rat adipocytes, there was essentially no effect in hypothyroid rat cells. In addition, T$_3$ treatment of the hypothyroid rats restored the responsiveness to epinephrine. Thus, thyroid hormone regulates LPL in rat adipose tissue posttranscriptionally, resulting in parallel changes in LPL synthetic rate, immunoreactive mass, and activity. Although thyroid hormone had direct effects on adipose LPL, it also altered the responsiveness of adipocytes to epinephrine.—**Saffari, B., J. M. Ong, and P. A. Kern.** Regulation of adipose tissue lipoprotein lipase gene expression by thyroid hormone in rats. *J. Lipid Res.* 1992. **33:** 241–249.

Supplementary key words translation regulation • triiodothyronine • hypothyroidism • lipid metabolism

Lipoprotein lipase (LPL) is a central enzyme in lipid metabolism. The enzyme is synthesized and secreted by adipocytes, and is important for the transfer of triacylglycerol fatty acids from the circulating blood into adipocytes (1). The cellular regulation of LPL is complex. Previous studies have described the effects of numerous hormones and physiologic conditions on the level of LPL catalytic activity, and more recent studies have identified a number of different mechanisms of LPL cellular regulation (1, 2).

Among the hormonal regulators of LPL is thyroid hormone. Adipose tissue levels of LPL have consistently been increased in hypothyroid rats (3–7), although plasma triglycerides have been either decreased (3, 4) or unchanged (8, 9). One study examined the interaction between diabetes and hypothyroidism in rats, and suggested that the effects of thyroid hormone and insulin occurred through different mechanisms (4). Several studies have examined the effects of T$_3$ added to cultures of preadipocytes. Spooner et al. (10) observed no effect to 10^{-8}M T$_3$ when added to cultures of mature 3T3-L1 cells, whereas studies in Ob17 adipocytes revealed an increase in LPL activity in response to T$_3$ (11). Most of the above studies measured only LPL catalytic activity, and thus could not precisely identify the mechanism of regulation of LPL. The studies described herein were intended to examine the mechanism of regulation of adipose tissue LPL in rats by thyroid hormone. Experiments were conducted in vivo and in vitro (using primary cultures of adipocytes) and LPL synthesis and cellular processing were examined using a specific anti-LPL antibody as well as the LPL cDNA.

METHODS

Treatment of rats and preparation of adipocytes

Both normal and hypothyroid male Sprague-Dawley rats were purchased from Harland Laboratories (Gilmore, CA), and weighed between 210 and 230 g. Hypothyroid rats had been surgically thyroidectomized 6 weeks prior to delivery, and pooled sera from these rats at the time of killing uniformly demonstrated elevated levels of TSH. For treatment with thyroid hor-

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Abbreviations: LPL, lipoprotein lipase; SDS, sodium dodecyl sulfate; T$_3$, triiodothyronine; TSH, thyroid-stimulating hormone; PBS, phosphate-buffered saline.
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mone, hypothyroid rats were given daily intraperitoneal injections of 10 µg/100 g triiodothyronine (T_3) for 10 days, which has been shown previously to reduce rat pituitary level of TSH mRNA (12).

Isolated rat adipocytes were prepared using a collagenase digestion of epididymal fat pads, as described previously (13). Cells were cultured for 24 h in Medium 199 (Irvine Scientific, Santa Ana, CA) without serum, and were then washed in the same medium, followed by the addition of the indicated concentration of T_3.

Measurement of LPL activity

In some experiments, LPL was measured in the heparin-releasable and extractable fractions of whole epididymal adipose tissue, as described previously (14). In brief, adipose tissue pieces were incubated in phosphate-buffered saline (PBS) containing 13 µg/ml heparin (Fisher Scientific Co.) for 30 min at 37°C. An aliquot of this solution was then assayed as described below. The tissue was then washed and the extractable fraction was prepared by homogenizing the tissue in detergent-containing buffer, as described previously (14). LPL activity was then measured in the aqueous layer after centrifugation, and fat cell number was determined using the method of DiGirolamo, Mendlinger, and Fertig (15). For isolated adipocytes, LPL activity was measured using the same methods, as previously described (16).

LPL activity was determined using an emulsified [^{3}H)triolein-containing substrate containing normal human serum as a source of apoC-II (17). After incubating the sample with substrate for 45 min at 37°C, the reaction was stopped and liberated ^{3}H-labeled free fatty acids were measured as described previously (16). Activity was expressed as nEq FFA released/min per 10^6 cells.

LPL immunoreactive mass

LPL immunoreactive mass was measured using an enzyme-linked immunosorbent assay (ELISA), as described previously (16, 18) using affinity-purified anti-LPL antibodies and bovine LPL as a standard. Samples for LPL immunoreactive mass were prepared as described above for LPL activity, except for the presence of protease inhibitors in all the buffers.

Pulse-labeling and immunoprecipitation

To assess LPL synthesis in adipose tissue, isolated adipocytes were prepared with a collagenase digestion (13). Cells were then pulse-labeled by incubating cells with 50 µCi of [^{35}S]methionine for 30 min in methionine-free Medium 199 containing heparin. The cells were lysed and immunoprecipitated with affinity-purified anti-LPL antibody, followed by analysis of the

samples on a 10% polyacrylamide-SDS gel, as described previously (13, 19). To account for any differences in [^{35}S]methionine labeling, an aliquot of cell lysate was precipitated with TCA, and the protein gels were loaded in proportion to the total TCA-precipitable counts in the samples. However, this procedure resulted in only small adjustments in gel loading, because there was no significant difference in [^{35}S]methionine incorporation into total protein between adipocyte primary cultures.

To study the effects of T_3 in vitro, adipocytes were incubated for 24 h with the indicated concentration of T_3, and then labeled with [^{35}S]methionine for 30 min (except for Fig. 7, see text), as described above, followed by cell lysis, immunoprecipitation, and SDS-PAGE. Pulse-chase experiments were also performed, in which cells were pulse-labeled as described above, and then chased with complete medium (containing 30 mg/l methionine) for the times indicated, in the presence of 13 µg/ml heparin. At each time during the chase, cells and medium were immunoprecipitated.

Quantitation of the autoradiographic image was accomplished using a laser scanning densitometer (Biomed Instruments, Model SLR-1D/2D) which subtracts background and integrates the image produced by an autoradiographic band.

RNA extraction and Northern analysis

RNA was extracted using the method of Chomczynski and Sacchi (20). Equal amounts of total RNA were resolved on a 2.2 M formaldehyde–1% agarose gel, transferred to a nylon membrane, and blotted with the ^{32}P-labeled (21) cDNA probes for human LPL (22) and gamma-actin (23) as described previously (14, 19). Images were quantitated by laser densitometry, as described above. When serial RNA dilutions were slot-blotted and probed with LPL and gamma-actin, the images were linear to the dilution, and the LPL/actin ratio remained constant.

Transcriptional run-off assays

Animals were treated with T_3 as described in the text, and fat pads were then removed. Tissue was washed once with a solution containing 0.25 M sucrose, 10 mM Tris-HCl (pH 7.5), 0.1 M KCl, 5 mM $MgCl_2$, and 5 mM DTT; the tissue was then resuspended in the above solution containing 0.1% Triton X-100 and 20 U/ml RNasin (Promega) and lysed with ten strokes in a Dounce homogenizer at room temperature. After allowing the disrupted cells to incubate on ice for 5 min, nuclei were isolated by centrifugation at 1000 g for 5 min at 4°C. The nuclei were washed once in 20 mM Tris-HCl (pH 8.0), 50 mM $(NH_4)_2SO_4$, 2 mM $MnCl_2$, 5 mM $MgCl_2$, 2.5 mM DTT,

20 U/ml RNasin, and 20% glycerol and then resuspended in the same solution, frozen immediately in liquid nitrogen, and stored at –70°C.

For transcriptional run-off reactions, nuclei from 8 x 10^6 cells were thawed and added to a reaction mixture yielding final concentrations of 4 mM each of ATP, CTP, and GTP and a total of 250 µCi [^{32}P]UTP. After incubation at 28°C for 45 min, the reaction was stopped and RNA was extracted as described previously (20). Two µg of linearized LPL cDNA plasmid, gamma-actin cDNA plasmid, and pGEM DNA plasmid (Promega) were heat-denatured and slot-blotted onto a nylon membrane. The nylon membrane was prehybridized at 55°C for at least 3 h in 40 mM sodium phosphate buffer (pH 7.2), 5% SDS, 1% BSA, and 100 µg/ml yeast tRNA. The membrane was then hybridized with equal counts of extracted radiolabeled RNA that was heat-denatured prior to use. Hybridization was performed at 55°C for 48 h followed by four washes with 40 mM sodium phosphate buffer (pH 7.2) and 5% SDS. Autoradiography was performed using Kodak XAR-5 X-ray film at –70°C.

Statistics

All data were expressed as the mean ± SEM, and were analyzed using the Student's t-test.

RESULTS

LPL activity and immunoreactive mass were measured in epididymal fat pads from control and hypothyroid rats. As shown in **Fig. 1A**, the levels of LPL activity in the heparin-releasable and extractable fractions of hypothyroid rats were 448% and 252%, respectively, of the levels in control rats. To determine whether there were similar changes in LPL protein, immunoreactive mass was measured by ELISA. LPL immunoreactive mass was also increased in both the heparin-releasable and extractable fractions of hypothyroid rats to 300% and 162%, respectively, of the level in control fat pads (Fig. 1B). Because the changes in LPL activity and mass occurred in parallel, there was no difference in LPL specific activity between control and hypothyroid rats. To determine whether the changes in LPL were reversible, hypothyroid rats were injected with T$_3$ for 10 days. LPL activity in these rats decreased by 57% and 51% in heparin-releasable and extractable, respectively. Although heparin-releasable LPL mass decreased by 70% in the T$_3$-treated hypothyroid rats, there was no significant change in extractable LPL mass.

RNA was extracted from the fat pads of the control, hypothyroid, and T$_3$-treated hypothyroid rats, and then analyzed by Northern blotting. The blots were
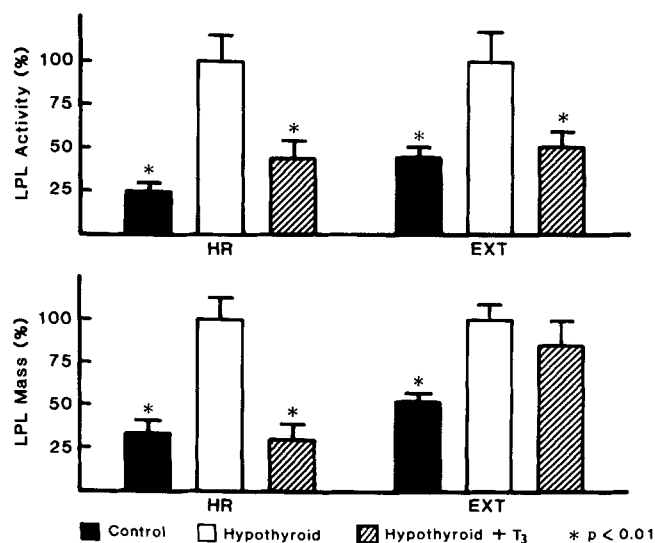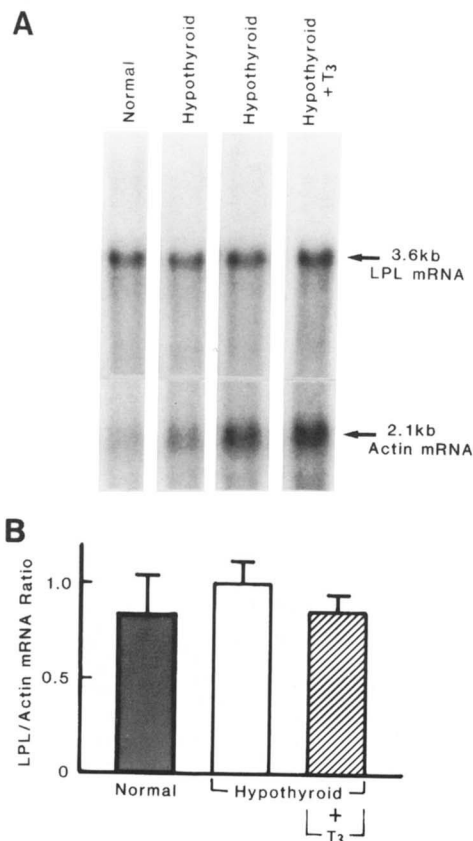

Fig. 1. Effect of hypothyroidism on LPL. The epididymal fat pads of hypothyroid and control rats were removed, followed by measurement of LPL catalytic activity (A) and immunoreactive mass (B). Data are expressed as the mean ± SE; *P < 0.05 versus control rats; **P < 0.01 versus control rats; n = 12 for control, and n = 16 for hypothyroid rats.

probed with the cDNA for LPL, as well as the cDNA to gamma-actin, which served as a constitutive probe. As shown in **Fig. 2A** (first two lanes), there was no difference in the level of the LPL mRNA in the fat pads of hypothyroid versus normal rats when compared to the level of gamma-actin. Additional experiments were performed with hypothyroid rats that were injected with T$_3$. As shown in the second two lanes of Fig. 2A, treatment of hypothyroidism did not change LPL mRNA levels, in spite of the changes in LPL activity and mass described above. As shown in Fig. 2B, the LPL/actin ratios from all hypothyroid, control, and T$_3$-treated hypothyroid rats were analyzed, and there was no significant difference between the three groups of animals.

To further assess LPL expression in hypothyroid rats, transcription rate was measured using run-off assays. Nuclei were prepared from the adipose tissue of hypothyroid rats, control rats, and hypothyroid rats that were treated with T$_3$ for 10 days. After allowing elongation of the initiated transcripts with [^{32}P]UTP, RNA was isolated, and the ^{32}P-labeled nuclear RNA was hybridized to filters containing the human LPL cDNA cloned in pGEM, mouse gamma-actin, or the pGEM vector alone without the LPL insert. As shown in **Fig. 3**, there was no difference in transcriptional rate of either LPL or gamma-actin from the adipose tissue of any of the three groups of rats.

To determine whether hypothyroidism affected LPL synthesis, isolated adipocytes were prepared from control and hypothyroid rats and pulse-labeled with

A

B

Fig. 2. Effect of hypothyroidism on LPL mRNA levels. RNA was extracted from fat pads from control rats, hypothyroid rats, and T_3-treated hypothyroid rats, and equal quantities of total RNA were used for Northern blotting with the cDNA probes for LPL and gamma-actin. A: Representative Northern blots of RNA extracted from the fat pads of a normal and hypothyroid rat, and of a hypothyroid rat and a T_3-treated hypothyroid rat. B: Densitometric quantitation of all the LPL/actin ratios (n = 7 for control, n = 13 for hypothyroid, and n = 5 for hypothyroid-T_3 treated rats).
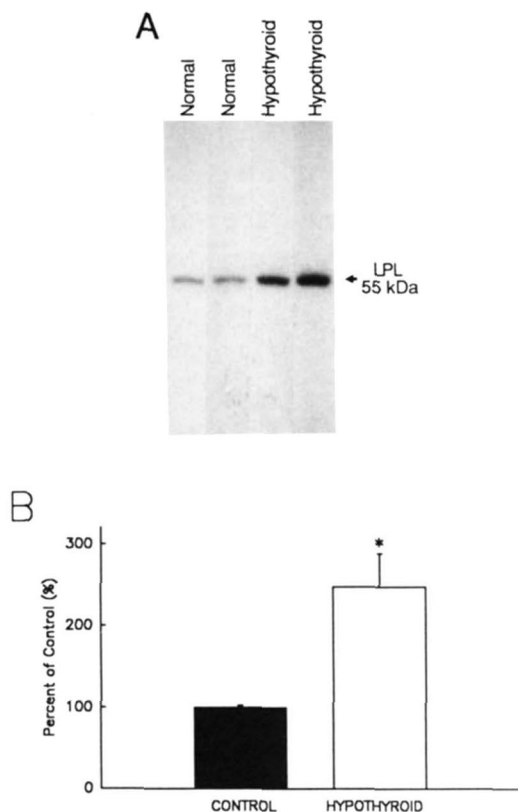
[^{35}S]methionine, followed by immunoprecipitation and SDS-PAGE. As shown in **Fig. 4A**, LPL synthetic rate was increased in the adipocytes prepared from the hypothyroid rats. When [^{35}S]methionine incorporation was quantitated by densitometry and data from all experiments pooled, [^{35}S]methionine incorporation into LPL from hypothyroid rat adipocytes was 250% of incorporation of control cells (Fig. 4B). Although the [^{35}S]methionine labeling occurred over a short time period (30 min), the increase in incorporation of isotope into LPL could have been due to a decrease in LPL degradation. To assess LPL degradation, cells from control and hypothyroid animals were pulse-labeled for 30 min with [^{35}S]methionine, and then chased for 135 min. As shown in **Fig. 5**, there was no difference between control and hypothyroid adipocytes in LPL degradation. Thus, the increased [^{35}S]methionine incorporation into LPL was due to increased LPL synthesis.
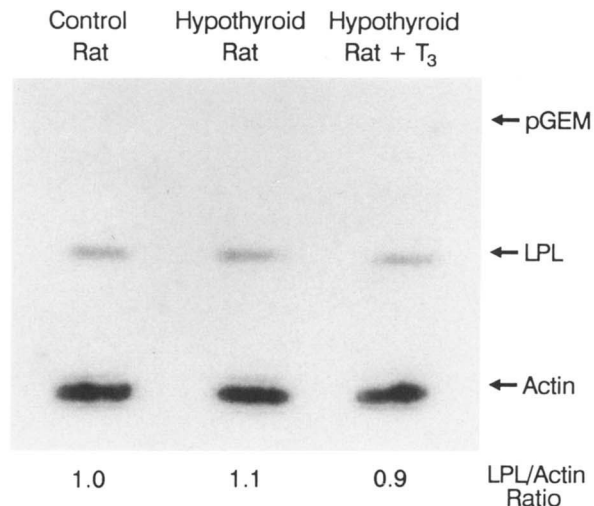
Fig 3. Effect of hypothyroidism on LPL transcription. Eight hypothyroid rats were injected with T_3, as described in Methods, and compared to seven hypothyroid and eight control rats that were injected with vehicle. After 10 days, fat pads were removed, and nuclei were isolated, followed by transcriptional run-off, as described under Methods. The membrane was prehybridized with the cDNAs for LPL and gamma-actin, and the pGEM vector alone, as a control. Equal counts of extracted, radiolabeled RNA from the run-off assays were added to each prehybridized slot, followed by washing, and autoradiography.

To determine whether thyroid hormone had direct effects on adipocyte LPL, isolated adipocytes were prepared from normal rats and cultured in the presence and absence of T_3. As shown in **Fig. 6**, 2 nM T_3 resulted in a decrease in both LPL activity and immunoreactive mass in the heparin-releasable and extractable fractions of the adipocyte. Heparin-releasable and extractable activities were decreased to 20% and 58% of control, and heparin-releasable and extractable masses were 60% and 64% of control, respectively. T_3-induced changes in LPL may have been due to changes in LPL mRNA level. Therefore, RNA was extracted from control and T_3-treated adipocytes and studied with Northern analysis. As shown in Fig. 6B, the T_3-treated adipocytes demonstrated no change in LPL mRNA level when compared to the level of the constitutive mRNA gamma-actin.

To determine the effects of T_3 in vitro on LPL synthesis, adipocytes from normal rats were treated overnight with 2 nM T_3, and then pulse-labeled with [^{35}S]methionine, followed by immunoprecipitation and SDS-PAGE. When cells were pulse-labeled for 15, 30, and 45 min, the incorporation of [^{35}S]methionine into LPL was linear in the presence of T_3 (**Fig. 7**). In Fig. 7B, the results of five experiments are shown using the 30 min time point of [^{35}S]methionine incorporation. Together, these data indicate that T_3 decreased [^{35}S]methionine incorporation into LPL to 60% of incorporation into control cells.

A

LPL
55 kDa

B

Fig. 4. LPL synthetic rate in hypothyroid rats. After preparation of isolated adipocytes from the fat pads of hypothyroid and control rats, the cells were pulsed-labeled for 30 min with [^{35}S]methionine and immunoprecipitated. A sample of the original [^{35}S]methionine-labeled extract was precipitated with TCA, and the immunoprecipitates were loaded onto an SDS-gel in proportion to the total TCA counts. A: Representative protein gel of the immunoprecipitated LPL from two control rats and two hypothyroid rats. B: Summary of the densitometric analysis of the immunoprecipitated LPL from all control and hypothyroid rats (n = 7 for control, n = 13 for hypothyroid rats); *$P < 0.05$ versus control.

T_3 may have affected LPL activity and mass through posttranslational changes in degradation or secretion. To better understand the mechanism of the decrease in LPL in response to T_3, cells were pulse-labeled with [^{35}S]methionine and then chased with unlabeled methionine (**Fig. 8**). Cells that were treated with T_3 demonstrated a decrease in [^{35}S]methionine incorporation into LPL, as shown in Fig. 7, and in the time 0 lanes of the cell fractions of Fig. 8. During the chase, there was a gradual decrease in the intensity of the band for cellular LPL, and an increase in medium LPL due to LPL secretion (Fig. 8). However, the decrease in cellular LPL and the increase in medium LPL were consistently proportional to the initial rate of LPL synthesis; in both the presence and absence of T_3, $t_{1/2}$ of cellular LPL was about 30 min. In addition, the decreased secretion of LPL into the medium was proportional to the decreased LPL synthesis.
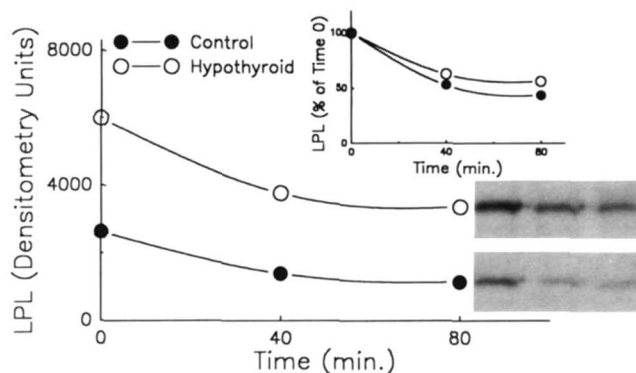

Fig. 5. Effect of hypothyroidism on LPL degradation. Isolated adipocytes were prepared from control and hypothyroid rat pads. After pulse-labeling the adipocytes with [^{35}S]methionine for 30 min, cells were chased with medium containing unlabeled methionine for the indicated times. The immunoprecipitated bands were then scanned using densitometry. The autoradiographic image is shown beside the densitometry data.

Thyroid hormone may change the response of LPL to other hormones, as well as have independent effects. To examine the effects of hypothyroidism on the response of adipocyte LPL synthesis to epinephrine, isolated adipocytes were prepared from control and hypothyroid rats, and were cultured in the presence and absence of epinephrine for 2 h, followed by pulse-labeling with [^{35}S]methionine and immunoprecipitation. As shown in **Fig. 9A**, incorporation of [^{35}S]methionine into LPL in the control cells was decreased at an epinephrine concentration of 10^{-5}M. Incorporation into LPL in the adipocytes from hypothyroid rats was higher than the control cells in the absence of epinephrine exposure, as demonstrated above. However, there was virtually no response to epinephrine in the hypothyroid cells. Thus, thyroid hormone deficiency not only resulted in an increase in LPL, but also blunted the inhibitory response of LPL to epinephrine. To prove that this unresponsiveness to epinephrine was due to the thyroid hormone deficiency, another group of animals were studied. In addition to control and hypothyroid rats, a group of hypothyroid rats was treated with T_3 for 10 days, followed by adipocyte preparation and pulse-labeling in response to epinephrine. As shown in Fig. 9B, treatment of rats with T_3 resulted in decreased [^{35}S]methionine incorporation into LPL compared to hypothyroid rats, and completely restored the responsiveness to epinephrine.

DISCUSSION

Because of the importance of LPL to lipid metabolism, much attention has been focused on the nutritional and hormonal regulation of this enzyme.

A

B

■ Control □ T₃-Treated

C
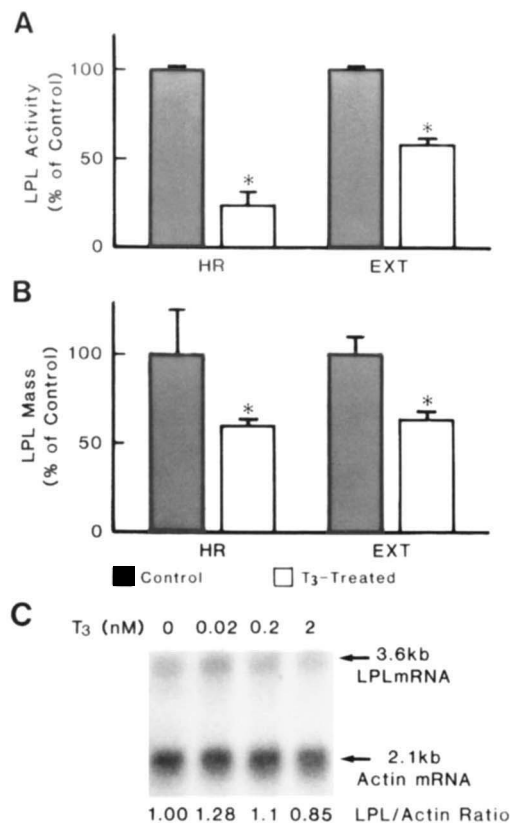
Fig. 6. Effect of T₃ treatment in vitro on adipocyte LPL. Isolated adipocytes were treated with the indicated concentration of T₃ for 24 h prior to measurement of LPL in the heparin-releasable (HR) and extractable (EXT) fractions (see Methods). A: LPL catalytic activity in response to 2 nM T₃. Control LPL activity was 1.64 and 9.48 nEq/min per 10^6 cells for HR and EXT, respectively. B: LPL immunoreactive mass in response to 2 nM T₃. Control LPL mass was 3.45 and 56.0 ng/10^6 cells for HR and EXT, respectively. Data are the mean of five separate experiments. C: LPL mRNA levels, one of three representative experiments. Below each lane is the LPL/actin ratio; *$P < 0.05$; **$P < 0.01$ versus control cells.

Previous studies have examined the effects of thyroid hormone in rats by rendering the rats hypothyroid by surgery or anti-thyroid drugs. Although the plasma triglyceride levels of hypothyroid rats varied, adipose tissue LPL activity was consistently increased in these studies (1). Thus, the present studies were undertaken to examine the mechanism of the change in LPL activity in response to thyroid hormone.

As described previously (1), LPL catalytic activity in hypothyroid rats was higher than in weight-matched controls. Using an ELISA to measure LPL immunoreactive mass, there was a concomitant increase in LPL protein, suggesting that chronic thyroid hormone depletion resulted in the increased production of LPL protein with normal specific activity. In addition, there was an increase in LPL synthesis in the hypothyroid rat adipocytes, using [35S]methionine labeling followed by immunoprecipitation. In spite of this increase in LPL synthesis, however, there was no

A

B
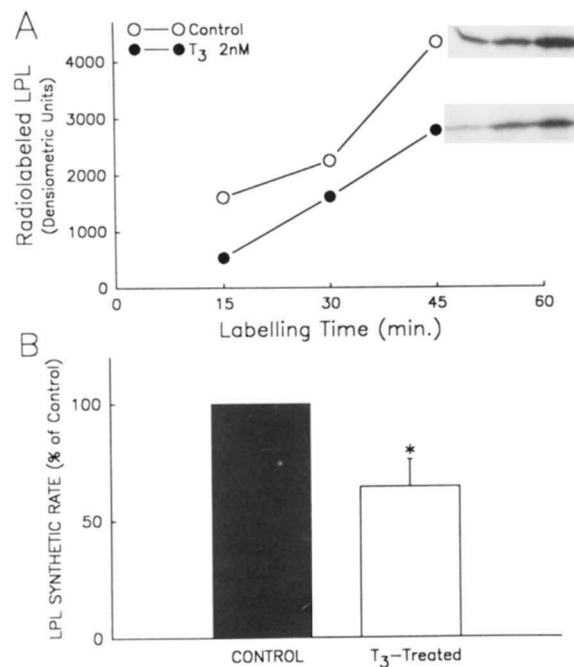
Fig. 7. Effect of T₃ treatment on [35S]methionine incorporation into LPL. A: Control cells, and cells treated with T₃ (2 nM), were pulse-labeled with [35S]methionine for 15, 30, and 45 min, followed by immunoprecipitation. The immunoprecipitated material added to the gel was normalized to the TCA precipitable counts in the control sample at each time point. One of two representative experiments. B: Summary of all experiments of the effects of T₃ on LPL synthetic rate using 30 min of labeling; n = 5; *$P < 0.05$ versus control cells.

change in the level of LPL mRNA in the hypothyroid rats, suggesting that regulation was occurring at the level of translation. The transcriptional run-off data confirmed the data with mRNA levels, and further suggested that LPL was not regulated by thyroid hormone at the transcriptional level. These changes in LPL activity and mass were reversible with treatment of hypothyroid rats with T₃, and studies in isolated adipocytes were consistent with the studies in vivo.

Rather than a direct effect on LPL translation, the changes in [35S]methionine incorporation into LPL could have been due to changes in posttranslational processing or degradation, as has been demonstrated for other regulators of LPL (14, 24, 25). In addition, the changes in LPL mass were not as great as the changes in LPL activity in some instances. However, a number of control experiments were performed to specifically examine LPL translation and posttranslational processing. In all experiments that examined [35S]methionine incorporation, the gels were loaded in proportion to total [35S]methionine incorporation, such that the changes in LPL synthesis were not due to global changes in protein synthesis. In addition, [35S]methionine incorporation into adipocyte LPL was linear, and degradation of LPL was unaffected by
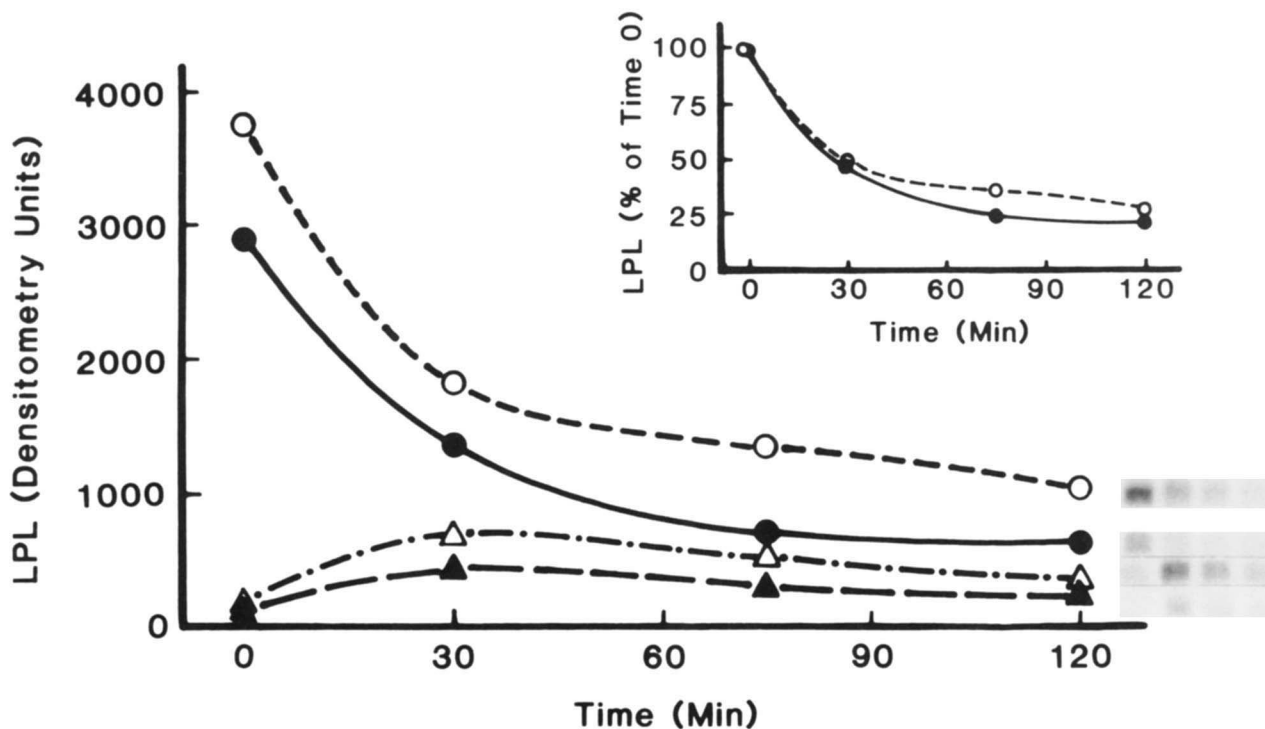
Fig. 8. Effect of T₃ in vitro on LPL synthesis and processing. Adipocytes from normal rats were cultured overnight in the presence and absence of 2 nM T₃, followed by pulse labeling with [³⁵S]methionine, followed by a "chase" in medium containing unlabeled methionine. LPL was then immunoprecipitated from the cells (○, ●) and medium (△, ▲), from both control (○, △) and T₃-treated (●, ▲) cells. Time 0 represents the end of the "pulse" and the beginning of the "chase." After exposing the gel, the LPL band, shown to the right of the figure, was quantitated by laser densitometry. In the insert, the densitometry data for cellular LPL are expressed as a percentage of the value at time 0.

thyroid hormone. Thus, these data suggest that the rate of LPL translation is altered by thyroid hormone, and LPL translation determined the level of LPL protein, which in turn controlled LPL catalytic activity.

Other studies have demonstrated that the actions of thyroid hormone and catecholamines are interrelated (26, 27), and previous data (1, 28) have demonstrated a decrease in LPL in response to catecholamines. Because [³⁵S]methionine incorporation into LPL synthetic rate is sharply reduced in response to catecholamines (29), we examined the effects of hypothyroidism on the response of adipocyte LPL to epinephrine. Adipocytes from hypothyroid animals were minimally responsive to epinephrine, whereas normal adipocytes demonstrated the expected inhibition of LPL synthesis, and treatment of hypothyroid animals with T₃ then restored the responsiveness of adipocyte LPL to epinephrine.

The binding of thyroid hormone to specific nuclear receptors is known to stimulate the expression of numerous proteins (30). In addition to the increased expression of genes coding for hepatic lipogenic enzymes and proteins related to lipogenesis, thyroid hormone also augments catecholamine-mediated stimulation of lipolysis (30, 31), which has led to the suggestion that thyroid hormone makes tissues more sensitive to catecholamines (26, 27). Although altered

catecholamine sensitivity may be the primary mechanism of thyroid hormone action in some tissues, thyroid hormone can also produce independent physiologic effects (27). For example, thyroid hormone can directly stimulate heart rate in rabbit heart preparations (32), and in vivo studies in humans found no increase in catecholamine sensitivity after subjects were made thyrotoxic (33).

In adipose tissue, hypothyroidism resulted in decreased responsiveness of lipolysis to catecholamines (34–36) even though there was no change in beta-adrenergic receptor levels (26, 36). This impairment in lipolytic responsiveness was reflected in decreased cellular cAMP levels due to an increase in the low K_m, GDP-inhibitable, cAMP phosphodiesterase, which degrades cAMP (34, 35, 37). In addition, some studies have suggested some impairment in adenylate cyclase activity in hypothyroid adipose tissue (26, 36). Thus, the decreased responsiveness of LPL to epinephrine in cells from hypothyroid rats is consistent with previous data on adipocyte lipolysis, and suggests that a second messenger common to both hormones, such as cyclic AMP, is important for LPL translation.

This study, as well as others (3–7), have shown that hypothyroidism results in an increase in the level of rat adipose LPL activity. In one study, the combined effects of hypothyroidism and diabetes were studied in
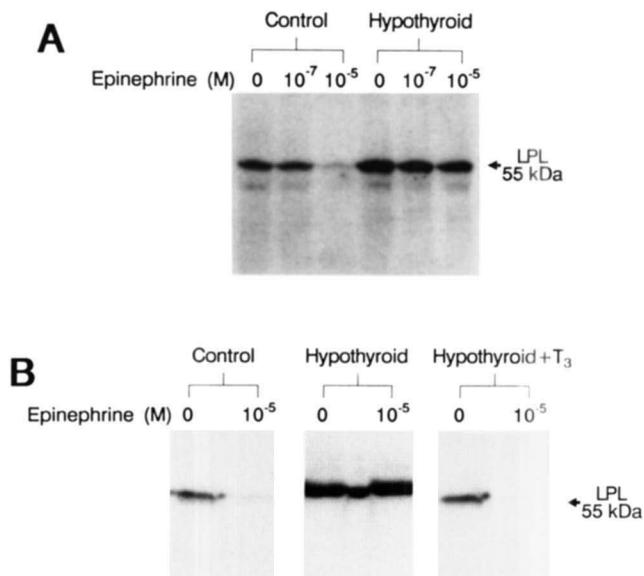
Fig. 9. Effect of thyroid hormone on the response to epinephrine. A: Adipocytes were prepared from normal and hypothyroid rats, treated with epinephrine for 2 h, followed by pulse-labeling with [^{35}S]methionine and immunoprecipitation. B: Repeat of the above experiment with a group of rats that were treated with T$_3$ for 10 days prior to adipocytes preparation and epinephrine treatment. In both experiments, adipocytes in each group were pooled from three to five rats.

rats (4). Hypothyroidism tended to counterbalance the decrease in LPL activity induced by diabetes, and insulin treatment of diabetes was synergistic with hypothyroidism in increasing LPL activity. The data described herein, along with previous studies (13, 28), provide a possible explanation for this synergism. Hypothyroidism increased LPL through an increase in translation, and previous studies demonstrated that insulin increased LPL mRNA levels (13, 28). Because of these different cellular sites of regulation, it is not surprising that the combination of hypothyroidism and insulin would greatly stimulate LPL activity.

In contrast to the studies in rats, thyroid-deficient humans demonstrated a decrease in LPL activity (38–40), leading to accumulation of remnant lipoproteins and hyperlipidemia. Thyrotoxic humans have normal levels of LPL activity in both postheparin plasma and adipose tissue (40, 41). Because of this fundamental species difference in LPL responsiveness, speculation on the mechanism of thyroid hormone's effect on human LPL must await further study.

In summary, these studies of the mechanism of regulation of adipose LPL by thyroid hormone observed parallel changes in LPL catalytic activity, immunoreactive mass, and synthetic rate. However, no changes in LPL mRNA levels were observed, suggesting that thyroid hormone regulates LPL at the level of translation. In addition, cells from hypothyroid rats

were not responsive to epinephrine, suggesting that part of the regulatory effects in vivo may be from a decrease in sensitivity to catecholamines. ▮

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