Translational regulation of lipoprotein lipase by thyroid hormone is via a cytoplasmic repressor that interacts with the 3′ untranslated region

Philip A. Kern,[1,*] Gouri Ranganathan,[*] Ada Yukht,[†] John M. Ong,[†] and Richard C. Davis[§]

Department of Medicine,* Division of Endocrinology, University of Arkansas for Medical Sciences and the John L. McClellan Memorial Veterans Hospital, Little Rock, AR 72205; Cedars-Sinai Medical Center,[†] Los Angeles, CA 90048; and Department of Medicine,[§] University of California, Los Angeles CA 90024

Abstract To better characterize the increase in lipoprotein lipase (LPL) translation by hypothyroidism, adipocytes were prepared from control and hypothyroid rats. Whereas LPL synthesis was higher in hypothyroid adipocytes, with no change in mRNA levels, there was no increase in hormone-sensitive lipase (HSL) synthesis. To determine whether a transacting translation regulatory factor was present, a cytoplasmic fraction was prepared from control and hypothyroid adipocytes, and added to an in vitro translation system containing the hLPL mRNA. The hypothyroid cell fraction from adipose and heart yielded an increase in LPL translation, when compared to control extracts. Further experiments determined that the control adipocyte extract contained a translation-inhibitory factor that was 8-fold lower in activity in the hypothyroid extract. Using different LPL mRNA constructs in the in vitro translation reaction, the region that controlled translation was localized to nucleotides 1599 to 1638 (proximal 3′ untranslated region (UTR)). To confirm the presence of a transacting factor, a sense RNA strand corresponding to this region was added to the in vitro translation reaction. This sense strand competed for the transacting factor in the control cell extract, yet had no effect on the hypothyroid cell extract. Thus, there is a translation repressor factor in the cytoplasm of rat adipocytes, and this factor is greatly reduced in activity in hypothyroid rat adipocytes. Because a similar mechanism of LPL regulation occurs in response to epinephrine, the absence of the translation repressor may be a mechanism for the loss of sensitivity of hypothyroid cells for catecholamines.—**Kern, P. A., G. Ranganathan, A. Yukht, J. M. Ong, and R. C. Davis.** Translational regulation of lipoprotein lipase by thyroid hormone is via a cytoplasmic repressor that interacts with the 3′ untranslated region. *J. Lipid Res.* 1996. **37:** 2332–2340.

Supplementary key words lipoprotein lipase • hypothyroidism • adipose tissue • hormone sensitive lipase • translation • RNA binding protein • catecholamines

Lipoprotein lipase (LPL) hydrolyzes the triglyceride core of chylomicrons and very low density lipoproteins, and is the enzyme responsible for adipose tissue lipid accumulation. LPL is regulated by a number of physiologic conditions, and in response to a variety of hormones (1). However, the mechanism of LPL regulation is complex, involving multiple sites in transcriptional and post-transcriptional processing. For example, in response to feeding, the increase in LPL activity occurs through post-translational changes, resulting in an activation of the LPL enzyme (2, 3). In response to other hormonal changes, LPL is regulated translationally. When epinephrine was added to primary cultures of rat adipocytes, there was a decrease in LPL synthesis with no corresponding change in LPL mRNA level (4). Recent studies have examined the translational regulation of LPL by epinephrine in more detail, and have suggested that epinephrine stimulated the production or activation of a transacting factor that interacted with sequences on the 3′ UTR of the LPL mRNA, and thereby inhibited LPL translation (5).

LPL is also regulated at the level of translation by thyroid hormone. LPL activity is increased in hypothyroid rat adipocytes (1), and this increase in LPL activity is accompanied by an increase in LPL immunoreactive mass and LPL synthetic rate, but no change in LPL mRNA level (6). Furthermore, the rather high level of LPL synthesis in hypothyroid rat adipocytes is not inhibited by treatment with epinephrine, suggesting that hypothyroidism yields adipocytes that are relatively insensitive to catecholamines. This study was intended to further characterize LPL translational regulation in the

Abbreviations: LPL, lipoprotein lipase; HSL, hormone-sensitive lipase; UTR, untranslated region; GAPD, glyceraldehyde phosphate dehydrogenase.

[1]To whom correspondence should be addressed.

adipocytes from hypothyroid rats. We sought to determine whether the increased LPL translation found in hypothyroid adipocytes could be due to the presence of a factor that augmented translation or due to the absence of an inhibitory factor. Our data suggest that an inhibitory factor is present in normal adipocytes and is greatly reduced in the adipocytes from hypothyroid rats.

METHODS

Animals and cells

Male Sprague-Dawley rats weighing between 180 and 220 g were killed after an overnight fast, and the epididymal adipose tissue, heart, liver, and kidneys were removed immediately. For hypothyroid rats, thyroids were removed surgically 6 to 8 weeks prior to killing the animals, and pooled serum uniformly demonstrated elevated thyroid-stimulating hormone levels. Control rats were weight-matched; because hypothyroid rats tend to gain weight more slowly, the control rats were slightly younger than the hypothyroid rats. Isolated adipocytes were then prepared from the adipose tissue by a collagenase digestion (6).

LPL and HSL synthetic rate

The synthetic rates of LPL and hormone-sensitive lipase (HSL) were measured in cultured cells using a 30-min pulse of [^{35}S]methionine (50 μCi), followed by immunoprecipitation with specific antibodies and analysis on a 10% polyacrylamide-SDS gel (7). The anti-LPL antibodies and the anti-HSL antibodies have been described previously (8, 9); the anti-HSL antibodies were a generous gift from Dr. F. B. Kraemer. The immunoprecipitations were performed with equal TCA-precipitated counts and there was no difference in TCA counts between control and hypothyroid adipocytes.

RNA extraction and Northern blotting

RNA was extracted from adipocytes using the method of Chomczynzki and Sacchi (10). RNA was quantitated spectrophotometrically, and the quality of RNA was verified by ethidium bromide staining of rRNA bands on a minigel. Equal amounts of total RNA were resolved on a 2.2 M formaldehyde–1% agarose gel, and Northern blotting with the human LPL cDNA (11) and human HSL cDNA (12) was performed as described previously (7). For some experiments, RNA loading was normalized to glyceraldehyde phosphate dehydrogenase (GAPD).

Preparation of cytoplasmic cell extract

An S-100 fraction was prepared from adipose tissue, heart, liver, and kidney, as a modification of a method of Walden, Patino, and Gaffield (13), and as described previously (5) for adipocytes. Control and hypothyroid cells or tissue, each from 5 animals, were homogenized in 2 volumes of lysis buffer (50 mM Tris-HCL, pH 7.4, 250 mM sucrose, 35 mM KCl, 10 mM $MgCl_2$, 0.5 mM EDTA, 7 mM β-mercaptoethanol), using 10 strokes of a glass homogenizer. Homogenates were centrifuged at 10,000 *g* for 15 min at 4°C. Five ml of the post-nuclear extract was used to prepare a high speed supernatant fraction (S-100) by centrifugation at 100,000 *g* for 2 h at 4°C. Solid ammonium sulfate was added to the S-100 fraction to 60% saturation and precipitated for 0.5 h on ice. Precipitated proteins were collected by centrifugation at 6,000 *g* for 10 min at 0°C, redissolved, and dialyzed against buffer A (20 mM Tris-HCL, pH 7.4, 20 mM KCl, 7 mM β-mercaptoethanol, 0.1 mM EDTA, and 10% glycerol). Protein concentration in the cell extract was determined with the Bio-Rad Protein Assay, using BSA as a standard. Equal quantities of the cell extract (0.1 μg) were used in the rabbit reticulocyte lysate, except as noted for Fig. 4.

In vitro translation

For in vitro translation, RNA transcripts from a variety of human LPL cDNA constructs were used (described below). In addition, RNA transcripts were made from the cDNAs for HSL (12), and neomycin phosphotransferase (Neo) (14). Template DNA was linearized with a suitable restriction enzyme to obtain a complete transcript of the cloned DNA. One μg of linearized DNA was transcribed with either SP6 or T7 Polymerase using SP6/T7 Transcription Kit (Boehringer Mannheim). Equal quantities of RNA transcripts (0.1 μg) were translated in a rabbit reticulocyte lysate system (Promega) in the presence of [^{35}S]methionine for 60 min, and the translation products were analyzed by SDS-PAGE and autoradiography. In previous studies, the in vitro translation of LPL was linear for up to 90 min. For in vitro translation of poly A-enriched RNA, total RNA was extracted from control and hypothyroid adipocytes. Poly A-enriched RNA was prepared using the Poly A tract mRNA isolation system III (Promega). Equal quantities (1 μg) of RNA were added to the rabbit reticulocyte lysate system in the presence of [^{35}S]methionine of 60 min. Translation products were immunoprecipitated (8), and proteins were analyzed by SDS-PAGE and autoradiography. The images from the in vitro translation were quantitated by densitometry within the linear range of the autoradiogram using a Soft Laser Scanning Densitometer SLR-2D/1D (Zeineh).

Preparation of constructs

Clone B in Fig. 5 is LPL35, described by Wion et al. (11). It contained 174 nucleotides of 5′ untranslated sequence, the complete coding sequence (1428 nucleotides), and 822 nucleotides of the 1950-nucleotide 3′ UTR.

The full length 3.6 kb LPL construct (clone A in Fig. 5) was prepared as described previously (15) using clones LPL35 (in pGEM4Z), LPL37, and LPL46, which were described by Wion et al. (11).

For clone C, LPL35 (clone B) was cut at the EcoRI site at nucleotide 1638.

For clone D, the entire 5′ UTR of LPL35 was replaced with 6 nucleotides encoding a BamHI site, and all but 44 nucleotides of the 3′ UTR were removed. This was accomplished using PCR to amplify the appropriate region of LPL35. To minimize nucleotide misincorporation, amplification was carried out using VENT polymerase with proofreading activity. The resulting fragment was cloned into pGEM2 using the BamHI and EcoRI sites.

Clone E was a generous gift from Dr. Robert H. Eckel. Clone B served as a template for the introduction of EcoRI and Hind III restriction sites by PCR and the elimination of the 5′ and 3′ UTRs. The upstream and downstream primers were CTTAAGCTTCCCGAGATG GAGAGC and ATGAGAATT CAGCCTG ACTTCTT, respectively. The construct was confirmed by double-stranded DNA sequencing, and was cloned into pGEM2 for in vitro transcription.

Clone F represented a shortened coding sequence, and was created using PCR to insert a stop codon after codon 302. The upstream primer (GACT GAATTC GCCACC ATG GAGAGC AAA GCC CTG CTC) contained 4 flanking nucleotides, an EcoRI site, 6 nucleotides representing a consensus Kozak sequence for translation initiation (16), and the first 21 nucleotides of coding sequence. The downstream primer (ACGT GGATCC TCA GTA CAT TTT GCT GCT TCT TT) contained 4 flanking nucleotides, a BamHI site, and reverse complement for a stop codon and 20 to 21 nucleotides of coding sequence preceding codon 303. After PCR using the proofreading polymerase, the fragment was cloned into the EcoRI and BamHI sites of pGEM2.

The ''sense'' RNA strand described in Fig. 6 was generated from clone B by PCR, using the appropriate primers, except for the addition of T7 polymerase sequences on the upstream primer. This construct was confirmed by double-stranded DNA sequencing.

For expression, purified plasmid DNA was digested at a polylinker restriction site downstream of the LPL insert, and in vitro RNA transcription was carried out using the appropriate upstream promoter for viral RNA polymerase.

RESULTS

The regulation of LPL translation is suggested by the increase in LPL synthetic rate, but with no change in LPL mRNA level. Adipocytes from control and hypothyroid rats were labeled in vitro with [^{35}S]methionine, followed by immunoprecipitation with antibodies to LPL and HSL, followed by SDS-PAGE, as described in Methods. In addition, RNA was extracted from the same adipocytes and Northern blots were performed using equal quantities of total RNA. As shown in **Fig. 1,** the hypothyroid rat adipocytes demonstrated an increased LPL synthetic rate, with no change in mRNA level. On average, there was a 2.5-fold increase in LPL synthetic rate. In previous studies, there was no change in LPL protein degradation with hypothyroidism (6), demonstrating that regulation was occurring at the translational level.

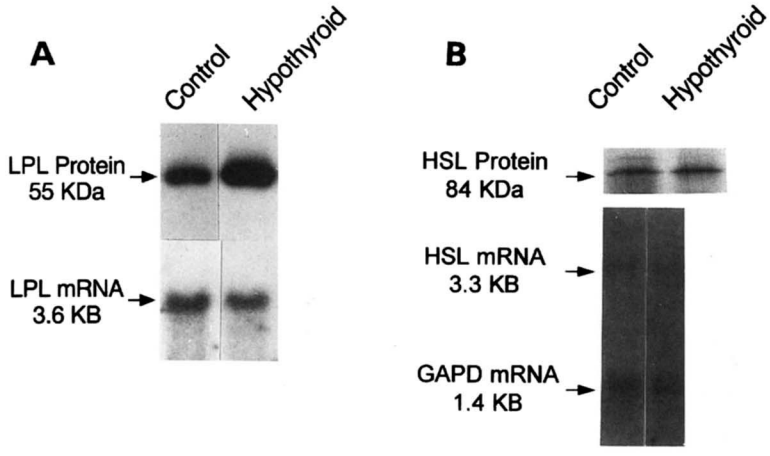

Fig. 1. Increase in LPL translation in hypothyroid adipocytes. Pooled adipocytes from control and hypothyroid rats were pulse-labeled with [^{35}S]methionine. Immunoprecipitation with anti-LPL (A) and anti-HSL (B) antibodies was performed on equal TCA counts from the control and hypothyroid cells. RNA was extracted from the same cells and equal quantities of total RNA were added to gels for Northern blotting with the [^{32}P]-LPL-labeled cDNA.

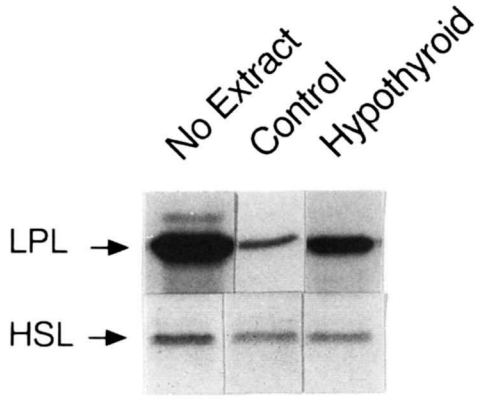

Fig. 2. Effects of a cytoplasmic extract on LPL translation. As S-100 fraction was prepared from control and hypothyroid adipocytes, and added to an in vitro translation system, consisting of rabbit reticulocytes, [^{35}S]methionine, and either the in vitro transcribed human LPL mRNA (full-length 3.2 kb), or human HSL mRNA. Five μl of extract from control and hypothyroid cells, corresponding to 0.1 μg of protein, was added to the reticulocyte lysates, and translation was performed for 60 min. The translation products were analyzed by SDS-PAGE and autoradiography.

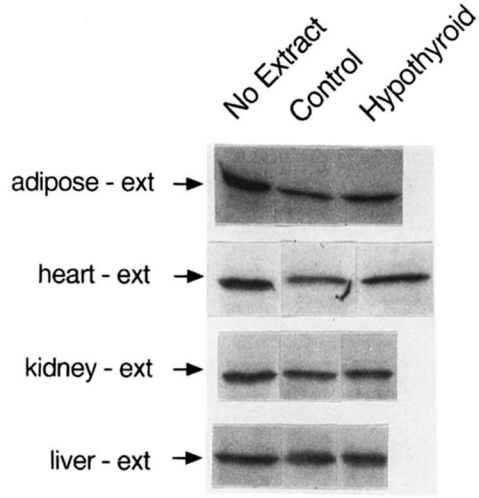

Fig. 3. Tissue specificity of hypothyroidism. Cytoplasmic extracts were prepared from adipose tissue, heart, kidney, and liver of control and hypothyroid rats. These extracts were added to the in vitro translation system using the LPL mRNA.

Hypothyroidism resulted in no change in the synthesis of HSL and no change in HSL mRNA levels (Fig. 1), suggesting that the effects on LPL are somewhat specific. The increase in LPL translation by hypothyroidism was reversed by treatment of rats with T_3, and the opposite effect (decrease in LPL synthesis) was demonstrated by treatment of normal cells with T_3 (6). An unlikely possibility for these observations could be hypothyroidism-mediated mRNA structural alterations, perhaps due to editing. However, there was no change in size of the LPL message from hypothyroid adipose tissue by Northern blotting (Fig. 1), and the translatability of the poly (A)-enriched mRNA from adipocytes was intact (data not shown), suggesting that there were no large structural changes in the LPL mRNA.

To determine whether LPL translation in hypothyroid adipocytes was controlled by a transacting binding factor, adipocyte cytoplasmic extracts containing 0.1 μg protein were added to an in vitro translation system containing the LPL mRNA. As described in Methods, this cellular extract (S-100 fraction) was added to a reticulocyte lysate system along with the in vitro transcribed full-length LPL mRNA in the presence of [^{35}S]methionine. As shown in **Fig. 2,** the addition of an S-100 fraction from control cells inhibited LPL translation in this in vitro system by 90%. However, the addition of the S-100 fraction from the hypothyroid adipocytes had less inhibitory effect (50% in this experiment, see below for further quantitation). These effects on translation were specific for LPL, as there were no differences in the effects of the hypothyroid and control extracts on the translation of hormone-sensitive lipase (HSL), or neomycin phosphotransfersase (data not shown).

To assess the tissue specificity of this effect of hypothyroidism, we determined whether cytoplasmic extracts from other hypothyroid rat tissues affected LPL translation. S-100 fractions from adipose tissue, heart, liver, and kidney were prepared using the same methods described above for adipocytes, and added to the in vitro translation system containing the LPL mRNA and [^{35}S]methionine. As shown in **Fig. 3,** the extract from control adipose tissue yielded a 75% decrease in translation, whereas hypothyroid adipose tissue yielded only a 40% decrease in translation. This difference was not as great as with adipocyte extracts (Fig. 2), probably due to the presence of cytoplasmic proteins from non-adipose cells. The cytoplasmic extract prepared from hypothyroid rat heart resulted in similar increases in LPL translation, compared to the extract from control heart. However, cytoplasmic extracts from liver and kidney of hypothyroid rats had no effect on LPL translation, when compared to liver and kidney cytoplasmic extracts from control rats.

The effect of thyroid hormone on translation could have been due to the presence of a factor in the hypothyroid cells that stimulated LPL translation. Alternatively, there may have been an inhibitory factor present in control cells that was absent in hypothyroid adipocytes. To distinguish between these possibilities, and to better quantitate the differences in translation, increasing amounts of the cytoplasmic cell extract from both control and hypothyroid adipocytes were added to the in vitro translation system. As shown in the inset of **Fig.**

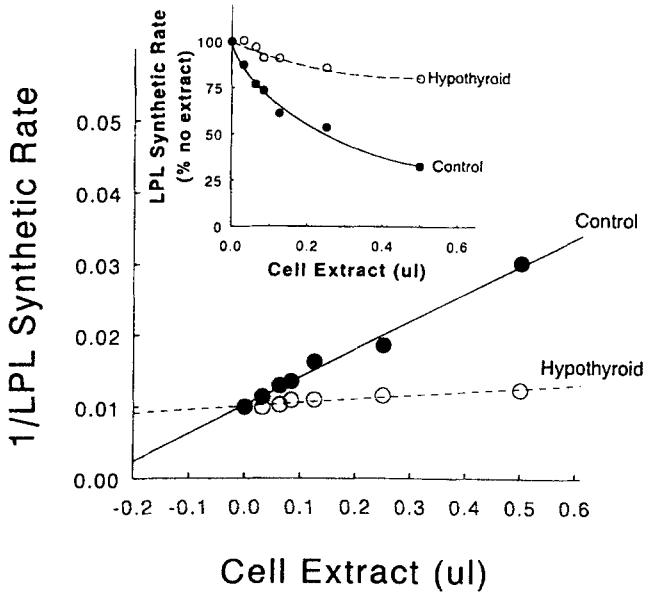

Fig. 4. Effects of different quantities of cell extract on in vitro translation. Cytoplasmic cell extracts were prepared from control and hypothyroid cells and added in increasing quantities (diluted in buffer A, see Methods) to the in vitro translation reaction. Data are expressed in relation to the addition of no cell extract. Using a Dixon plot, the quantity of cell extract yielding half-maximal synthetic rate was 0.25 μl for control cells, and 1.97 μl for hypothyroid cells. Inset: Inhibition of LPL in vitro translation by the control and hypothyroid extracts.

4, the addition of increasing amounts of the control cell extract resulted in an inhibition of LPL translation. However, the addition of increasing amounts of the hypothyroid extract had less effect than the control extract, suggesting that there was not a translation-stimulatory factor in the hypothyroid extract. When these data were plotted in a Dixon plot (Fig. 4), control cell extract appeared to contain about 8-fold more LPL synthesis inhibitor activity than the hypothyroid cell extract.

To determine what region of the LPL mRNA was involved with the altered translation, various LPL cDNA constructs were transcribed and translated in vitro in the reticulocyte lysate system in the presence of the control and hypothyroid extracts. As shown in **Fig. 5A,** constructs were prepared that were deficient in either the 5′ UTR or various lengths of the 3′ UTR. In vitro translation with these constructs revealed that the hypothyroid cell cytoplasmic extract fraction had a mild to moderate inhibition of translation, when compared to the addition of no extract (Fig. 5B). However, the control cell extract almost completely inhibited translation in all constructs except constructs E and F, which were lacking the entire 3′ UTR. These data suggest that at least 39 nucleotides of 3′ UTR were necessary for the regulation of LPL translation by thyroid hormone. Of note, constructs C and D were identical except for the presence and absence of the 5′ UTR, demonstrating that the 5′ UTR was not involved in LPL translation regulation under these circumstances.

If a transacting RNA binding protein were present in the control cell extract, the addition of a sequence of "sense" RNA to the in vitro translation reaction would be expected to compete for this binding protein, and restore translation towards normal. As shown in **Fig. 6,** the indicated sequences of LPL mRNA "sense" strands, corresponding to the terminal 87 nucleotides of the coding sequence and the proximal 235 nucleotides of the 3′ UTR, were added to the in vitro translation reaction in increasing quantities. In the presence of the control extract, increasing quantities of the sense RNA strand resulted in an increase in LPL translation, suggesting competition with a transacting binding factor. In contrast, the addition of sense RNA strand had no effect on LPL translation in the presence of the hypothyroid extract, and had no effect on LPL translation in the absence of any extract. Thus, this experiment provided additional evidence for the presence of an inhibitory binding protein in the control extract and its relative absence in the hypothyroid extract.

DISCUSSION

The regulation of LPL is complex. In some instances, changes in LPL mRNA levels have been demonstrated, whereas in other cases, regulation in LPL translation and post-translational processing have been demonstrated (17). Much important physiologic regulation of LPL occurs at the posttranscriptional level. Glycosylation of LPL at the first N-linked glycosylation site is essential for catalytic activity, intracellular transport, and secretion (18–21), and these posttranslational changes are responsible for the increase in LPL activity with feeding (2, 3). We have described changes in LPL translations (i.e., an increase in LPL synthetic rate, with no corresponding change in LPL mRNA levels) in response to several important physiologic stimuli, including the addition of glucose, epinephrine, and T_3 in vitro (4, 6, 22), and the treatment of diabetes in humans (23). In rats, the induction of hypothyroidism resulted in an increase in LPL activity (1), which was shown to be at the level of translation (6). This study was undertaken to better characterize the translational regulation of LPL by thyroid hormone.

To obtain evidence for the presence of a transacting factor, a cytoplasmic extract was prepared from control and hypothyroid adipocytes. LPL translation in vitro was inhibited by increasing concentrations of control extract, but much less inhibited by addition of increas-

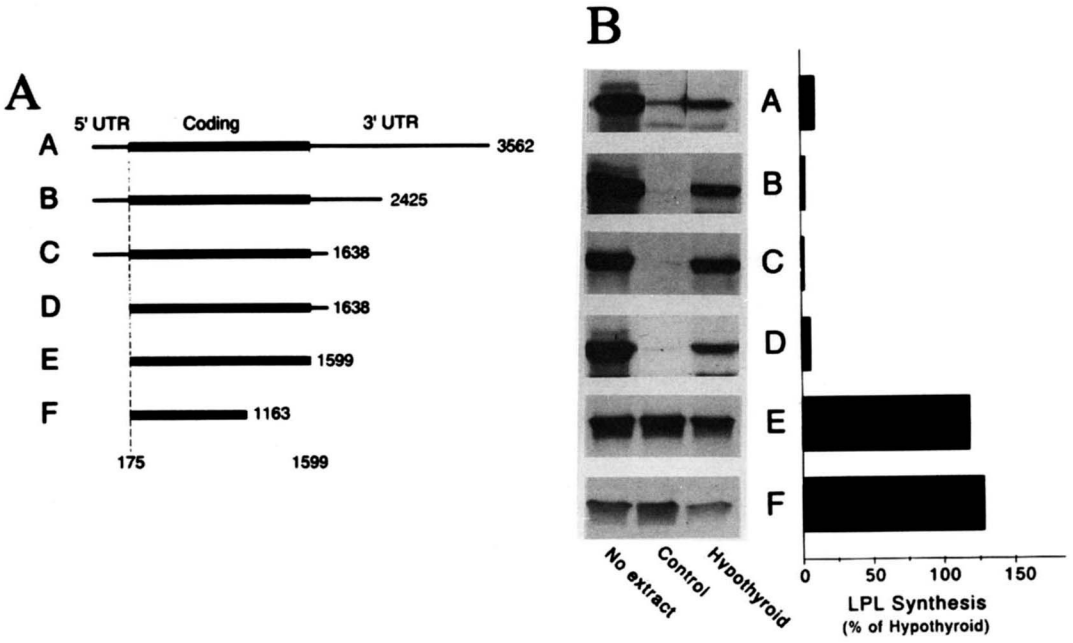

Fig. 5. Effect of the hypothyroid cell extract on different LPL mRNA constructs. A: LPL constructs used for analysis of translational regulation. B: Effect of the cytoplasmic extract on the different LPL constructs. S-100 cytoplasmic extracts derived from control and hypothyroid cells were added to reticulocyte lysates in the presence of each of the constructs in A. Autoradiograms are from representative experiments, and at least 3 experiments were performed with each construct. The bar graph for each construct represents the mean of the densitometric images, expressed as a percent of the image obtained from the hypothyroid cell extract.

ing concentrations of hypothyroid extract. These data suggested that an inhibitory factor was present in control extracts, and that the increase in translation in the hypothyroid cells was due to the absence of this inhibitory factor. In addition, the thyroid hormone-mediated decrease in this transacting factor only occurred in heart and adipose tissue, which are LPL-producing tissues, and only affected the LPL message. Using a Dixon plot, we estimated that the hypothyroid extract of adipose tissue was 8-fold deficient in this inhibitory factor. Although there are many examples of translation inhibitory factors, there are relatively few examples of translation augmenting factors, especially involving the 3′ UTR (24). Thus, these data correspond with many previous studies of translational regulation.

The region of the LPL mRNA involved in controlling translation was determined using in vitro translation to examine the expression of variety of LPL constructs. Deletion of the 5′ UTR had no effect on the translational changes observed with the control and hypothyroid cell extracts. However, the inhibitory effect of the control extract required the first 39 nucleotides of the 3′ UTR. Constructs lacking this region showed similar translation rates in the presence or absence of either control or hypothyroid extracts, and showed similar translation rates to the addition of "no cell extract." Although these data indicate the importance of the first 39 nucleotides of the 3′ UTR, they do not exclude the possibility of other regulatory elements on the distal 3′ UTR.

Further evidence for the absence of an inhibitory factor in the hypothyroid extract was obtained from the use of sense strand competition in the in vitro translation assays. The addition of an RNA sense strand (corresponding to nucleotides 1512 to 1834) to the reticulocyte lysate reactions restored translation towards normal when control extract was added, but had no effect on the reaction when the hypothyroid extract was added. These sense strand competition experiments also made it unlikely that translation inhibition is due to a nonspecific process, such as the differential presence of a protease, and suggest the presence of an RNA binding protein. These data, however, cannot determine whether hypothyroidism results in a decreased quantity of this RNA binding protein, or a decrease in activity of a protein that may change in activity (e.g., due to phosphorylation).

In previous studies, we noted that LPL translation was inhibited by the addition of epinephrine (4). In addition, adipocytes from hypothyroid rats demonstrated increased levels of LPL translation, and maintained this high level of LPL translation when treated with epinephrine (6). Thus, hypothyroid adipocytes were insensitive to treatment with epinephrine. Recently, we have characterized the translational inhibition of LPL by epi-

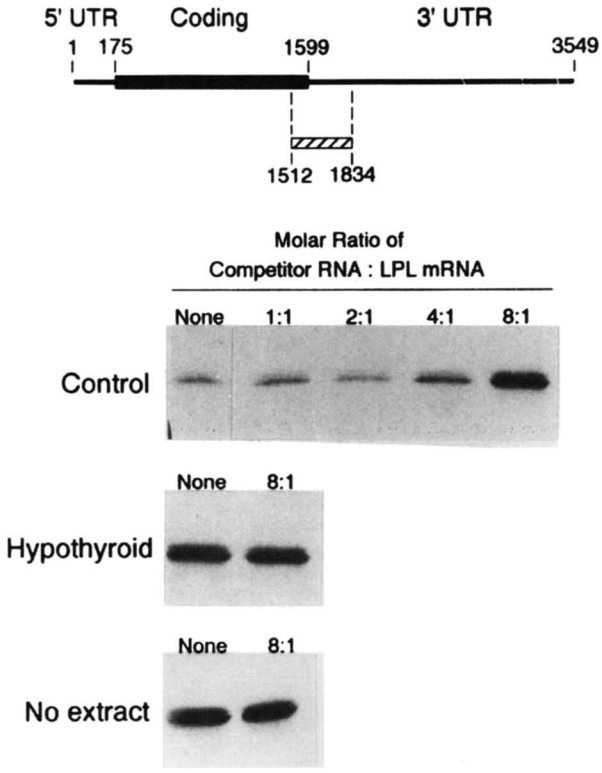

Fig. 6. Competition for transacting factor with sense RNA strand. An RNA sense strand, corresponding to nucleotides 1512 to 1834, was added to the in vitro translation system along with cell extracts from control and hypothyroid cells. Increasing quantities of sense RNA were added, up to a sense RNA:LPL mRNA molar ratio of 8:1. [^{35}S]methionine was then added and in vitro translation then proceeded for 60 min.

nephrine in 3T3-L1 cells, and found evidence for the presence of a transacting substance that was produced in response to epinephrine, and bound to the 3′ UTR of the LPL mRNA between nucleotides 1599 to 1640 (5). This transacting substance was present at a high level of activity in epinephrine-treated cells, but was also present in control cells, suggesting that this factor played a constitutive role in the regulation of LPL expression. The data described herein suggest that this same factor is absent in the cells from hypothyroid adipocytes. Such a conclusion is supported by previous data (6), which demonstrated that hypothyroid adipocytes were unresponsive to epinephrine. If hypothyroid adipocytes do not elaborate this constitutive LPL mRNA binding factor, then these cells may remain unresponsive to catecholamines.

Many studies have examined the interactions between thyroid hormone and catecholamines (25, 26). The binding of thyroid hormone to nuclear receptors stimulates the expression of genes coding for hepatic lipogenic enzymes (27) and also augments catecholamine-mediated stimulation of lipolysis (27, 28), suggesting that thyroid hormone makes adipose and other tissues more sensitive to catecholamines (25, 26). Although altered catecholamine sensitivity may be an important mechanism for thyroid hormone action in some tissues, thyroid hormone can also produce independent physiologic effects. For example, GAPD hormone can directly stimulate heart rate in rabbit heart preparations (29).

Previous studies of translational regulation have suggested that sequences in the 5′ UTR or 3′ UTR may be important in controlling translation (30). A well-characterized system of translational regulation involved the inhibition of ferritin translation due to the binding of a transacting factor to the 5′ UTR, leading to inhibition of mRNA initiation and dissociation of the mRNA from polysomes (31–33). In a recent study, we found no evidence for an inhibition of LPL initiation by epinephrine and much evidence for regulation at the 3′ UTR. Numerous studies have demonstrated the importance of the 3′ UTR in mRNA stability, as with the regulation of the transferrin receptor (34), and several studies have shown that specific sequences in the 3′ UTR can control translational efficiency as well (24, 35–38). Translational regulation due to changes at the 3′ UTR may affect translation initiation or may primarily affect translation elongation. In addition, the motif recognized by the RNA binding protein may be short and devoid of obvious secondary structure. For example, the RNA recognition motif family of RNA binding proteins recognize short sequences (3 to 11 nucleotides) of uridylates on the 3′ UTR. Several members of this family are TIA-1 and TIAR, which may play a role in apoptotic cell death (39).

Whereas numerous previous studies have demonstrated increased adipose LPL activity in hypothyroid rats, hypothyroid humans have decreased LPL activity (reviewed in ref. 1). The human LPL cDNA was used for the studies with rat adipose extracts described herein, suggesting that the differences between rats and humans do not lie with LPL but with the RNA binding protein.

In summary, adipocytes from hypothyroid rats express higher levels of LPL due to an increase in LPL translation. This is due to the absence of a constitutive factor that exerts an inhibitory effect on LPL translation by binding to the 3′ UTR of the LPL mRNA. LPL expression from hypothyroid adipocytes has previously been shown to be resistant to the effects of catecholamines, and catecholamines have been shown to stimulate the activity of an LPL mRNA binding protein in adipocytes. These data suggest that the resistance to catecholamine-mediated inhibition of LPL translation is due to the absence of this same transacting factor in hypothyroid adipocytes, which then cannot be stimulated by the addition of catecholamines.

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