The Translational Regulation of Lipoprotein Lipase in Diabetic Rats Involves the 3′-Untranslated Region of the Lipoprotein Lipase mRNA*

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Adipose tissue lipoprotein lipase (LPL) activity is decreased in patients with poorly controlled diabetes, and this contributes to the dyslipidemia of diabetes. To study the mechanism of this decrease in LPL, we studied adipose tissue LPL expression in male rats with streptozotocin-induced diabetes. Heparin releasable and extractable LPL activity in the epididymal fat decreased by 75–80% in the diabetic group and treatment of the rats with insulin prior to sacrifice reversed this effect. Northern blot analysis indicated no corresponding change in LPL mRNA levels. However, LPL synthetic rate, measured using [35S]methionine pulse labeling, was decreased by 75% in the diabetic adipocytes, and insulin treatment reversed this effect. These results suggested regulation of LPL at the level of translation. Diabetic adipocytes demonstrated no change in the distribution of LPL mRNA associated with polysomes, suggesting no inhibition of translation initiation. Addition of cytoplasmic extracts from control and diabetic adipocytes to a reticulocyte lysate system demonstrated the inhibition of LPL translation in vitro. Using different LPL mRNA transcripts in this in vitro translation assay, we found that the 3′-untranslated region (UTR) of the LPL mRNA was important in controlling translation inhibition by the cytoplasmic extracts. To identify the specific region involved, gel shift analysis was performed. A specific shift in mobility was observed when diabetic cytoplasmic extract was added to a transcript containing nucleotides 1818–2000 of the LPL 3′-UTR. Thus, inhibition of translation is the predominant mechanism for the decreased adipose tissue LPL in this insulin-deficient model of diabetes. Translation inhibition involves the interaction of a cytoplasmic factor, probably an RNA-binding protein, with specific sequences of the LPL 3′-UTR.

Lipoprotein lipase (LPL)hydrolyzes the core of triglyceride-rich lipoproteins (chylomicrons and very low density lipoprotein) into free fatty acids and monoacylglycerol, facilitating the removal of triglyceride-rich lipoproteins from the bloodstream. Patients with diabetes, especially insulin-deficient diabetes, often manifest a decrease in adipose tissue LPL activity, and this is accompanied by an increase in plasma triglycerides (1). With insulin treatment, there is an improvement in both LPL activity and triglycerides (2, 3). The regulation of LPL activity is closely linked to insulin levels and nutritional state, as demonstrated by the changes in LPL during cycles of feeding and fasting (4–6).

Both in rat models of diabetes and human diabetes, the use of drugs to improve diabetes control resulted in increased adipose tissue LPL activity (1, 7, 8). However, recent studies demonstrated that the treatment of diabetes resulted in increases in LPL protein and LPL synthesis with no change in LPL mRNA levels, suggesting posttranscriptional regulation, possibly at the level of LPL translation (3, 9).

Translational regulation has been identified as an important mechanism for the regulation of LPL in response to catecholamines and thyroid hormone (10, 11). In response to catecholamines, cultured adipocytes demonstrate a 4-fold decrease in LPL synthesis mediated by the presence of a RNA-binding protein, which interacts with a region on the proximal 3′-UTR of LPL mRNA (12). Hypothyroid rats demonstrate an increase in LPL translation, and this is thought to be related to the absence of the RNA-binding protein that binds to the same region (11). The expression of many other genes is regulated by translation, and this can occur through RNA-binding proteins that bind to specific regions of either the 5′- or 3′-UTRs of the mRNA, and interfere with translation (13, 14).

We have studied the mechanism involved in the regulation of LPL activity in the adipose tissue of diabetic rats. This inhibition of LPL activity was accompanied by a corresponding decrease in LPL synthetic rate with no significant change in LPL mRNA. To further characterize the mechanism involved, we made cytoplasmic extracts from adipocytes and studied the effect of cytoplasmic trans-acting factors on translation of various LPL constructs. We have identified a region of the 3′-UTR UPL mRNA that is involved in an RNA protein interactions, resulting in inhibition of LPL translation in diabetes.

**MATERIALS AND METHODS**

**Animals**—Male Harlan Sprague-Dawley rats (175–200 g) were purchased from Harlan Sprague-Dawley (Indianapolis, IN). Three groups with insulin; PAGE, polyacrylamide gel electrophoresis; RT-PCR, reverse-transcription polymerase chain reaction.
of rats were used in these studies: control (C) rats, diabetic (D) rats, and diabetic rats that were treated with insulin (DI). The rats were made diabetic by tail vein injection of Streptozotocin (80 mg/kg body weight) dissolved in 50 mM citrate buffer, pH 4.5. Control rats were injected with the same volume of buffer. Insulin-treated diabetic rats were treated identically to the diabetic rats except that they received 8 units of neutral protamine Hagedorn human insulin subcutaneously on each of the last 2 days before sacrifice. All the animals were sacrificed 14 days after streptozotocin injection. At the time of sacrifice, blood glucose levels in the diabetic group were greater than 375 mg/dl, insulin-treated diabetic rats were higher than the control but considerably lower than the diabetic group (Table I). The epididymal fat pads were immediately removed and processed as described below.

Measurement of LPL Activity—Heparin releasable and extractable LPL activities were determined (15). To measure heparin releasable LPL, 10 mg of minced adipose tissue was incubated in 1 ml of Dulbecco’s modified Eagle’s medium containing 10 units/ml heparin for 45 min at 37 °C. After collecting the heparin released fraction, tissue LPL was extracted in 50 mM phosphate-buffered saline, pH 7.4, containing 1% Triton X-100, 0.1% SDS, and 0.5% deoxycholate, as described previously (6). LPL catalytic activity was measured as described previously (16), using a substrate containing [3H]triolein and human serum as a source of apoC-II. LPL activity was expressed as nanomoles of free fatty acid released per minute per 10^9 cells. Cell number was determined using the method of DiGirolamo (17).

LPL Synthetic Rate—The synthetic rate of LPL was measured in adipocytes using a 30-min pulse with [35S]methionine (100 μCi/ml), as described previously (18). Previous studies have demonstrated the linearity of [35S]methionine incorporation into adipocytes for up to 90 min in the presence and absence of insulin and thyroid hormone (18, 19). The unincorporated label was aspirated, and the total cellular proteins were extracted in lysis buffer containing 50 mM phosphate buffer, pH 7.4, 2% deoxycholate, 1% SDS, 20 mM phenylmethylsulfonyl fluoride, 2 mM leupeptin, and 2 mM EDTA. The extracts were immunoprecipitated using specific polyclonal antibodies as described previously (20). Immunoprecipitated samples were analyzed on 10% SDS-PAGE followed by autoradiography.

RNA Extraction and Northern Blotting—RNA was extracted from adipocytes using the method of Chomczynski and Sacchi (21). Equal quantities of total RNA from the various treatment groups were analyzed using 2.2 μg formaldehyde, 1% agarose gels. Northern blots were probed using [32P]dCTP-labeled human LPL (22) and actin cDNA probes followed by autoradiography. The intensity of the image was quantitated using Gelbase/Gelblot software (Ultraviolet Products, Ltd., San Gabriel, CA). The bar graph represents the mean arbitrary units generated from the densitometric analysis of the images. B, analysis of LPL/Actin mRNA ratio in C, D, and DI treated rat adipocytes. The bar graph represents the mean of arbitrary units generated from the densitometric analysis of the Northern blots of total RNA from control, diabetic, and insulin-treated adipocytes, probed for LPL and actin mRNA.

Preparation of Cytoplasmic Cell Extract—An S-100 fraction was prepared from adipocytes isolated from control, diabetic, and insulin-treated diabetic rats, as described previously (10, 26). Cells were homogenized in 2 volumes of lysis buffer (50 mM Tris-HCl, pH 7.4, 250 mM sucrose, 35 mM KCl, 10 mM MgCl₂, 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, and the postnuclear extract was used to prepare S-100 fraction by centrifugation at 100,000 × g for 30 min on ice. Proteins were fractionated using 60% ammonium sulfate, and the precipitated proteins were 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). Equal quantities of cytoplasmic extracts (0.1 μg of protein) were used to analyze effects on in vitro translation using the rabbit reticulocyte lysate system (Promega).

Preparation of Constructs—Clone 2 in Fig. 3 is LPL35, described by Wiss et al. (27). It contains 174 nucleotides of 5′-untranslated sequence, the complete coding sequence (1428 nucleotides), and 822 nucleotides of the 3′-UTR of LPL. Clone 1 is identical to clone 2 except that it includes the 3′-UTR up to the first polyadenylation site at nucleotide 3155, as described earlier (28). Clone 3 was made by cloning clone 2 described above with EcoRI.

In Vitro Translation—RNA transcripts from human LPL cDNA constructs were used for in vitro translation studies, as described previously (12). Template DNA was linearized with a suitable restriction enzyme to obtain a complete transcript of the cloned DNA. Linearized DNA was transcribed; RNA transcripts (0.1 μg) were translated using a rabbit reticulocyte lysate system (Promega) in the presence of [35S]methionine for 60 min. Cytoplasmic extracts from adipocytes were added to the in vitro translation reaction. The translation reaction products were analyzed by SDS-PAGE and autoradiography.

Gel Shift Analysis—Four overlapping DNA segments were synthesized using PCR with a T7 transcription promoter added to the 5′ primer, corresponding to LPL nucleotides 1512–1663, 1645–1833, 1158–1177, and the downstream primer was nucleotides 1369–1389. An equal volume of each fraction (containing 0.1–1 ng of RNA) was reverse-transcribed, followed by PCR for 35 cycles at 55°C. The resulting ethidium bromide-stained gel was imaged using an Imagestore 5000 scanner and analyzed using the Gelbase/Gelblot software (Ultraviolet Products, Ltd., San Gabriel, CA).

Table I

| Heparin released | Extracted | Glucose |
|------------------|-----------|---------|
| C                | 22 ± 0.4g | 22 ± 6.0 | 108 ± 5 |
| D                | 3.2 ± 0.8g| 5.9 ± 1.0g| 405 ± 24g|
| DI               | 73 ± 12g  | 34 ± 7.0g| 165 ± 7g|

*p < 0.01 versus control.
The data shown represent one of two similar experiments. RT-PCR and expressed as a percentage of the maximum LPL mRNA. The amount of LPL mRNA present in each fraction was quantitated using Fractions 1–20 contain both 18 and 28 S ribosomal subunits. The fractions 10 and 15, which contains monosomes and short polysomes, mRNA, associated with the polysomes. The second peak was between numbered 1–24 from the gradient fractionation of postmitochondrial supernatants. Fractions are Total RNA was extracted from C and D adipocytes after sucrose somes.

To study the mechanism of regulation by diabetes and insulin treatment, LPL mRNA levels and LPL synthetic rate in the DI adipocytes was similar to that in control adipocytes. To compare LPL mRNA levels, Northern blots were performed on the adipocytes from C, D, and DI rats. When expressed as the LPL/actin ratio, LPL mRNA in the D rat adipose tissue was no different from LPL mRNA in the C adipose tissue (Fig. 1B). In a similar manner, the LPL/actin mRNA ratio in the DI adipocytes was not significantly different from the C group (Fig. 1B). Thus, these data show that large changes in LPL activity can be explained by changes in LPL synthesis and are not reflected in changes in LPL mRNA. These data suggest that the major step of regulation is at the level of LPL translation.

One mechanism of translational regulation involves the interaction of transacting factors with the 5'-UTR, leading to the dissociation of mRNA from the polysomes and an inhibition of translational initiation (30). To determine whether such a mechanism also occurred with LPL, we studied the distribution of LPL mRNA on polysome preparations from C and D rat adipocytes. As shown in Fig. 2, we detected no change in the distribution of LPL mRNA on polysomes from C and D rat adipocytes. In a similar manner, the LPL/actin mRNA ratio in the DI adipocytes was not significantly different from the C group (Fig. 1B). Thus, these data show that large changes in LPL activity can be explained by changes in LPL synthesis and are not reflected in changes in LPL mRNA. These data suggest that the major step of regulation is at the level of LPL translation.

Another site for regulation by transacting binding proteins is the 3'-UTR, which is involved in the regulation of LPL by catecholamines (10). To determine whether a similar mechanism is involved with diabetes, we prepared cytosolic extract from control and diabetic rat adipocytes as described under “Materials and Methods” and added these cytosolic extracts to a rabbit reticulocyte lysate in vitro translation system containing LPL transcript. If cytoplasmic factors were present in the adipocyte extracts that bound to the LPL transcript and regulated translation, we would expect to see a change in LPL translation by the reticulocyte lysate sys-
tem. In this in vitro translation reaction, we used three different constructs (Fig. 3). Each of these constructs contained the full 5'-UTR and coding sequence. The first 3.2-kilobase construct contained essentially the whole LPL mRNA sequence up to the first consensus polyadenylation sequence on the 3'-UTR (27). The second and third constructs contained progressive deletions of the 3'-UTR of the LPL mRNA and terminated at nucleotide 2435 and 1640, respectively. As shown in Fig. 3, translation of constructs 1 and 2 was inhibited by the addition of diabetic adipocyte extracts. Although there was some inhibition of LPL translation by the control extracts, as described by us previously (11), the diabetic adipocyte extract significantly inhibited LPL translation in constructs 1 and 2. However, construct 3, which terminated at nucleotide 1640, was not inhibited by the addition of the extracts, indicating that an inhibitory factor was interacting with a sequence on the 3'-UTR beyond nucleotide 1640.

To obtain further evidence for the interaction of a protein with the LPL 3'-UTR, we performed gel retardation assays. As described under “Materials and Methods,” RNA transcripts were made to fragments of LPL 3'-UTR spanning the region between nucleotides 1512 and 2144 (the end of the coding region is at nucleotide 1599). Equal amounts of adipocyte extract from C, D, and DI adipose tissue were then added to each of the 32P-labeled transcripts. Four overlapping RNA sequences were transcribed corresponding to LPL nucleotides 1512–1663, 1645–1833, 1818–2000, and 1981–2144 (transcripts 1–4, Fig. 4). Whereas RNA transcripts 1, 2, and 4 formed no complex with the diabetic adipose extract, RNA transcript 3, corresponding to nucleotides 1818–2000, formed a complex with the diabetic extract which caused a mobility shift (Fig. 4). Addition of the DI extract caused a mobility shift; however, the complex formed was 10-fold less intense with the same amount of extract protein, indicating a decrease in amount of complex generated. To demonstrate the specificity of this complex formation, we added an excess of unlabeled transcript 3. As shown in Fig. 5, an excess of transcript corresponding to nucleotides 1818–2000 prevented the gel shift, whereas the addition of an excess of irrelevant RNA transcript did not compete for complex formation.

DISCUSSION

Lipoprotein lipase is a central enzyme in lipid metabolism, and the adipose tissue enzyme is important in the regulation of plasma triglyceride levels and in the accumulation of adipose tissue lipid stores (31, 32). The regulation of LPL in adipose tissue is complex and occurs at multiple cellular sites (33). Insulin is an important regulator of adipose tissue LPL, but the mechanism of regulation by insulin is dependent on the species and system studied. For example, in humans and rats LPL activity is increased in the hyperinsulinemic postprandial state, and this increase is due to increased LPL posttranslational processing (5, 6). Changes in LPL activity are accompanied by increases in LPL mRNA levels in other animal models (34). In vitro studies of the effect of insulin on LPL in adipocytes have demonstrated increases in LPL mRNA levels in rat
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adipocyte primary cultures (18) and increased posttranscriptional processing in 3T3-L1 adipocytes (35).

Patients with both type 1 and type 2 diabetes manifest decreased adipose LPL activity, which increases following treatment of the diabetes (2, 3, 7, 8). In humans, this increase in LPL activity was not accompanied by an increase in mRNA levels, but was associated with an increase in LPL protein and synthetic rate (3). Similar observations were made in rats, which were rendered diabetic with streptozocin (9). Since these data suggested a translational mechanism of regulation, we examined the regulation of LPL by streptozocin-induced diabetes in greater detail.

LPL activity was greatly decreased in the D rats compared with the C and DI rats. Of interest, the DI rats demonstrated LPL activity that was even higher than that of the control rats, which appeared to reflect the rapid rate of new LPL synthesis and processing that accompanied the administration of insulin. Similar finding were observed previously (9). When the adipose tissues of these rats were examined for LPL gene expression, there were minimal changes in LPL mRNA levels despite over 5-fold changes in LPL activity, indicating that the changes in LPL activity could not be explained based on the changes in LPL transcription. On the other hand, the changes in LPL synthesis, based on [3H]methionine labeling, closely paralleled the changes in LPL activity between the D and C rats. The considerable increase in LPL activity in the DI rats (compared with C) could not be accounted for by the small increase in LPL/actin mRNA and the increase in LPL synthetic rate, suggesting that posttranslational regulation was important, as has been described previously in other hyperinsulinemic states (5). Overall, these data suggest that translational regulation is the primary means of LPL regulation in this setting of insulin deficiency.

Translational regulation of protein synthesis can occur through the actions of trans-acting binding proteins and can involve sequences on either the 5′-UTR or 3′-UTR of the mRNA (14). Involvement of the 5′-UTR has been described in the regulation of ferritin translation, where the binding of a trans-acting protein to the 5′-UTR resulted in the dissociation of ferritin mRNA from the polysomes (29, 30). To examine this mechanism in our system, we performed an analysis of polysomes prepared from control and diabetic adipocytes. We found no change in the distribution of LPL mRNA with the polysomes, suggesting that the translational regulation was not due to a change in initiation of translation.

Translational regulation can also occur through interactions of transacting factors with the 3′-UTR (13, 36). We reported previously the involvement of trans-acting proteins that inhibited LPL translation by binding to the proximal 3′-UTR between nucleotides 1599 and 1638 (12). We prepared cytoplasmic extracts from adipocytes derived from the C, D, and DI rats and added these extracts to an in vitro translation system. The cytoplasmic extract from the D rats inhibited translation of LPL in the reticulocyte lysate system. However, when the transcript used in the assay terminated at nucleotide 1640, the D extract did not inhibit translation, indicating that the motif on the 3′-UTR involved in regulating translation was located beyond nucleotide 1640.

To further identify the motif, a gel shift analysis was performed, using overlapping 180–200 nucleotide RNA fragments corresponding to the 3′-UTR. These data indicated the presence of an RNA binding factor in the diabetic extract that interacted specifically with the RNA fragment corresponding to nucleotides 1818–2000. Taking into account the overlapping sequences of the other fragments, the motif for this RNA binding factor probably involves nucleotides 1834–1980.

plasmic extract from DI rats did not inhibit LPL translation in vitro, but the gel shift reaction still demonstrated some residual binding. One explanation for this observation is the formation of an RNA-binding protein or complex that involves enzyme (e.g. kinase) activity. The adipose tissue from DI rats may still contain RNA binding protein, which may bind to the RNA, but it may be catalytically inactive due to the insulin treatment.

Translational regulation of LPL has been identified previously following the depletion of PFKC from adipocytes (37) and following treatment of adipocytes with epinephrine and thyroid hormone (11, 38). In the studies involving epinephrine and thyroid hormone, similar methods were used to identify the RNA binding motif, which was on the proximal 3′-UTR of LPL between nucleotides 1599 and 1638 (12). Thus, the RNA binding motif in the diabetic adipose tissue (between nucleotides 1834 and 1980) is different from that of epinephrine treated cells and suggests that translation inhibition from diabetes may involve a different RNA-binding protein.

In summary, insulin-deficient diabetic rats manifest low levels of adipose tissue LPL predominantly due to an inhibition of LPL translation. This decrease in translation is due to the interaction of a cytoplasmic substance with cis-acting sequences on the 3′-UTR of the LPL mRNA. The further characterization of this trans-acting substance will be important in the characterization of the abnormalities associated with the diabetic condition.

Acknowledgments—We are pleased to acknowledge Annadell Fowler for technical assistance and Kimberly Henning for assistance with animal maintenance and experimental procedures.

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