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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 [^{35}S]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)[1] 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 of LPL 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

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[1] The abbreviations used are: LPL, lipoprotein lipase; UTR, untranslated region; C, control rats; D, diabetic rats; DI, diabetic rats treated with insulin; PAGE, polyacrylamide gel electrophoresis; RT-PCR, reverse-transcription polymerase chain reaction.

TABLE I
Effect of diabetes and insulin treatment on LPL activity
All data are expressed as mean ± S.E., n = 10–12 for each group.

	LPL activity		Glucose
	Heparin released	Extracted	
	nmol/min/10^6 cells		*mg%*
C	22 ± 0.4	22 ± 6.0	109 ± 5
D	3.2 ± 0.8[a]	5.9 ± 1.0[a]	405 ± 24[a]
DI	73 ± 12[a]	34 ± 7.0[a]	165 ± 7[a]

[a] $p < 0.01$ *versus* control.

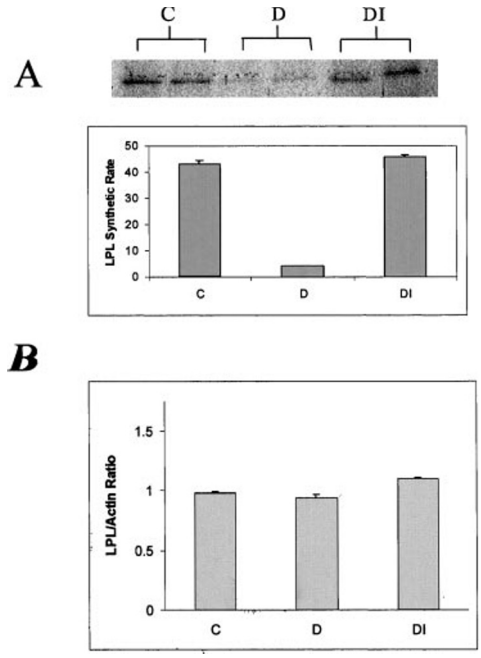

FIG. 1. *A*, LPL synthetic rate in C, D, and DI rat adipocytes. Adipocytes were pulse-labeled with [^{35}S]methionine and immunoprecipitated. The autoradiograph represents one of three representative experiments. The bar graph represents the mean arbitrary units generated from the densitometric analysis 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.

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 veil injection of Streptozotocin (60 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, 100 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 [^{3}H]triolein and human serum as a source of apoC-II. LPL activity was expressed as nanomoles of free fatty acid released per minute per 10^6 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 [^{35}S]methionine (100 μCi/ml), as described previously (18). Previous studies have demonstrated the linearity of [^{35}S]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 amounts of total RNA from the various treatment groups were analyzed using 2.2 M formaldehyde, 1% agarose gels. Northern blots were probed using [^{32}P]dCTP-labeled human LPL (22) and actin cDNA probes followed by autoradiography. The intensity of the image was quantitated using the Eagle sight™ 3.0 Image Capture and analysis software (Stratagene 2, La Jolla, CA), and the ratio of LPL/actin mRNA was calculated.

Polysome Preparation and RT-PCR—Polysome profiles were obtained as described previously (10, 23). Postmitochondrial supernatants were prepared from adipocytes isolated from control and diabetic rats. Cytoplasmic extracts were reconstituted with 0.25 M sucrose and layered over 10–50% sucrose gradients. Gradients were centrifuged at 180,000 × g for 3 h at 4 °C. To demonstrate the release of all LPL mRNA into the free fraction, 1 mM EDTA was added to a control gradient. Gradients were collected in 12 1-ml fractions, and polysome profiles were recorded by reading UV absorption at 260 nm. Each fraction was precipitated, and RNA was extracted. Measurement of LPL mRNA levels in each fraction was done using RT-PCR, as described previously (24). The primers for this reaction were derived from the mouse LPL cDNA sequence, and the upstream primer was nucleotides 1158–1177, and the downstream primer was nucleotides 1369–1389 (25). 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).

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 $MgCl_2$, 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, 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 Wion *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 cutting clone 2 described above with *Eco*RI.

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 [^{35}S]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, 1818–2000, and 1981–2144. The template sizes were verified on agarose gels and used to generate [α-^{32}P]UTP-labeled transcript

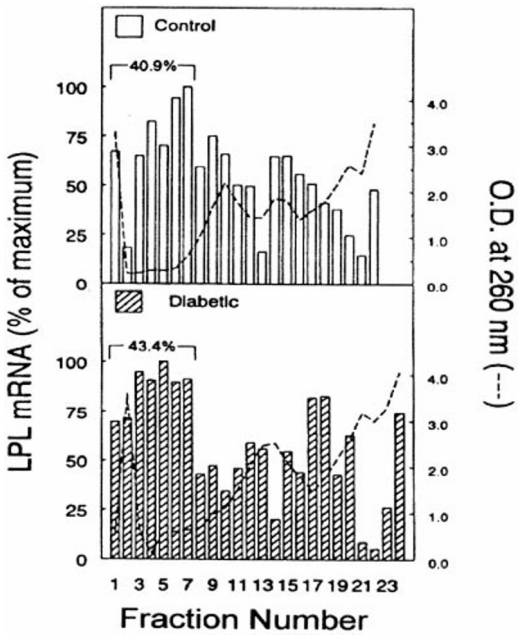

FIG. 2. **Effect of diabetes on LPL mRNA distribution in polysomes.** Total RNA was extracted from C and D adipocytes after sucrose gradient fractionation of postmitochondrial supernatants. Fractions are numbered 1–24 from the *bottom* to the *top* of the gradient. The first 260 nm absorption peak was between fractions 1 and 3, which contains the mRNA, associated with the polysomes. The second peak was between fractions 10 and 15, which contains monosomes and short polysomes. Fractions 1–20 contain both 18 and 28 S ribosomal subunits. The amount of LPL mRNA present in each fraction was quantitated using RT-PCR and expressed as a percentage of the maximum LPL mRNA. The data shown represent one of two similar experiments.

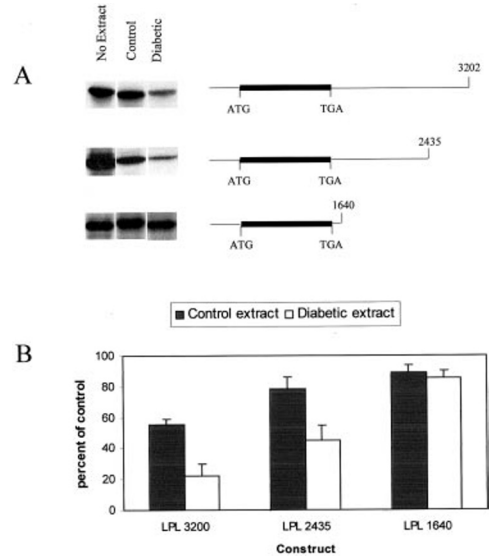

FIG. 3. **Effect of control and diabetic cytoplasmic extracts on LPL translation.** *A*, cytosolic extracts were prepared from C and D adipocytes as described under "Materials and Methods." The specified extract was added to the *in vitro* translation assay containing [^{35}S]methionine and each LPL mRNA transcript. Translation reactions were all done for 60 min. The products were analyzed by SDS-PAGE. *B*, autoradiographic analysis of three experiments. Data are expressed as percentage of control, where control is the autoradiographic image from "no extract" added to the *in vitro* translation reaction. *LPL 3200*, *LPL 2435*, and *LPL 1640* refer to the constructs shown in *A*. *, $p < 0.05$ *versus* the control extract from the same construct, and also $p < 0.05$ versus the diabetic extract from construct LPL 1640. Statistical analysis was performed with a Student's *t* test.

(transcripts 1–4, Fig. 4), using T7 RNA polymerase (Promega). RNA was transcribed in the presence of [α-^{32}P]UTP, 10 mCi/ml (PerkinElmer Life Sciences), 1 mM ATP, CTP, GTP, and 0.1 mM UTP. Transcripts were purified using phenol extraction and ethanol precipitation. Gel shift analysis was performed as described previously (29). Labeled transcript (50,000 cpm) was incubated with cytosolic extracts (5–10 μg of protein) from adipocytes at 22 °C for 20 min in the presence of yeast tRNA (25 ng) and buffer containing 20 mM Tris, pH 7.4, 50 mM KCl, 10% glycerol, and 1 mm dithiothreitol. Heparin was added to a final concentration of 5 μg/ml, and incubation was continued for an additional 10 min. Samples were separated on 5% polyacrylamide in 1× TBE (0.09 M Tris base, 0.09 M boric acid, 0.002 M EDTA) and analyzed by autoradiography.

RESULTS

Rats were sacrificed 14 days after streptozotocin injection. Table I shows the blood glucose and adipose tissue LPL activity in the three groups of rats. Because diabetes results in accelerated lipolysis and a reduction in fat cell size, LPL activity data were expressed per 10^6 cells (Table I). Blood glucose was uniformly elevated (>375 mg %) in the D rats, and LPL activity was decreased over 75% *versus* C. In the DI rats, blood glucose was lower than in the D rats, although still elevated compared with C. However, in the DI group of rats, LPL activity was greatly increased, and was higher than in the C group, especially in the heparin released fraction.

To study the mechanism of regulation by diabetes and insulin treatment, LPL mRNA levels and LPL synthetic rate were measured. To examine LPL synthetic rate, adipocytes from C, D, and DI rats were pulse-labeled with [^{35}S]methionine followed by immunoprecipitation of LPL. As shown in Fig. 1A, there was a 75% inhibition of LPL synthesis in the cells isolated from diabetic rat adipose tissue, whereas 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. 1*B*). In a similar manner, the LPL/actin mRNA ratio in the DI adipocytes was not significantly different from the C group (Fig. 1*B*). 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 associated with the polysomes. In both C and D rat adipose tissue, 40–45% of LPL mRNA was associated with polysomes, suggesting that the 5′-UTR was not be involved in the regulatory mechanism.

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 cytoplasmic extracts 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-

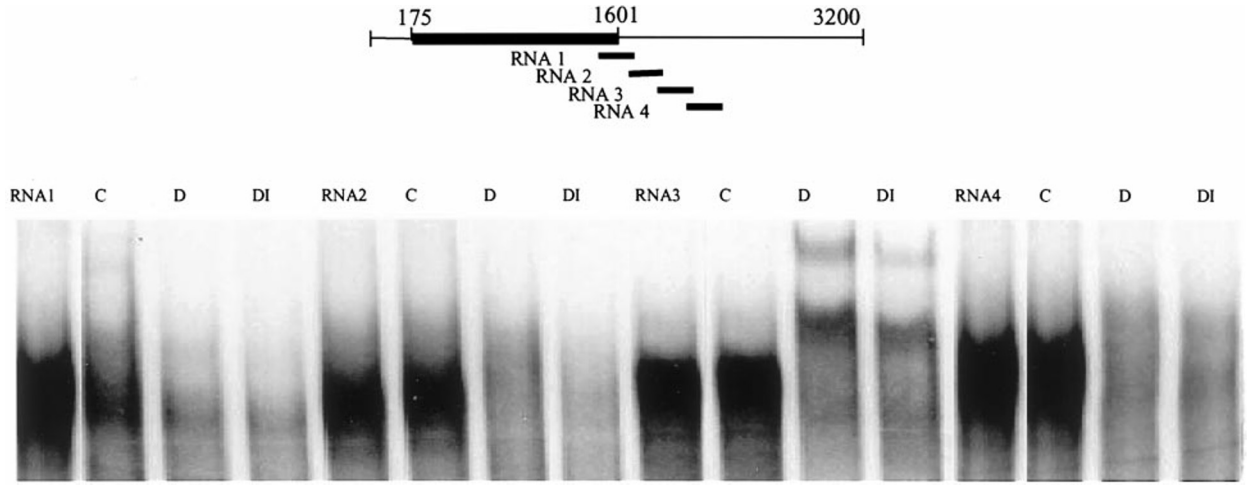

FIG. 4. **Gel shift analysis cytosolic extracts from control, diabetic, and insulin-treated rat adipocytes.** Four overlapping transcripts corresponding to LPL nucleotides 1512–1663, 1645–1833, 1818–2000, and 1981–2144 were generated as described under "Materials and Methods" and incubated with C, D, or DI cytoplasmic extracts. Samples were separated on 5% polyacrylamide in 1× TBE and analyzed by autoradiography. The figure represents one of three typical experiments.

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 ^{32}P-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.

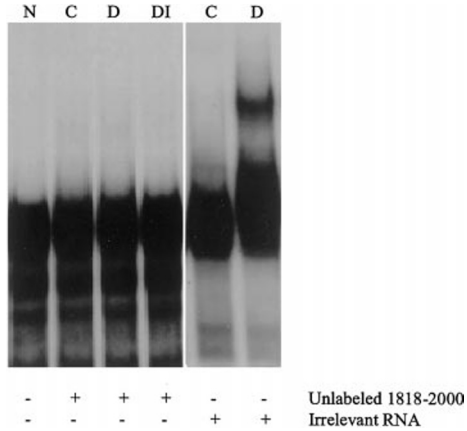

FIG. 5. **Gel shift competition analysis on cytosolic extracts from control, diabetic, and insulin-treated rat adipocytes.** Transcripts corresponding to LPL nucleotides 1818–2000 were incubated with C, D, or DI cytoplasmic extracts in the presence of unlabeled transcript, or irrelevant RNA transcript, as illustrated in the figure. Samples were separated on 5% polyacrylamide in 1× TBE and analyzed by autoradiography. The figure represents one of two typical experiments.

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

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 streptozotocin (9). Since these data suggested a translational mechanism of regulation, we examined the regulation of LPL by streptozotocin-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 [^{35}S]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 group of 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. The cytoplasmic 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 PKC 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.

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