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Tissue-specific Expression of Human Lipoprotein Lipase

EFFECT OF THE 3′-UNTRANSLATED REGION ON TRANSLATION*

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Lipoprotein lipase (LPL) is a central enzyme in lipoprotein metabolism and is expressed predominantly in adipose tissue and muscle. In these tissues, the regulation of LPL is complex and often opposite in response to the same physiologic stimulus. In addition, much regulation of LPL occurs post-transcriptionally. The human LPL cDNA is characterized by a long 3′-untranslated region, which has two polyadenylation signals. In this report, human adipose tissue expressed two LPL mRNA species (3.2 and 3.6 kb) due to an apparent random choice of sites for mRNA polyadenylation, whereas human skeletal and heart muscle expressed predominantly the longer 3.6-kb mRNA form. To determine whether there was any functional significance to this tissue-specific mRNA expression, poly(A)-enriched RNA from adipose tissue and muscle were translated *in vitro*, and the poly(A)-enriched RNA from muscle was more efficiently translated into LPL protein. The increased translatability of the 3.6-kb form was also demonstrated by cloning the full-length 3.2- and 3.6-kb LPL cDNA forms, followed by *in vitro* translation of *in vitro* prepared transcripts. To confirm that this increased efficiency of translation occurred *in vivo*, Chinese hamster ovary cells were transfected with the 3.2- and 3.6-kb LPL cDNAs. Cells transfected with the 3.6-kb construct demonstrated increased LPL activity and synthesis, despite no increase in levels of LPL mRNA. Thus, human muscle expresses the 3.6-kb form of LPL due to a non-random choice of polyadenylation signals, and this form is more efficiently translated than the 3.2-kb form.

Lipoprotein lipase (LPL)[1] is an enzyme produced predominantly by adipose tissue and muscle and is a central enzyme in the metabolism of plasma triglyceride-rich lipoproteins (1). In addition, LPL may be important in the development of obesity by promoting partitioning of dietary lipids into either adipose tissue for storage or into muscle for use as an energy substrate.

Although there is only one gene for LPL (2), there are important differences between adipose tissue and muscle LPL gene expression. In response to regulatory influences such as feeding, exercise, insulin, and catecholamines, adipose tissue and muscle LPL activity are regulated inversely (3). This tissue-specific regulation fits the physiologic role of this enzyme; in adipose tissue, LPL provides lipid for storage, whereas in muscle, LPL provides lipid for oxidative metabolism. Conditions that promote adipose lipid storage (*e.g.* feeding, insulin) stimulate adipose LPL and inhibit muscle LPL. However, the mechanism for these tissue-specific differences in LPL regulation are not known.

Studies of the mechanism of regulation of LPL in response to nutritional and hormonal factors have disclosed changes in LPL mRNA levels (4–8) and post-translational processing (9–12). In addition, the regulation of adipose LPL translation has been demonstrated *in vitro* in response to catecholamines, thyroid hormone, and glucose (13–15) and in response to improved glycemic control in human diabetics (16). Translational regulation often involves the participation of sequences in the untranslated regions of mRNA (17). The human LPL cDNA sequence is known and is characterized by a 2-kb 3′-untranslated region (UTR), with two consensus sequences for addition of the poly(A) tail (18). If these polyadenylation sites were used randomly during post-transcriptional modification of the primary LPL RNA transcript, one would expect to detect the presence of two LPL mRNA species upon Northern blotting. In previous reports, human LPL mRNA transcripts were identified at approximately 3.2 and 3.6 kb, suggesting that the choice of polyadenylation sites was not regulated. Previous studies, however, have not examined LPL mRNA from human muscle. In this report, we examined the tissue-specific expression of LPL mRNA from human adipose tissue and compared it with human skeletal and cardiac muscle.

MATERIALS AND METHODS

Human Adipose Tissue and Muscle—Human adipose tissue was obtained either from the subcutaneous abdominal region by incisional biopsy or from the omental region from patients having elective surgery. All human skeletal muscle samples were obtained by needle biopsy from the vastus lateralis muscle of 14 subjects. All patients gave informed consent, and all procedures were approved by the Cedars-Sinai Medical Center Internal Review Board. Human heart was obtained from the left ventricular myocardium (interventricular septum, to avoid contamination by epicardial fat) of the explanted heart of 2 different transplant recipients.

Measurement of LPL Activity and Synthesis—LPL catalytic activity was measured in the medium as well as in the cell homogenate of cultured cells. The cell homogenate was prepared by scraping cells in buffer containing deoxycholate and heparin, as previously described (19). An aliquot of this buffer was then assayed, as described below, after dilution to remove any inhibitory effects of the detergent. LPL activity was determined as previously described (20) using a [^{3}H]-triolein-containing substrate containing normal human serum as a source of apoC-II. After incubating the samples with substrate for 45 min at 37 °C, the reaction was stopped by the addition of a mixture of chloroform/methanol/heptane (21), and liberated ^{3}H-free fatty acids

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[1] The abbreviations used are: LPL, lipoprotein lipase; UTR, untranslated region; PCR, polymerase chain reaction; RT, reverse transcriptase; CHO cells, Chinese hamster ovary cells; kb, kilobase(s).

were separated and quantitated by liquid scintillation. Activity was expressed as neq free fatty acid released/min/10^6 cells.

LPL synthetic rate was measured in cultured cells as previously described (22). In brief, cells were cultured in methionine-deficient medium for 2 h prior to the addition of 50 μCi of [^{35}S]methionine for 30 min. Cells were then scraped in lysis buffer (22), followed by immunoprecipitation with anti-LPL antibodies (23), analysis on a 10% SDS-polyacrylamide gel, and autoradiography.

Northern Blotting—Northern blotting of human adipose tissue and muscle with the human LPL cDNA probe was performed as previously described (10). Total RNA was extracted using the methods of Chomczynski and Sacchi (24), and the quality of RNA was verified by ethidium bromide staining of rRNA bands on a minigel. After analysis on a 1% agarose gel, the RNA was transferred to nylon and blotted with the random-primed (25) ^{32}P-labeled hLPL cDNA (18). Northern blots were performed either with total RNA or poly(A)-enriched RNA (Promega poly(A) tract mRNA isolation system).

Use of Polymerase Chain Reaction to Confirm 3′-UTR Terminus—The identification of the 3.2- and 3.6-kb LPL mRNA species was performed using reverse transcriptase (RT) and polymerase chain reaction (PCR), with the rapid amplification of cDNA ends protocol of Frohman *et al.* (26) (Fig. 2*A*). The strategy uses a hybrid primer consisting of oligo(dT) (20 residues) linked to a unique 21-base oligonucleotide adapter primer (primer 1). After the initial cDNA synthesis by RT, PCR was performed using the adapter primer (primer 3) and an LPL-specific 20-base oligonucleotide primer (primer 2) located upstream of the first polyadenylation site. Following reverse transcriptase, the PCR reaction was carried out using ^{32}P-labeled dCTP for 13, 15, and 17 cycles at 68 °C as shown. The resulting amplified PCR products were 322 and 717 base pairs corresponding to the 3.2- and 3.6-kb LPL mRNA species, respectively. With additional PCR cycles, some of the smaller PCR product was observed in muscle. In the process of optimizing the PCR reaction, we found that the 3.2-kb transcript was more efficiently amplified and continued to be generated in a linear fashion for up to 35 cycles, whereas the 3.6-kb transcript was no longer linear after 20 cycles. Therefore, it is difficult to estimate the precise proportion of 3.6- to 3.2-kb transcript using this strategy because the smaller PCR product was amplified more efficiently.

Cloning and Transcription of Full-length 3.2- and 3.6-kb LPL cDNAs—Clones LPL 35 (in pGEM4Z), LPL 37, and LPL 46, as previously described (18), were generously provided by Dr. Richard Davis. To create the 3.2-kb LPL cDNA, the distal 3′-end of LPL 37, which extends to the first consensus polyadenylation site at nucleotide 3155, was cloned into the *Acc*I site of LPL 35, which contains the complete 5′-UTR, coding sequence, and 813 nucleotides of 3′-UTR. To generate the 3.6-kb LPL cDNA, the distal 3′-end of LPL 46, which contains the 3′-UTR of LPL to the second polyadenylation site at nucleotide 3562, was cloned into the *Bgl*II site of the 3.2-kb cDNA at nucleotide 2798. These constructs were confirmed by informative restriction cuts, and the 3′-ends of these constructs were confirmed by double-stranded DNA sequencing (U. S. Biochemicals, sequenase sequencing kit). Both clones contained short sequences of polylinker from the vector (pGEM4Z). After *in vitro* transcription with RNA polymerase T7 (Boeringer Mannheim), the actual lengths of the transcripts were 3202 and 3599 bases, respectively.

Quantitative RT-PCR—Quantitative competitive RT-PCR was performed as previously described (27–29). 0.5 pg of RNA from the 3.2 and 3.6 constructs were added to increasing quantities of a commercially available cRNA construct (Gene Amplimer PAW 109 RNA, Perkin-Elmer Corp.) that contained primer sites for human LPL. These primer sites are located at nucleotides 1261–1281 and 1516–1536 of the LPL cDNA, which generates a product that is 23 nucleotides longer than the PCR product generated from native human LPL mRNA. Because the above primer sites span an intron in the LPL gene, contamination of the PCR reaction with genomic DNA is avoided. Following the reverse transcriptase reaction, PCR was carried out 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). The ratio of cRNA standard/LPL product was plotted against the number of copies of cRNA added to yield the equivalence point between cRNA and LPL mRNA.

In Vitro Translation—*In vitro* translation of poly(A)-enriched RNA and *in vitro* transcribed LPL mRNA were carried out using a rabbit reticulocyte lysate system (Promega). The full-length LPL 3.2 and 3.6 mRNAs were transcribed, and equal quantities of RNA (0.1 μg) were added to the lysate in the presence of [^{35}S]methionine and translated for 10, 20, and 40 min, followed by SDS-polyacrylamide gel electrophoresis and autoradiography. For *in vitro* translation of mRNA from adipose tissue and heart, RNA was extracted, and poly(A)-enriched RNA was prepared (Promega poly(A) tract mRNA isolation system). Equal quantities (0.9 μg) of RNA were added to the rabbit reticulocyte lysate system, followed by translation for 60 min. After immunoprecipitation with anti-LPL antibodies (23), the proteins were separated on a protein gel, followed by autoradiography. The ~50-kDa band immunoprecipitated from poly(A)$^+$ RNA competed away with the addition of unlabeled LPL.

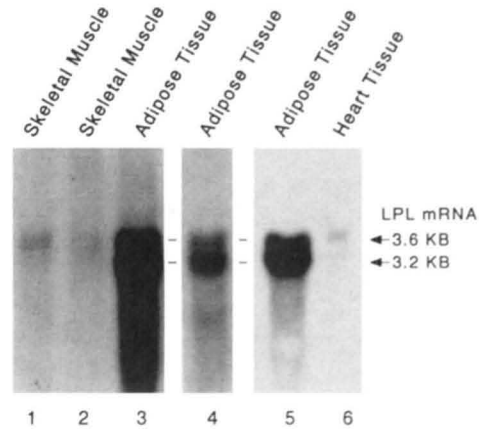

FIG. 1. **Northern blot of human adipose tissue and muscle with the human LPL cDNA probe.** *Lanes 1* and *2*, 20 μg of human skeletal muscle total RNA. *Lanes 3* and *4*, 20 μg of human adipose tissue total RNA; *lane 4* is a shorter exposure of *lane 3*. *Lane 5*, 20 μg of total RNA from adipose tissue. *Lane 6*, 2 μg of poly(A) RNA from heart. Adipose tissue was obtained from normal subjects from the abdominal subcutaneous region. Adipose tissue samples from omental adipose tissue yielded similar results, as did RNA extracted from isolated adipocytes (data not shown).

Transient Transfection of Cells with LPL-pcDNA-1 Constructs—The vector pcDNA-1 (Invitrogen) is designed for cDNA cloning in *Escherichia coli* MC1061P3 and expression in eukaryotic cells. The complete 3.2- or 3.6-kb LPL cDNAs were cloned into pGEM4Z as described above and cut out using *Pst*I and *Bam*HI. This fragment was gel purified and sub-cloned into pcDNA1. Colonies were screened for the presence of a correct size insert using restriction analysis. Large scale plasmid preparations were purified using a plasmid extraction kit (Quigen Inc.) and further purified using cesium chloride gradient centrifugation. Transient transfections were performed using calcium phosphate co-precipitation in Chinese hamster ovary (CHO) cells, as previously described (30).

LPL expression was assessed in the CHO cells 24 h following transfection. LPL activity was measured in the medium, as well in the cell homogenate, as described above. In addition, LPL synthesis was measured by pulse-labeling cells with [^{35}S]methionine for 30 min, followed by immunoprecipitation and electrophoresis, as described above. Quantitation of the human transfected LPL mRNA was performed using quantitative RT-PCR as described above. As described in the text, the primers used for quantitating human LPL mRNA did not detect the endogenous CHO LPL mRNA. In addition, PCR without the reverse transcriptase detected nothing, demonstrating that the RT-PCR was not detecting any untranscribed transfected human LPL cDNA. To demonstrate that there was no change in expression of CHO LPL mRNA, PCR was performed with primers that do not recognize human LPL sequence. Since the sequence of hamster LPL has not been reported, we used primers derived from the mouse LPL cDNA. The upstream primer was nucleotides 1158–1177, and the downstream primer was nucleotides 1369–1389 (2). Using these primers in the RT-PCR reaction under conditions described above, human LPL mRNA was not detected.

Statistics—All statistical comparisons were made using the Student's *t* test.

RESULTS

Previous studies have identified 3.2- and 3.6-kb LPL mRNA transcripts in human tissues, but such studies have not included muscle. Fig. 1 shows the results of Northern blotting samples from human adipose tissue and muscle. As in previous studies, adipose tissue from humans yielded two LPL mRNA transcripts of approximately 3.2 and 3.6 kb. In contrast, human

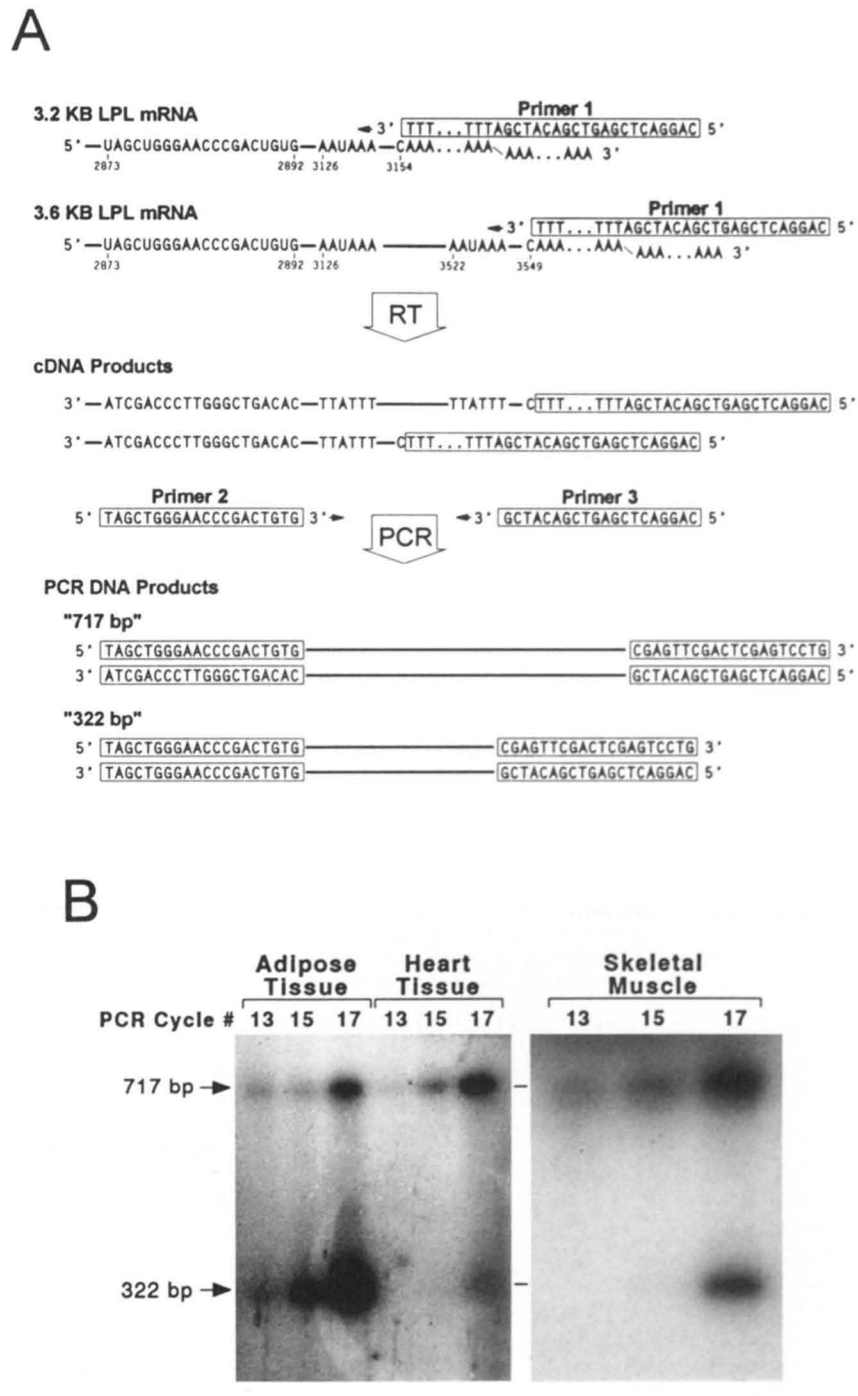

FIG. 2. **Identification of LPL transcripts in adipose tissue and muscle.** *A*, the identification of the 3.2- and 3.6-kb LPL mRNA species was performed using RT and PCR with the rapid amplification of cDNA ends protocol (see "Materials and Methods"). *B*, RT-PCR of human adipose tissue and muscle (heart) using the above strategy. Following reverse transcriptase, the PCR reaction was carried out using ^{32}P-labeled dCTP for 13, 15, and 17 cycles at 68 °C as shown.

skeletal and heart muscle yielded predominantly the larger 3.6-kb transcript. In some Northern blots, a faint image of the smaller 3.2-kb LPL transcript was visible in muscle with longer exposure. Overall, adipose tissue appeared to express higher levels of LPL mRNA than muscle, although the gels were loaded with equal quantities of total RNA, and muscle is more dense and contains more total RNA per gram of tissue.

To confirm the presence of the different transcripts, RT-PCR was used to amplify the distal segments of the LPL mRNA. The PCR strategy is described in Fig. 2*A*. By employing the rapid amplification of cDNA ends method (26), as described under "Materials and Methods," primers were chosen that permitted the PCR amplification of the 3′-ends of the 3.2- and 3.6-kb LPL mRNA species. This strategy would be expected to yield PCR products of 322 and 717 bases in the presence of the 3.2- and 3.6-kb mRNA transcripts, respectively. As shown in Fig. 2*B*, RT-PCR of human adipose tissue RNA yielded two PCR products corresponding to the two LPL mRNA transcripts identified by Northern blotting. Whereas the smaller PCR product predominated in analyses of adipose tissue mRNA, in both human heart and skeletal muscle, the larger PCR product, corresponding to the 3.6-kb mRNA transcript, predominated.

The above data demonstrated that muscle and adipose tissue express different relative amounts of LPL mRNA transcripts. To determine whether LPL transcripts with different 3′-UTRs have functional significance, the cDNAs of the full-length 3.2- and 3.6-kb transcripts were cloned, transcribed *in vitro*, and translated in the reticulocyte lysate system. As shown in Fig. 3, equal quantities of *in vitro* transcribed LPL mRNA, differing only in the presence or absence of the distal 3′ 394 nucleotides, were added to an *in vitro* translation system. After 10, 20, and 40 min of translation in the presence of [^{35}S]methionine, the

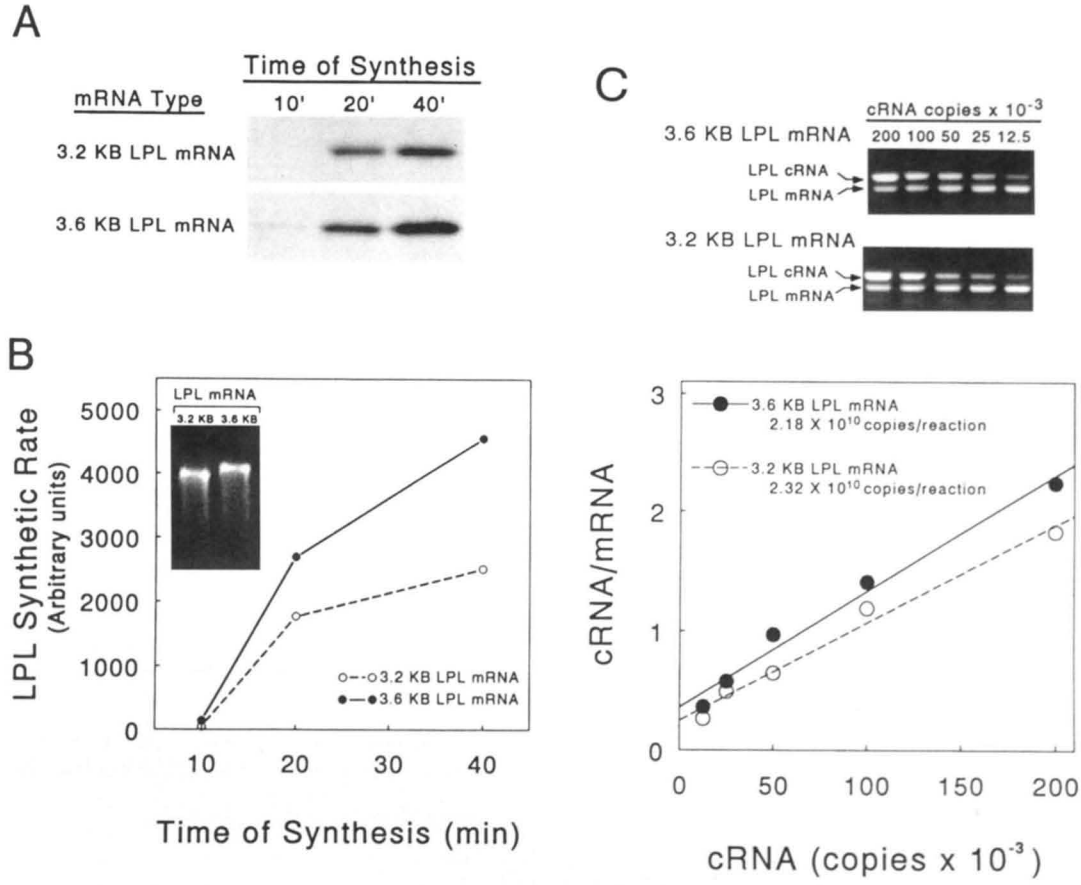

FIG. 3. **In vitro translation of LPL mRNA constructs.** The full-length LPL 3.2- and 3.6-kb mRNAs were cloned, transcribed, and translated in a rabbit reticulocyte *in vitro* translation system. Equal quantities of RNA (0.1 μg) were added to a rabbit reticulocyte lysate system, followed by translation for the indicated times in the presence of [^{35}S]methionine. *A*, autoradiograph of the *in vitro* translation. The 3.2- and 3.6-kb mRNAs were translated for 10, 20, and 40 min, followed by SDS-polyacrylamide gel electrophoresis and autoradiography. This is one of four representative experiments. *B*, densitometric analysis of the image from *A*. *Inset*, ethidium bromide-stained gel of the RNA used for the *in vitro* translation. *C*, quantitation of 3.2- and 3.6-kb LPL mRNAs using RT-PCR. 0.5 pg of RNA from the 3.2 and 3.6 constructs were added to increasing quantities of a commercially available cRNA construct that contained primer sites for human LPL (see "Materials and Methods"). Following the RT reaction, PCR was carried out for 35 cycles at 55 °C. The ratio of cRNA standard/LPL product was plotted against the number of copies of cRNA added to yield the equivalence point between cRNA and LPL mRNA.

3.6-kb mRNA consistently produced more LPL (Fig. 3, *A* and *B*). When the transcripts were left at 30 °C for up to 90 min, no difference in stability of the 3.2- and 3.6-kb mRNAs were observed by analysis on an agarose gel. The *in vitro* translation reactions utilized equal quantities of LPL mRNA, as determined by absorbance at 260 nm and as confirmed by staining with ethidium bromide. To confirm that equal quantities of LPL mRNA were used, quantitative RT-PCR was performed with the *in vitro* transcribed LPL mRNA that was used for the *in vitro* translation. As shown in Fig. 3*C*, the amount of PCR product for the 3.2- and 3.6-kb mRNAs was compared with a cRNA standard that utilized the same primers. The number of copies of the 3.2-kb LPL mRNA used in the *in vitro* translation reaction was 2.32×10^{10}, and the number of copies of the 3.6-kb mRNA was essentially the same, at 2.18×10^{10}. Therefore, when the same quantity of LPL mRNA was used for *in vitro* translation, the 3.6-kb mRNA yielded more translation product than the 3.2-kb mRNA.

Although *in vitro* transcribed LPL mRNA demonstrated differences in translation, we wished to determine whether LPL mRNA extracted from adipose tissue and muscle yielded the same results. To accomplish this, poly(A)-enriched RNA was isolated from human adipose tissue and heart muscle and translated in a rabbit reticulocyte *in vitro* translation system, followed by immunoprecipitation with anti-LPL antibodies. As shown in Fig. 4*A*, the poly(A) mRNA from muscle translated more efficiently than the mRNA from adipose tissue. In the above *in vitro* translation reaction, equal quantities of poly(A)-enriched RNA were added to the reticulocyte lysate. However, the relative levels of specific LPL mRNA message may differ between the two RNA preparations. To confirm that equal quantities of LPL mRNA were added to the *in vitro* translation system, quantitative RT-PCR was performed. As shown in Fig. 4*B*, each sample of poly(A)-enriched RNA contained equal quantities of LPL mRNA.

The above data demonstrated that the 3.6-kb LPL mRNA was more efficiently translated *in vitro* using the reticulocyte lysate system. To determine whether the 3.6-kb mRNA was also more efficiently expressed *in vivo*, we performed transient transfection into CHO cells. Both the 3.2- and 3.6-kb LPL cDNAs were cloned into the pcDNA vector. Transfection of each of these constructs, along with vector alone, was performed as described under "Materials and Methods." After 24 h, LPL activity was measured in the medium, as well as in the cell homogenate, as shown in Fig. 5*A*. CHO cells are known to express LPL (31), and LPL activity was detected in the medium of cells that were transfected with vector alone. When compared with cells transfected with vector alone, cells transfected with the constructs containing LPL sequence contained higher levels of LPL activity. However, when comparing levels of LPL activity in the cells transfected with the two LPL constructs, cells transfected with the 3.6-kb LPL cDNA demonstrated levels of LPL activity in the medium and cell homogenate that was 57 and 125% (respectively) higher than in cells transfected

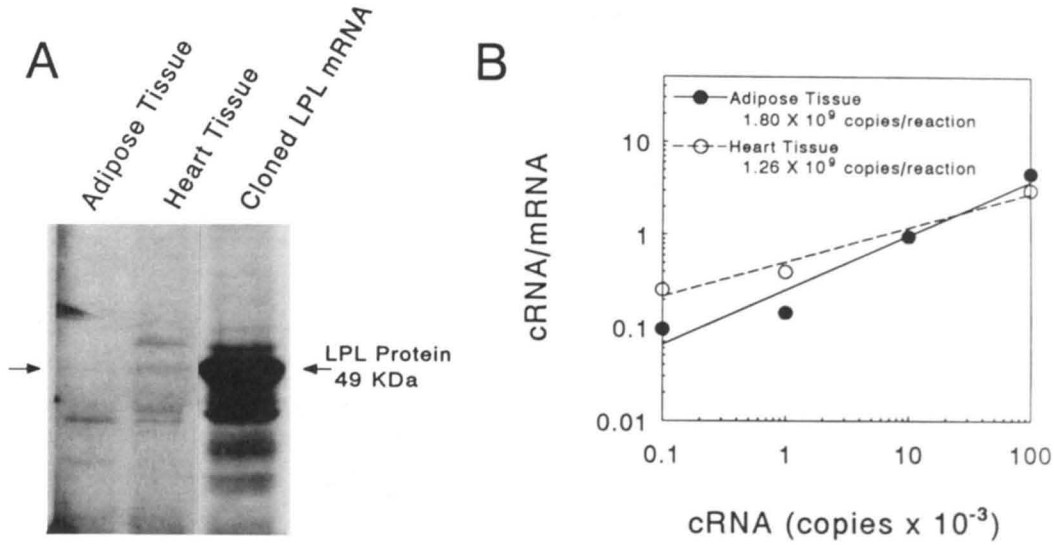

FIG. 4. **In vitro translation of mRNA from adipose tissue and heart.** RNA was extracted, and poly(A)-enriched RNA was prepared. Equal quantities (0.9 μg) of RNA was added to a rabbit reticulocyte lysate system, followed by translation for 60 min in the presence of [^{35}S]methionine. Following immunoprecipitation with anti-LPL antibodies, the proteins were separated on a protein gel, followed by autoradiography. *A*, protein gel of immunoprecipitated LPL from poly(A)-enriched RNA extracted from human adipose tissue and heart. *Lane 3* contains the protein that was generated from the *in vitro* transcribed LPL mRNA. *B*, RT-PCR of poly(A)-enriched RNA from adipose tissue and muscle. Quantitative competitive PCR was performed as described under "Materials and Methods" using 5 pg of poly(A)-enriched RNA from adipose tissue and muscle.

with the 3.2-kb LPL cDNA. To demonstrate that this increased expression was due to an increase in LPL translation, CHO cells were pulse labeled with [^{35}S]methionine, followed by immunoprecipitation. As shown in Fig. 5*B*, LPL synthesis was higher in the cells transfected with the 3.6-kb LPL cDNA. The increased expression of LPL in the cells transfected with the 3.6-kb cDNA could have been due to a higher level of the 3.6-kb LPL mRNA in these cells or possibly to a higher level of endogenous CHO LPL mRNA. To distinguish between these possibilities, RT-PCR was performed using primers that distinguish between the transfected human LPL cDNA and CHO LPL mRNA. Using the primers described under "Materials and Methods" for quantitation of human LPL mRNA, LPL message was only detected in cells transfected with the human LPL cDNA constructs and not in cells transfected with vector alone (Fig. 5*C*, *inset*). In addition, the use of mouse primers, which detected CHO LPL mRNA but not human LPL mRNA, demonstrated equal quantities of LPL mRNA in cells transfected with vector alone or with the two human LPL constructs (data not shown, see "Materials and Methods"). When the levels of LPL mRNA were quantitated using the human primers and compared with the cRNA standard, there was no difference in the level of transfected LPL mRNA in the cells transfected with the two human LPL constructs (Fig. 5*C*). These data confirmed that the increase in LPL synthesis and activity was due to increased mRNA translation. In addition, this experiment demonstrates no difference in mRNA stability 24 h following transfection of the 3.2- and 3.6-kb mRNA species.

DISCUSSION

The cDNA sequence of human LPL has been previously characterized (18), and the 3′-UTR of human LPL was noted to contain two consensus polyadenylation sites. Because two corresponding LPL transcripts were expressed in human adipose tissue, the data suggested that the choice of polyadenylation signals was random. In this study, we demonstrated by Northern blotting that human skeletal and cardiac muscle expressed predominantly the 3.6-kb LPL mRNA form, whereas adipose tissue expressed both forms. The potential significance of this tissue-specific gene expression was illustrated by the differences between the translational efficiencies of the two LPL mRNA species. Using both *in vitro* translation and transient transfection into CHO cells, the 3.6-kb LPL mRNA transcript, which is the species more abundant in muscle, was more efficiently translated into LPL protein than the 3.2-kb LPL mRNA transcript. There was no evidence for a difference in mRNA stability conferred by the longer 3′-UTR. Thus, the tissue-specific differences in choice of polyadenylation sites led to functional differences in LPL expression.

When the cDNA sequence of human LPL is compared with LPL from other species, there are a number of observations relevant to this study. The sequence of the human LPL 3′-UTR is about 75% homologous with the 3′-UTR of bovine and mouse LPL (32), and there are two polyadenylation signals in all species examined (18, 33–35) corresponding to the human sequence at nucleotides 3155 and 3550. However, only the second polyadenylation signal is used in rat tissues, and one LPL mRNA species is expressed in rat muscle and adipose tissue. In the mouse, however, both of the polyadenylation sites are used in all tissues (2). Although the 3′-UTR for guinea pig LPL has not been reported, one report identified one LPL mRNA species in heart and two species in adipose tissue (36). In separate reports, bovine LPL mRNA size was different in different tissues (34, 37). Therefore, the polyadenylation site corresponding to 3155 in humans has been shown to be variably recognized among different tissues and different species. Whether or not the choice of polyadenylation sites leads to the differences in LPL expression in non-human tissues is not known.

Numerous studies have implicated changes in the 3′-UTR of mRNA in the regulation of translation (17). Although the 3′-UTR is important for mRNA stability (38), numerous studies have shown that the 3′-UTR can control translational efficiency (17, 38). Examples of inhibition of translation by interactions with the 3′-UTR include δ-interferon in *Xenopus* oocytes (39) and creatine kinase in the U937 cell line (40). The 3′-UTR may function to increase protein translation. Studies with ornithine decarboxylase, which is under translational control by polyamines (41), have demonstrated coordinated regulation of translation by both the 5′- and 3′-UTRs. Whereas ornithine decarboxylase translation initiation is inhibited by sequences in the 5′-UTR of the mRNA, the 3′-UTR functions to

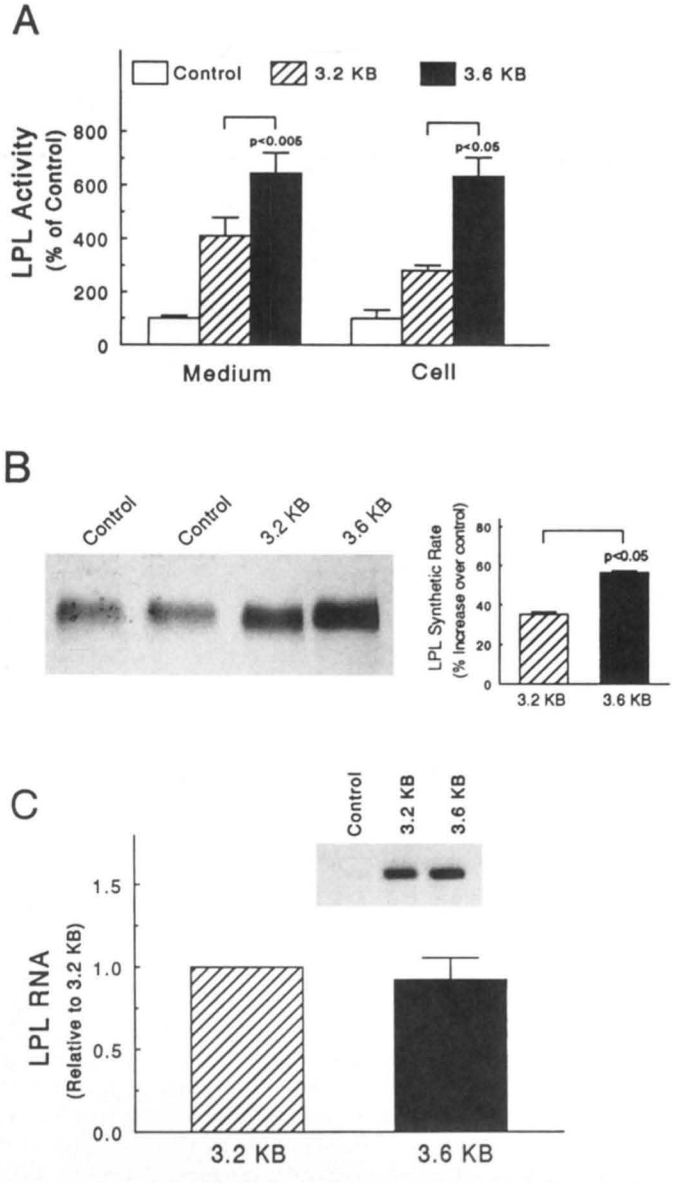

FIG. 5. **Expression of LPL in CHO cells transfected with the 3.2- and 3.6-kb constructs.** CHO cells were transfected with either vector alone or vector containing the 3.2- or 3.6-kb human LPL cDNAs, as described under "Materials and Methods." 6–8 separate transfection experiments were performed with each construct. *A*, LPL activity in the medium and cell extract. Enzyme activity was performed in duplicate from each transfection. *B*, LPL synthesis in the cells was assessed using pulse labeling with [^{35}S]methionine, followed by immunoprecipitation, as described under "Materials and Methods." *Inset*, the data from 2–3 experiments were analyzed by densitometry and expressed relative to the synthetic rate in control cells (*bar graph*). *C*, human LPL mRNA levels in the transfected cells using quantitative RT-PCR. *Inset*, RT-PCR under the same conditions for 35 cycles, demonstrating no detection of CHO LPL mRNA using the human primers.

augment, and partially negate, this inhibition of translation (42). The amyloid protein precursor mRNA, like LPL, has two sites of polyadenylation, and the longer of the two mRNAs is translated more efficiently in *Xenopus* oocytes (43). The mechanism for the increased translation of the 3.6-kb LPL mRNA is not known, but it could involve changes in mRNA secondary structure, permitting interaction with a trans-acting binding protein.

If the choice of polyadenylation sites were regulated in a tissue leading to different transcript expression, this could provide a mechanism for the translational regulation of LPL. For example, previous studies in humans have demonstrated that LPL in adipose tissue is regulated at the translational level in response to improved diabetes control (16). One possible mechanism for this translational regulation of human adipose LPL could be regulation of the choice of polyadenylation sites. When LPL translation is increased in the adipose tissue of treated diabetic patients, this could be due to a preferential recognition of the second polyadenylation signal and the transcription of the 3.6-kb LPL mRNA, which would be more efficiently translated. Conversely, a decreased expression of the 3.6-kb LPL mRNA would be predicted to result in a decrease in LPL translation.

Although there is a single gene for LPL, muscle and adipose tissue LPL are regulated in an inverse manner in response to feeding (3), insulin-glucose infusion (44), and exercise (10). All of these inverse changes in LPL expression occur post-transcriptionally, yet the mechanism for this inverse regulation is unexplained. Because of this difference in LPL transcript expression, it is tempting to hypothesize a possible role of the distal 3′-UTR in the inverse regulation of LPL between adipose tissue and muscle. However, it is important to note that rat adipose tissue and muscle LPL also display inverse regulation in response to feeding, even though the mRNA transcripts are the same in adipose tissue and muscle. Thus, any role of the 3′-UTR of the LPL mRNA in the tissue-specific inverse regulation of LPL must remain speculative.

In summary, we have demonstrated that human adipose tissue and muscle express different mRNA forms due to regulation of polyadenylation at different sites. This difference in length of the 3′-UTR may be of functional significance because the longer mRNA, which is preferentially expressed in muscle, is more efficiently translated. These data illustrate an important tissue-specific difference in LPL gene expression and an interesting mechanism for translational regulation.

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