Post-transcriptional mechanisms are responsible for the reduction in lipoprotein lipase activity in cardiomyocytes from diabetic rat hearts

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Lipoprotein lipase (LPL) activity is reduced in cardiomyocytes from rat hearts following the acute (4–5 day) induction of diabetes with 100 mg/kg streptozotocin. The molecular basis for this inhibitory effect of diabetes on LPL activity was investigated by measuring steady-state LPL mRNA content and the synthesis and turnover of LPL protein ($[^{35}\text{S}]$ methionine incorporation into immunoprecipitable LPL protein in pulse and pulse–chase experiments) in control and diabetic cardiomyocytes. LPL activity was reduced to approx. 50% of control in diabetic cardiomyocytes, but LPL mRNA levels and turnover (degradation) of newly synthesized LPL were unchanged. Synthesis of total protein and LPL were reduced to 72% and 71% of control

respectively; therefore, relative rates of LPL synthesis were the same in control and diabetic cardiomyocytes. The diabetes-induced reduction in LPL synthesis was accompanied by a decrease in LPL mass to 78% of control, and a decrease in enzyme specific activity (0.48 to 0.33 m-unit/ng of LPL protein) since the decline in catalytic activity was greater than the decrease in LPL synthesis and mass. Thus, post-transcriptional mechanisms involving a reduction in LPL synthesis as part of a generalized decrease in total protein synthesis, together with a post-translational mechanism(s) that result in accumulation of inactive LPL protein, are responsible for the decreased LPL activity in cardiomyocytes from diabetic rat hearts.

INTRODUCTION

The triacylglycerol (TG) component of circulating lipoproteins is hydrolysed by lipoprotein lipase (LPL), located at the luminal surface of the vascular endothelium in extrahepatic tissues, including the heart [1,2]; the non-esterified fatty acid product is then available for tissue (parenchymal cell) utilization (e.g. oxidation in cardiomyocytes). Displacement of the endothelium-bound enzyme by perfusing hearts with heparin resulted in a marked reduction in the degradation of TG-rich lipoproteins [3]. Although heparin-releasable LPL (HR-LPL) from the coronary vasculature of perfused hearts represents the functional pool of enzyme, vascular endothelial cells do not synthesize LPL [4,5]. In the adult heart, LPL is synthesized and processed by glycosylation in cardiomyocytes, and then is translocated to its functional site on the surface of the vascular endothelium [1].

Hypertriglyceridaemia is a characteristic feature of insulin-dependent diabetes mellitus, principally because of reduced catabolism of TG-rich lipoproteins [6,7]. The diabetes-induced reduction in degradation of TG-rich lipoproteins *in vivo* is the result of two mechanisms, involving both the lipoprotein substrate and the catalytic activity of LPL. Alterations in apoprotein content make the TG-rich lipoprotein particle a poorer *in vivo* substrate for LPL [8–10]. In addition, LPL activity measured *in vitro* is decreased in heart, skeletal muscle and adipose tissue preparations from diabetic animals [11–14].

HR-LPL activity was decreased in perfused hearts from insulin-deficient diabetic rats [15–17]; this reduction in functional endothelium-bound LPL accounts for the observation that the degradation of TG-rich lipoproteins is diminished in perfused diabetic hearts [10,15,18]. In addition, LPL activity was reduced in cardiomyocytes isolated from diabetic rat hearts [17,19,20]. Since LPL synthesized in cardiomyocytes is the precursor of the functional, endothelium-bound enzyme in the heart, it is important to establish the mechanism(s) responsible for regulating LPL in cardiomyocytes isolated from diabetic rat hearts. The

objective of this investigation, therefore, was to determine whether the molecular basis for the diabetes-induced decrease in LPL activity in cardiomyocytes [17,19,20] was the result of transcriptional (reductions in steady-state levels of LPL mRNA) and/or post-transcriptional (alterations in rates of LPL synthesis and/or turnover) mechanisms.

EXPERIMENTAL

Experimental animals

Male Sprague–Dawley rats (180–220 g) were fed a laboratory chow diet *ad libitum*, allowed free access to water, and maintained under a 12 h light (0700–1900 h)–dark cycle. An acute (4–5 day) and severe model of insulin-deficient diabetes was induced by administration of streptozotocin (100 mg/kg in 50 mM citrate buffer, pH 4.5), as described previously [16,17,19,20]. Control rats gained 31 ± 1 g (mean $\pm$ S.E.M.; $n = 21$) in this time interval, whereas diabetic rats lost 14 ± 4 g ($n = 21$). The plasma glucose concentrations were 7.2 ± 0.4 mM ($n = 21$) and 26.4 ± 1.7 mM ($n = 20$) in control and diabetic rats respectively.

Preparation and incubation of cardiac myocytes

Calcium-tolerant cardiomyocytes were isolated as described previously [21]. The viability (percentage of elongated cells with clear cross striations that excluded 0.4% Trypan Blue) was identical ($78 \pm 1\%$) for myocyte preparations from control ($n = 23$) and diabetic ($n = 22$) rat hearts. Myocytes were incubated with low-molecular-mass heparin (LMMH; $5 \mu\text{g/ml}$) for the indicated times and then centrifuged [22]. LPL activity in the supernatant (medium) is referred to as heparin-releasable LPL (HR-LPL). For determinations of cellular LPL activity, cell pellets were resuspended in 0.2 ml of 50 mM ammonia buffer (pH 8.0) containing 0.125% (v/v) Triton X-100, sonicated and then made up to 1 ml with buffer consisting of 0.25 M sucrose,

Abbreviations used: TG, triacylglycerol; LPL, lipoprotein lipase; LMMH, low-molecular-mass heparin; HR-LPL, heparin-releasable lipoprotein lipase; CHX, cycloheximide; SSC, 0.15 M NaCl/0.015 M sodium citrate, pH 7.0; TCA, trichloroacetic acid.

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10 mM Hepes, 1 mM EDTA, and 1 mM dithiothreitol (pH 7.5). In some experiments, cycloheximide (CHX) treatment [23] was utilized to deplete LPL activity in isolated cardiomyocytes. Control and diabetic rats were administered CHX (2 mg/kg) 2.5 h before death and removal of the hearts, and 50 μ M CHX was added to the isolation solutions during the preparation of cardiomyocytes. The repletion of cellular LPL and HR-LPL activities was then measured during an incubation of CHX-treated cardiomyocytes for 120 min in the absence of CHX.

LPL activity

The activity of LPL in the incubation medium of cardiomyocytes (HR-LPL) and in cell homogenates was determined by measuring the hydrolysis of a sonicated [3 H]triolein substrate emulsion [21]. The standard assay conditions were 0.6 mM glycerol tri[9,10- 3 H]oleate (1 mCi/mmol), 25 mM Pipes (pH 7.5), 0.05 % (w/v) fatty acid-free BSA, 50 mM MgCl₂, 2 % (v/v) heat-inactivated chicken serum as LPL activator, with appropriate quantities of the medium (60–100 μ l), or sonicated cell homogenate (20 μ l) in a total volume of 400 μ l. Previous investigations have established that the pH optimum for cellular LPL and HR-LPL was 7.5 [24,25]. When LPL activity was measured in myocyte homogenates, heparin (2 units/ml) was added to the assay. The activity of LPL is routinely expressed as nmol of oleate released/h per 10⁶ cells.

LPL mRNA

An LPL cDNA plasmid (868-bp fragment), obtained from Drs. R. H. Eckel and M. Raynolds (University of Colorado Health Sciences Center, Denver, CO, U.S.A.) [26], and a β -actin plasmid (Dr. G. Schultz, University of Calgary, Calgary, Canada), were labelled with [32 P]dCTP by random oligonucleotide-priming. Total RNA was isolated from hearts (0.3 g of ventricular tissue) and cardiomyocytes (10⁷ cells) from control and diabetic rats by the guanidinium thiocyanate–acid phenol–chloroform method [27]. The RNA content of each sample was determined by absorbance at 260 nm; the A_{260}/A_{280} ratio was 1.9 for RNA preparations from control and diabetic hearts. RNA (10 μ g) was separated on a 1 % agarose/formaldehyde gel and then transferred to Hybond-N⁺ membrane in 20 $\times$ SSC (1 $\times$ SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0). The RNA in gels was detected with ethidium bromide and photographed by UV transillumination to ensure the integrity of RNA samples and to check that equivalent amounts of total RNA were loaded. A typical ethidium bromide-stained gel for RNA from control and diabetic cardiomyocytes is shown in Figure 1; equal amounts of 28 S and 18 S rRNA bands are evident. If significant quantities of DNA were detected, the samples were re-extracted.

Total LPL mRNA was quantified by slot-blot hybridization. Briefly, total RNA was serially diluted (0.5–0.0625 μ g per sample) in 40 μ l of diethylpyrocarbonate-treated water, mixed with 120 μ l of 0.66 % (v/v) formamide/0.2 % (v/v) formaldehyde/26 mM Mops (pH 7.0)/6.5 mM sodium acetate/1.3 mM EDTA and denatured at 65 $^{\circ}$ C for 5 min. Cold 20 $\times$ SSC was added and the samples were slotted on to Hybond-N⁺ membrane. Blots were then fixed with 0.05 M NaOH, rinsed with 2 $\times$ SSC and pre-hybridized in 0.9 M NaCl/50 mM sodium phosphate buffer (pH 7) containing 5 mM EDTA, 0.8 % (w/v) SDS, 5 $\times$ Denhardt's (1 $\times$ Denhardt's solution: 0.02 % polyvinylpyrrolidone/0.02 % Ficoll/0.02 % BSA), 0.8 $\times$ SSC, and 100 μ g/ml denatured salmon sperm DNA, for 1 h at 68 $^{\circ}$ C. Hybridization was carried out for 24 h under the same conditions with the addition of radiolabelled LPL or β -actin cDNA probes at a concentration of 1.5 $\times$ 10⁶ c.p.m./ml. Blots were then washed

Figure 1 Electrophoresis of RNA from control and diabetic cardiomyocytes

Samples of RNA from a control (C) and diabetic (D) cardiomyocyte preparation were electrophoresed and stained with ethidium bromide; the 28 S and 18 S rRNA bands are indicated by the arrows.

at room temperature for 20 min in 1 $\times$ SSC/0.1 % SDS, followed by two washes at 68 $^{\circ}$ C in 0.2 $\times$ SSC/0.1 % SDS, before being exposed to Kodak X-Omat XAR film for 24–48 h at -70° C. Autoradiographic signals for LPL and β -actin mRNA were quantified by scanning densitometry.

LPL synthesis and turnover

Monoclonal antibody to bovine milk LPL (5D2; [28]) was coupled to Affigel 10 beads (0.7 μ g of antibody/ μ l of gel matrix). Control and diabetic cardiomyocytes (10⁶ cells/ml in Joklik medium) were suspended into a modified Krebs–Ringer buffer [29], and labelled with 0.33 mCi/ml [35 S]methionine at 37 $^{\circ}$ C under O₂/CO₂ (19:1). After the indicated pulse times, aliquots (1 ml) were removed and centrifuged at 15000 g for 10 s; the cell pellets were then homogenized in 1 ml of lysis buffer [29]. After centrifugation, 0.5 ml of the clear supernatants were immunoprecipitated overnight with 20 μ g of Affigel-5D2 monoclonal antibody matrix. A second immunoprecipitation of the cell lysate did not reveal any radiolabelled LPL. The immune complexes were precipitated with Protein A from *Staphylococcus aureus* cells, and subjected to SDS/PAGE [29]. LPL was identified as a protein band with an apparent M_r of 56000 [29] on the fluorograms. 35 S-labelled LPL bands were dissolved in 50 % hydrogen peroxide and radioactivity was determined by liquid scintillation spectrometry. Addition of excess purified milk LPL with the 5D2 monoclonal antibody markedly reduced the radioactivity in the LPL band. Total 35 S radioactivity in trichloroacetic acid (TCA)-precipitable protein was determined by liquid scintillation spectrometry [29].

Turnover of newly synthesized LPL was measured in pulse-chase experiments. Control and diabetic cardiomyocytes were labelled with 0.33 mCi/ml [35 S]methionine for 20 min at 37 $^{\circ}$ C under O₂/CO₂ (19:1). Aliquots (1 ml) were then centrifuged at 15000 g for 10 s and the pellets were stored on ice. The remaining cells were washed twice and resuspended into Joklik minimal

essential medium supplemented with 1.2 mM MgSO₄, 1 mM DL-carnitine, 1 mM CaCl₂, 1 % (w/v) defatted albumin and 10 mM methionine, and incubated for 90 min as above [29]. At the end of the chase, 1 ml aliquots were centrifuged. All the pellets were then processed for determination of radioactivity in TCA-precipitable total protein and immunoprecipitable LPL. Heparin was not added to the pulse or chase solutions to avoid any loss of radioactive LPL to the medium; a radiolabelled band corresponding to LPL could not be detected on fluorograms when the medium after the chase incubation was immunoprecipitated (results not shown).

LPL mass and LPL specific activity

The mass of LPL in homogenates of cardiomyocytes (2, 4 and 8 µl aliquots, in duplicate) from control and diabetic rat hearts was determined by ELISA as described by Goers et al. [30]. The mass of LPL is expressed as ng of LPL protein/ml, based on a standard curve generated with purified bovine milk LPL. LPL activity was measured in the homogenates, and is expressed as m-units/ml where 1 m-unit is defined as the amount of enzyme catalysing the release of 1 nmol of fatty acid (oleate)/min. The specific activity of LPL in the homogenates from control and diabetic cardiomyocytes was calculated as m-units/ng of LPL protein.

Materials

Collagenase (Worthington Type II) was obtained from Technicon Canada Ltd. (Richmond, British Columbia, Canada). Joklik minimal essential medium was purchased from Gibco Canada (Burlington, Ontario, Canada). [³H]Triolein and [³²P]dCTP were purchased from Amersham Canada (Oakville, Ontario, Canada); Trans³⁵S-label (L-[³⁵S]methionine, L-[³⁵S]cysteine) was from ICN Biomedicals (Mississauga, Ontario, Canada). LMMH (bovine intestinal mucosa; average *M_r* approx. 3000), fatty acid-free BSA, streptozotocin and other biochemicals were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Heparin (Hepalean; 1000 units/ml) was obtained from Organon Teknika (Toronto, Ontario, Canada). Monoclonal antibody to LPL (5D2) was a generous gift from Dr. J. D. Brunzell, University of Washington, Seattle, WA, U.S.A.; mouse IgG was from BioCan Science (Mississauga, Ontario, Canada).

RESULTS

Cardiomyocytes from diabetic rat hearts had reduced cellular LPL and HR-LPL activities (Table 1). LPL activity in cardiomyocytes can be reduced to approx. 10 % of control by CHX

Figure 2 Effect of diabetes on the repletion of LPL activity in CHX-treated myocytes

CHX-treated cardiomyocytes from a control (left-hand panel) and diabetic (right-hand panel) rat heart were incubated with 5 units/ml heparin. At the indicated times, LPL activity in cells (○, ●) and in the incubation medium (△, ▲) was measured.

treatment [23]. Incubation of CHX-treated myocytes from control rat hearts resulted in a time-dependent increment in cellular LPL and HR-LPL activities (Figure 2, left-hand panel); this repletion of enzyme activity reflects the cellular processes of enzyme synthesis, processing and translocation to the cell surface. The repletion of cellular LPL and HR-LPL activities in CHX-treated cardiomyocytes from a diabetic rat heart was reduced relative to control cardiomyocytes (Figure 2, right-hand panel). In four experiments, the net increment in cellular LPL and HR-LPL activities in CHX-treated cardiomyocytes from diabetic rat hearts was $40 \pm 4\%$ and $42 \pm 7\%$, respectively, of the increment in control cardiomyocytes incubated for 120 min. The molecular basis for this diabetes-induced reduction in LPL activity in both normal and CHX-treated cardiomyocytes was investigated by determining the effects of diabetes on LPL mRNA levels, and on rates of LPL synthesis and turnover in pulse and pulse-chase experiments, where the incorporation of [³⁵S]methionine into immunoprecipitable LPL protein was measured.

Northern blots of RNA from a control rat heart hybridized to a rat LPL cDNA clone [26] showed a single 3.6 kb mRNA species (Figure 3a) consistent with previous results with rat heart

Table 1 Effect of diabetes on cellular and heparin-releasable LPL activities in cardiomyocytes

Cardiomyocytes were isolated from control (*n* = 5) and diabetic (*n* = 4) rat hearts. Aliquots of the cell suspensions were centrifuged at zero-time and after 10 min and 60 min incubation with 5 µg/ml LMMH; LPL activity was measured in the supernatants (HR — LPL in the medium) and cell pellets (cellular LPL). Results are mean ± S.E.M. Probability values were calculated by a Student's *t* test; **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Incubation time (min)	LPL activity (nmol/h per 10 ⁶ cells)			
	Medium		Cells	
	Control	Diabetic	Control	Diabetic
0	150 ± 17	54 ± 9*	2770 ± 220	1271 ± 164**
10	730 ± 66	208 ± 30***	2046 ± 314	1068 ± 150*
60	1032 ± 80	317 ± 81***	1953 ± 164	747 ± 130***

Figure 3 Effect of diabetes on LPL mRNA in whole heart and cardiomyocytes

(a) Northern-blot analysis of LPL mRNA in a heart from control (CON) and diabetic (DIA) rats. The LPL mRNA size is 3.6 kb, as indicated by the arrow. (b) The content of LPL mRNA and β -actin mRNA in cardiomyocytes from control (CON) and diabetic (DIA) rat hearts was determined by slot-blot hybridization, using the indicated amounts of total RNA (μ g/sample).

Table 2 Effect of diabetes on LPL activities, on LPL mRNA content, and on LPL synthesis and turnover in cardiomyocytes

LPL activities (total cellular at zero-time and HR-LPL measured after 10 min incubation with 5 μ g/ml LMMH), mRNA levels for LPL and β -actin, total protein and LPL synthesis (20 min pulse), and LPL turnover (20 min pulse–90 min chase) were measured in control and diabetic cardiomyocytes. The results for diabetic cardiomyocytes are expressed as the percentage of control (mean $\pm$ S.E.M.) for the number of preparations indicated in parentheses. Probability values were calculated by a paired Student's *t*-test; **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

	Diabetic (% of control)
(A) LPL activity (16)	
Total cellular	54 $\pm$ 3***
HR-LPL	45 $\pm$ 4***
(B) mRNA content (4)	
LPL mRNA	98 $\pm$ 2
β -Actin mRNA	40 $\pm$ 2***
(C) Translation (7)	
Total protein synthesis	72 $\pm$ 7**
LPL synthesis	71 $\pm$ 9*
(D) Post-translation (6)	
Turnover	81 $\pm$ 12

[31]. Northern-blot analyses of a diabetic rat heart are also shown in Figure 3(a); diabetes had no apparent effect on LPL mRNA content. The steady-state levels of LPL mRNA were quantified by slot-blot dilution series for total RNA preparations from control and diabetic cardiomyocytes (Figure 3b). LPL mRNA in diabetic myocytes was 98 $\pm$ 2% of control (Table 2); in comparison, total cellular LPL and HR-LPL activities were reduced to approx. 50% of control activity in diabetic cardiomyocyte preparations. Similar results (not shown) were obtained in experiments with whole hearts from diabetic rats, where LPL mRNA was 99 $\pm$ 5% (*n* = 3) of control. The expression of β -actin is frequently used as a reference mRNA for Northern- and slot-blot analyses. However, levels of β -actin mRNA measured by slot-blot (Figure 3b) were decreased to 40 $\pm$ 2% in diabetic

Figure 4 Effect of diabetes on synthesis of total protein and LPL in cardiomyocytes

Cardiomyocytes from control (O) and diabetic (●) rat hearts were incubated with [35 S]methionine for the indicated pulse times, and the incorporation of radioactivity into total TCA-precipitable protein (top panel) and into immunoprecipitable LPL (bottom panel) was measured. The portion of the fluorogram corresponding to LPL is shown in the insert to the lower panel; the incorporation of radioactivity into immunoprecipitable LPL was measured by liquid scintillation spectrometry. Similar results were obtained in a second pulse-time experiment.

cardiomyocytes (Table 2). Similar results were reported by Camps et al. [32] for β -actin mRNA expression in diabetic rat hearts. Since β -actin mRNA could not be used as a reference or loading control for slot blots, great care was taken to establish that equivalent amounts of RNA were electrophoresed based on ethidium bromide staining of rRNA (Figure 1).

The incorporation of [35 S]methionine into total TCA-precipitable protein and into immunoprecipitable LPL as a function of pulse-time is presented in Figure 4. The total protein synthesis rate was linear in control cardiomyocytes. Incorporation of radioactivity into total protein was reduced in diabetic cardiomyocytes, particularly at the longer pulse times of 40 and 60 min. A reduction in total protein synthesis in diabetic cardiomyocytes was observed previously by Braun and Severson [17]. Rates of LPL synthesis were linear up to 40 min pulse time (Figure 4, lower panel). Using a pulse time of 20 min, rates of general protein synthesis and LPL synthesis in diabetic cardiomyocytes (*n* = 7) were reduced to 72 $\pm$ 7% and 71 $\pm$ 9% of control (Table 2). Because of the parallel change in both total protein and LPL synthesis, the relative rate of LPL synthesis (incorporation of

Figure 5 Effect of diabetes on turnover of LPL in cardiomyocytes

Cardiomyocytes from diabetic and control rat hearts were pulse-labelled for 20 min with [35 S]methionine (lanes 1 and 3), and then chased for 90 min with unlabelled methionine (lanes 2 and 4). Immunoprecipitable LPL was subjected to SDS/PAGE and autoradiography to obtain the fluorogram.

Table 3 Effect of diabetes on LPL activity, mass and enzyme specific activity in cardiomyocytes

Total cellular LPL activity and LPL mass by ELISA was measured in homogenates of control and diabetic cardiomyocytes. Results are from two separate experiments.

Cardiomyocyte preparation	LPL activity (m-units/ml)	LPL mass (ng/ml)	LPL specific (m-units/ng)
Control	96.8, 99.1	211, 197	0.46, 0.50
Diabetic	55.7, 48.1	158, 160	0.35, 0.30

[35 S]methionine into LPL as a percentage of radioactivity in total TCA-precipitable protein) was the same in control ($0.060 \pm 0.008\%$) and diabetic ($0.059 \pm 0.009\%$) cardiomyocyte preparations. This value for relative LPL synthesis is similar to results obtained with mouse heart (0.046% ; [33]) but is somewhat less than the value of 0.11% reported for perfused guinea-pig heart [34].

The turnover of newly synthesized LPL in control and diabetic cardiomyocytes was assessed in pulse-chase experiments, where the reduction in [35 S]methionine incorporated into immunoprecipitable LPL after the 20 min pulse was determined following a chase incubation of 90 min in the presence of unlabelled methionine. A representative fluorogram is shown in Figure 5. In control cardiomyocytes, the radioactivity in immunoprecipitable LPL was reduced during the chase incubation to $62 \pm 7\%$ ($n = 6$) of the initial radioactivity measured after the 20 min pulse. A similar extent of LPL turnover was reported previously by Carroll et al. [29] for CHX-treated cardiomyocytes and by Liu and Olivecrona [34] with perfused guinea-pig hearts. The turnover of LPL in diabetic cardiomyocytes was not significantly different from the value for control cardiomyocytes (Table 2).

The effect of diabetes on the mass of LPL in homogenates of control and diabetic cardiomyocytes is shown in Table 3. LPL

activity and mass in diabetic cardiomyocytes was reduced to 53% and 78% of control respectively; as a consequence, the specific activity for LPL (m-units/ng of LPL protein) was reduced from an average of 0.48 in control cardiomyocytes to 0.33 in diabetic cardiomyocytes.

DISCUSSION

Insulin-deficient models of diabetes result in a reduction in LPL activity in both heart [11,12,15–17,19,20] and adipose tissue [11,13,14] preparations that is reversed by *in vivo* administration of insulin [11,13,15,17,19,20]. It should be noted, however, that adipose tissue LPL is more sensitive to diabetes than the cardiac enzyme. The induction of diabetes with 45 mg/kg streptozotocin resulted in a fall in adipose tissue LPL activity to 25% of control, but heart LPL activity was unchanged [13]. A reduction in myocardial LPL activity has been observed at higher diabetogenic doses of streptozotocin: 65 [15], 75 [11,12] or 100 mg/kg [16,17,19,20].

The molecular basis for the diabetes-induced fall in adipose tissue LPL activity has been investigated by Tavangar et al. [13]. The 75% reduction in LPL activity was accompanied by only a 33% decrease in LPL mRNA content, and a 47% reduction in specific activity (total activity divided by immunoreactive mass). Therefore, the diabetes-induced reduction in adipose tissue LPL activity must involve both transcriptional and post-transcriptional mechanisms [13]. Interestingly, chronic insulin-treatment of diabetic rats increased adipose tissue LPL activity, LPL mRNA and LPL mass, whereas acute administration of insulin increased adipose tissue LPL activity and mass without any change in LPL mRNA levels. Experiments where adipocytes are incubated with insulin *in vitro* have provided evidence that LPL can be activated by both transcriptional and post-transcriptional mechanisms [35].

Total cellular and HR-LPL activities were reduced in diabetic cardiomyocytes (Table 1), as reported previously by Braun and Severson [17,19]. In addition, the repletion of LPL activity in CHX-treated cardiomyocytes from diabetic rat hearts was markedly diminished relative to control myocytes (Figure 2), indicating that diabetes may have inhibited any or all of the cellular processes (synthesis, processing, translocation) responsible for the recovery of LPL activity in this model system. The decrease in LPL activity observed in diabetic cardiomyocytes was not, however, accompanied by any change in LPL mRNA content (Table 2). Similarly, LPL mRNA levels were not reduced in whole diabetic hearts. Therefore, post-transcriptional mechanisms must be responsible for the regulation of myocardial LPL by diabetes.

The synthesis of LPL (incorporation of [35 S]methionine into immunoprecipitable LPL protein) was reduced to 71% of control in diabetic cardiomyocytes (Table 2). Diabetes produced a comparable decrease in total protein synthesis (TCA-precipitable radioactivity); as a result, the relative rate of LPL synthesis was unchanged in diabetic cardiomyocytes. It is well known that diabetes results in a generalized fall in total protein synthesis because of decreased translational efficiency [36,37]. Gouni et al. [38] have reported recently that endotoxin decreased LPL activity in heart without any change in LPL mRNA levels. Thus, these results with endotoxin [38] are very similar to the effects of diabetes on LPL in cardiomyocytes (Table 2). The stimulatory effect of insulin on LPL synthesis in adipocytes from obese Zucker rats was entirely due to a generalized increase in total protein synthesis [39]. In experiments with adipocytes from non-obese rats, however, insulin stimulation of total protein synthesis was significantly less than the increase in LPL synthesis [40,41],

so that insulin did produce an increase in the relative rate of LPL synthesis.

Newly synthesized LPL undergoes rapid turnover (degradation) in adipose tissue [1]. In contrast, pulse-chase experiments with perfused hearts [34] and isolated cardiomyocytes [29] have revealed much lower turnover rates for myocardial LPL. Therefore, an increase in the turnover of LPL in diabetic cardiomyocytes could have contributed to the observed diabetes-induced reduction in LPL activity, along with reduced rates of synthesis. However, LPL turnover as assessed by 20 min pulse-90 min chase experiments (Figure 5) was not significantly different in diabetic cardiomyocytes relative to control (Table 2).

The reduction in LPL synthesis with no change in LPL turnover (degradation) in diabetic cardiomyocytes resulted in the expected decrease in LPL mass (Table 3). However, the decrease in total cellular LPL and HR-LPL activities in diabetic cardiomyocytes to approx. 50 % of control was much greater than the reduction in LPL synthesis (Table 2) or mass (Table 3). As a result, the specific activity for LPL in diabetic cardiomyocytes was reduced, suggesting that post-translational mechanisms which result in the accumulation of inactive LPL protein must also contribute to the regulation of myocardial LPL activity in the diabetic heart. LPL is a glycoprotein and catalytic activity in cardiomyocytes is dependent on dimerization subsequent to processing of oligosaccharide chains by glucosidases in the endoplasmic reticulum [1,29]. Thus, diabetes could also reduce LPL activity by: (i) influencing the assembly of the dolichol-linked oligosaccharide precursor that is transferred to the nascent polypeptide chain during translation; (ii) regulating the glucosidase(s) that remove terminal glucose residues in the N-linked oligosaccharides; and (iii) modulating the conversion of the inactive monomeric enzyme to the active homodimer. These potential mechanisms are currently under investigation.

Finally, although *in vivo* administration of insulin rapidly normalized the reduced LPL activity in diabetic cardiomyocytes, *in vitro* incubations with insulin had no stimulatory effect [17,19,20]. Therefore, myocardial LPL activity may not be regulated directly by insulin, in contrast to adipose tissue [35]. Rather, other hormonal and/or metabolic factors that are perturbed in acute, insulin-deficient models of diabetes may mediate the reduction in myocardial LPL activity. Identification of these regulatory factors will provide another example of a tissue-specific mechanism for regulating LPL.

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