Effect of improved diabetes control on the expression of lipoprotein lipase in human adipose tissue

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Abstract Patients with diabetes commonly manifest hypertriglyceridemia along with decreased adipose tissue lipoprotein lipase (LPL) activity, and improved diabetes control tends to reverse these abnormalities. To better understand the mechanism of regulation of LPL in diabetes, 11 diabetic patients (3 type I, 8 type II) were brought under improved glycemic control, and adipose tissue LPL gene expression was assessed by performing paired fat biopsies. Six of the 11 patients attained improved control with insulin, with a decrease in glycohemoglobin (glyc Hgb) from 13.8 ± 0.9 to 10.4 ± 0.6%; 5 patients attained improved control with glyburide (glyc Hgb fell from 14.2 ± 2.4 to 8.8 ± 0.6%), and together they demonstrated a lowering of serum triglycerides and total cholesterol. No changes were observed in HDL cholesterol. Improved diabetes control resulted in a significant increase in LPL activity in both the heparin-releasable (HR) and extractable (EXT) fractions of adipose tissue, as well as in LPL immunoreactive mass. The change in LPL activity with improved control was variable, and showed a positive correlation with the HDL levels prior to treatment ($r = 0.74$, $P < 0.02$). When adipose tissue was pulse-labeled with [^{35}S]methionine, there was an increase in isotope incorporation into LPL after treatment, indicating an increase in LPL synthetic rate. However, improved diabetes control resulted in no significant change in LPL mRNA levels. Thus, improved glycemic control resulted in an increase in LPL activity which correlated with each patient's basal high density lipoprotein. This increase in LPL activity was accompanied by an increase in LPL immunoreactive mass, and an increase in LPL synthesis. Since there was no change in LPL mRNA levels, these data suggest that LPL regulation in these patients occurs at a translational level or through a possible change in degradation.—**Simsolo, R. B., J. M. Ong, B. Saffari, and P. A. Kern.** Effect of improved diabetes control on the expression of lipoprotein lipase in human adipose tissue. *J. Lipid Res.* 1992. **33:** 89–95.

Supplementary key words insulin • glyburide • high density lipoprotein • messenger RNA • translation

Lipoprotein lipase (LPL) is present mainly in adipose tissue and muscle, and is the rate-limiting enzyme in triglyceride-rich lipoprotein metabolism (1). Deficiencies in LPL play an important role in the pathophysiology of hyperlipidemia in a number of important clinical conditions, among the most common of which is poorly controlled diabetes. Numerous studies have described decreases in LPL catalytic activity in the adipose tissue (2–4) or the post-heparin plasma (5, 6) of both insulin-dependent (IDD) and non-insulin-dependent diabetics (NIDD) who are under poor glycemic control. Subsequent treatment of these patients has been shown to increase the level of lipoprotein lipase, coincident with a fall in plasma triglycerides. However, previous studies have studied only LPL catalytic activity and have not examined the mechanism of regulation of LPL. With the recent development of high quality anti-LPL antibodies (7) and the LPL cDNA (8), more detailed studies of LPL gene expression are now possible.

Recent studies from our laboratory and that of others have demonstrated that the mechanism of regulation of LPL varies with different regulatory events. For example, insulin added to isolated rat adipocytes in vitro increased LPL activity through an increase in LPL mRNA levels (9). On the other hand, feeding increased LPL activity due to posttranslational effects (10, 11), whereas weight loss in obese subjects increased LPL due to an increase in LPL mRNA levels (12). Therefore, to study the defect in LPL regulation in diabetes, we examined the mechanism of regulation of adipose tissue LPL after improvement of glycemic control either with insulin or sulfonylurea drug treatment.

METHODS

Human Subjects

Eleven diabetic patients who were under suboptimal glycemic control were recruited from the outpatient clinic and gave informed consent to the procedure. No

Abbreviations: LPL, lipoprotein lipase; PBS, phosphate-buffered saline; HR, heparin-releasable LPL; EXT, extractable LPL; ELISA, enzyme-linked immunosorbent assay; IDD, insulin-dependent diabetes; NIDD, non-insulin-dependent diabetes; BMI, body mass index; Glyc Hgb, glycohemoglobin; HDL, high density lipoprotein; VLDL, very low density lipoprotein.

patient was taking any medication or had any concurrent illness likely to affect lipid metabolism. The day prior to the biopsy the patients were placed on an isocaloric diet, consisting of 50% carbohydrate, 20% protein, and 30% fat, and they presented to the clinic after fasting for 12 h. A subcutaneous fat biopsy was performed from the lower abdominal wall. The biopsy was performed as described previously (10) by infiltrating 1% lidocaine (without epinephrine) in a square pattern, followed by a 3-cm incision and removal of 3 to 10 g fat. A portion of the excised tissue was placed into iced phosphate-buffered saline (PBS) and processed immediately for LPL activity, immunoreactive protein, and synthetic rate as described below. A second portion of the adipose tissue was immediately frozen in liquid nitrogen and stored at −70°C for subsequent RNA isolation. All subjects tolerated the procedure well. In addition, blood samples were obtained for glucose, insulin, glycated hemoglobin, and lipids. Patients were then treated either with insulin or glyburide, depending on clinical circumstances, to achieve improved glycemic control. After 3 months of improved glycemic control, patients were again placed on the same standardized diet and reported in the fasting state for an adipose tissue biopsy from the contralateral lower abdominal wall.

Six of the eleven patients were treated with insulin after the first biopsy was performed. This group consisted of three type I diabetics who were on a suboptimal insulin regimen and three type II diabetics who were failures on oral agents. The patients on oral agents were withdrawn from the drug 48 h prior to the first fat biopsy, which did not result in any acute change in fasting blood glucose. The remaining five patients were newly discovered type II diabetics and were treated with glyburide. The characteristics of all the patients recruited for this study are shown in **Table 1**.

LPL catalytic activity

LPL activity was separated into two fractions, as described previously (10): activity released with heparin (HR) and activity extracted from the tissue after the heparin release (EXT). Adipose tissue was minced and incubated with 13 μg/ml heparin (Fisher Scientific Co.) in PBS for 30 min at 37°C. An aliquot of buffer was then assayed for HR LPL activity as described below. The heparin-treated tissue was then washed once with PBS and the extractable fraction was prepared by homogenizing the tissue in buffer containing deoxycholate and heparin, as described previously (10, 13). The sample was centrifuged, and the aqueous layer was collected and assayed for EXT LPL activity in duplicate. Total adipose LPL activity was computed as the sum of HR and EXT, which has been validated previously (14).

The LPL activity assay was performed as described previously (15) using a [^{3}H]triolein-containing substrate emulsified with lecithin, and containing normal human serum as a source of apoC-II. Samples were incubated with the substrate solution for 45 min at 37°C, and ^{3}H-labeled free fatty acids were separated by the method of Belfrage and Vaughan (16). LPL activity was expressed in nEq FFA released/min per 10^6 cells, and cell number was determined according to the method of DiGirolamo et al. (17).

TABLE 1 Clinical characteristics of the patients

Patient No.	Sex	Age	R_x	BMI		Glyc Hb		Glucose		TG		HDL-C		Chol	
Diabetes Type				B	A	B	A	B	A	B	A	B	A	B	A
		yr		*kg/m²*		*%*		*mg%*		*mg%*		*mg%*		*mg%*	
1 I	M	42	INS	23.4	24.3	13.1	9.0	253	97	106	74	35	30	155	132
2 I	M	44	INS	23.5	24.4	10.0	9.4	238	122	266	111	43	36	209	195
3 I	F	31	INS	21.9	24.1	14.9	9.1	259	181	137	177	58	56	177	179
4 II	F	32	INS	58.2	59.8	15.0	11.1	255	124	148	140	59	43	230	206
5 II	F	65	INS	31.0	31.0	15.8	12.3	200	169	56	53	49	59	142	159
6 II	M	46	INS	38.9	41.1	14.2	11.6	242	237	234	175	45	60	280	238
7 II	M	57	GLY	43.1	43.1	8.7	7.7	169	157	271	216	40	40	226	217
8 II	M	36	GLY	23.9	26.1	23.1	7.9	355	80	163	133	30	29	198	143
9 II	M	54	GLY	34.7	36.1	11.9	8.3	235	142	259	211	22	24	194	176
10 II	M	41	GLY	22.5	21.7	13.9	10.8	165	140	139	102	44	48	167	140
11 II	M	59	GLY	34.0	34.0	14.4	9.5	236	104	535	242	25	28	229	212
Mean ±		46		32.3	33.2*	14.1	9.7*	237	141*	205	145*	41	41	201	182*
SEM		3		3.4	3.4	1.1	0.5	15	13	40	18	4	4	12	11

Abbreviations: BMI, body mass index; Glyc Hb, glycated hemoglobin; TG, triglyceride; HDL-C, HDL cholesterol; Chol, cholesterol; B, before diabetes control; A, after diabetes control.

*$P < 0.05$ versus before diabetes control.

LPL immunoreactive mass

For determining LPL immunoreactive mass, tissue samples were prepared as described above for the LPL activity assay, except for the addition of protease inhibitors (18). The enzyme-linked immunosorbent assay (ELISA) for measuring LPL immunoreactive mass was described previously, and data were expressed as ng immunoreactive LPL/10^6 cells (18). In brief, a microtiter plate was coated with affinity-purified chicken anti-LPL antibody, and samples and bovine LPL standards were applied in a buffer containing 1 M NaCl, 0.1% Triton X-100, 0.1% albumin, protease inhibitors, and 25 mM Tris-HCl (pH 7.4). Biotinylated anti-LPL antibody was then added, followed by the addition of streptavidin-horseradish peroxidase (Bethesda Research Laboratories). The plate was developed and quantitated on an ELISA plate reader at 490 nm. Samples from before and after diabetes control were assayed in duplicate on the same ELISA plate to minimize interassay variation.

LPL synthetic rate

Adipose tissue was minced, washed three times with methionine-free Medium 199 (Irvine Scientific, Santa Ana, CA), and incubated in the same medium for 1 h at 37°C in preparation for radiolabeling. After this, the tissue was minced, and metabolically labeled with 50 μCi of [^{35}S]methionine for 30 min. Lysis buffer was then added to give a final lysate concentration of 0.02 M sodium phosphate (pH 7.5), 0.2 mM NaCl, 2% (v/v) Triton X-100, 1% (w/v) sodium deoxycholate, 0.2% (w/v) sodium dodecyl sulfate, 5 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride. The samples were immunoprecipitated, as described previously (9), and analyzed using a 10% SDS-polyacrylamide gel. After autoradiography the intensity of the bands was quantitated by laser densitometry.

To control for minor differences in labeling conditions between biopsies, as well as for isotopic decay of the ^{35}S, an aliquot of adipose tissue extract was precipitated with trichloroacetic acid (TCA) at the time of immunoprecipitation and SDS PAGE. The immunoprecipitates were then loaded onto the gel in proportion to the total TCA-precipitable counts.

Extraction of RNA and Northern analysis

Isolation of RNA from adipose tissue was performed according to the guanidinium–phenol–chloroform method (19) and RNA was quantitated spectrophotometrically. As described previously (9), the extracted RNA samples were electrophoresed on a 2.2 M formaldehyde–1% agarose gel and transferred onto a nylon membrane (Hybond-N; Amersham Corp.) for Northern blot analysis. Northern blots were loaded with equal quantities of total RNA. The blotted membranes were hybridized to ^{32}P-labeled cDNA probes coding for human LPL (8) and gamma-actin (20). Hybridization and washing of the membranes were as described previously (10) and the images were then quantitated by autoradiography and laser densitometry.

Statistics

All data were expressed as the mean ± SEM and were analyzed nonparametrically using the Wilcoxon matched-paired singed-rank test for paired data, and the Mann-Whitney rank sum test for nonpaired data, and the Spearman Rank Correlation Coefficient for trends.

RESULTS

Treatment with either insulin or glyburide led to similar improvements in glycemic control, as shown by the significant fall in glycohemoglobin and fasting glucose (Table 1). In addition, there was a significant decrease in plasma triglycerides and cholesterol, without change in HDL cholesterol. There was a slight increase in body weight in most of the patients.

Improved glycemic control resulted in a significant increase in HR, EXT, and total LPL activity (**Fig. 1**, panel A). Prior to improved control, HR LPL activity was 0.97 ± 0.52 nEq FFA released/min per 10^6 cells, and increased to 1.64 ± 0.69; and EXT LPL activity was 0.28 ± 0.17 and increased to 1.08 ± 0.33 nEq FFA released/min per 10^6 cells following treatment. The levels of both HR and EXT LPL immunoreactive mass also increased from 2.39 ± 0.62 to 5.15 ± 1.76 ng/10^6 cells, and from 6.24 ± 1.06 to 17.0 ± 4.89 ng/10^6 cells, respectively, suggesting that the increase in LPL activity was due to an increase in LPL protein (**Fig. 1**, panel B).

Although diabetes control resulted in overall increases in LPL activity, there was variability among patients in the LPL changes. This variability could not be accounted for by the type of diabetes, the degree of obesity, or the magnitude of change in glycohemoglobin. However, a significant relationship was observed between the change in adipose LPL activity and the basal HDL level. As shown in **Fig. 2**, there was a significant correlation between HDL prior to treatment, and the change in LPL with treatment.

To further examine the mechanism of the increase in LPL with treatment, [^{35}S]methionine pulse-labeling of adipose tissue was performed, along with mRNA extraction and Northern blotting. **Fig. 3** illustrates the data of two representative patients who demonstrated large increases in LPL activity and mass. As can be seen, there were increases in [^{35}S]methionine incorporation into LPL, indicating an increase in LPL synthetic

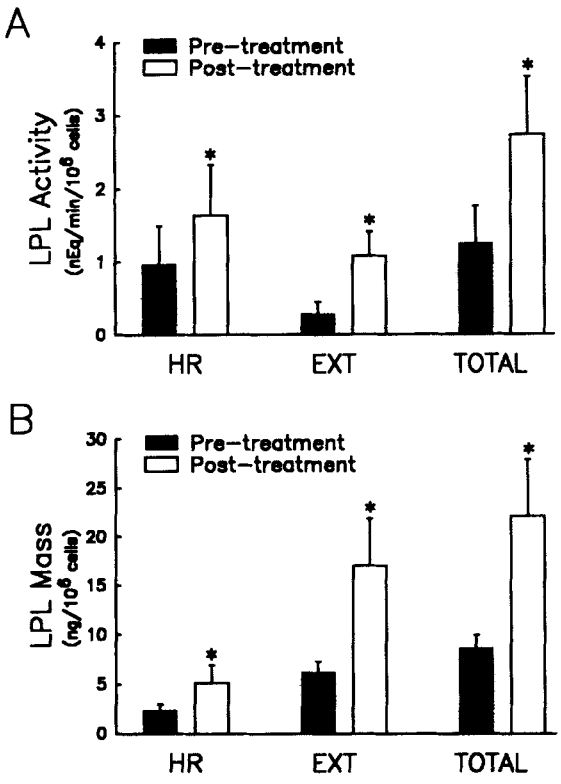

Fig. 1. Effect of diabetes control on LPL activity and immunoreactive mass. Fasting adipose tissue biopsies were performed on 11 subjects before and 3 months after attainment of improved glycemic control of their diabetes. $^{*}P < 0.05$ versus before treatment.

rate. As shown in the Northern blots from the same patients (second panel) there were no changes in LPL mRNA levels after improved glycemic control, when compared to the gamma-actin mRNA level.

RNA was extracted and Northern blots were performed on all 11 patients, and the results are summarized in **Fig. 4**. The data are expressed as the LPL/gamma-actin ratio, using laser densitometry to quantitate the image, standardized to the LPL/gamma-actin ratio from the first biopsy. Although some patients showed small increases in LPL mRNA after improved glycemic control, these changes were not consistently seen, were not statistically significant, and could not explain the changes in synthetic rate and mass. Thus, the increase in LPL synthesis in response to treatment was not accompanied by a corresponding change in mRNA levels, suggesting that glycemic control in diabetic patients regulates LPL post-transcriptionally.

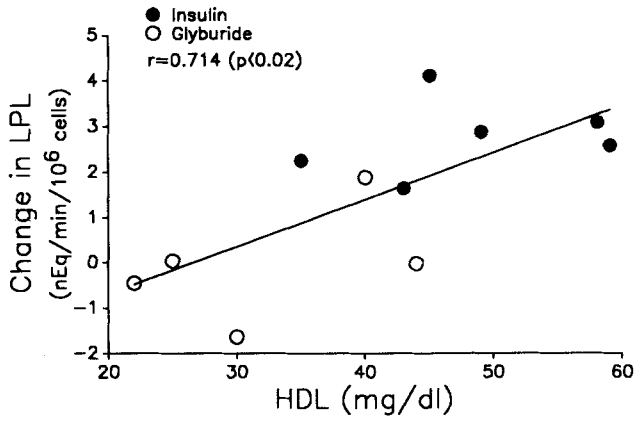

Fig. 2. Relationship between change in LPL with improved diabetes control and basal HDL levels prior to treatment.

DISCUSSION

Hypertriglyceridemia is a common lipid abnormality in patients with diabetes (21), and can result from a combination of two abnormalities: overproduction of VLDL by the liver, and diminished removal of triglyceride-rich lipoprotein, caused by reduced LPL activity (22, 23). Patients who are hyperinsulinemic, including obese nondiabetic patients, tend to manifest hypertriglyceridemia predominantly due to VLDL overproduction, whereas insulinopenic diabetics tend to have more of a triglyceride removal defect. Nevertheless, patients with poorly controlled diabetes uniformly demonstrate a reduction in plasma triglycerides with improved glycemic control.

Numerous previous studies have measured LPL activity in diabetic patients under a variety of conditions. When diabetic patients were compared to a suitable control group, there was a decreased activity of the enzyme in both post-heparin plasma (5, 6, 24–26) and adipose tissue (2–4, 26–28). In addition, when improved diabetes control was achieved with insulin, there were consistent increases in LPL activity in adipose tissue both in IDD and NIDD, as well as increases in post-heparin plasma LPL (2–6, 25, 28, 29). Several studies have also examined improved diabetes control with sulfonylurea drugs in type II diabetics. Similar increases in adipose or post-heparin plasma LPL activity were observed (2, 6, 27), although one study observed no significant change in post-heparin plasma LPL activity with both glibenclamide and phenformin (5).

Previous studies have not examined the mechanism of regulation of LPL in diabetic patients before and after glycemic control. With the recent development of the LPL cDNA (8) and high quality antibodies to LPL (7), we attempted to determine whether the increase in adipose tissue LPL after diabetes control was due to an increase in LPL mRNA levels, or secondary to post-transcriptional effects.

In this study, all patients were studied while under suboptimal glycemic control, and again after 3 months of improved glycemic control, achieved either with in-

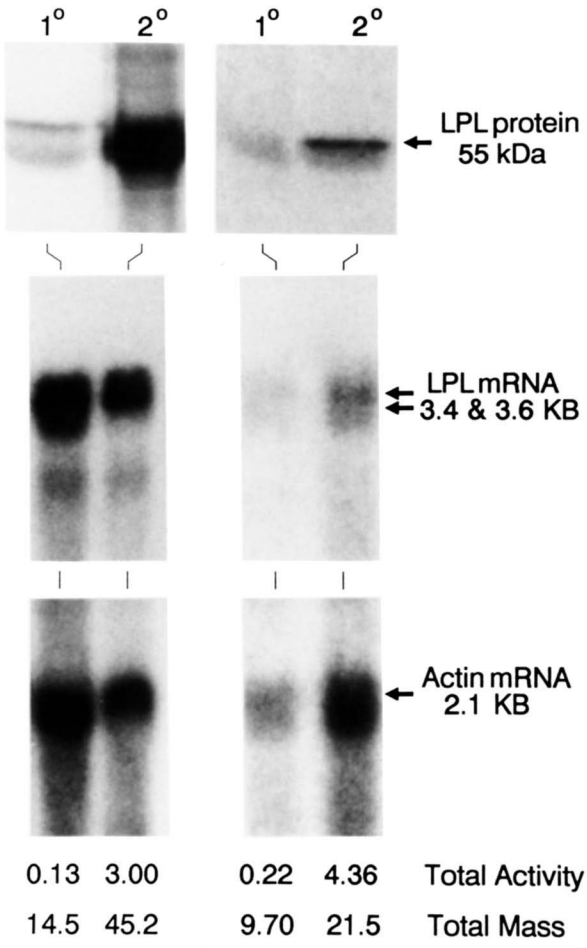

Fig. 3. Effect of diabetes control on LPL synthesis and LPL mRNA levels. LPL synthetic rate (top panel) was assessed by pulse-labeling with [^{35}S]methionine for 30 min, followed by immunoprecipitation, as described in Methods. In the lower panels are the Northern blots for LPL and gamma-actin from the same two patients. Ethidium bromide staining of the agarose gels demonstrated rRNA bands that corresponded closely with the gamma-actin mRNA level. Data from two representative patients are shown, and the data on LPL activity and mass are shown at the bottom.

sulin or glyburide. Improved glycemic control resulted in a significant increase in LPL activity, as has been previously described. Even though the patients as a whole increased LPL activity, there was variability in the LPL changes. These changes in LPL with treatment were significantly correlated with the levels of HDL prior to treatment, suggesting that HDL levels may be predictive of the LPL response to improved diabetes control. This is of interest because of the intimate relationship between HDL and LPL-mediated triglyceride metabolism (30). Previous studies have noted a positive correlation between HDL cholesterol and LPL activity in the basal state (31–33). In addition, a recent study described a polymorphism in the LPL gene that correlated with HDL levels (34), raising the possibility that LPL responsiveness and HDL levels may be genetically regulated.

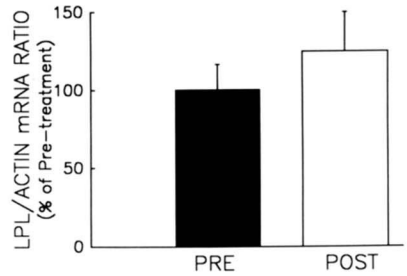

Fig. 4. Effect of diabetes control on LPL mRNA. The bars (mean ± SEM) show the LPL/actin mRNA ratio, standardized to the LPL/actin ratio from the first biopsy, for all 11 patients. Although some patients demonstrated either an increase or decrease in LPL/actin ratio after improved diabetes control, these changes were minor, did not correspond with changes in LPL activity or mass, and were not statistically significant.

The increase in LPL activity in these patients was accompanied by a significant increase in both immunoreactive mass and synthetic rate. However, even though some patients showed small increases in LPL mRNA levels, no overall significant change was found in LPL/gamma-actin mRNA levels, suggesting that glycemic control in diabetic patients resulted in an increase in LPL translation, or perhaps a decreased degradation of the LPL protein.

A number of studies have examined the regulation of LPL in relation to insulin and carbohydrate metabolism. In normal subjects, a direct correlation was found between the increase in adipose tissue HR LPL activity and insulin levels after a carbohydrate-rich meal (2). In addition, LPL activity was stimulated when an insulin-glucose infusion was administered to normal subjects (35). Obese, hyperinsulinemic subjects have higher adipose tissue LPL activity when compared to lean subjects (36), and the mechanism of this increased LPL, as well as the post-prandial increase in LPL, is a post-translational activation of a previously inactive LPL protein (10). In diabetic patients, however, insulin or improved glycemic control seem to operate at a translational level, since we were able to demonstrate an increase not only in LPL activity but in the total protein mass and synthetic rate with no changes in LPL mRNA.

In vitro studies using adipocytes have examined LPL regulation by insulin and glucose. Insulin increased LPL activity in isolated rat adipocytes (37) through an increase in mRNA levels (9). A recent study has observed no changes in LPL transcription rate with insulin, suggesting that insulin affects LPL mRNA stability (38). In human adipocytes, high concentrations of insulin are required to stimulate LPL, suggesting an effect mediated by the IGF-I receptor (14). Other in vitro studies examined the effects of high glucose on LPL activity in human adipocytes, and found an inhibition of LPL secretion, along with a diminished response to several regulations (39).

In summary, improvement in diabetes control resulted in increased adipose tissue LPL activity, due to an increase in LPL synthesis. Because there was no increase in LPL mRNA levels, these data suggest that improved diabetes control regulates LPL at the level of translation, or possibly through a change in LPL degradation. These data emphasize the complex nature of the metabolic regulation of LPL by multiple factors involved in carbohydrate metabolism.

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