

# Alteration in lipoprotein lipase activity bound to triglyceride-rich lipoproteins in the postprandial state in type 2 diabetes

Valérie Pruneta-Deloche,<sup>1,\*</sup> Agnès Sassolas,<sup>†</sup> Geesje M. Dallinga-Thie,<sup>§</sup> François Berthezène,<sup>\*\*</sup> Gabriel Ponsin,<sup>\*</sup> and Philippe Moulin<sup>\*,\*\*</sup>

UMR 585 INSERM/INSA Physiopathologie des Lipides et Membranes,<sup>\*</sup> Villeurbanne, France; Laboratoire de Biochimie,<sup>†</sup> Hôpital Neurologique, Lyon-Bron, France; Department of Internal Medicine,<sup>§</sup> University Medical Center Utrecht, Utrecht, The Netherlands; and Unité 11, Hôpital Cardiovasculaire et Pneumologique Louis Pradel,<sup>\*\*</sup> Lyon-Bron, France

**Abstract** Postprandial lipid metabolism is largely dependent upon lipoprotein lipase (LPL), which hydrolyses triglycerides (TGs). The time course of LPL activity in the postprandial state following a single meal has never been studied, because its determination required heparin injection. Recently, we have shown that LPL activity could be accurately measured in nonheparinized VLDL using a new assay aiming to determine the LPL-dependent VLDL-TG hydrolysis. Based on the same principle, we used in this study TG-rich lipoprotein (TRL)-bound LPL-dependent TRL-TG hydrolysis (LTTH) to compare the time course of LPL activity of 10 type 2 diabetics to that of 10 controls, following the ingestion of a lipid-rich meal. The peak TG concentration, reached after 4 h, was 67% higher in diabetics than in controls ( $P < 0.005$ ). Fasting LTTHs were  $91.3 \pm 15.6$  in controls versus  $70.1 \pm 4.8$  nmol NEFA/ml/h in diabetics ( $P < 0.001$ ). LTTH was increased 2 h postprandially by 190% in controls and by only 89% in diabetics, resulting in a 35% lowering of the LTTH area under the curve in diabetics. Postprandial LTTH was inversely correlated with TG or remnant concentrations in controls but not in diabetics, and with insulin resistance in both groups. These data show that TRL-bound LPL activity increases in the postprandial state and is strongly reduced in type 2 diabetes, contributing to postprandial hypertriglyceridemia.—Pruneta-Deloche, V., A. Sassolas, G. M. Dallinga-Thie, F. Berthezène, G. Ponsin, and P. Moulin. **Alteration in lipoprotein lipase activity bound to triglyceride-rich lipoproteins in the postprandial state in type 2 diabetes.** *J. Lipid Res.* 2004. 45: 859–865.

**Supplementary key words** lipoprotein lipase • type 2 diabetes • postprandial period • triglyceride-rich lipoprotein • remnant

Postprandial hypertriglyceridemia is a prominent feature of dyslipidemia in type 2 diabetes and is considered

to be proatherogenic (1–3). Accumulation of remnants in the postprandial state is thought to constitute a cardiovascular risk factor in insulin-resistant subjects (4, 5). Postprandial plasma triglyceride (TG) concentration depends upon the balance between intestinal and hepatic production of TG-rich lipoproteins (TRLs) and plasma clearance of remnants and VLDL. However, the precise mechanisms in TG clearance are difficult to explore, because little is known concerning the time course of the changes in the postprandial activity of lipoprotein lipase (LPL), the main enzyme involved in plasma TG hydrolysis. Because the bulk of LPL is bound to the endothelium, the release of the enzyme through heparin injection must be achieved to permit the ex vivo measurement of total LPL activity (6). In addition to being cumbersome, this process causes a prolonged release of LPL stores, which precludes any relevant time course study of LPL activity. However, several reports have described the presence of LPL protein in nonheparinized plasma (7–10). The mass concentration of the preheparinic LPL appeared to be negatively related to plasma TG concentration and positively related to HDL-cholesterol (HDL-C) (11, 12). In addition, preheparinic LPL concentration was found to be increased after treatment with an insulin sensitizer, troglitazone (13), and decreased in situations in which TG catabolism was defective (14, 15). Previous studies have shown that this plasma circulating LPL was bound to TRLs and exhibited some lipolytic activity (10, 16). Taking these findings into consideration, we developed a new method that allows the measurement of the VLDL-bound LPL-dependent VLDL-TG

Abbreviations: FPLC, fast-protein liquid chromatography; HOMA, homeostasis model assessment; LPL, lipoprotein lipase; RLP, remnant-like lipoprotein particle; TG, triglyceride; TRL, triglyceride-rich lipoprotein.

<sup>1</sup> To whom correspondence should be addressed.  
e-mail: valerie.pruneta-deloche@insa-lyon.fr

Manuscript received 22 October 2003 and in revised form 2 February 2004.

Published, JLR Papers in Press, February 16, 2004.  
DOI 10.1194/jlr.M300435JLR200

Copyright © 2004 by the American Society for Biochemistry and Molecular Biology, Inc.

This article is available online at <http://www.jlr.org>

hydrolysis (LVTH). This assay is considered to be a relevant marker of the functional LPL pool in the fasting state on the basis of three considerations (17). First, in contrast to conventional postheparin LPL activity, LVTH is tightly correlated with VLDL clearance in human subjects. Second, it allows efficient discrimination of patients with heterozygous LPL deficiency. And finally, LVTH is clearly lowered in type 2 diabetes, a condition known to be frequently accompanied by defective lipolysis. Inasmuch as our assay does not require a heparin injection, it has the definitive advantage of easily permitting repetitive time course measurements of LPL activity in humans. In the present study, we used this new tool to further investigate the alterations in TG lipolysis in type 2 diabetes. Fasting LPL activity is lowered in type 2 diabetic patients, which raises the question of its fate in the postprandial state. To answer this question, we had to take into account that postprandial TRLs include both VLDL and chylomicrons. On the basis of the same concept as LVTH, we developed a TRL-bound LPL-dependent TRL-TG hydrolysis (LTTH), in which VLDL was replaced by total TRLs. In the present work, we analyzed the time course of LPL activity after a high-fat meal in both diabetic patients and matched control subjects.

## MATERIALS AND METHODS

### Subjects

A total of 20 male subjects were investigated in the present study. Ten patients with clinically defined type 2 diabetes mellitus were compared with 10 nondiabetic and normolipidemic healthy control subjects. None of the diabetic patients had proteinuria or hypothyroidism. All received either sulphonylurea or biguanides or both, but no insulin therapy. Relevant clinical and physiological characteristics of the subjects are presented in **Table 1**. All control subjects and patients gave written informed

TABLE 1. Subject characteristics

	Control Subjects, n = 10	Type 2 Diabetics, n = 10	<i>P</i>
Age (years)	48 ± 5	56 ± 2	ns
Body mass index (kg/m <sup>2</sup> )	23.4 ± 0.9	28.9 ± 1.5	0.0059
Hemoglobin A <sub>1c</sub> (%)	ND	7.7 ± 0.8	—
Fasting glucose (mmol/l)	5.3 ± 0.1	8.0 ± 0.7	0.0017
Fasting insulin (mU/l)	8.7 ± 1.3	15.3 ± 2.4	0.0322
HOMA index	2.0 ± 0.3	5.2 ± 0.8	0.0057
Triglycerides (mmol/l)	0.9 ± 0.1	1.4 ± 0.2	0.0103
Total cholesterol (mmol/l)	5.1 ± 0.3	4.7 ± 0.3	ns
HDL-C (mmol/l)	1.16 ± 0.05	0.85 ± 0.07	0.0015
LDL-C (mmol/l)	3.54 ± 0.31	3.24 ± 0.21	ns
RLP-C (mmol/l)	0.25 ± 0.03	0.33 ± 0.04	ns
ApoB (g/l)	0.98 ± 0.08	1.12 ± 0.08	ns
ApoA-I (g/l)	1.39 ± 0.06	1.19 ± 0.07	0.0433
ApoE (mg/l)	36 ± 1	41 ± 2	0.0471
ApoE phenotype (n)			
3/3	7	6	ns
3/4	3	4	ns

ApoB, apolipoprotein B; HDL-C, HDL-cholesterol; HOMA, homeostasis model assessment; RLP-C, remnant-like lipoprotein particle-C; ND, not determined; ns, not significant. Results are given as mean ± SEM.

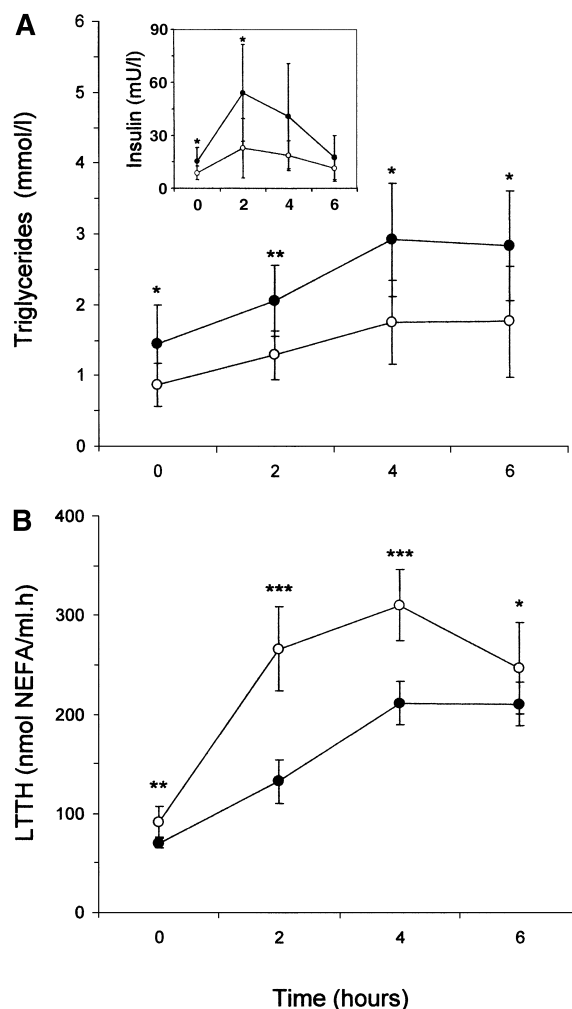
consent to the study protocol, which was approved by our local ethical committee.

### Oral fat load and blood samples

After 12 h overnight fasting, each participant was given a semi-liquid test meal containing 40 g fat/m<sup>2</sup> body surface area in the form of ice cream. The total energy content of the meal was 730 Kcal/m<sup>2</sup> (60% from fat, 30% from carbohydrate, and 10% from protein). The ice cream was ingested within 20 min and was well tolerated by all subjects. After the test meal, only water was allowed for drinking during the oral fat test. Blood samples were collected in prechilled EDTA-containing tubes before the meal and every 2 h after the meal over a 6 h period. After separation of plasma, aliquots were either stored frozen at -80°C for subsequent measurement of LTTH or immediately used for determinations of lipids.

### Laboratory measurements

TG, cholesterol (Sigma Diagnostics), and NEFA (NEFA C, Wako Chemicals) plasma concentrations were measured using commercial kits. HDL-C was determined after precipitation of



**Fig. 1.** Line plots show the postprandial response of plasma triglycerides (TGs) and insulin concentrations (A) and TRL-bound LPL-dependent TRL-TG hydrolysis (LTTH) (B) in type 2 diabetic patients (closed circles) and in control subjects (open circles). Values are mean ± SD; n = 10. \* *P* < 0.05, \*\* *P* < 0.001, and \*\*\* *P* < 0.0001 versus type 2 diabetes.

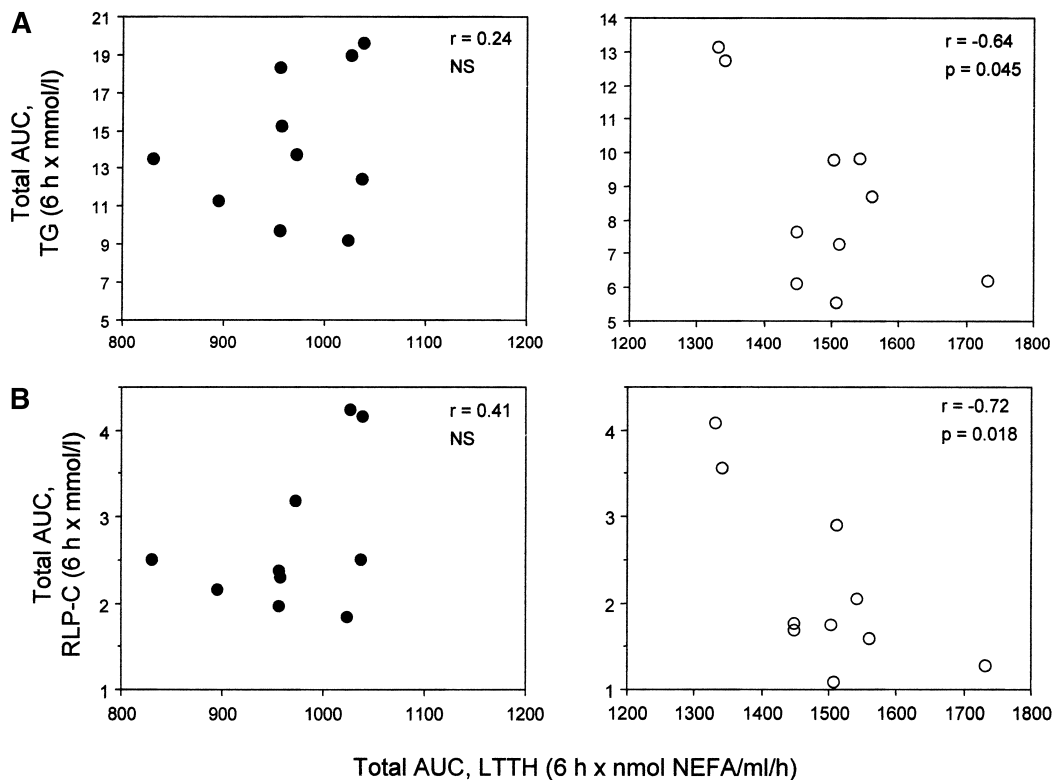


Fig. 2. Relationships between LTTH and plasma TG (A) or remnant-like lipoprotein particle-C (RLP-C) (B) in type 2 diabetic patients (closed circles) and in control subjects (open circles). Data are expressed as 0 to 6 h AUCs.

apolipoprotein B (apoB)-containing lipoproteins using the phosphotungstate-MgCl<sub>2</sub> procedure. Fasting plasma LDL-C was calculated according to the formula of Friedewald, Levy, and Fredrickson (18). ApoB, apoA-I, and apoE were measured by immunonephelometry, and apoC-II and apoC-III by immunoturbidimetry. Hemoglobin A<sub>1c</sub> was determined by HPLC analysis. Glucose and insulin were measured in fasting plasma. Insulin was determined using an immunoenzymatic commercial kit (Insulin IRMA BSE, Brahms), and glucose was measured using a glucose oxidase enzymatic assay (Randox Laboratories). Insulin sensitivity was estimated by the homeostasis model assessment (HOMA) index (19).

#### Determination of TRL-TG hydrolysis

Spontaneous lipolytic activity in TRLs resulting from LPL bound on their surface was measured using the LTTH assay. LTTH was similar to our previously described method for LVTH (17) except that VLDL was replaced by whole TRLs. Briefly, TRLs were isolated by fast-protein liquid chromatography (FPLC) using a Superose 6 HR 10/30 column (Pharmacia). On the basis of preliminary calibration experiments, chylomicrons

and VLDL isolated by sequential ultracentrifugations were shown to elute in fractions 9 to 10 and in fractions 11 to 18, respectively. Thus, 1 ml of filtered plasma was applied to the column and chromatographed at a flow rate of 0.3 ml/min. Fractions corresponding to total TRLs, including both chylomicrons and VLDL (fractions 9 to 18), were pooled and immediately assayed for LPL activity (17). Aliquots of the pooled TRLs corresponding to 0.3  $\mu$ mol of TG were incubated at 37°C. The resulting amounts of NEFA released were then measured, and after correction for plasma TG concentrations, LTTH was finally expressed as the amount of NEFA released per milliliter of plasma per hour.

#### Remnants determination

The fraction containing remnant-like lipoprotein particles (RLPs) was prepared using the immunoseparation technique described by Nakajima et al. (20). Briefly, 5  $\mu$ l of plasma was added to 300  $\mu$ l of mixed immunoaffinity gel suspension containing monoclonal anti-human apoA-I and anti-human apoB-100 antibodies (Japan Immunoresearch Laboratories). The reaction mixture was gently shaken for 2 h at room temperature. Af-

TABLE 2. Plasma concentrations of apoC-II and apoC-III in the fasting state and 2 h following a test meal

	Control Subjects		Type 2 Diabetics		<i>P</i> (Diabetics vs. Controls)	
	Fasting	Postprandial	Fasting	Postprandial	Fasting	Postprandial
apoC-II (g/l)	31.4 $\pm$ 3.5	31.2 $\pm$ 3.6	45.6 $\pm$ 4.1	45.5 $\pm$ 3.7	0.017	0.013
apoC-III (g/l)	93.4 $\pm$ 5.5	93.6 $\pm$ 5.5	123.2 $\pm$ 15.8	123.3 $\pm$ 14.0	ns	ns
apoC-II/C-III	0.35 $\pm$ 0.04	0.34 $\pm$ 0.04	0.40 $\pm$ 0.04	0.38 $\pm$ 0.03	ns	ns

Results are given as mean  $\pm$  SEM.

ter the mixture had been standing for 15 min, the cholesterol content of the unbound fraction (RLP-C) was measured in the supernatant.

### Dot blot analysis of LPL

Fractions eluted from the FPLC column in the TRL size range were pooled and applied to a nitrocellulose sheet. Dilutions of purified bovine LPL were used as standards. The membrane was incubated with the monoclonal mouse antibody 5D2 (a gift from Dr. J. Brunzell, Seattle, WA). 5D2 Mabs bound to LPL on the membrane were then detected using a horseradish peroxidase-linked secondary antibody against mouse and with the ECL-Western blotting analysis system (Amersham).

### Statistical analysis

Data are expressed as mean  $\pm$  SD, except where indicated. Statistical analyses were performed using the Statview statistical package. Correlation coefficients were calculated assuming a linear relationship between parameters. For all analyses, the level of statistical significance was taken as  $P < 0.05$ . To quantify the total increases of LTTH, TG, and NEFA in plasma during the 6 h postprandial period, areas under the curve (AUCs) were calculated by the trapezoidal method.

## RESULTS

The clinical characteristics of participants are shown in Table 1. Patients and controls were matched for age. Patients were mildly obese, and plasma glucose and insulin concentrations were significantly increased, resulting in a significantly higher HOMA index. Type 2 diabetic patients had a typical moderate dyslipidemia characterized by elevated fasting plasma TG (+67%) and decreased fasting HDL-C (-27%) and apoA-I concentrations (-14%) compared with controls. A mild, nonsignificant increase in RLP-C (+31%) was also observed in the fasting state.

Following the ingestion of the lipid-rich meal, plasma triglyceridemia increased in controls and type 2 diabetics, with a maximum reached in both groups after 4 h (Fig.

1A). At this time point, the elevation in plasma TG concentration was higher in type 2 diabetics (+67% vs. controls,  $P < 0.005$ ). The AUC of plasma TG concentrations over the 6 h time course was significantly higher in type 2 diabetics than in controls ( $14.2 \pm 0.6$  vs.  $8.7 \pm 2.7$  mmol/h/l,  $P < 0.005$ ). This difference was maintained when only the TRLs isolated by FPLC were considered (AUC:  $10.9 \pm 2.6$  vs.  $7.3 \pm 1.8$  mmol/h/l,  $P < 0.005$ , data not shown). As shown in the insert in Fig. 1A, a postprandial increase in plasma insulin was observed in both type 2 diabetics and controls, although it was more pronounced in the former group. A strong postprandial increase of LTTH was observed in control subjects (Fig. 1B). LTTH also increased in type 2 diabetic patients, but to a lesser extent (AUC LTTH:  $969 \pm 67$  vs.  $1491 \pm 115$  nmol NEFA/ml in control subjects,  $P < 0.0001$ ). LTTH activity, which differed by 23% between the two groups in the fasting state, reached its maximal difference 2 h after the test meal (-50%,  $P < 0.0001$ ).

In both groups, fasting plasma TG concentrations exhibited an inverse relationship to LTTH ( $r = -0.65$ ,  $P < 0.005$ , data not shown). However, in the postprandial state, inverse correlations between either TG or RLP-C and LTTH AUCs were found in control subjects but not in diabetic patients (Fig. 2).

Plasma apoC-II and apoC-III concentrations, known to stimulate and inhibit LPL activity, respectively, are shown in Table 2. The concentrations of apoC-II were moderately more elevated in diabetics than in controls, in both the basal and in the postprandial state. No differences were observed in the apoC-II/apoC-III ratio.

To determine the best predictor of LTTH when combining control and diabetic groups, we performed two multivariate analyses. The first included fasting and postprandial triglyceridemia, body mass index, and insulin and glucose concentrations. In the second analysis, insulin and glucose were replaced by HOMA, which results from a combination of these two parameters. Insulinemia was the only significant predictor of LTTH in the first

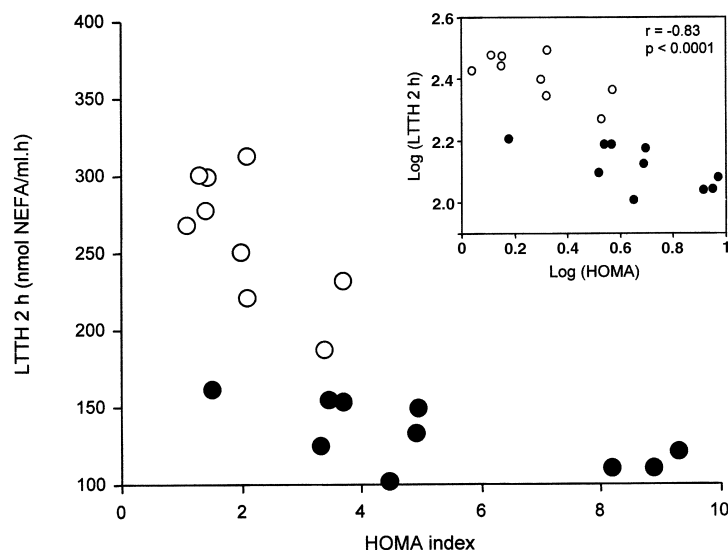
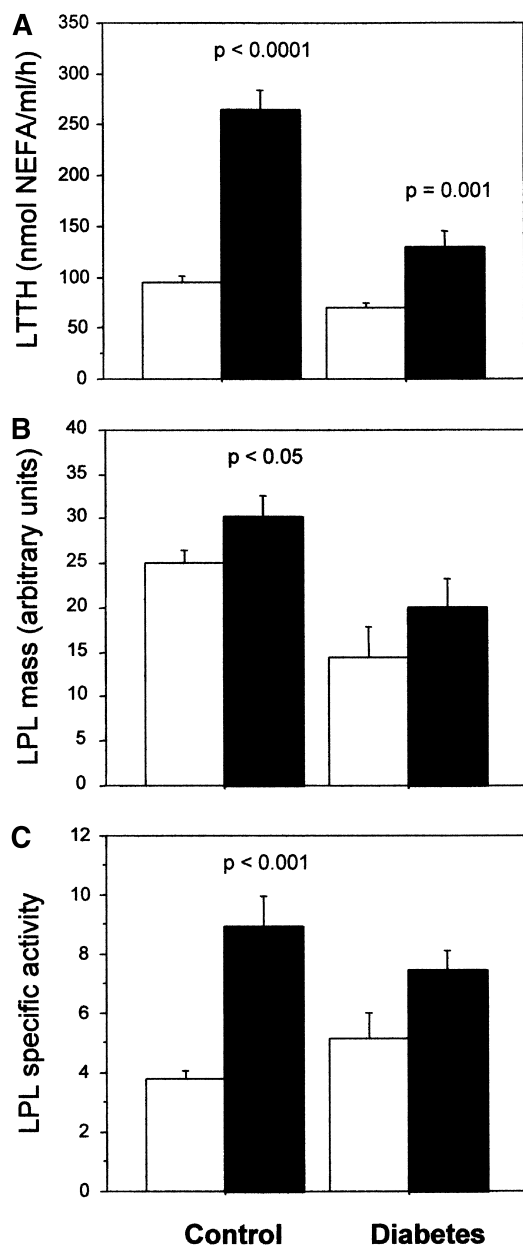


Fig. 3. Relationship between postprandial LTTH, measured 2 h after meal, and insulin resistance (homeostasis model assessment index) in type 2 diabetic patients (closed circles) and in control subjects (open circles). In the inset, the data were linearized by double log transformation.





**Fig. 4.** Triglyceride-rich lipoprotein-bound lipoprotein lipase activity (A), mass (B), and specific activity (C) in type 2 diabetic patients and in control subjects, measured in the fasted state (open bars) and 2 h after the meal (closed bars). The data are shown as the mean  $\pm$  SEM. *P* values refer to comparisons between postprandial and fasting states for each group.

analysis, but its predictive power was improved when HOMA was used (data not shown). Thus, LTTTH appeared to be related to insulin resistance. We therefore studied the direct correlation between fasting or postprandial (time 2 h) LTTTH and HOMA in more detail. Under these two conditions, consistent inverse correlations were found in both control and diabetic groups, whether combined or considered separately. The data obtained in the postprandial state are shown as an example in **Fig. 3** ( $r = -0.69$ ,  $P < 0.05$ ;  $r = -0.66$ ,  $P < 0.05$  for control subjects and type 2 diabetic patients, respectively). When com-

bined data were considered, the plot was linearized after log transformation (**Fig. 3**, inset;  $r = -0.83$ ,  $P < 0.0001$ ).

Finally, we compared the alterations of LTTTH to those of LPL mass concentrations and expressed our data in terms of specific activity (**Fig. 4**). In the basal state, the LPL-specific activities were similar in both control subjects and diabetic patients. In the postprandial state, a highly significant 130% increase was noted in the control group. In contrast, in the diabetic group, that increase was only 30% and did not reach statistical significance.

## DISCUSSION

To our knowledge, we provide the first study on the time course of LPL activity in the postprandial state, taking advantage of the measurement of LTTTH. This new assay that we recently developed (17) is based on measuring the naturally in vivo circulating LPL activity, thereby avoiding the requirement of heparin injection. Until now, conventional postheparin LPL activity measurements precluded any sequential analysis in the postprandial state. In humans, measurements in the fed and the fasted states on separate days led to conflicting results. A mild increase in preheparin LPL activity was found in the postprandial state in control subjects, while a 40% decrease in postheparin activity was noticed by Ruge et al. (21). However, no significant attenuation in the postprandial increase in LPL activity was observed in diabetic patients, although they were expected to have a decrease in LPL activity (22). The measurement of LTTTH unambiguously shows that circulating LPL activity is increased in the postprandial state and is hampered in type 2 diabetes. Previous gene expression studies have led to various results. In healthy humans and in animal models, LPL gene expression in adipose tissue was reported to be either increased (23–25) or unchanged (26–28) in the postprandial state, whereas in skeletal muscle, postprandial gene expression was found to be rather lowered (29, 30). In insulin-resistant subjects, the elevated LPL gene expression measured in biopsies of adipose tissue in the postprandial state was found to be either reduced or unchanged (22, 31–33). Overall, these studies do not permit the establishment of a clear relationship between LPL gene expression and plasma LPL activity in the postprandial state. The inverse relationship that we observed between the HOMA index and postprandial LTTTH strongly suggests that insulin resistance is an important determinant of decreased LPL activity in type 2 diabetes. The mechanism behind this observation, however, remains to be identified in additional studies. Thus, our study confirms a previous work in patients with type 2 diabetes that showed that fasting postheparin LPL activity was reduced (34), but it extends this finding to the postprandial state.

In principle, alterations of LPL activity are expected to induce corresponding changes in lipolysis. This was effectively observed in control subjects, where both postprandial TG and remnant concentrations were inversely correlated with LTTTH activity. Interestingly, these correlations

were not found in type 2 diabetic patients, leading to the conclusion that additional metabolic alterations are involved in the homeostasis of plasma TG and remnant particle concentrations. In agreement with this concept, an increase in hepatic VLDL production in the postprandial state has already been described in type 2 diabetes as well as a decrease in hepatic clearance (35, 36).

To understand the extent to which the alterations of LTH reflected those of LPL mass concentrations, we analyzed our data in terms of specific activity. In the control group, the LPL-specific activity clearly increased by 130% in the postprandial state, whereas in the diabetic group, that increase, if any, was minor. From a theoretical standpoint, two main explanations for the modification in LPL-specific activity may be considered. The first would be the alteration of the apoC-II/apoC-III ratio. ApoC-II and apoC-III have been shown to stimulate and inhibit LPL activity, respectively. However, no differences in the apoC-II/apoC-III balance were observed between the basal and postprandial states in control subjects or diabetic patients, ruling out the possibility of any significant effect of these apolipoproteins. The second parameter that could explain the alteration of LPL-specific activity relates to the physico-chemical characteristics of the lipoprotein substrates of LPL. TRLs constitute a family of highly heterogeneous particles that may vary in size, composition, and physical properties. These various particles do not necessarily have the same substrate efficiency with respect to LPL. For example, it has been established that large lipoproteins are better substrates than are small particles (37). Interestingly, previous studies have reported that the profiles of TRLs could be profoundly modified in the postprandial state, showing, in particular, an enhancement in the proportion of large particles. Thus, one can hypothesize that the postprandial modifications of lipoprotein composition may result in alterations of LPL-specific activity. Additional studies are required to compare compositional changes in postprandial TRL subclasses in patients with type 2 diabetes mellitus and in control subjects. ■

The authors thank Dr. J. Brunzell for providing anti-human LPL antibody and Drs. R. Cohen and P-J. Bondon for performing the insulin RIA and glucose assay. The authors also thank C. Jacobs and C. Lestra for expert technical assistance. This work was supported by grants from ALFEDIAM (Association de Langue Française pour l'Étude du Diabète et des Maladies Métaboliques), Fondation de France, and Laboratoires Fournier.

## REFERENCES

1. Karpe, F., and A. Hamsten. 1995. Postprandial lipoprotein metabolism and atherosclerosis. *Curr. Opin. Lipidol.* **6**: 123–129.
2. Austin, M. A., J. E. Hokanson, and K. L. Edwards. 1998. Hypertriglyceridemia as a cardiovascular risk factor. *Am. J. Cardiol.* **81**: 7B–12B.
3. De Man, F. H., M. C. Cabezas, H. H. van Berlingen, D. W. Erkelens, and T. W. de Bruin. 1996. Triglyceride-rich lipoproteins in non-insulin-dependent diabetes mellitus: postprandial metabolism and

relation to premature atherosclerosis. *Eur. J. Clin. Invest.* **26**: 89–108.

4. Takeichi, S., N. Yukawa, Y. Nakajima, M. Osawa, T. Saito, Y. Seto, T. Nakano, A. R. Saniabadi, M. Adachi, T. Wang, and K. Nakajima. 1999. Association of plasma triglyceride-rich lipoprotein remnants with coronary atherosclerosis in cases of sudden cardiac death. *Atherosclerosis.* **142**: 309–315.
5. Watanabe, N., T. Taniguchi, H. Taketoh, Y. Kitagawa, H. Namura, N. Yoneda, Y. Kurimoto, S. Yamada, and Y. Ishikawa. 1999. Elevated remnant-like lipoprotein particles in impaired glucose tolerance and type 2 diabetic patients. *Diabetes Care.* **22**: 152–156.
6. Boberg, J. 1970. Quantitative determination of heparin-released lipoprotein lipase activity in human plasma. *Lipids.* **5**: 452–456.
7. Goldberg, I. J., J. J. Kandel, C. B. Blum, and H. N. Ginsberg. 1986. Association of plasma lipoproteins with postheparin lipase activities. *J. Clin. Invest.* **78**: 1523–1528.
8. Vilella, E., and J. Joven. 1991. Lipoprotein lipase binding to plasma lipoproteins. *Med. Sci. Res.* **19**: 111–112.
9. Vilella, E., J. Joven, M. Fernandez, S. Vilaro, J. D. Brunzell, T. Olivecrona, and G. Bengtsson-Olivecrona. 1993. Lipoprotein lipase in human plasma is mainly inactive and associated with cholesterol-rich lipoproteins. *J. Lipid Res.* **34**: 1555–1564.
10. Zambon, A., I. Schmidt, U. Beisiegel, and J. D. Brunzell. 1996. Dimeric lipoprotein lipase is bound to triglyceride-rich plasma lipoproteins. *J. Lipid Res.* **37**: 2394–2404.
11. Tornvall, P., G. Olivecrona, F. Karpe, A. Hamsten, and T. Olivecrona. 1995. Lipoprotein lipase mass and activity in plasma and their increase after heparin are separate parameters with different relations to plasma lipoproteins. *Arterioscler. Thromb. Vasc. Biol.* **15**: 1086–1093.
12. Totsuka, M., Y. Miyashita, Y. Ito, H. Watanabe, T. Murano, and K. Shirai. 2000. Enhancement of preheparin serum lipoprotein lipase mass by bezafibrate administration. *Atherosclerosis.* **153**: 175–179.
13. Shirai, K., Y. Itoh, H. Sasaki, M. Totsuka, T. Murano, H. Watanabe, and T. Miyashita. 1999. The effect of insulin sensitizer, troglitazone, on lipoprotein lipase mass in preheparin serum. *Diabetes. Res. Clin. Pract.* **46**: 35–41.
14. Watanabe, H., Y. Miyashita, T. Murano, Y. Hiroh, Y. Itoh, and K. Shirai. 1999. Preheparin lipoprotein mass: the effect of age, gender, and types of hyperlipidemias. *Atherosclerosis.* **145**: 45–50.
15. Hitsumoto, T., H. Ohsawa, T. Uchi, H. Noike, M. Kanai, M. Yoshinuma, Y. Miyashita, H. Watanabe, K. Shirai, and Y. Saito. 2000. Preheparin serum lipoprotein lipase mass is negatively related to coronary atherosclerosis. *Atherosclerosis.* **153**: 391–396.
16. Kern, P. A., R. A. Martin, J. Carty, I. J. Goldberg, and J. M. Ong. 1990. Identification of lipoprotein lipase immunoreactive protein in pre- and postheparin plasma from normal subjects and patients with type I hyperlipoproteinemia. *J. Lipid Res.* **31**: 17–26.
17. Pruneta, V., D. Autran, G. Ponsin, C. Marçais, L. Duvillard, B. Vergès, F. Berthezène, and P. Moulin. 2001. Ex vivo measurement of lipoprotein lipase-dependent very low density lipoprotein (VLDL)-triglyceride hydrolysis in human VLDL: an alternative to the postheparin assay of lipoprotein lipase activity? *J. Clin. Endocrinol. Metab.* **86**: 797–803.
18. Friedewald, W. T., R. I. Levy, and D. S. Fredrickson. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin. Chem.* **18**: 499–502.
19. Matthews, D. R., J. P. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Treacher, and R. C. Turner. 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* **28**: 412–429.
20. Nakajima, K., T. Saito, A. Tamura, M. Suzuki, T. Nakano, M. Adachi, A. Tanaka, N. Tada, H. Nakamura, E. Campos, and R. J. Havel. 1993. Cholesterol in remnant-like lipoproteins in human serum using monoclonal anti apoB-100 and anti apoAI immunoaffinity mixed gels. *Clin. Chim. Acta.* **223**: 53–71.
21. Ruge, T., A. Svenson, J. W. Eriksson, T. Olivecrona, and G. Olivecrona. 2001. Food deprivation increases post-heparin lipoprotein lipase activity in humans. *Eur. J. Clin. Invest.* **31**: 1040–1047.
22. Eriksson, J. W., J. Buren, M. Svensson, T. Olivecrona, and G. Olivecrona. 2003. Postprandial regulation of blood lipids and adipose tissue lipoprotein lipase in type 2 diabetes patients and healthy control subjects. *Atherosclerosis.* **166**: 359–367.
23. Taskinen, M-R., and E. A. Nikkila. 1987. Basal and postprandial lipoprotein lipase activity in adipose tissue during caloric restriction and refeeding. *Metabolism.* **36**: 625–630.

24. Bessesen, D. H., A. D. Robertson, and R. H. Eckel. 1991. Weight reduction increases adipose but decreases cardiac LPL in reduced-obese Zucker rats. *Am. J. Physiol. Endocrinol. Metab.* **261**: E246–E251.
25. Tavangar, K., Y. Murata, M. E. Pedersen, J. F. Goers, A. R. Hoffman, and F. B. Kraemer. 1992. Regulation of lipoprotein lipase in the diabetic rat. *J. Clin. Invest.* **90**: 1672–1678.
26. Ong, J. M., and P. A. Kern. 1989. Effect of feeding and obesity on lipoprotein lipase activity, immunoreactive protein, and messenger RNA levels in human adipose tissue. *J. Clin. Invest.* **84**: 305–311.
27. Doolittle, M. H., O. Ben-Zeev, J. Elovson, D. Martin, and T. G. Kirchgessner. 1990. The response of lipoprotein lipase to feeding and fasting. Evidence for posttranslational regulation. *J. Biol. Chem.* **265**: 4570–4577.
28. Bergo, M., G. Olivecrona, and T. Olivecrona. 1996. Forms of lipoprotein lipase in rat tissues: in adipose tissue the proportion of inactive lipase increases on fasting. *Biochem. J.* **313**: 893–898.
29. Ong, J. M., R. B. Simolo, M. Saghizadeh, A. Pauer, and P. A. Kern. 1994. Expression of lipoprotein lipase in rat muscle: regulation by feeding and hypothyroidism. *J. Lipid Res.* **35**: 1542–1551.
30. Ladu, M. J., H. Kapsas, and W. K. Palmer. 1991. Regulation of lipoprotein lipase in adipose and muscle tissues during fasting. *Am. J. Physiol.* **260**: R953–R959.
31. Taskinen, M-R. 1987. Lipoprotein lipase in diabetes. *Diabetes Metab. Rev.* **2**: 551–570.
32. Panarotto, D., P. Remillard, L. Bouffard, and P. Maheux. 2002. Insulin resistance affects the regulation of lipoprotein lipase in the postprandial period and in an adipose tissue-specific manner. *Eur. J. Clin. Invest.* **32**: 84–92.
33. Maheux, P., S. Azhar, P. A. Kern, Y. D. Chen, and G. M. Reuven. 1997. Relationship between insulin-mediated glucose disposal and regulation of plasma and adipose tissue lipoprotein lipase. *Diabetologia.* **40**: 850–858.
34. Knudsen, P., J. Eriksson, S. Lahdenperä, J. Kahri, L. Groop, and M-R. Taskinen. 1995. Changes of lipolytic enzymes cluster with insulin resistance syndrome. *Diabetologia.* **38**: 344–350.
35. Duvillard, L., F. Pont, E. Florentin, C. Galland-Jos, P. Gamber, and B. Verges. 2000. Metabolic abnormalities of apolipoprotein B-containing lipoproteins in non-insulin-dependent diabetes: a stable isotope kinetic study. *Eur. J. Clin. Invest.* **30**: 685–694.
36. Howard, B. V. 1987. Lipoprotein metabolism in diabetes mellitus. *J. Lipid Res.* **28**: 613–628.
37. Xiang, S. Q., K. Cianflone, D. Kalant, and A. D. Sniderman. 1999. Differential binding of triglyceride-rich lipoproteins to lipoprotein lipase. *J. Lipid Res.* **40**: 1655–1662.