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Hyperglycemia Downregulates Total Lipoprotein Lipase Activity in Humans

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Summary

To address the question whether an increase in insulinemia and/or glycemia affects the total activity of lipoprotein lipase (LPL) in circulation, the enzyme activity was measured after periods of hyperinsulinemia (HI), hyperglycemia (HG), and combined hyperinsulinemia and hyperglycemia (HIHG) induced by euglycemic hyperglycemic clamp, hyperglycemic clamp with the infusion of somatostatin to inhibit endogenous insulin secretion, and hyperglycemic clamp, respectively. The results obtained were compared to those after saline infusion (C). Twelve healthy normolipidemic and non-obese men with normal glucose tolerance were included in the study. At the end of each clamp study, LPL activity was determined first *in vivo* using an intravenous fat tolerance test and then *in vitro* in postheparin plasma. Whereas isolated HI had no effect on LPL activity in postheparin plasma, both HG and HIHG reduced LPL activity to 60 % and 56 % of that observed after saline infusion. Similarly, the k_2 rate constant determined in intravenous fat tolerance test was reduced to 95 %, 84 %, and 54 % after periods of HI, HG, and HIHG, respectively. The activity of hepatic lipase, another lipase involved in lipoprotein metabolism, was not affected by hyperinsulinemia and/or hyperglycemia. In conclusion, our data suggest that hyperglycemia *per se* can downregulate the total LPL activity in circulation.

Key words

Lipoprotein lipase • Hepatic lipase • Glucose • Insulin • Triglyceride

Introduction

Lipoprotein lipase (LPL) hydrolyses triglycerides of chylomicrons and very low density lipoproteins (VLDL). The enzyme is widely expressed in most of the extrahepatic tissues and transported to the capillary endothelium of those tissues, where hydrolysis of chylomicron and VLDL triglycerides takes place (Goldberg 1996, Olivecrona and Olivecrona 1999, Mead *et al.* 2002). The LPL activity is a key element determining the triglyceride concentration. Importantly, its activity is finely regulated to deliver fatty acids released by its action into tissues that utilize them at a given moment (Eckel 1989, Zechner 1997, Fielding and Frayn 1998). It has been repeatedly demonstrated that insulin markedly increases LPL activity in adipose tissue and has an opposite effect on the enzyme activity in the muscle (Lithell *et al.* 1978, Sadur and Eckel 1982, Yki-Järvinen *et al.* 1984, Farese *et al.* 1991). Fatty acids from circulating triglycerides are thus postprandially targeted into the adipose tissue to be reesterified and stored as triglycerides. However, it is not entirely clear yet how the

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total LPL activity in the circulation is affected by changes in insulinemia. Such a question is of considerable interest because it should be expected that the changes in total LPL activity would be a principal factor determining the magnitude of postprandial lipemia. Moreover, under physiologic conditions, the increase of insulinemia results from the body response to enhanced glycemia and it is also not clear whether hyperglycemia interferes with the LPL activity regulation in any way.

To find whether changes in insulinemia and/or glycemia affect total LPL activity in circulation we measured LPL activity both *in vitro* and *in vivo* using intravenous fat tolerance test (IVFTT) after periods of isolated hyperinsulinemia (HI), isolated hyperglycemia (HG) and hyperinsulinemia and hyperglycemia (HIHG) induced by clamp techniques. The results were compared to those obtained after saline infusion as controls (C).

Methods

Subjects

Twelve healthy men (age: 27.2±5.3 years; BMI: 24.8±2.6 kg/m^2) were recruited for the study. They were normolipidemic (cholesterol 3.99±0.47 mmol/l; triglycerides 0.96±0.35 mmol/l; HDL-cholesterol 1.44±0.35 mmol/l) and normoglycemic (fasting glucose 4.6±0.4 mmol/l). All of them had normal glucose tolerance as determined by the oral glucose tolerance test during the screening and normal levels of glycosylated hemoglobin (HbAlc 4.8±0.3 %).

Experimental protocol

The four consecutive studies – hyperglycemic clamp, control infusion of saline, hyperinsulinemic euglycemic clamp, and hyperglycemic clamp with insulin secretion blockade lasting for 5 h - were carried out in all the subjects. There were at least 2-week intervals between the individual studies. Each of the examinations was done on an outpatient basis. The subjects were asked to adhere to their ordinary lifestyle and avoid changes in food intake, alcohol consumption and exercise before each of the examinations. To further standardize the conditions of each examination, identical food on the day preceding the examination was provided to the subjects. All the studies were conducted after 12 h of fasting.

Each of the studies lasted 5 h and was combined with indirect calorimetry. Before the end of the studies, the intravenous fat tolerance test was conducted, and, finally, each subject was injected with heparin and blood was drawn for lipoprotein lipase (LPL) and hepatic lipase (HL) activities determination.

Throughout each of the clamps the urine was collected to determine urinary urea excretion.

The study protocol was approved by the Ethical Committee of the Institute for Clinical and Experimental Medicine, Prague, and the informed consent was obtained from all the subjects.

Clamps

To simulate combined hyperinsulinemia and hyperglycemia (HIHG), the hyperglycemic clamp study was conducted as previously described (DeFronzo *et al.* 1979, Pelikánová *et al.* 2001) with a slight modification. Briefly, a teflon cannula (Venflon; Viggo, Helsinborg, Sweden) was inserted into the antecubital vein for the infusion of all test substances. A second cannula was inserted retrogradely into a wrist vein for blood sampling and the hand was placed in a heated box (65 ºC) to achieve venous blood arterialization. The clamp was designed to raise plasma glucose concentration acutely to fixed hyperglycemic plateau at 17 mmol/l and to maintain glycemia at adjusted level for 5 h. That was accomplished by 40 % glucose bolus dose insertion lasting 15 min and glucose concentration was then maintained at desired hyperglycemic level by continuous 15 % glucose infusion at rates adjusted according to results of glucose concentration measurements in blood samples taken in 5- or 10-min intervals.

Control experiment (C) consisted of a saline infusion controlled to be time- and volume-compatible with the hyperglycemic clamp.

To simulate isolated hyperinsulinemia (HI), the hyperglycemic euglycemic clamp study was conducted as previously described (DeFronzo *et al.* 1979, Beatty *et al.* 1993, Pelikánová *et al.* 2001) with a slight modification. A clamp technique was the same as that described above. To raise and maintain the plateau of insulin plasma concentration at 60 mU/l, a primed constant insulin infusion (1 mU/kg/min of Actrapid HM, NovoNordisk, Bagsvaerd, Denmark) was administered to all subjects. Euglycemia was maintained at the fasting level by continuous infusion of 15 % glucose at variable rates adjusted according to the glucose concentration. To keep fluid intake at the same level as that in the hyperglycemic clamp, saline was infused to subjects so as to be volume- and time-compatible with the hyperglycemic clamp.

To simulate isolated hyperglycemia (HG), the hyperglycemic clamp with insulin secretion blockade was performed as described previously (Del Prato *et al.* 1997)

with a minor modification to achieve isolated hyperglycemia of 17 mmol/l. Endogenous insulin secretion was blocked by continuous somatostatin infusion (0.5 mg/h; Somatostatin, UCB s.a. Pharma, Braine L'Alleut, Belgium). A hyperglycemic plateau was achieved by an intravenous glucose infusion consisting of two phases – the primary 40 % glucose bolus dose lasting 15 min followed by continuous 15 % glucose infusion at variable rates adjusted according to the glucose concentration measurements in blood samples taken in 5- or 10-min intervals. Again, saline was infused to subjects to be volume- and time-compatible with the hyperglycemic clamp.

Indirect calorimetry

To determine substrate utilization during the studies, indirect calorimetry measurements were performed 190 to 230 min after the start of the studies as previously described (Pelikánová *et al.* 1993) using W_{max} metabolic monitor (Sensormedics, Ltd., Anaheim, USA).

Intravenous fat tolerance test

Intravenous fat tolerance test was carried out 240 min after the start of the study as described earlier (Rössner 1974). Briefly, bolus of 20 % Intralipid (FreseniusKabi AB, Uppsala, Sweden; 0.5 ml/kg) was given intravenously to the subjects through inserted cannula. Blood for determination of serum turbidity was collected before Intralipid application and then in 5-min intervals for 40 min. The turbidity of serum samples was determined on MARK IV nephelometer (Scientific Furnishings Ltd, England). The measured values of light scattering index were logarithmically transformed to obtain the rate constant k_2 reflecting LPL activity *in vivo*.

Determination of lipoprotein lipase (LPL) and hepatic lipase (HL) activities

Before the end of each study (290 min after the start), heparin (Léčiva, Prague, Czech Republic; 100 IU/kg) was intravenously applied to each subject and plasma for measurement of LPL and HL activity was obtained from blood collected 10 min later. The plasma was stored at −70 °C before determination. The activity of both LPL and HL was then measured as previously described (Bengtsson-Olivecrona and Olivecrona 1992). Briefly, the activity of LPL is measured using Intralipid labeled with glycerol-^{3}H-oleate (Amersham Pharmacia Biotech) as a substrate; HL activity is inhibited using specific antibody (kindly provided by Prof. Hans Jansen, Erasmus University Rotterdam, the Netherlands). The activity of HL is measured using triolein emulsion stabilized with arabic gum and labeled with glycerol-^{3}H-oleate as a substrate; LPL activity is inhibited by high salt concentration. To eliminate the interassay variation, samples from all four studies in one subject were run in the same assay.

Other analyses

Plasma glucose concentrations during studies were measured on a Beckman analyzer (Beckman Instruments, Fullerton, CA) using glucose oxidase method. Blood samples for determination of cholesterol, triglycerides (TG), HDL-cholesterol and for isolation of VLDL were drawn at 0 and 240 min. To isolate VLDL, aliquots of plasma were subjected to ultracentrifugation at density 1.006 g/ml (Beckman 50.4 rotor, 39 000 rpm, 4 ºC, 18 h), tubes were sliced and top floating fractions collected. Cholesterol and triglycerides in plasma and VLDL and HDL cholesterol in plasma were measured on a Roche COBAS MIRA autoanalyzer (Hoffmann-La Roche, Switzerland) using enzymatic kits from the same manufacturer. Blood samples for insulin determination were taken before (0 min) and at 60, 120, 180, 210, 225, and 240 min after the start of the studies; insulin concentration was determined using IRMA kits (Immunotech, Prague, Czech Republic). Free fatty acid concentrations in the plasma were assessed colorimetrically (Novák 1965) in samples collected at 0, 60, 120, 180, and 240 min.

Statistical analysis

Willcoxon test or, where appropriate, non-parametric Friedman statistic followed by a variant of Student-Newman-Keuls procedure for non-parametric multiple comparisons was used (Glantz 1992).

Results

Glycemia and insulinemia during clamps

There was no significant change in glucose and insulin concentration during saline infusion reaching concentrations 4.8±0.4 mmol/l and 3.4±1.6 mIU/l at 4 h of infusion. In the course of the other studies, glucose concentration was kept at the required levels 4.7±0.3, 17.0±1.2, and 16.4±2.5 mmol/l during HI, HG, and HIHG, respectively. Insulin concentration was kept at 57.0±17.4 mIU/l during HI and steadily rose during HIHG reaching concentration 352±229 mIU/l at 4 hour. During HG, insulin concentration was kept at the relatively stable level 9.9±12.8 mIU/l up to 210 min when

it started to rise to reach concentration 23.8±12.4 mIU/l at240 min.

Utilization of substrates for oxidation

Both HI and HIHG were associated with the significantly higher values of respiratory quotient (RQ) and shift to utilization of carbohydrates instead of lipids as a substrate for oxidation (Table 1). Importantly, isolated HG had no effect on RQ and substrate utilization, although insulin concentration was higher than that during saline infusion (Table 1).

Table 1. Respiratory quotients (RQ) and percentage of energy utilized for oxidation from carbohydrates and lipids after saline infusion (C) and after periods of hyperinsulinemia (HI), hyperglycemia (HG), and combined hyperinsulinemia and hyperglycemia (HIHG).

	C	HI	HG	HIHG	p
RQ	0.76 ± 0.05 [a]	0.87 ± 0.05 [b]	0.78 ± 0.05 [a]	0.91 ± 0.05 [b]	< 0.05
Oxidative utilization of					
carbohydrates (%)	20.0 ± 13.5 [a]	53.0 ± 19.2 [b]	18.1 ± 15.6 [a]	62.4 ± 14.9 [b]	< 0.05
lipids (%)	59.3 ± 16.3 [a]	26.5 ± 19.0 [b]	63.1 ± 11.6 [a]	25.0 ± 11.6 [b]	< 0.05

Data are means ± S.D. Friedman test followed by multiple comparisons (Student-Newman-Keuls test) was used to evaluate the differences between groups. Identical letters denote the results that are not different.

Table 2. Change of lipids and lipoproteins during 4 hours of saline infusion (C) and 4 hours of hyperinsulinemia (HI), hyperglycemia (HG), and combined hyperinsulinemia and hyperglycemia (HIHG).

	C		HI		HG		HIHG	
Time (hours)	**0**	**4**	**0**	**4**	**0**	**4**	**0**	**4**
Cholesterol	3.83	4.04	3.71	3.69	3.75	3.85	3.78	3.77
(mmol/l)	± 0.66	± 0.76**	± 0.52	± 0.44	± 0.54	± 0.62	± 0.57	± 0.66
HDL-C	1.16	1.32	1.13	1.17	1.21	1.25	1.04	1.08
(mmol/l)	± 0.28	± 0.52	± 0.27	± 0.30	± 0.38	± 0.39	± 0.24	± 0.23
TG	0.97	1.17	1.06	0.66	1.07	0.82	1.05	0.85
(mmol/l)	± 0.44	± 0.54	± 0.49	± 0.45***	± 0.49	± 0.54**	± 0.41	± 0.49**
VLDL-TG	0.59	0.55	0.62	0.35	0.58	0.48	0.60	0.51
(mmol/l)	± 0.37	± 0.35	± 0.48	± 0.40***	± 0.48	± 0.46	± 0.36	± 0.40*
VLDL-C	0.25	0.25	0.25	0.18	0.22	0.21	0.25	0.23
(mmol/l)	± 0.12	± 0.15	± 0.19	± 0.19***	± 0.17	± 0.17	± 0.13	± 0.15

Data are means ± S.D. The data obtained 4 hours after start of the clamps were corrected for hemodilution. Wilcoxon test was used to evaluate differences between 0 and 4 hours: *p< 0.05, ** p< 0.01, ***p< 0.001.

Lipid and lipoprotein changes during clamps

No changes in concentration of cholesterol and HDL-cholesterol were observed during clamps with exception of the 5 % increase in cholesterolemia during saline infusion (Table 2). On the other hand, concentration of TG at 4 hours went down to 62 %, 77 % and 81 % of that at the baseline, no change of triglyceridemia was observed under control conditions (Table 2).

Similarly, concentrations of VLDL-TG dropped to 56 % in HI and to 85 % in HIHG, the decrease to 83 % in HI did not reach statistical significance; no change was observed during saline infusion. VLDL-cholesterol concentration dropped down to 72 % only during HI, no significant changes were observed under the other experimental conditions (C, HG, HIHG).

Concentration of free fatty acids was reduced to the same level during HI, HG, and HIHG, and was not affected during saline infusion (Fig. 1).

LPL and HL activities

Whilst HI alone had no significant effect on LPL activity measured *in vitro*, both isolated HG and combined HIHG were associated with 40 % and 44 % reduction of LPL activity, respectively (Table 3). No significant effect of hyperglycemia and/or hyperinsulinemia on HL activity was observed (Table 3).

Intravenous fat tolerance test (LPL activity in vivo)

The k_2 rate constant determined in intravenous fat tolerance test that should reflect LPL activity *in vivo*, was decreased to 95 %, 84 % and 54 % in HI, HG, and HIHG, respectively, in comparison to saline infusion (Table 3).

To determine whether LPL activity measurements *in vitro* and *in vivo* are correlated, the univariate correlation between LPL activity and k_2 values under all four conditions was analyzed. Positive correlation between both measurements was found in HI ($r = 0.671$, $p<0.02$), whilst no significant correlation was found in the control experiment (C) ($r = 0.141$), HG ($r = 0.470$), and HIHG ($r = 0.287$).

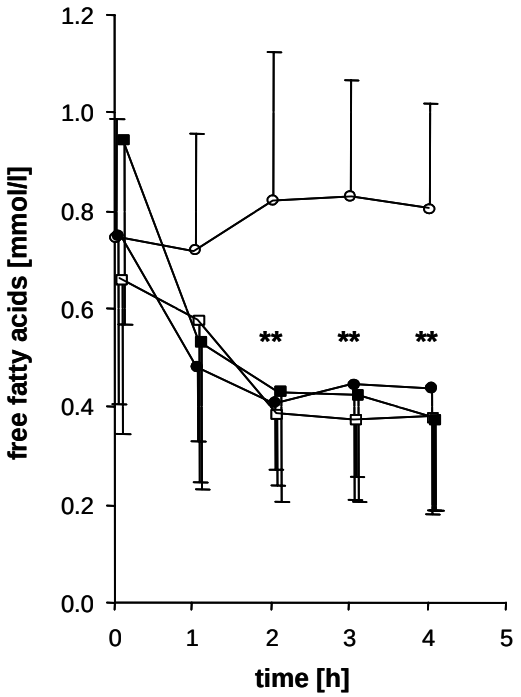

Fig. 1. Free fatty acids concentration during saline infusion (C) and during periods of hyperinsulinemia (HI), hyperglycemia (HG), and combined hyperinsulinemia and hyperglycemia (HIHG). ○ - C, ● - HI, □ - HG, ■ - HIHG. Data are means ± S.D. Friedman test followed by multiple comparisons (Student-Newman-Keuls test) was used to evaluate differences between free fatty acid concentrations at each time point, ** $p < 0.01$ (HI, HG, and HIHG vs C).

Table 3. Lipoprotein lipase (LPL) and hepatic lipase (HL) activities determined in vitro and k_2 rate constant determined in vivo using intravenous fat tolerance test in subjects after saline infusion (C) and after periods of hyperinsulinemia (HI), hyperglycemia (HG), and combined hyperinsulinemia and hyperglycemia (HIHG)

	C	HI	HG	HIHG	p
LPL (mmol/l/h)	7.13 ± 2.65^{a}	6.46 ± 2.25^{a}	4.31 ± 1.67^{b}	3.99 ± 1.39^{b}	< 0.01
HL (mmol/l/h)	5.76 ± 1.80^{a}	5.63 ± 1.86^{a}	5.23 ± 1.79^{a}	5.45 ± 2.02^{a}	n.s.
k_2 (%/min)	6.06 ± 2.05^{a}	5.77 ± 3.52^{b}	5.07 ± 2.22^{b}	3.28 ± 1.26^{c}	< 0.01

Data are means ± S.D. Friedman test followed by multiple comparisons (Student-Newman-Keuls test) was used to evaluate the differences between groups. Identical letters denote the results that are not different.

Discussion

It can be summarized that the total LPL activity in circulation (determined *in vitro* in postheparin plasma) is depressed after both hyperglycemia and combined hyperinsulinemia and hyperglycemia induced by clamp techniques. However, it is not affected when only isolated hyperinsulinemia is induced. The LPL activity measurement *in vitro* reflects the total amount of active enzyme present in circulation and, therefore, our results suggest that lipoprotein lipase activity is downregulated by 5 hours of hyperglycemia.

The data reflecting LPL activity measured directly *in vivo* by the intravenous fat tolerance test are in support of such a conclusion; however, significant but rather minor inhibition of LPL activity was also observed

in isolated hyperinsulinemia. Moreover, the suppression of the k_2 rate constant is not so pronounced as the suppression of LPL activity measured *in vitro* after a period of isolated hyperglycemia.

Such results are quite surprising because it was shown that insulin markedly upregulates LPL activity in adipose tissue (Sadur and Eckel 1982, Yki-Järvinen *et al.* 1984, Farese *et al.* 1991) and only modestly downregulates LPL activity in the muscle (Lithell *et al.* 1978, Farese *et al.* 1991). Therefore, it could be expected that the effect of hyperinsulinemia on LPL in adipose tissue should prevail and lead to higher total LPL activity in the circulation. However, it cannot be excluded that the effects on LPL in different tissues could be counterbalanced and may result in no changes or even inverse change in total LPL activity in circulation.

Our data do not provide any information with respect to mechanism by which glucose affects the amount of active LPL present in circulating blood. Although it was shown that glucose regulates lipoprotein lipase gene expression (Sartippour and Renier 2000), other posttranscriptional mechanisms including changes in mRNA stability, translation, protein degradation, processing, secretion, translocation to its site of action and even its displacement from the vascular endothelium (Mead *et al.* 2002) are likely to be involved.

Interestingly, when the relationship between both measurements of LPL activity was analyzed, a significant positive correlation between LPL activity in postheparin plasma and k_2 rate constant was found only after isolated HI. Our results suggest that the other mechanisms such as the rate of blood perfusion through different tissues (and thus a chance that lipoprotein lipase meets its substrate in circulating lipoproteins) or locally increased free fatty acids concentration (that inhibits LPL activity and may not be detected in the whole circulation – Karpe *et al.* 1992) can operate *in vivo* and modulate LPL activity measured using IVFTT. It can even be speculated that the relationship between k_2 rate constant and LPL activity is revealed just under non-physiological conditions associated with isolated HI when LPL activity is upregulated in adipose tissue and, at the same time, there is a limited availability of glucose as an oxidative substrate.

If the increase in both glycemia and insulinemia downregulates LPL activity, it could be expected that LPL activity measured in the postprandial state is lower than that measured in postabsorptive state. Indeed, Ruge *et al.* (2001) found that LPL activity in postprandial state is lower than that in the fasting state. It was also demonstrated that LPL activity measured during postprandial lipemia displays a diurnal variation with higher activity in the evening than in the morning (Arasaradnam *et al.* 2002). This could also reflect the exposure to periods of hyperglycemia during the day. Similarly, Cohen and Berger (1990) who studied the effect of glucose ingestion on LPL activity measured *in vivo* using intravenous fat tolerance test observed 10 % decrease, albeit non-significant, of k_2 rate constant 2 h after ingestion of 50 g of glucose.

It is well documented that LPL activity is lower in patients with diabetes mellitus 2 (Nikkilä *et al.* 1977). LPL activity is inversely related to the level of insulin resistance (Pollare *et al.* 1991, Maheux *et al.* 1997, Panarotto *et al.* 2002) and could be increased by improved control of diabetes throughout the insulin therapy (Simsolo *et al.* 1992, Geltner *et al.* 2002). Our data suggest that, in addition to documented effect of insulin resistance on LPL, hyperglycemia *per se* could contribute to defective LPL activity in diabetes and insulin resistance.

If the LPL activity is depressed, it could be expected that triglyceride concentration in circulation should be higher in states associated with hyperglycemia. However, we observed consistent decrease in TG or VLDL-TG during HG, HIHG, or even HI. Such results could be easily explained by the fact that under these conditions the concentration of free fatty acids in circulation is suppressed to the same level (Fig. 1) and that the availability of circulating free fatty acids is a principal determinant of VLDL secretion rate from the liver (Lewis *et al.* 1995). The findings that NEFA concentration is depressed even in isolated HG that has no effect on utilization of substrate for oxidation seem to be rather unexpected but it was repeatedly proven that hyperglycemia *per se* is associated with a decrease in circulating FFA concentration, at least when somatostatin is used to inhibit insulin secretion from pancreas (Park *et al.* 1990, Carlson *et al.* 1991). Hence, our observation that the magnitude of TG or VLDL-TG decrease during HG and HIHG is approximately half of that observed in isolated HI is in accordance with the idea that LPL activity is downregulated in HG and HIHG, not in hyperinsulinemia *per se*.

It should also be stressed that the activity of hepatic triglyceride lipase (HL), another postheparin lipase involved in the regulation of lipoprotein catabolism, is not affected by changes in insulinemia and/or glycemia.

When interpreting our results we should be cautious to draw premature conclusions. It remains to corroborate our conclusions in more physiological setting than that of hyperglycemic clamps. It has to be confirmed in such studies that hyperglycemia exerts its effect on LPL activity at glucose concentrations close to those typical for postprandial state. Moreover, it has to be tested whether hyperglycemia *per se* contributes to defective regulation of LPL in patients with insulin resistance and type 2 diabetes, metabolic conditions characterized by the presence of hyperglycemia.

In conclusion, we found that total LPL activity in circulation is downregulated by hyperglycemia and combined hyperinsulinemia and hyperglycemia induced by clamp techniques and that it is not affected when only isolated hyperinsulinemia is induced. To best of our knowledge, this is the first evidence that LPL activity in circulation can also be regulated by changes in glucose concentration. However, it remains to be clarified which mechanisms are involved in this effect and what is the exact role of hyperglycemia in regulation of triglyceridemia in states like postprandial lipemia or insulin resistance.

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