Glucose Administration Downregulates Lipoprotein Lipase Activity in vivo: a Study Using Repeated Intravenous Fat Tolerance Test

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Summary
Lipoprotein lipase (LPL) is a key factor determining the clearance of triglycerides from the circulation. The enzyme activity is tissue-specifically regulated by insulin, but it is not clear yet how insulin regulates the total LPL activity in the circulation. To answer such question, we measured LPL activity using the intravenous fat tolerance test (IVFTT) that was carried out 1 h before as well as 2 h and 4 h after oral administration of glucose (75 g) in eleven healthy male volunteers. In control experiments, no glucose was given to the subjects. Glucose administration resulted in an expected increase in plasma glucose and insulin and in a suppression of non-esterified fatty acid concentration. The LPL activity assessed in IVFTT as a $k_2$ rate constant did not change in control experiments and decreased to 78 % and 73 % of baseline values 2 h and 4 h after glucose administration, respectively (p=0.01). Similarly, LPL activity measured in the plasma after intravenous injection of heparin at the end of the experiments was 16 % lower (p<0.05) after glucose administration. In conclusion, LPL activity is already downregulated in vivo 2 h after glucose administration. The results of our study indicate that repeated IVFTT is a promising approach for studying acute changes in LPL activity.

Key words
Lipoprotein lipase • Glucose • Insulin • Triglyceride • Intravenous fat tolerance test

Introduction
Lipoprotein lipase (LPL) plays a key role in the regulation of plasma triglyceride (TG) concentration (Olivecrona and Olivecrona 1999, Mead et al. 2002). It hydrolyses triglycerides of triglyceride-rich lipoproteins (TRL) - very low density lipoproteins (VLDL) and chylomicrons. Therefore, its activity determines the rate of triglyceride clearance from the circulation. The enzyme activity is regulated in a tissue-specific manner so that fatty acids are released and then taken up in the tissues that utilize them at a given time (Eckel 1989, Zechner 1997). Insulin has been shown to upregulate LPL activity in the adipose tissue and downregulate it in the muscle (Lithell et al. 1978, Sadur and Eckel 1982, Yki-Järvinen et al. 1984, Farese et al. 1991) so when the supply of glucose for oxidation is sufficient, fatty acids are stored in adipose tissue. However, it is not entirely clear yet what is the effect of insulin on the change of total LPL activity in the circulation (that should represent
a combination of changes in all the tissues). This can be of considerably great importance for the regulation of postprandial lipemia magnitude due to the fundamental role of insulin in the postprandial phase.

However, the study of changes in LPL activity is limited due to the intricate complexion of LPL. The enzyme is synthesized in parenchymal cells of extrahepatic tissues and transported to capillary endothelium where it binds to heparan sulphate and performs its catalytic function (Goldberg 1996, Olivecrona and Olivecrona 1999). To measure its activity 

in vitro, enzyme has to be released into the circulation by heparin which displaces the enzyme from the endothelium. However, such a measurement cannot be repeated at short-time intervals and this seriously limits the study of changes in LPL activity. Importantly, the LPL activity response to insulin should be rather fast to accomplish its metabolic role.

To overcome such a limitation, we tested whether the intravenous fat tolerance test (IVFTT) (Lewis et al. 1972, Rössner 1974), that is believed to be a measure of LPL activity 
in vivo, can be repeated at short-time intervals to study the acute effects of glucose administration on changes of the total LPL activity in the circulation. In this test, the rate constant of the first order (k2) for the rate of intravenously administered fat emulsion disappearance is determined. The k2 then reflects the LPL activity 
in vivo. The IVFTT was performed three times within 6 h: one hour before as well as 2 and 4 hours after the oral administration of 75 g of glucose to healthy volunteers.

**Methods**

**Subjects**

The study group consisted of 11 healthy male volunteers without any known abnormality in lipid metabolism (age: 28.2±7.3 years; BMI: 27.1±2.1 kg/m²; cholesterol: 3.84±0.58 mmol/l; HDL-cholesterol: 1.17±0.23 mmol/l; TG: 1.10±0.38 mmol/l; glucose: 4.9±0.5 mmol/l). No medication was regularly taken by the subjects. Normal glucose tolerance was confirmed by the oral glucose tolerance test (oGTT) according to the American Diabetes Association criteria (Genuth et al. 2003) during the screening phase of the study. The study protocol was approved by the Ethical Committee of the Institute for Clinical and Experimental Medicine, Prague, and informed consent was obtained from all the subjects.

**Study design**

Briefly, intravenous fat tolerance test (IVFTT) was carried out 1 h before and 2 and 4 h after glucose loading during an oGTT (Fig. 1). Glucose (75 g) was dissolved in 300 ml of black tea. In the control experiment, exactly the same design was used, except that black tea without glucose was given to the subjects. The time period between glucose and control experiments without glucose given to the subjects was at least one week. Subjects were examined on an outpatient basis after an overnight fast (12 h) and were instructed to avoid alcohol consumption, tobacco smoking and vigorous exercise the day before the examination. All subjects completed both studies; the order of the studies was randomized.

**Intravenous fat tolerance test (IVFTT)**

The test was performed as previously described (Rössner 1974) with slight modifications. First, one indwelling catheter was inserted into the antecubital vein in the right arm for Intralipid® infusion and blood sampling. The baseline blood sample (0 min) was withdrawn and 20 % Intralipid® (FreseniusKabi AB, Uppsala, Sweden; 0.5 ml/kg of weight) was then injected within 2 min. Eight blood samples were then withdrawn.
the k₂ rate constant was collected into vacutainers with serum clot activator and separation gel. The vacutainers were then kept at room temperature till centrifugation (10 min, 3000 x g, 4 °C). The separated serum was kept in the same vacutainer till the analysis and then mixed by inverting the tube. Fifty microliters of serum were diluted in 5 ml of saline and the turbidity of diluted serum was measured on MARK IV nephelometer (Scientific Furnishings Ltd, England). The measured values of light scattering index (LSI) were logarithmically transformed and the rate constant k₂ (100*slope of regression line between log(LSI) and time (min)) was calculated.

Three IVFTTs were performed during each experiment: First one 1 hour before and two others 2 and 4 h after the subject had received the load of glucose. Consequently, three k₂ values were obtained (k₂-0, k₂-1, k₂-2, respectively) (Fig. 1).

**Determination of LPL activity**

At the end of the study, 10 minutes after the third IVFTT (time: 350 min), heparin (Léčiva, Prague, Czech Republic; 100 IU/kg) was i.v. injected to the subjects and 10 min later blood for in vitro lipoprotein lipase activity assessment was withdrawn. Postheparin plasma was stored at –80 °C before determination. LPL activity was determined as described earlier (Bengtsson-Olivecrona and Olivecrona 1992). Briefly, the samples of postheparin plasma were preincubated with antibody against hepatic lipase (kindly provided by Prof. Hans Jansen, Erasmus University Rotterdam, the Netherlands) and then the activity was measured using Intralipid® labeled with glycerol-3H-oleate (American Radiolabeled Chemicals, Inc., St Louis, MO, USA) as substrate. To eliminate the interassay variation, all the samples were run in the same assay.

**Other methods**

The blood for examination of plasma glucose, immunoreactive insulin, non-esterified fatty acids (NEFA) and TG was taken before and at the end of the first IVFTT (time 0 and 40 min), before and 30, 60 and 120 min after the glucose load (time 60, 90, 120, and 180 min, respectively) as well as before and at the end of the second and the third IVFTT (180, 220, 300, and 340 min, respectively). Plasma glucose concentrations were measured on a Beckman analyzer (Beckman Instruments, Fullerton, CA) using glucose oxidase method, insulin was determined using IRMA kits (Immunotech, Prague, Czech Republic), NEFA and TG concentrations were measured on Roche COBAS MIRA autoanalyzer (Hoffmann-La Roche, Switzerland) using enzymatic kits from Wako, Japan, and Hoffmann-La Roche, Switzerland, respectively.

**Statistical analysis**

Data are presented as means ± S.D. The ANOVA for repeated measures (SYSTAT, SPSS Inc., Chicago, IL, USA) was used to analyze the differences between oGTT and control experiments. Alternatively, paired t-test was used when appropriate.

**Results**

The oral glucose administration resulted in the expected increase in glucose and insulin concentrations and suppression of non-esterified fatty acids (NEFA) concentration (Fig. 2). No such changes were observed in control experiments. The course of glycemia after glucose load did not differ from that observed during screening oGTT. Importantly, there were no significant differences in the course of triglyceridemia between both experiments. It can be noted that after each IVFTT, the transient increase in NEFA and TG concentrations was observed. However, the increase in TG concentrations after each IVFTT may reflect an increase not only in serum TG but also in glycerol concentration because Intralipid® contains an approximately equimolar amount of triglycerides and glycerol and the method used for TG determination does not include a correction for glycerol concentration.

The baseline TG concentration in serum was 40 % lower in the oGTT experiment likely due to random day-to-day variation and, accordingly, k₂ value (k₂-0) was 30 % higher (Figs 2 and 3, respectively); these differences were not significant (p=0.08, p=0.28, respectively). Importantly, when glucose was given to subjects, the k₂ values decreased to 78 % of baseline at 2 h after glucose administration (k₂-1) and did not change further after 4 h (k₂-2) (Fig. 2). No change of k₂ was observed in a control experiment. The difference between k₂ changes in both experiments did not reach a statistical significance (p=0.06). However, when the k₂ values were expressed as a percentage of the baseline (to adjust to the difference in the k₂-0 values) the difference became significant (Fig. 3).
In accordance with such findings, the LPL activity measured \textit{in vitro} in the plasma obtained 10 min after intravenous heparin application at the end of each experiment was 16\% lower after glucose administration than in the control experiment (2.88±0.77 mmol/l/h vs. 3.42±1.02 mmol/l/h, p<0.05, paired t-test).
Discussion

Using a repeated intravenous fat tolerance test, we demonstrated that an increase in insulinemia and/or glycemia downregulates LPL activity already 2 h after glucose administration and that such a downregulation persists for at least another 2 h. The decrease in LPL activity was observed even when measured in vitro in postheparin plasma 5 h after glucose administration.

Such findings are in good agreement with our recent observation that LPL activity measured both in postheparin plasma and as the k2 rate constant is downregulated by hyperinsulinemia and/or hyperglycemia induced in 4-h clamp studies (Kovář et al. 2004). However, the design of the present study allowed us to study the regulation of LPL activity in an experimental setting that is much closer to physiological conditions than that of clamp studies in which hyperglycemia and/or hyperinsulinemia are kept at the adjusted level for several hours.

Contrary to our findings, Cohen and Berger (1990) did not find any significant effect of glucose administration on the k2 rate constant measured in IVFTT. However, they carried out the IVFTTs on different days and it cannot be excluded that intraindividual variations in the k2 rate constant (that may be rather high based on our data (see Fig. 3)), overrode the effect of glucose. In this respect it is worth to mention that k2 rate constant was 10% lower (though not significantly) 2 h after glucose administration in Cohen and Berger’s experiments.

Lipoprotein lipase is ubiquitously expressed in extrahepatic tissues with adipose tissue and muscle being quantitatively of the primary importance (Olivecrona and Olivecrona 1999). It has repeatedly been demonstrated that insulin downregulates LPL activity in the muscle and upregulates it in the adipose tissue when the LPL activity is measured in tissue samples obtained by biopsy (Lithell et al. 1978, Sadur and Eckel 1982, Yki-Järvinen et al. 1984, Farese et al. 1991). However, until now no data are available on the effect of insulin on the change in total LPL activity in the circulation that is critical with respect to the rate of TRL-triglyceride hydrolysis. It can be assumed that the change in LPL activity in circulation is a sum of opposite changes in different tissues – our results therefore suggest that the insulin effect on LPL activity in the circulation reflects rather its lowering effect in the muscle than its raising effect in the adipose tissue.

In this respect, our findings are in quite good agreement with the observation that 30-h fasting enhance postheparin LPL activity and that such a change reflects the increased activity in muscles rather than decreased LPL activity in adipose tissue (Ruge et al. 2001, 2005). It was also noted that postheparin LPL activity measured postprandially is lower in the evening than in the late morning (Arasaradnam et al. 2002) and that may reflect the repeated exposures to periods of hyperinsulinemia and hyperglycemia during the day.

Given that LPL activity decreases after a glucose load, TG concentration would be expected to increase. This is not likely due to a coincidental lowering effect of insulin on NEFA concentration and hepatic VLDL production (Lewis et al. 1993). Furthermore, the LPL activity is unlikely to be a rate limiting factor under conditions of this study.

It is estimated that the capacity of lipoprotein lipase for triglyceride hydrolysis in the circulation is around 40 g/h and that should be well above the rate of TG transport in the circulation after a normal meal (Olivecrona and Olivecrona 1999). The fact that triglyceridemia increases postprandially, in spite of such a capacity, is interpreted as a result of local accumulation of fatty acids that release the LPL from the endothelium into the circulation (Saxena et al. 1989, Peterson et al. 1990, Karpe et al. 1992). Our observations provide an alternative explanation that the total LPL activity in the circulation is downregulated postprandially or more precisely that the downregulation of LPL in tissues (such as muscle) is quantitatively more important than the upregulation in other tissues (adipose tissue). The fact that serum NEFA concentrations were even higher in our control experiments, where no change of LPL activity was observed, is in agreement with such an explanation.

We do not have enough data to speculate on the mechanism(s) of such regulations. However, the fact that LPL activity changes occur already 2 h after glucose administration support the idea that insulin and/or glucose regulate the enzyme activity predominantly at the posttrascriptional level (Ong and Kern 1982, Semenovitch et al. 1989).

Our results suggest that the use of IVFTT repeated at short time intervals is a very promising tool to study the changes of total LPL activity in the circulation. For the first time it allowed us to demonstrate the changes in LPL activity in circulation within 2-3 h in a given individual. This is impossible when LPL activity is measured in plasma obtained after heparin application due to an extensive release of LPL from tissues and its
loss from circulation into the liver for degradation (Hultin et al. 1992). It is possible to measure LPL activity repeatedly in samples obtained by biopsy of the skeletal muscles or subcutaneous adipose tissue but such a measurement does not provide information on total LPL activity in the circulation.

Therefore, repeated IVFTT may be a very useful tool for studying the effect of different stimuli (such as hormones, nutrients, drugs, physical activity, etc.) on the acute changes in lipoprotein lipase activity in circulation in vivo. Importantly, results of repeated IVFTT will provide important information on the changes of LPL capacity to hydrolyze triglycerides.

On the other hand, it has to be noted that the repeated IVFTT has a certain limitation. The $k_2$ values obtained using IVFTT correlate inversely with triglyceridemia and this may be partially due to a competition of Intralipid® with endogenous TRL. It can be estimated that the injection of Intralipid® (0.1 g of fat/kg of weight) should result in an approximately 2 mmol/l increase of TG concentration and, therefore, the test should not be performed when significant changes of triglyceridemia are expected (such as in postprandial state after a fat load).

In conclusion, using the intravenous fat tolerance test repeated in short-time intervals, we demonstrated that concomitant increase in glycemia and insulinemia results in decrease of LPL activity in circulation of healthy male volunteers already 2 h after oral glucose administration. The results of our study also indicate that repeated IVFTT is a promising tool for studying acute changes in total LPL activity in circulation.

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References


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