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The majority of lipoprotein lipase in plasma is bound to remnant lipoproteins: A new definition of remnant lipoproteins

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ABSTRACT

Background: Lipoprotein lipase (LPL) is a multifunctional protein and a key enzyme involved in the regulation of lipoprotein metabolism. We determined the lipoproteins to which LPL is bound in the pre-heparin and post-heparin plasma.
Methods: Tetrahydrolipstatin (THL), a potent inhibitor of serine lipases, was used to block the lipolytic activity of LPL, thereby preventing changes in the plasma lipoproteins due to *ex vivo* lipolysis. Gel filtration was performed to obtain the LPL elution profiles in plasma and the isolated remnant lipoproteins (RLP).
Results: When *ex vivo* lipolytic activity was inhibited by THL in the post-heparin plasma, majority of the LPL was found in the VLDL elution range, specifically in the RLP as inactive dimers. However, in the absence of THL, most of the LPL was found in the HDL elution range as active dimers. Furthermore, majority of the LPL in the pre-heparin plasma was found in the RLP as inactive form, with broadly diffused lipoprotein profiles in the presence and absence of THL.
Conclusions: It is suggested that during lipolysis *in vivo*, the endothelial bound LPL dimers generates RLP, forming circulating RLP-LPL complexes in an inactive form that subsequently binds and initiates receptor-mediated catabolism.

1. Introduction

Lipoprotein lipase (LPL) hydrolyzes the triglyceride-rich core of chylomicrons (CM) and very low density lipoproteins (VLDL). It is also known as a ligand, *in vitro* or *in vivo*, for the binding of lipoproteins to the low density lipoprotein receptor-related protein 1 (LRP-1) and may play a central role in the receptor-mediated removal of triglyceride rich lipoproteins [1–3]. After synthesis in parenchymal cells, primarily in adipose tissue and skeletal muscle, LPL is transported to the intimal surface of the vascular endothelium, where it is non-covalently anchored to the heparan sulfate side chains of membrane proteoglycans and the recently discovered GPI-HBP1 [1,4,5]. At this site, LPL is responsible for the hydrolysis of the TG-rich core of circulating CM and VLDL, generating free fatty acids that can be either used immediately for energy or stored, primarily in adipose tissue under normal circumstances. The enzyme is known to be active only in a dimeric configuration [6,7]. To carry out its functions, LPL binds the interface of TG-rich lipoproteins [8,9]. After reduction in TG content and size, CM and VLDL remnants are believed to detach from the endothelium and released into the circulation by mechanism(s) that have yet to be fully understood [10,11].

The aim of this study was to clarify the characteristics of the interaction between LPL and remnant lipoproteins (RLP) in both pre-heparin

Abbreviations: CM, chylomicrons; VLDL, very low density lipoproteins; HTGL, hepatic triglyceride lipase; LRP-1, low density lipoprotein receptor-related protein 1; LPL, lipoprotein lipase; RLP, remnant-like lipoprotein particles; RLP-C, remnant-like lipoprotein particles-cholesterol; RLP-TG, remnant-like lipoprotein particles-triglyceride; TC, total cholesterol; TG, triglyceride; THL, tetrahydrolipstatin; VLDL, very low density lipoprotein.

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and post-heparin plasma. Tetrahydrolipstatin (THL) was used to block the *ex vivo* lipolytic activity of LPL in the post-heparin plasma in this study. The relationship between LPL activity and its inhibitors (apoC1 and apoC3) in RLP was also investigated.

There is growing evidence that LPL not only affects lipolysis, but also acts as a ligand for the binding of lipoprotein particles to receptors on the cell surface. A number of studies have reported that LPL is associated with lipoproteins in human pre- and post-heparin plasma, in both its inactive and active form [12–14]. Felts, ltakura, and Crane [15] in 1975 suggested that LPL binds to lipoprotein remnants and may be a marker for uptake of remnant lipoproteins by the liver. However, the isolation of LPL bound remnants from TG-rich lipoproteins or VLDL, namely the LPL-remnant complex, in plasma has not been demonstrated at this time.

Previous studies showed that LPL bound to apolipoprotein E-rich lipoproteins enhances their binding to the LRP-1 *in vitro*, the putative receptor for CM remnants [16], as well as to the VLDL receptor [17,18]. Moreover, Nykjaer et al. [19] suggested that only the LPL dimer, and not the monomer, was able to mediate this binding. Goldberg et al. [12] reported that lipolytic activity in the pre- and post-heparin plasma is associated with cholesterol-rich particles slightly larger than LDL. Other reports confirmed this observation by showing that the LPL protein after gel filtration is associated with LDL and HDL in both pre-heparin and post-heparin plasma [13,14]. In these studies, however, no steps were taken to inhibit lipolytic activity *ex vivo*. Zambon, Hashimoto, and Brunzell [20] demonstrated that a significant amount of the TG-rich core of CM and VLDL can be hydrolyzed in the post-heparin plasma stored *ex vivo* even at 4 °C if the LPL activity is not effectively inhibited. It is, therefore, possible that when heparin is administered, LPL is initially released associated with VLDL particles that undergo further hydrolysis by LPL *ex vivo*, resulting in lipoproteins with an LDL and HDL size range. However, more recently Zambon et al. [21] reported that dimeric LPL is bound to triglyceride-rich plasma lipoproteins in the presence of THL [22,23] as the result of an inhibition of *ex vivo* lipolysis.

The present study, we analyzed LPL binding to RLP isolated from post-heparin and pre-heparin plasma by immunoaffinity gel separation. By inhibiting lipolytic activity *ex vivo*, this study aimed to examine: 1) lipoprotein subclass with which LPL is associated *in vivo* before and after heparin administration as well as in the presence and absence of THL, 2) whether the LPL bound to RLP isolated by immunoaffinity gel are dimers or monomers and with an active or inactive form, and 3) whether the association between LPL and lipoproteins previously observed in the post-heparin plasma reflects a physiological or non-physiological condition. To clarify these biochemical and physiological characteristics of the LPL and RLP interaction will provide the critical new insight into the definition of remnant lipoproteins.

2. Materials and methods

2.1. Subjects

Randomly selected type 2 diabetes mellitus (T2DM) and metabolic syndrome (MetS) patients (aged 23–77 y; males 101, females 94) who were under treatment at Gunma University Hospital, Maebashi, Japan were studied in cases with plasma TG levels above and below 150 mg/dl. The study had the approval of the Ethical Committee of the Gunma University School of Medicine. Written informed consent was obtained from all of the participants.

The post-heparin plasma study was conducted as part of a trial investigating the metabolic effects of sugar consumption at University of California, Davis [24,25]. The participants, who were young and generally healthy, received intravenous administration of heparin for measurements of LPL and hepatic triglyceride lipase (HTGL) activity. The 29 samples analyzed for the current study were from 15 male and 14 females who were overweight or obese and susceptible to be postprandial remnant hyperlipoproteinemia (median age 24 y, with an average BMI of 27 kg/m^2). The post-heparin plasma was collected in the fasting state at 8:00 a.m. in the morning. The UC Davis Institutional Review Board approved the experimental protocol and all of the subjects provided written informed consent to participate in the study. All of the plasma samples were kept frozen at −80 °C until analysis.

2.2. Plasma handling

The pre- and post-heparin plasma samples were obtained after 12-h fasting. The post-heparin plasma was collected at 15 min after i.v. administration of 50 U/kg body weight of heparin at UC Davis. In addition, a normotriglyceridemic (male aged 60 y) and a hypertriglyceridemic Japanese subject (male aged 32 y) were injected with 30 U/kg of heparin and the plasma was collected at 0, 15, 30, 60 min at Hidaka Hospital, Takasaki, Japan.

To inhibit lipolytic activity *ex vivo*, 1 aliquot of post-heparin blood was mixed with tetrahydrolipstatin (THL) (Orlistat, Sigma-Aldrich) immediately after blood withdrawal at a final concentration of 1 μg/ml [21]. Plasma was separated from the THL-added blood by centrifugation (1800*g*, 10 min at room temperature).

2.3. Assay procedure for sandwich LPL-ELISA

The LPL concentration in plasma was measured using the LPL-ELISA newly developed at Immuno-Biological Laboratories (IBL). The assay used 2 different monoclonal antibodies against human recombinant LPL (57A5 and 88B8) for the sandwich ELISA. Tetra methyl benzidine (TMB) was used as the coloring agent (chromogen). Briefly, 100 μl of plasma or standard LPL diluted >100 fold was incubated with a solid phase antibody (57A5) for 60 min at 37 °C using a plate lid. After washing the plate with phosphate buffer, another antibody (88B8) labelled with horse radish peroxidase was added and incubated for 30 min at 4 °C with the plate lid. After washing, chromogen was added and incubated for 30 min at room temperature. The plate was read at 450 nm against a reagent blank within 30 min of the addition of 1 N H_2SO_4 solution to stop the reaction. The measurement range of the assay was 0.02–1.5 ng/ml. The CV was <10% in both intra- and inter-assay

2.4. LPL activity assay

The LPL activities were determined by an assay developed by Imamura et al. [26] for measuring the increase in absorbance at 546 nm due to the quinoneine dye. Reaction mixture-1 (R-1) contained dioleoylglycerol solubilized with lauryldimethylaminobetaine, monoacylglycerol-specific lipase, glycerolkinase, glycerol-3-phosphate oxidase, peroxidase, ascorbic acid oxidase and apolipoprotein C-II (apoC-II). R-2 contained Tris-HCl (pH 8.7) and 4-aminoantipyrine. Assay of lipase activity was performed with a chemistry analyzer (H7700P). In the assay for LPL activity, 160 μl of R-1 was incubated at 37 °C with 2 μl of sample for 5 min, and 80 μl of R-2 was added and incubated for additional 5 min. HL activities were measured under the same conditions without apoC-II.

2.5. Isolation of remnant lipoproteins with the immunoaffinity gel

Immuno-separation method [27] was used to isolate RLP containing both apoB-48 and apoB-100 from the plasma using specific antibodies so as to isolate the RLP as an unbound fraction. Briefly, 0.5 ml aliquots of plasma were applied to 5 ml of immunoaffinity mixed gel containing 2 monoclonal antibody clones, Mab JI-H raised against human apoB-100 and Mab H-12 raised against human apoA-I (JIMRO) with gentle shaking for 2 h at room temperature. The unbound fraction was concentrated to the same volume of plasma used for the preparation of RLP with an Amicon Ultra filter (Millipore) for the gel filtration analysis.