Clinica Chimica Acta 440 (2015) 193–200

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Comparison of the effect of post-heparin and pre-heparin lipoprotein lipase and hepatic triglyceride lipase on remnant lipoprotein metabolism

Takashi Shirakawa [a,d,*], Katsuyuki Nakajima [a,b,c], Younosuke Shimomura [a], Junji Kobayashi [b], Kimber Stanhope [c], Peter Havel [c], Tetsuo Machida [d], Hiroyuki Sumino [d], Masami Murakami [d]

[a] Diabetes and Metabolic Disease Research Center, Hidaka Hospital, Takasaki, Gunma, Japan
[b] Department of General Medicine, Kanazawa Medical University, Kanazawa, Ishikawa, Japan
[c] Department of Molecular Biosciences, School of Veterinary Medicine and Department of Nutrition, University of California, Davis, CA, USA
[d] Department of Clinical Laboratory Medicine, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan

ARTICLE INFO

Article history:
Received 24 February 2014
Received in revised form 27 May 2014
Accepted 17 July 2014
Available online 17 September 2014

Keywords:
Post-heparin plasma
Pre-heparin plasma
Lipoprotein lipase (LPL)
Hepatic triglyceride lipase (HTGL)
Remnant lipoproteins (RLP)
RLP-TG/RLP-C ratio

ABSTRACT

Background: A comparison of post-heparin and pre-heparin plasma lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) on the metabolism of remnant lipoproteins (RLPs) has not been reported yet.
Methods: Healthy volunteers were injected with heparin for LPL and HTGL determination in the fasting (8:00) and postprandial (20:00) plasma on the same day. Plasma total cholesterol (TC), triglycerides (TG), LDL-C, HDL-C, small dense LDL (sdLDL)-C, remnant lipoprotein (RLP)-C, RLP-TG, the RLP-TG/RLP-C ratio, adiponectin and apoCIII were measured.
Results: LPL activity and concentration in the post-heparin plasma exhibited a significant inverse correlation with TG, RLP-C, RLP-TG, and RLP particle size estimated as RLP-TG/RLP-C ratio and sdLDL-C, and positively correlated with HDL-C. HTGL was only inversely correlated with HDL-C. LPL concentration in the pre-heparin plasma was also inversely correlated with the RLP-TG/RLP-C ratio and other lipoprotein parameters. Adiponectin was inversely correlated with RLP-TG/RLP-C ratio and apoC III was positively correlated with RLP-TG/RLP-C ratio, but not correlated with LPL activity.
Conclusion: LPL activity and concentration were inversely and significantly correlated with the particle size of RLP in both the post-heparin and pre-heparin plasma. Those results suggest that LPL concentration in pre-heparin plasma can take the place of LPL activity in the post-heparin plasma.

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1. Introduction

Remnant lipoprotein (RLP) metabolism is known to be regulated by lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) [1–3]. LPL plays a central role in triglyceride-rich lipoproteins (TRLs) metabolism by catalyzing the hydrolysis of triglycerides (TG) in chylomicrons (CMs) and very low-density lipoprotein (VLDL) particles and is a useful biomarker in diagnosing Type I hyperlipidemia [4] and also prediction of cardiovascular diseases [5]. HTGL has been recognized to play a role in catalyzing the hydrolysis of the smaller remnants into LDL [6]. We previously reported that postprandial RLPs in a 4 h period after a fat load are significantly larger in particle size compared to the fasting state, with a TG increase in RLPs [7]. The interaction of those lipase activities with the associated lipoproteins and RLP particle size in the fasting and postprandial plasma was the focus of this investigation.

The accumulation of RLPs of large particle size after an oral fat load is mainly due to the delayed metabolism of VLDL by LPL [7,8]. LPL and HTGL activities and concentrations, both with and without a heparin injection, were pursued in order to elucidate the plasma TRL metabolism, especially in terms of the effect on the RLP particle size, estimated by RLP-TG/RLP-C ratio compared with HPLC assay by Okazaki et al. [9]. If there is a close similarity between the RLP particle size in pre- and post-heparin plasma, it may be possible to eliminate the heparin injection that is commonly used to measure LPL and HTGL activities and concentrations for clinical diagnostic purposes. Non-heparinized plasma (pre-heparin plasma) is known to contain a considerably large amount of LPL, but the activity of TG hydrolysis is very low or non-detectable. Watson et al. [10] tried to measure this low level of LPL activity by increasing the serum volume and prolonging the incubation time, but they still did not find a meaningful association between the pre-heparin LPL activity and the lipoprotein concentrations, suggesting that the plasma LPL concentration does not reflect a significant role in lipid metabolism, at least via its lipolytic activity.

However, recent studies have revealed that catalytically inactive LPL in pre-heparin plasma can act as a ligand for lipoprotein receptors and glucosaminoglycans in the liver [11–15]. Thus, catalytically inactive LPL might participate in lipoprotein metabolism via its ligand function rather than its lipolytic function. Because it is

* Corresponding author at: Diabetes and Metabolic Disease Research Center, Hidaka Hospital, 886 Nakao-machi, Takasaki, Gunma, Japan 370-0001. Tel.: +81 90 2893 6429.
E-mail address: takahsi830530@yahoo.co.jp (T. Shirakawa).

http://dx.doi.org/10.1016/j.cca.2014.07.020
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catalytically inactive, the measurement of pre-heparin LPL concentration has not received much attention as a diagnostic marker in clinical laboratories, despite the extensive studies reported by Shirai and et al. [16–23] and others [24,25].

2. Materials and methods

2.1. Study subjects

The study in relatively healthy young volunteers (some cases were overweight or obese) in a male ($n = 36$) and female ($n = 40$) population (Caucasian 45, Asian 10, Hispanic 9, African American 7, others 5) with a median age of 24 and BMI of 24 at the University of California, Davis (Table 1). Inclusion criteria included age from 18 to 40 y and BMI of 18–35 kg/m^2 with a self-report of stable body weight during the prior 6 months. Exclusion criteria included evidence of diabetes, renal disease, or hepatic disease; fasting serum TG concentrations greater than 400 mg/dl; hypertension (>140/90 mm Hg); and history of surgery for weight loss. Individuals who smoked, reported exercise of >3.5 h/week at a level more vigorous than walking, or reported having used thyroid, lipid-lowering, glucose-lowering, antihypertensive, antidepressant, or weight-loss medications were also excluded. Seventy six volunteers were injected with heparin for the LPL and HTGL activity assays. The University of California at Davis Institutional Review Board approved the experimental protocol and the subjects provided written informed consent to participate in the study. Baseline blood samples of fructose and glucose study by Stanhope et al. [26] were provided for this study and all the parameter analysis were performed at Gunma University.

Fasting blood samples were collected at 08:00 h before breakfast and postprandial blood samples were collected at 20:00 h after dinner on the same day. During the day, standardized meals were provided as breakfast, lunch and dinner to all of the volunteers. The energy content of the meals was based on each subject's energy requirements as determined by the Mifflin equation [27].

2.2. Determination of lipids and lipoproteins as well as lipase activities and concentrations

The plasma samples for the measurement of TC, TG, HDL-C, LDL-C, RLP-C, RLP-TG and sdLDL-C were withdrawn before (pre-heparin plasma) and after heparin injection (post-heparin plasma) and kept frozen at −80 °C until analysis. As LPL and HTGL activities were not detectable in the pre-heparin plasma, all of the lipase activities in this study were determined in the post-heparin plasma by the method of Imamura [28]. Briefly, the post-heparin plasma was withdrawn 15 min after the intravenous injection of 50 units of heparin/kg body weight for the assay of LPL, HTGL activity and concentration. The LPL and HTGL activity assays were an automated kinetic colorimetric method in post-heparin plasma by using a natural long-chain fatty acid, 1,2-diglyceride as substrate for assaying HL activity and with the presence of apoC-II for assaying LPL activity. LPL activity was calculated after subtracted HTGL activity from total lipase activity after adding apo CII. LPL and HTGL hydrolyzed the clear substrate solution to produce a 2-monoglyceride, which in turn releases glycerol by the action of a 2-monoglyceride lipase. The glycerol produced is then assayed by a sequence of enzymatic actions that produce a violet quinone monoimine dye. The accuracy and stability of this activity assay were reported previously by Imamura et al. [28]. The pre-heparin LPL and HTGL concentrations were determined by the highly sensitive and specific ELISA kit (IBL, Fujioka, Japan) recently developed by Miyashita et al. [29]. The TC and TG concentrations were determined enzymatically. The LDL-C and HDL-C concentrations were measured using a homogenous method (Kyowa Medex, Tokyo). Glucose and insulin were determined by PolyChem (Polymedco, NY). RLP-C and RLP-TG were determined by an immunoseparation method (JIMRO II, Otsuka, Tokyo) [30]. Small dense LDL-C was determined by the method of Ito et al. [31].

Table 1
Clinical characteristics of 76 volunteers.

	Median	(25%tile–75%tile)
Age (y)	24	(22–30)
Gender male/female	36/40	
Body weight (kg)	73	(61–82)
BMI	24	(22–27)
Abdominal circumference (cm)	77	(67–81)
Blood pressure		
Systolic (mm Hg)	117	(110–130)
Diastolic (mm Hg)	72	(67–76)

2.3. Statistical analysis

Data were analyzed with Dr. SPSS II (SPSS).The data are presented as median values with 25th and 75th percentile values, rather than as mean values with standard deviation. The statistical significance of difference was determined by Mann–Whitney *U*-test. Pearson's correlation coefficients (r = value) were determined and single linear regression analysis was performed to detect associations between variables. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Post-heparin plasma lipids, lipoproteins, LPL and HTGL activity and concentration analyses in the fasting and postprandial states.

Table 1 indicates the demographic data on 76 volunteers recruited at UC Davis, CA. Table 2 indicates that the mean total cholesterol (TC), LDL-C and HDL-C levels were within a normal range in the fasting and postprandial plasma. TG, RLP-C, and RLP-TG were significantly elevated in the postprandial plasma, but TC, LDL-C, HDL-C and sdLDL-C did not change. RLP-TG/RLP-C ratio in the postprandial plasma increased 2.6 fold compared to the fasting plasma both in pre-heparin and post-heparin plasma. LPL activity was significantly reduced in the postprandial plasma, but LPL concentration was not. HTGL activity and concentration were unchanged in the postprandial plasma. Both LPL and HTGL activities were not detected in pre-heparin plasma. HTGL concentration was detected negligible level compared to LPL concentration in pre-heparin plasma.

3.2. A single linear regression analysis of these parameters in the post-heparin and pre-heparin plasma.

A). TG correlations with the post-heparin and pre-heparin LPL and HTGL activities and concentrations in the fasting and postprandial plasma.

In post-heparin plasma (Table 3), TG concentration was inversely and significantly correlated with LPL activity and concentration in both the fasting and postprandial plasma, respectively, while no correlation was found with HTGL activity or concentration in either the fasting or postprandial plasma.

In pre-heparin plasma (Table 4), TG concentration was inversely correlated with LPL concentration in both the fasting and significantly correlated in postprandial plasma, while no correlation was found with HTGL concentration in either the fasting or postprandial plasma.

B). RLP-C correlations with the post-heparin and pre-heparin LPL and HTGL activities and concentrations in the fasting and postprandial plasma

In post-heparin plasma (Table 3), RLP-C was inversely and significantly correlated with the LPL activity and concentration in the fasting and postprandial plasma respectively, while no correlation was found with HTGL activity in either.

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