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# An automated method for measuring lipoprotein lipase and hepatic triglyceride lipase activities in post-heparin plasma

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ABSTRACT

*Background:* Lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) play a central role in triglyceride-rich lipoprotein metabolism by catalyzing the hydrolysis of triglycerides. Quantification of LPL and HTGL activity is useful for diagnosing lipid disorders, but there has been no automated method for measuring these lipase activities.

*Methods:* The automated kinetic colorimetric method was used for assaying LPL and HTGL activity in the post-heparin plasma using the natural long-chain fatty acid 2-diglyceride as a substrate. LPL activity was determined with apoCII and HTGL activity was determined without apoCII with 2 channel of auto-analyzer.

*Results:* The calibration curve for dilution tests of the LPL and HTGL activity assay ranged from 0.0 to 500 U/L. Within-run CV was obtained within a range of 5%. No interference was observed in the testing of specimens containing potentially interfering substances. The measurement range of LPL activity in the post-heparin plasma was 30–153 U/L, while HTGL activity was 135–431 U/L in normal controls.

*Conclusions:* The L PL and HTGL activity assays are applicable to quantitating the LPL and HTGL activity in the post-heparin plasma. This assay is more convenient and faster than radiochemical assay and highly suitable for the detection of lipid disorders.

## 1. Introduction

Lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) play a major role in the metabolism and transport of lipids and lipoproteins [1–3]. We developed and evaluated the automated kinetic colorimetric method for assaying LPL and HTGL activity in the post-heparin plasma using the natural long-chain fatty acid 2-diglyceride as a substrate and the determination of produced 2-monoglyceride by lipases enzymatically. Although the principle of this assay is the same previously reported by Imamura et al. [4,5], we have modified and improved the assay system (analytical accuracy and assay performance) in order to satisfy for the clinical laboratory use and evaluated it in patient plasma who were under coronary angiography with heparin administration.

LPL is the enzyme responsible for the hydrolysis of core triglycerides (TG) in chylomicrons (CM) and very low density lipoproteins (VLDL), producing CM remnants and VLDL remnants, respectively. Determination of LPL activity in plasma has typically been carried out after the intravenous injection of heparin together with a determination of the LPL protein concentration. It is known that a comparatively high LPL concentration (the range; approximately 30–100 ng/ml in normal controls) is found in the pre-heparin serum or plasma, along with an undetectable level of LPL activity [6–8].

However, it has not been clarified enough on this the discrepancy between the activity and concentration.

The LPL activity and concentration in the post-heparin plasma has been clinically used for the detection of LPL deficiency [1–3], but is not generally used for the diagnosis of lipid disorders or the risk of cardiovascular disease because of the necessity of heparin administration

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and the lack of assay methods easy to handle. Considering the assay time and the technical steps required for the quantitative measurement by radiochemical LPL activity assay [9], the method is not suitable for either large-scale epidemiological studies or routine clinical laboratory use. Therefore, there remains a need for a reliable, rapid and automated assay for LPL activity that has both good sensitivity and calibrator stability. Brunzell et al. [10,11] showed that there were patients in whom the LPL activity deviated from the LPL concentration because of a gene mutation. Therefore, the determination of LPL activity along with the LPL concentration in the post-heparin serum is essentially required for the detection of LPL deficiency.

HTGL, a lipolytic enzyme that is a secreted glycoprotein, is synthesized by hepatocytes and binds to heparin sulfate proteoglycans on the surface of liver sinusoidal capillaries. The function of HTGL is recognized not as simple as that of LPL. It is widely accepted that HTGL plays a major role in lipoprotein metabolism as a lipolytic enzyme that hydrolyzes TG and phospholipids in chylomicron remnants, intermediate-density lipoproteins (IDL) and high-density lipoproteins (HDL) [12,13]. Patients with HTGL deficiency present with hypercholesterolemia or hypertriglyceridemia and accumulated VLDL, chylomicron remnants, IDL, TG-rich low-density lipoproteins (LDL), and HDL [14–19]. Although Ikeda et al. [20,21] developed a HTGL-ELISA which made it possible to determine the post-heparin plasma HTGL levels, there is still a need to develop an HTGL activity assay that has an easy and accurate automated assay method for clinical use. The assay presented here exhibits promise in determining the clinical significance of HTGL activity more clearly in cardiovascular and metabolic disease, including the HTGL deficiency which has been difficult to screen among patients.

The performance of both LPL and HTGL activity was evaluated on a Hitachi H7700 P automated analyzer. We compared its analytical power of determining the LPL and HTGL activities with the LPL-latex assay [22] and HTGL–ELISA [23] in normal controls and patients who were treated heparin administration under cardiac catheter test.

## 2. Materials and methods

### 2.1. Reagents

Reaction mixture-1 (R1A and R1B) contained dioleoylglycerol (natural long-chain fatty acid 2-diglyceride) solubilized with lauryldimethylaminobetaine, monoacylglycerol-specific lipase, glycerolkinase, glycerol-3-phosphate oxidase, peroxidase, ascorbic acid oxidase, N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidone (TODB). R1B contained apolipoprotein C-II (apoC-II), but without apoCII in R1A. All these reagents were obtained from Imamura Enzyme Technology Laboratory (Shizuoka). R-2 contained Tris-HCl (pH 8.7) and 4-aminoantipyrine for colorization. The interfering reagents containing bilirubin F and C, hemoglobin, TG and rheumatoid factor were purchased from Sysmex. All of the chemica1s and regents were of the highest available grade.

### 2.2. Preparation of blood samples

The plasma samples for the measurement of total cholesterol (TC), TG, HDL−C, LDL-C, RLP-C, RLP-TG and sdLDL-C were withdrawn from patients before and after heparin infusion and kept frozen at −80 °C until analysis. As the LPL and HTGL activities were not detectable in the pre-heparin plasma [24], all of the lipase activities in this study were determined in the post-heparin plasma. The normal controls (n = 4) (male; aged 35–70) and 100 patients who underwent coronary angiography with heparin administration for the cardiac catheter test (aged 65–79 y, male 87 and female 13 and BMI 21–27) [25] were enrolled at Hidaka Hospital. The post-heparin plasma was withdrawn 15 min after the intravenous injection of 30 units of heparin/kg body weight (BW) for the assay of the LPL and HTGL activities and concentrations. A statement of institutional approval of the study in accordance with the Declaration of Helsinki was provided. The study was approved by the ethical committees of Hidaka Hospital and written informed consent was obtained from all of the participants in this study.

The prepared plasma was used for the experiments on precision, sensitivity, dilution and recovery as well as the assay range in healthy volunteers and patients under cardiac catheter test.

### 2.3. Calibrator preparation

In-house calibrators for LPL measurement were prepared, with the recombinant LPL generated by CHO cells at IBL in Tris-HCl buffer (pH 8.0). To determine the LPL values on the in-house calibrator, calibrators of ELISA from Sekisui Medical with known concentrations,were used for comparison. The in-house calibrators for HTGL measurement were prepared by the same procedure as the LPL calibrators. The calibrator in R1B was used for the total of HTGL and LPL activity (584 U/L). The calibrator in R1A was used for HTGL activity alone (431 U/L). LPL activity is expressed as 153 U/L, which is the value of R1A subtracted from RIB.

### 2.4. Assay procedure

Fig. 1 shows the reaction sequence of the automated method. The LPL and HTGL activities were determined by measuring the increase in absorbance at 546 nm due to the quinoneine dye. LPL and HTGL hydrolyze the clear substrate solution (1, 2-Diolein; dioleoyglycerol) to produce a 2-monoglyceride, which in turn releases glycerol by the action of a 2-monoglyceride lipase. Produced glycerol is then reacted followed by a sequence of enzymatic actions (glycerol kinase, glycerol phosphate oxidase, and peroxidase) and the produced $H_2O_2$ reacted with 4-AA, peroxidase and TODB to form a violet quinone monoimine dye. Endogenous glycerol was deleted enzymatically using glycerol kinase, glycerol-3-phosphate oxidase, peroxidase, and TODS in R1A and R1B.

The LPL and HTGL activity assay was performed separately in the post-heparin plasma with apoCII (Reaction mixture-1; R1B) and without apoCII (Reaction mixture-1; R1A). Automated assay of lipase activities was performed with an automatic clinical analyzer (H7700P).

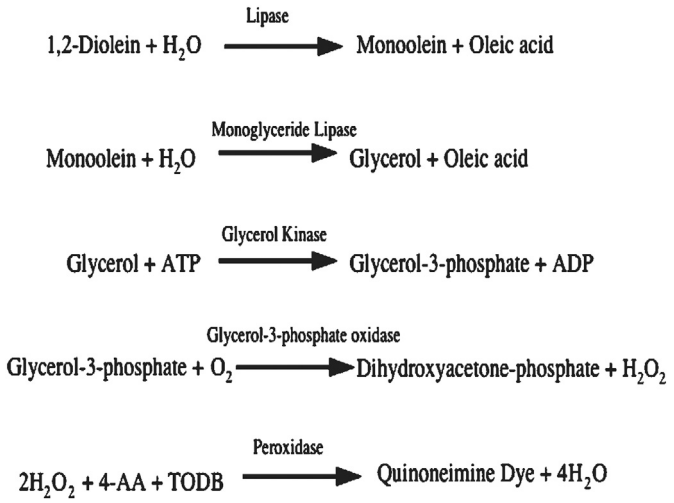


**Fig. 1.** The reaction sequence of the automated method. LPL or HTGL hydrolyzes the clear substrate solution to produce a 2-monoglyceride, which in turn releases glycerol by the action of a 2-monoglyceride lipase. Produced glycerol is then assayed by a sequence of enzymatic actions (glycerol kinase, glycerol phosphate oxidase, and peroxidase) that produce a violet quinone monoimine dye. AA, aminoantipyrine; TODB, N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidone. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)