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The role of circulating lipoprotein lipase and adiponectin on the particle size of remnant lipoproteins in patients with diabetes mellitus and metabolic syndrome

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ABSTRACT

Background: The factors regulating particle size of remnant lipoproteins (RLPs) in type 2 diabetes (T2DM) and metabolic syndrome (MetS) cases have not been well elucidated.
Methods: T2DM, MetS and healthy controls with and without a fatty liver were studied. Remnant lipoprotein (RLP)-cholesterol (RLP-C) and RLP-triglyceride (RLP-TG), small dense LDL-cholesterol (sdLDL-C), lipoprotein lipase (LPL), hepatic triglyceride lipase (HTGL) and adiponectin concentrations were measured in the fasting pre-heparin plasma. The RLP particle size was estimated by the RLP-TG/RLP-C ratio.
Results: The serum TG, RLP-C, RLP-TG, RLP-TG/RLP-C ratio and sdLDL-C were significantly greater in T2DM and MetS than in controls. Fatty liver and high serum TG were significantly associated with an increased RLP-TG/RLP-C ratio which was used to estimate the particle size of RLP in controls, T2DM and MetS. LPL and adiponectin in the pre-heparin plasma were inversely correlated with RLP-TG/RLP-C ratio in normal, T2DM and MetS. LPL was also positively correlated with adiponectin in all three cases.
Conclusions: RLP particle size in T2DM and MetS was significantly larger than in controls and was regulated by circulating LPL and adiponectin, but not HTGL. Fatty liver and high TG were significantly associated with the prevalence of the large RLP particle size.

1. Introduction

Type 2 diabetes (T2DM) and metabolic syndrome (MetS) are common disorders that consist of a cluster of clinical and metabolic factors accompanied by an increased risk for cardiovascular disease. The atherogenic dyslipidemia associated with these two conditions is characterized by elevated fasting triglycerides (TG) and remnant lipoproteins (RLPs) along with low-density lipoprotein cholesterol (LDL-C) levels, small dense LDL (sdLDL-C), and as a final factor, the postprandial accumulation of TG-rich lipoprotein (TRL) [1]. Of note, the accumulation of large TRL apoB-100 particles has been shown to be a particular characteristic for hypertriglyceridemic patients with coronary heart disease (CHD) compared with healthy hypertriglyceridemic subjects, suggesting a link between the accumulation of large very low density lipoproteins (VLDL) and the development of atherosclerosis [2]. However, the relationship between the large VLDL and the particle size of remnant lipoproteins has not been well elucidated.

We have already reported that the RLP particle size estimated by the RLP-TG/RLP-C ratio is significantly larger in the postprandial plasma of healthy volunteers in both Japanese and US populations [3], which has been proposed as a most common risk factor of cardiovascular diseases by Zilversmit [4]. However, the particle size of remnant lipoproteins in atherogenic diseases such as T2DM and MetS in fasting plasma has not been reported yet. Therefore, we sought to determine the particle size of RLP as the consequence of TG-rich lipoprotein hydrolysis by lipase activities. Therefore, the RLP particle sizes were determined among healthy controls, T2DM and MetS patients with and without the presence of a fatty liver, which is also known as a risk factor for CHD [5]. In order to elucidate the RLP particle size in T2DM and MetS, we used an immunoaffinity separation method for the isolation and determination of the RLP in plasma [6], and estimated the RLP particle size as the RLP-TG/RLP-C ratio previously reported by Okazaki et al. [7]. The relationships between RLP particle size and LPL [8] and adiponectin [9] were also targeted for investigation, because those factors are known to regulate the remnant lipoprotein metabolism associated with the risk of CHD.

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LPL plays a major role in the metabolism and transport of lipids and lipoproteins [10,11]. It is the enzyme that is responsible for the hydrolysis of core triglycerides in CM and VLDL, producing CM remnants and VLDL remnants, respectively. Measurement of LPL in the plasma is routinely carried out after the intravenous injection of heparin. However, it is also known that a comparatively large LPL concentration is found in the pre-heparin plasma, albeit with an undetectable level of activity, indicating that although the majority of circulating LPL is catalytically inactive, it is still a ligand for the receptors [12–17]. The LPL concentration in pre-heparin plasma was extensively investigated by Shirai and his colleagues in recent decades, demonstrating the clinical significance of measuring pre-heparin LPL concentration in a number of clinical studies [18–26]. Also, the key factor regulating RLP particle size has come to be considered to be mainly due to LPL. We have recently shown the possibility that the LPL concentration in the pre-heparin plasma is replaceable with LPL activity in the post-heparin plasma in a study which compared the details of the two [27]. From the result of the study, we found that the particle size most probably reflects the degree of LPL hydrolysis (the occurrence of LPL activity) in the plasma in the course of remnant metabolism under physiological conditions. As pre-heparin plasma LPL levels are much less compared to the post-heparin plasma levels, we have newly developed a highly sensitive and specific LPL enzyme linked immunosorbent assay (ELISA) to investigate the lower LPL levels for this study.

We also investigated the presence of fatty liver and elevated TG in T2DM and MetS patients as factors influencing RLP particle size. T2DM and MetS are closely associated with visceral fat accumulation and fatty liver elicitation of a pro-inflammatory state, which is characterized by a reduction in plasma adiponectin among other cytokine alterations. An inverse correlation between the adiponectin and RLP-C levels has been previously reported [28–30]. However, whether the serum adiponectin level in T2DM and MetS cases has any relationship with RLP particle size heterogeneity has not been reported yet.

2. Materials and methods

2.1. Subjects

The clinical and biochemical characteristics of the controls, as well as the T2DM and MetS cases were studied. There were 219 men and 101 women in the healthy controls without any significant diseases (median age 48), with 53 men and 8 women (median age 54 y) in DM, and 61 men and 6 women (median age 54 y) in MetS. These cases were selected consecutively for the present study from among subjects who attended the outpatient clinic service for an annual regular health-check at Hidaka Hospital, Takasaki, Japan. The statistical analysis of the plasma biochemical markers was conducted with the men and women combined because of the small number of women in all three groups. The study had the approval of the Ethical Committees of Hidaka Hospital and of the Gunma University School of Medicine. Written informed consent was obtained from all of the participants.

Most of the MetS cases were men, because the prevalence of MetS using the Japanese criteria is very low in women [31]. A total of 67 MetS patients who underwent screening were not receiving medication for hypertension or lipid abnormalities. In this population, we defined the MetS participants as those having abdominal obesity (a waist circumference of 85 cm or greater in males and 90 cm or greater in females) and ≥2 of the following: (1) a systolic blood pressure (SBP) of 130 mm Hg or higher or a diastolic blood pressure (DBP) of ≥85 mm Hg, (2) a serum triglyceride level of ≥150 mg/dl, 3) high density lipoprotein cholesterol (HDL-C) <40 mg/dl, and (4) a fasting plasma glucose (FPG) of ≥110 mg/dl. In addition, 320 normal control subjects were identified who had a waist circumference of <85 cm and none of the other components for MetS described above. Sixty one DM subjects were defined according to the Japan Diabetes Society (JDS) (criteria: HbA1c NGSP ≧6.5% and fasting plasma glucose ≧126 mg/dl or the use of a prescribed anti-diabetic agent).

The cases of fatty liver were identified as non-alcoholic fatty liver (NAFLD). All the patients were referred to the hepatology unit at Hidaka Hospital for ultrasound investigation of hepatic steatosis, which was performed as a part of routine clinical practice. According to the ultrasound results, subjects in the MetS, DM and controls were further divided into those with and without a fatty liver. The fatty liver positive group included the cases who presented with “bright liver” echo patterns according to standard criteria (i.e. evidence of a diffuse increase in echogenicity of the liver compared to the kidneys). The non-fatty liver in MetS, DM and controls was comprised of cases without any echogenic evidence of a fatty liver.

The weight and height of each participant were determined and body mass index (BMI) was calculated to check for the presence of obesity. The waist circumference measurement was made midway between the lateral lower rib margin and the superior anterior iliac crest in a standing position, with this measurement performed in all cases by the same investigator.

2.2. Sandwich LPL-ELISA

The LPL plasma concentration was measured using the LPL-ELISA newly developed by Miyashita et al. (Immuno-Biological Laboratories). The assay is a solid phase sandwich ELISA using 2 kinds of highly specific monoclonal antibodies against human recombinant LPL. The assay used two different monoclonal antibodies against LPL (57A5 and 88B8) for the sandwich ELISA, which can detect both monomeric and dimeric LPL. Tetramethylbenzidine (TMB) was used as the coloring agent (Chromogen). The strength of the coloring observed is proportional to the quantity of human recombinant LPL. Briefly, 100 μl of pre-heparin plasma or standard LPL diluted more than 100 fold were incubated with a solid antibody (57A5) for 60 min at 37 °C using a plate lid. After washing the plate with phosphate buffer, an antibody (88B8) labeled with horse radish peroxidase was added and incubated for 30 min at 4 °C. 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 sensitivity (detection limit) was 0.009 ng/ml in serum or plasma, with over a 100 fold dilution. The CVs were <5% in both intra- and inter-assays.

The plasma HTGL concentration was determined by the method of Miyashita et al. (Immuno-Biological) [32]. Briefly, a new sandwich based ELISA for measuring the protein mass of human hepatic triglyceride lipase (HTGL) using two distinct anti-human HTGL mouse monoclonal antibodies. The dynamic assay range of the HTGL ELISA was from 0.47 to 30 ng/ml, and the sensitivity was 0.08 ng/ml in serum or plasma with >8 fold dilution. The CVs were <7% in both intra- and inter-assays.

2.3. Other assay procedures and reagents

After a 12-h overnight fast, blood samples were taken, and serum was isolated and stored at 4 °C. The following analytes were measured within 48 h of drawing the blood: liver enzymes, glucose, HbA1c, total cholesterol (TC), triglycerides (TG), direct low density lipoprotein cholesterol (LDL-C) (Denka-Seiken), small dense LDL-C (sdLDL-C) (Denka-Seiken) and HDL-C (Denka-Seiken), as previously reported [30]. Aliquots of serum were stored at −80 °C for subsequent determination of parameters. RLP-C and RLP-TG (JIMRO II) were obtained from Otsuka. The adiponectin assay was a latex-based immunoturbidimetric method obtained from Otsuka, and the TSH and T4 assay used was a chemiluminescence immunoassay obtained from Fuji Rebio. Liver enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltranspeptidase (GGT) and cholinesterase (ChE), which were utilized as markers of hepatic