Small Dense LDL Cholesterol Measured by Homogeneous Assay in Japanese Healthy Controls, Metabolic Syndrome and Diabetes Patients with or without a Fatty Liver.

Koji Kikkawa 1) 3), Katsuyuki Nakajima 1) 2), Younosuke Shimomura 1), Yoshiharu Tokita 2), Tetsuo Machida 3), Hiroyuki Sumino 3), Masami Murakami)

1. Diabetes and Metabolic Disease Research Center, Hidaka Hospital, Takasaki, Gunma, Japan
2. Department of Laboratory Sciences, Gunma University Graduate School of Health Sciences, Maebashi, Gunma, Japan
3. Department of Clinical Laboratory Medicine, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan

Correspondence to; Koji Kikkawa, MD

Diabetes and Metabolic Disease Research Center,

Hidaka Hospital.

886 Nakao-machi Takasaki, Gunma, Japan 3701-0001

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Abstract

BACKGROUND: Serum small dense LDL-cholesterol (sdLDL-C) levels in healthy controls and the cases with diabetes (T2DM) and metabolic syndrome (MetS) with or without a fatty liver in a large, typical Japanese population was determined.

METHODS: The plasma lipids and lipoproteins, including sdLDL-C by homogeneous assay, were determined in controls, MetS and T2DM patients (n=5255). The cases with MetS and preliminary MetS (pre-MetS) as well as T2DM and preliminary T2DM (pre-DM) were selected based on the Japanese criteria for MetS and T2DM. Fatty liver was diagnosed using the ultrasonography.

RESULTS: The 75th percentile values for sdLDL-C were 27.5mg/dL for men and 23.3mg/dL for women and increased with age. The concentrations of sdLDL-C and sdLDL-C/LDL-C were significantly higher in pre-MetS and pre-T2DM patients than healthy controls as well as in MetS and T2DM patients. Significantly higher sdLDL-C was found in cases with a fatty liver than without a fatty liver in all five groups.

CONCLUSIONS: Significantly elevated sdLDL-C levels were found in pre-MetS, MetS and pre-T2DM, T2DM patients compared to the healthy controls. Fatty liver significantly enhanced serum sdLDL-C levels and the multiple regression analyses ascertained that fatty liver was an independent determinant for sdLDL-C.

Highlight

1. The normal range of sdLDL-C in Japanese population was determined.
2. sdLDL-C was significantly higher in pre-MetS and pre-T2DM than in controls
3. sdLDL-C was highest in MetS and T2DM when associated with a fatty liver

Introduction

The atherogenic lipid profile in patients with metabolic syndrome or glucose intolerance is characterized by hypertriglyceridemia, elevated apolipoprotein B levels, reduced high-density lipoprotein cholesterol (HDL-C) concentrations and an increased proportion of small, dense low-density lipoprotein (sdLDL) particles [1, 2]. The sdLDL particles exhibit increased penetration of the arterial wall, lower affinity for the LDL receptor, longer half-life in plasma, greater susceptibility to glycation and lower resistance to oxidative stress, suggesting that sdLDL is highly atherogenic [3]. Indeed, patients with high levels of sdLDL particles were shown to have an approximately 3-fold increase in the risk of developing coronary heart disease compared with individuals with primarily large, buoyant LDL particles [4, 5]. In addition, the sdLDL-cholesterol (sdLDL-C) concentration has been suggested to be a better surrogate marker than the LDL-C concentration for the severity of coronary heart disease [6-8].

LDL particles are heterogeneous with respect to size and density. Compared to large, buoyant LDL, sdLDL particles are known to exhibit atherogenic properties [9, 10]. Therefore, sdLDL particles possess an elevated atherogenic potential and are linked to premature cardiovascular disease (CVD), and a growing body of evidence supports their role as a CVD biomarker [11-14] determined by gradient gel electrophoresis (GGE) method (15, 16) and Nuclear Magnetic Resonance (NMR) spectroscopy (17). Recently, a simpler method for the determination of cholesterol in sdLDL was developed by Hirano et al. [18]. In it, the lipoproteins at a density <1.044 g/ml are precipitated using heparin and MgCl2, and cholesterol is measured by a homogeneous method in the supernatant fluid on a routine chemical analyzer, or ApoB is measured by immunotubidometry. More recently, a fully automated homogeneous assay for sdLDL cholesterol (sdLDL-C) has been developed by Ito et al. [19]. Using this method, the sdLDL-C values obtained by this new assay are compared with those obtained by isolation of the d =1.044–1.063 g/ml plasma fraction by sequential ultracentrifugation showed excellent agreement [20] and predicted incident cardiovascular disease (21). Therefore, we used this homogeneous sdLDL-C assay to establish a normal range of for sdLDL-C levels in a healthy Japanese population in order to compare with metabolic syndrome (MetS) and type 2 diabetes (T2DM).

As a means to finding an early diagnosis of cardiovascular disease, we investigated the cases with preliminary MetS (pre-MetS) and preliminary T2DM (pre-T2DM) patients, the presence of a fatty liver and associated lipid disorders. Pre-MetS and pre-T2DM are known to be associated with the impaired glucose tolerance (IGT) and high risk group of CVD (1). Also, a fatty liver has been proposed to be an independent predictor of coronary heart disease (CHD) [22-25]. A fatty liver is also a manifestation of metabolic syndrome, and is associated with obesity, T2DM and hypertriglyceridemia [25, 26]. In patients with T2DM or MetS, the prevalence of a fatty liver is significantly high and may enhance atherogenesis by increasing the level of sdLDL particles [27]. It has been reported that there is an overproduction and secretion of VLDL in the insulin resistance with a fatty liver, independent of liver fat deposits [28, 29]. De Vries et al reported that both lipoprotein lipase activity and adiponectin are inversely correlated with sdLD-C [30]. The precise role of a fatty liver in the pathogenesis of sdLDL, however, is still unclear. In the present study, we performed a cross-sectional analysis of a cohort of more than 5000 men and women to examine the potential associations between a fatty liver and the serum sdLDL levels, in particular in pre-MetS and pre-T2DM patients as an early detection of cardiovascular risk assessment by sdLDL-C.

The aims of this study were to determine the normal range of the plasma sdLDL-C concentration in a strictly selected healthy Japanese population and compared with the sdLDL-C level in patients with T2DM and MetS, in particular an early stage of these diseases. Furthermore, the metabolic pathway of formation of sdLDL-C and the driving mechanism of the interaction (i.e. which is the causative or primary factor) between the presence of a fatty liver and an elevated sdLDL-C were discussed. Which factor comes first, fatty liver or elevated sdLDL-C, and how influenced to the other and deteriorate the cardiovascular diseases was also discussed in this large population study.

Materials and Methods

Study subjects

The study subjects included 5,255 participants (3,199men and 2,056women) who underwent medical examination at Hidaka Hospital in Takasaki, Gunma prefecture, Japan. The subjects ranged in age from 28 to 83 in the men and from 27 to 75 in the women. The demographic data on age, height, weight, waist circumference, medications, systolic blood pressure, diastolic blood pressure, lipids and lipoproteins, including sdLDL-C, and other blood tests were collected at the time of the examination after an overnight fast. Abdominal ultrasonography was performed at the same time. The body mass index [BMI] was calculated as the weight in kilograms divided by the square of the height in meters. However, no information available with regard to menstruation. **From whole 5,255 participants, we excluded cases with a lipid-lowering agent because of the possible influence on the sdLDL-C levels. Total of 4,388 subjects (2,665 men and 1,723 women) were selected for this study.**

Written informed consent was obtained from all subjects and study was approved by the ethics committee of Hidaka Hospital.

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Healthy control subjects

The healthy control subjects (712 men, 915 women) were selected strictly on the basis of medical records from among the total subjects. Healthy subjects were defined as having normal blood pressure (systolic blood pressure < 130 mmHg and diastolic blood pressure < 85 mmHg), without impaired glucose tolerance (fasting plasma glucose < 110 mg/dL and HbA1c(NGSP) < 6.0 %), without any lipid metabolism disorder (LDL-C < 140 mg/dL and triglycerides(TG) < 150 mg/dL and HDL-C ≧ 40 mg/dL), without any thyroid disorder(TSH ≧ 0.4μIU/mL and < 4.0μIU/mL, FreeT4 ≧ 0.8ng/dL and < 1.9ng/dL) and not taking any medication.

For the establishment of the reference range interval, the cases with fatty liver detected by ultrasonography were excluded from this healthy control group (604 men, 864 women).

Metabolic syndrome (MetS)

MetS subjects were diagnosed according to the Japanese criteria [31], that is, visceral obesity (waist circumference ≧ 85 cm in men, 90 cm in women) plus two or more of the following components : (a)TG ≧ 150 mg/dL and/or HDL-C < 40 mg/dL, (b) systolic blood pressure ≧ 130 mmHg and/or diastolic blood pressure ≧ 85 mmHg, or the prescribed use of an antihypertensive agent and (c) a fasting plasma glucose ≧ 110mg/dL or the prescribed use of an anti-diabetic agent.

In addition, we defined pre-MetS subjects as those with visceral obesity and one of the components (hypertension, lipid metabolism disorder, or impaired glucose tolerance) used to define MetS mentioned above. The number of pre-MetS and MetS cases were 544 and 414 in men and 47 and 28 in women, respectively. Significantly larger number of pre-MetS and MetS cases were found in this study population followed by the definition of Japanese criteria.

Diabetes Mellitus (DM)

DM subjects were defined according to the Japan Diabetes Society (JDS) criteria: HbA1c(NGSP) ≧ 6.5% and fasting plasma glucose ≧ 126mg/dL or the use of a prescribed anti-diabetic agent. In addition, pre-DM subjects (mainly consisting of impaired fasting glucose (IFG)) were also defined according to the JDS criteria: HbA1c(NGSP) < 6.5% , fasting plasma glucose ≧ 110mg/dL and < 126mg/dL, and without the use of any prescribed anti-diabetic agent. “Suspected DM”, as defined by JDS criteria, was not included, since this cannot be determined in a single examination.

Fatty liver

A fatty liver was diagnosed based on ultrasonographic findings (Japanese Society of Sonographers), as follows: (a) Bright liver (increased echo level); (b) Liver-kidney contrast (an increased liver echo level compared to the kidney); (c) Deep attenuation (attenuation of the echo level in deep regions) ; (d) Vascular blurring (blurring of the hepatic vein).

Laboratory measurements

Total cholesterol (TC) and TG were measured with an enzymatic assay (Denka Seiken, Tokyo). HDL-C and LDL-C were measured by means of a direct assay (Denka seiken, Tokyo). SdLDL-C was measured with a newly developed homogeneous assay [19]. Glucose was measured with a hexokinase assay (Sekisui Medical, Tokyo). TSH and FT4 were measured by the CLEIA method (ADVIA Centaur XP, Siemens Healthcare Diagnostics). AST was measured by malate dehydrogenase assay (Sekisui Medical, Tokyo). ALT was measured with a lactate dehydrogenase assay (Sekisui Medical, Tokyo). γGT was measured by L-γ-glutamyl-3-carboxy-4-nitroanilide substrate(Sekisui Medical,Tokyo). ALP was measured by p-nitrophenylphosphate substrate (Wako Pure Chemical,Osaka). ChE was measured with a p-hydroxybenzoylcholine substrate assay (Serotec, Sapporo, Japan). All of the items except TSH and freeT4 were determined using a TBA-c8000 (Toshiba, Tokyo). Ultrasonography was performed with a TUS-A300 (Toshiba, Tokyo)

Statistical analysis

Statistical analyses were conducted with the Microsoft Office Excel 2007, Dr. SPSS II for Windows (11.0.1 J standard version) and StatFlex ver. 6 (Artech, Osaka, Japan). All values are expressed as the median. Linear relations between TG, LDL-C, HDL-C, sdLDL-C and large LDL-C were evaluated by linear regression models and Spearman's correlation coefficients in all of the subjects participating in this study. All the data were classified and the groups established, and then tested for significant differences between groups of 2 with Mann-Whitney's U test, and groups of 3 with the Kruskal-Wallis method (the P-value was calculated with Bonferoni's correction). Multiple logistic regression analysis and multiple regression analysis were conducted by normalization with power transformation (StatFlex). A significant difference was defined as $P< 0.05$.

Results

1. Diagnostic parameters among the control, pre-MetS and MetS cases and pre-T2DM and T2DM.

Relevant biochemical and anthropomorphic characteristics for the healthy subjects are presented in Table 1. The parameters that differed between men and women were BMI, blood pressure, serum glucose, triglycerides (TG), HDL-C, LDL-C, liver enzymes and thyroid hormones.

The data are presented as the median with 75^{th} percentile values, rather than as mean values with standard deviations, because almost none of the variables were normally distributed. The parameters in men and women with MetS and T2DM were divided into two groups, i.e. pre-MetS and MetS, as well as pre-T2DM and T2DM. The results show the stronger associations with BMI, blood pressure, lipoproteins (elevated TC, TG, LDL-C, sdLDL-C, low HDL-C), markers of glucose homeostasis (fasting glucose, HbA1c) significantly dependent on the waist circumference in MetS and pre-MetS, but not T2DM and pre-T2DM. A significantly increased sdLDL-C, sdLDL-C/LDL-C (%) level ($P<0.001$) was found in the pre-MetS, MetS and pre-DM, DM (both men and women) as compared to the control group. Significant differences in sdLDL-C, sdLDL-C/LDL (%) ($P<0.001$) were found between pre-MetS and MetS in men ($P<0.001$), but no significant differences were found between pre-DM and DM. Interestingly, no significant difference was found in LDL-C between pre-MetS and MetS and between pre-DM and DM in both men and women, although the number of MetS and pre-MetS patients was too small in women for the performance of an adequate statistical analysis. These results indicated that sdLD-C and sdLDL-C/LDL (%) increased significantly in the early stage of diseases such as pre-MetS and pre-T2DM and can distinguish between the early stage and late stage. However, LDL-C can not distinguish between pre-MetS and Mets and between pre-T2DM and T2DM. Also, the greater waist circumference significantly impacted the increase in sdLDL-C and sdLDL-C/LDL (%).

2. A comparison of sdLDL-C and other diagnostic parameters in patients with or without a fatty liver in the control, MetS and T2DM cases.

All of the parameters in the five groups were further divided into those with or without a fatty liver (Table 2). The prevalence of fatty liver was in normal controls (15.2%), pre-MetS (59.4%), MetS (71.0%), pre-T2DM (52.0%) and T2DM (60.9 %). Significantly higher prevalence of fatty liver was found in pre-MetS and pre-T2DM as well as in MetS and T2DM compared to the controls. The presence of fatty liver shows significantly stronger associations with lipoproteins in all five groups. A larger waist circumference was also significantly associated with a fatty liver than those without fatty liver. Significantly increased TG, LDL-C and sdlDL-C levels were found in all of the control, pre-MetS, MetS and pre-T2DM, T2DM cases with a fatty liver as compared to the groups without a fatty liver. Significantly decreased HDL-C was found in men in all five groups with a fatty liver ($P<0.001$). However, sdLDL-C/LDL-C (%) was not significantly increased in the control and MetS cases in men or women, but it was significantly increased in T2DM in men. The cases with a fatty liver mostly exhibited a trend towards higher levels of liver injury markers (ChE, ALT, AST, γGT, ALP) , although most of these levels were within the high normal range.

These results indicate that the presence of fatty liver significantly affected the increase of sdLDL-C in the controls, pre-MetS, MetS and pre-T2DM, T2DM cases, but the sdLDL-C/LDL-C (%) was only affected in T2DM in men.

3. Reference range analysis for sdLDL-C

The sdLDL-C concentrations increased with age and the gender difference was evident throughout all age categories. The concentrations of sdLDL-C were considered to trend upward with age in both men and women based on 10-year intervals. (Table 3). In 604 healthy men and 864 healthy women without fatty liver as described previously, the mean and standard deviation of the sdLDL-C concentration were 23.5 ± 7.8mg/dL (men), 20.3± 5.7mg/dL (women), respectively. The median was 22.6mg/dL in men and 19.6mg/dL in women. The 75^{th} percentile values for sdLDL-C were 27.5mg/dL for men and 23.3mg/dL for women. From these distribution ranges, cutoff points were also calculated for low, normal and increased concentrations. The ranges determined for sdLDL-C in Japanese men and women were: low, (<18.3; <16.3 mg/dL), normal, (18.4-27.4; 16.4- 23.3 mg/dL) and increased, (>27.5; > 23.3 mg/dL).

4. Univariate correlations among the lipid parameters.

Figure 1 shows the difference in the correlation with LDL-C between small and large buoyant LDL-C. The regression coefficient was determined for LDL-C vs sdLDL-C (0.314) and large LDL-C (0.686), and the correlation coefficient was 0.632 with sdLDL-C and 0.872 with large LDL-C, respectively.

Figure 2 shows the correlation with TG between small and large buoyant LDL-C. The regression coefficient was determined for TG vs sdLDL-C (0.142) and large LDL-C (-0.056), and the correlation coefficient was 0.703 with sdLDL-C and -0.176 with large LDL-C.

Figure 3 shows the correlation with HDL-C between small and large buoyant LDL-C. The regression coefficient was determined for HDL-C vs sdLDL-C (-0.285) and large LDL-C (-0.142) and the correlation coefficient was -0.303 with sdLDL-C and -0.095with large LDL-C, respectively.

Those univariate correlation coefficient analysis among the parameters showed that sdLDL-C is significantly and positively correlated with total cholesterol, triglyceride, LDL-C and inversely correlated with HDL-C. Large LDL was inversely correlated with TG and weakly correlated with HDL-C. These data indicate there is a clear difference between large LDL-C and sdLDL-C within the LDL fraction.

4. Multiple logistic regression analysis to identify the variables in diagnosing fatty liver and waist circumference.

To identify variables which are effective in diagnosing fatty liver from among the cardiovascular markers in Table 1 (age, HDL-C, LDL-C, sdLDL-C, ALT, ChE, HbA1c, etc.), multiple logistic regression analysis was performed using a backward elimination procedure. sdLDL-C was not an independent determinant for fatty liver. However, the standard partial regression coefficient with three explanatory variables (age, fatty liver and waist circumference) exhibited a significant correlation with sdLDL-C by multiple regression analysis; (age =0.048 (P<0.01) and the presence of a fatty liver =0.206 (P<0.001, waist circumference =0.225 (P<0.001).

Therefore, fatty liver was ascertained to be an independent determinant for sdLDL-C by multiple regression analyses.

Discussion

The normal range of serum sdLDL-C levels in the Japanese population using strict criteria has been determined using a novel homogeneous assay recently developed by Ito et al. [19] and the results compared with the serum sdLDL-C levels in patients with pre-MetS, MetS and/or pre-T2DM, T2DM. Significantly elevated sdLDL-C and sdLDL-C/LDL-C (%) levels were found in pre-MetS and pre-T2DM as well as in MetS and T2DM compared to normal controls. These results indicated that sdLD-C and sdLDL-C/LDL (%) increased significantly in the early stage of diseases such as pre-MetS and pre-T2DM and could distinguish the disease stage between preliminary and established phase. However, LDL-C could not distinguish the disease stage between pre-MetS and Mets and between pre-T2DM and T2DM. Further, the presence of fatty liver in the control, pre-Mets and pre-T2DM as well as MetS and T2DM cases was significantly associated with increased sdLDL-C and sdLDL-C/LDL-C (%) compared to the cases without a fatty liver. The different characteristics of small and large LDL-C was clearly evident, as sdLDL-C was positively correlated with TG, while large LDL-C was negatively correlated with TG.

The serum concentrations of sdLDL-C and total LDL-C were positively correlated and approximately 30% of LDL-C was comprised of sdLDL-C, as shown in Figure1. The major portion of LDL-C in normal controls was comprised of large LDL-C. However, the correlation between sdLDL-C and TG (r=0.703) shown to be significantly higher than that of large LDL-C and TG (r=-0,176). This means that sdLDL-C is derived from TG-rich lipoproteins, but large LDL-C may not be directly derived from TG-rich lipoproteins. This metabolic pathway for the formation of sdLDL is still controversial, but lipoprotein lipase (LPL), hepatic triglyceride lipase (HTGL) and cholesteryl ester transfer protein (CETP) activities are known to play roles in the formation of sdLDL-C [32]. Nakajima et al. reportedly demonstrated a significant inverse correlation between sdLDL-C and LPL activity, but no correlation was found between sdLDL-C and HTGL (33). Although the metabolic pathway to form sdLDL has been reported to be related to HTGL activity [34, 35], the mechanism has not been reportedly elucidated. One of the purposes of this study was to determine the mechanism of sdLDL-C formation under certain special circumstances, such as a fatty liver and/or a large waist circumference.

The analysis of sdLDL-C and fatty liver was conducted in a comparatively large Japanese population at Hidaka Hospital, which is located outside Tokyo, an urban area where the healthy control subjects comprise a typical Japanese population. Subjects who possessed a healthy medical profile without the ingestion of any medications or abnormal physiological markers were selected on the basis of strict criteria for the determination of 75th percentile normal range of sdLDL-C. These group excluded fatty liver and hyperlipidemia. The upper cut off value was 27.5mg/dL in men and 23.3mg/dL in women based on the 75th percentile method. The sdLDL-C/ LDL-C ratio was approximately 20 % in both men and women. Because sdLDL-C was correlated with both TG and LDL-C, it was increased in hypertriglyceridemia and hypercholesterolemia. As expected, sdLDL-C was markedly increased in combined hyperlipidemia, and the majority of LDL-C was recovered in the sdLDL-C fraction. Familial combined hyperlipidemia is characterized by high levels of LDL particles (hyper apoB) and a preponderance of small dense LDL, and it is a representative disease associated with a high incidence of CHD [36] Therefore, measurement of sdLDL-C may be useful when screening for familial combined hyperlipidemia [37]. However, the subjects with severe hypertriglyceridemia including chylomicronemic subjects, were found to have only slightly elevated sdLDL-C levels. It is likely that the subjects with a TG level >400 mg/dL had a low level of LPL activity, which impaired the conversion of TG-rich lipoproteins to LDL and thus also resulted in a disproportionately low sdLDL-C concentration relative to the TG level. Such a modest elevation of sdLDL-C in subjects with severe hypertriglyceridemia might explain why the incidence of CHD is reportedly not increased further by a massive increase in the TG level.

In this study, we found that the serum sdLDL-C and sdLD-C/LDL-C (%) were significantly increased in the early stage of both Mets and T2DM with impaired glucose tolerance. Both of the preliminary disease conditions were significantly associated with an increase in waist circumference and the prevalence of a fatty liver compared with normal controls. As waist circumference reflect the amount of visceral fat, we compared the role of a fatty liver and visceral fat in the increase of sdLDL-C. Although a fatty liver and visceral fat often co-exist, the fatty liver is known to be more strongly associated with dyslipidemia and dysglycemia and to be independent of visceral fat [38]. sdLDL-C was additively increased in cases with a fatty liver in all five groups compared to the non-fatty liver cases (Table 2). This may mean that the presence of a fatty liver enhances the formation of serum sdLDL-C in controls cases as well as in the MetS and T2DM cases. We speculate that the development of a fatty liver comes first and this enhances the formation of sdLDL-C. Hosoyamada et al. [39] reported a relationship between a fatty liver and LDL particle size. They demonstrated an independent association between the presence of a fatty liver and the serum sdLDL-C levels by logistic regression analysis, after adjustment for such potential confounders as BMI and impaired fasting glucose levels. Toledo et al. [26] showed a positive relationship between a fatty liver and sdLDL particle size in patients with T2DM. Sugino et al. [27] suggested that a fatty liver synergistically interacts with metabolic syndrome so as to affect sdLDL-C levels. Based on these studies, a fatty liver appears to affect LDL particle size, an effect that may be independent of visceral obesity and/or systemic insulin resistance. However, Yatsuzuka et al [40] reported that sdLDL-C is not significantly associated with a fatty liver by multiple regression analysis in MetS. In our current study, multiple regression analyses ascertained that fatty liver is an independent determinant for sdLDL-C, but sdLDL-C was not an independent determinant of the presence of a fatty liver. Fatty liver may have more strongly associated variables than sdLDL-C. Therefore we have concluded from this large population study that fatty liver and sdLDL-C are independent of each other as Hosoyamada et al. reported [39].

Visceral obesity (in MetS) and insulin resistance (in T2DM) have been recognized as major causes of increased levels of sdLDL particles, because these factors are major contributors to postprandial hypertriglyceridemia, in which one of the underlying mechanisms is an increased free fatty acid release from adipocytes that stimulates hepatic TG output in the form of VLDL. Additionally, if a fatty liver is present, upregulated de novo synthesis of fatty acids may increase hepatic TG production. Donnely et al. [41] reported that approximately 60% of the fat that accumulates in the liver and is incorporated into lipoprotein is derived from circulating free fatty acids, while approximately 25% results from de novo lipid synthesis in patients with nonalcoholic fatty liver disease. In addition to altered TG output, a fatty liver has been shown to be associated with an increased TG content in large VLDL [28, 29]. Large VLDL efficiently promotes the modification of LDL particles via CETP. Recent studies showed that the liver X receptor (LXR)-sterol regulatory element-binding protein (SREBP)-1c pathway governs the size of the VLDL particles secreted by the liver [42, 43]. It is noteworthy that the LXR-SREBP-1c pathway is a major causative factor in the development of a fatty liver, because several genes involved in de novo fatty acid synthesis are expressed in response to up-regulated LXR-SREBP-1c signaling [44]. Thus, a fatty liver affects VLDL particles both quantitatively and qualitatively, resulting in both an increased amount of VLDL remnants and sdLDL formation.

The activities of CETP, LPL and HTGL are known to correlate with the serum LDL particle size [35, 45]. Lipoprotein lipase is responsible for a major step in TG clearance. Interestingly, Li et al. [46] reported that hepatic macrophage content is increased in a fatty liver and the plasma CETP levels significantly increased in correlation with the increase in hepatic macrophage content, not adipose tissue. This indicates that a fatty liver accumulates macrophages, which in turn enhances the expression of hepatic CETP levels. Lucero et al. [47] reported that plasma CETP levels are significantly increased in cases of a fatty liver. The major pathway in the formation of sdLDL-C is reported to be the combination of CETP and HTGL activity [34, 35]. The overproduction of VLDL, delayed clearance of VLDL remnants, a decrease in LPL and increased CETP as well as HTGL may individually or jointly increase the formation of sdLDL-C associated with a fatty liver. These results suggest that a fatty liver enhances the formation of sdLDL-C along with increased CETP. Therefore, treating a fatty liver may be of primary importance in order to decrease the risk of atherogenesis, associated with a reduction in the sdLDL-C levels by statins or other drugs in patients with pe-MetS or pre-T2DM in order to prevent cardiovascular disease.

It should be noted that this study has certain limitations. First, the association between a fatty liver and sdLDL-C was examined with multivariate analysis using age, BMI, hypertension, hyperlipidemia, hyperglycemia and sdLDL-C levels as co-variables. It is possible that additional factors that were not analyzed may have affected the results. Second, the fatty liver diagnosis was made using abdominal ultrasonography to identify fatty steatosis. Ultrasonography may not detect a subset of advanced alcohol or nonalcoholic fatty liver diseases—referred to as “burnt-out steatohepatitis:—which are characterized by a less pronounced fatty steatosis. Furthermore, ultrasonography does not always detect a fatty liver parallel with computed tomography measurements, detecting a comparatively high rate of false positives from our preliminary observation (unpublished data).

In conclusion, this homogeneous sdLDL-C assay technology constitutes an important technological advance in determining a large number of clinical samples in the course of routine clinical practice. The measurement of sdLDL-C in conjunction with a routine lipid profile should prove useful for cardiovascular risk stratification and global risk assessment in select populations. The availability of a rapid and precise method also allows the determination of sdLDL-C in pre-MetS and pre-DM patient serum as a routine laboratory assay to prevent the CHD. The presence of a fatty liver associated with T2DM and/or MetS may enhance the formation of sdLDL-C through the up-regulation of CETP and HTGL activity and thus increase the risk of cardiovascular disease. Therefore, the treatment of a fatty liver should be the primary therapeutic target, followed by an inhibition of CETP, activation of LPL or statins to reduce the sdLDL-C levels in the patients with MetS or T2DM.

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Figure legends

Figure 1.Correlations with LDL-C between large LDL-C and sdLDL-C. The 4388 plots with an open circle (sdLDL-C) and X (large LDL-C) reflects the total number of normal controls, MetS and

T2DM. The concentration of large LDL-C is more than 2 fold that of sdLD-C.

Figure 2. Correlations with TG between large LDL-C and sdLDL-C. The 4388 plots with open circle (sdLDL-C) and X (large LDL-C) reflects the total number of normal controls, MetS and T2DM. sdLDL-C is positively correlated with TG, but inversely correlated with large LDL-C.

Figure 3. Correlations with HDL between large LDL-C and sdLDL-C. The 4388 plots with open circle (sdLDL-C) and X (large LDL-C) reflects the total number of normal controls, MetS and T2DM. sdLDL-C, but not large LDL-C, is more potently and inversely correlated with HDL-C.

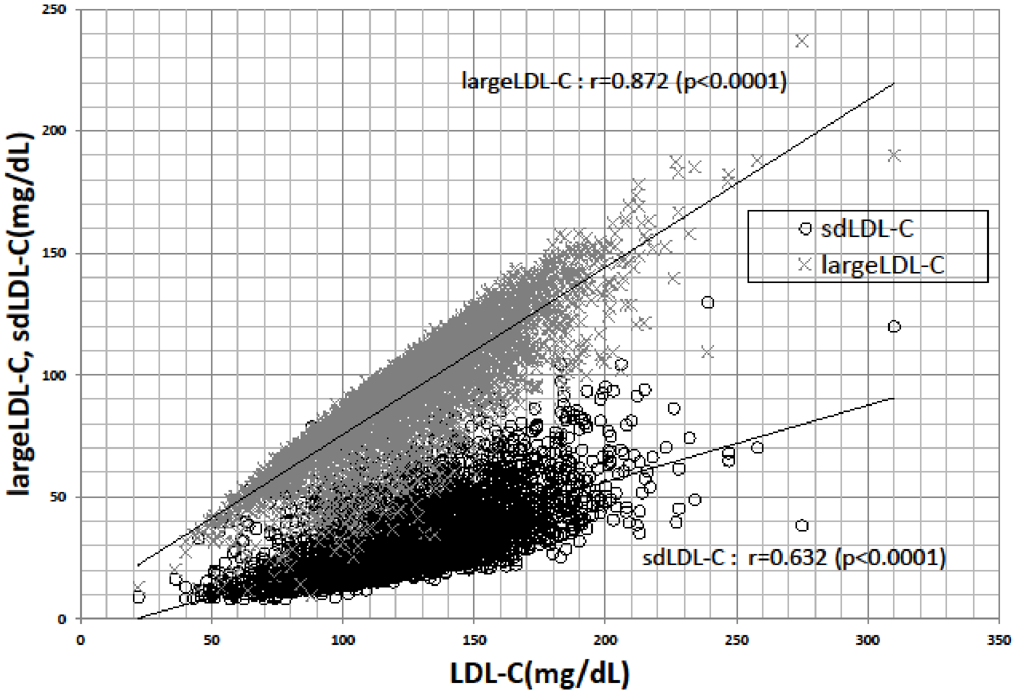

Fig. 1 Correlations between LDL-C and other cholesterols(n=4,388)

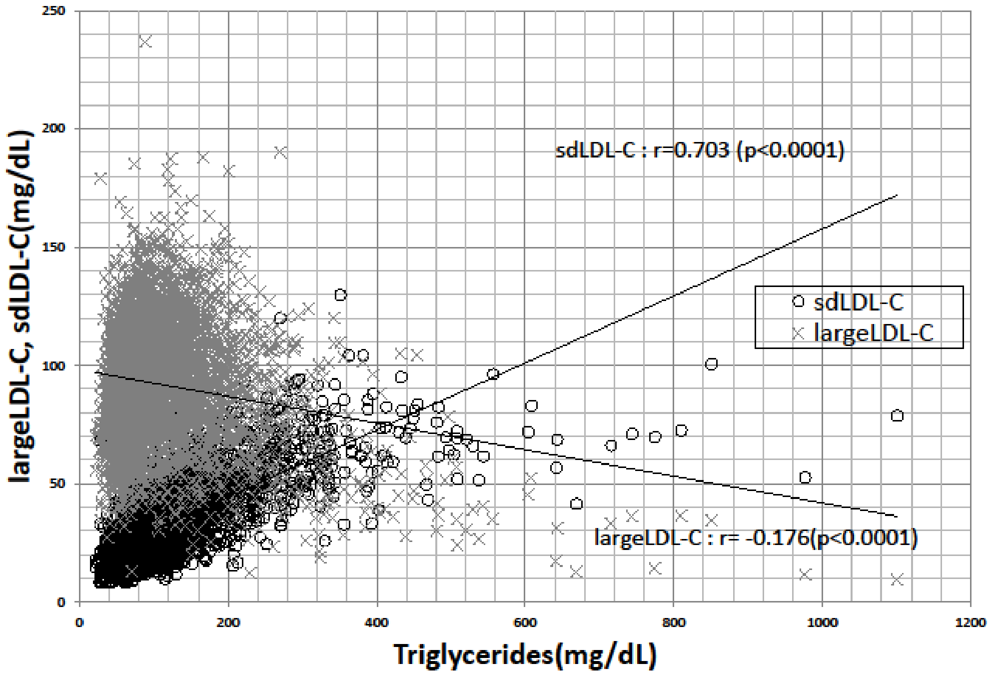

Fig. 2 Correlations between triglycerides and cholesterols(n=4,388)

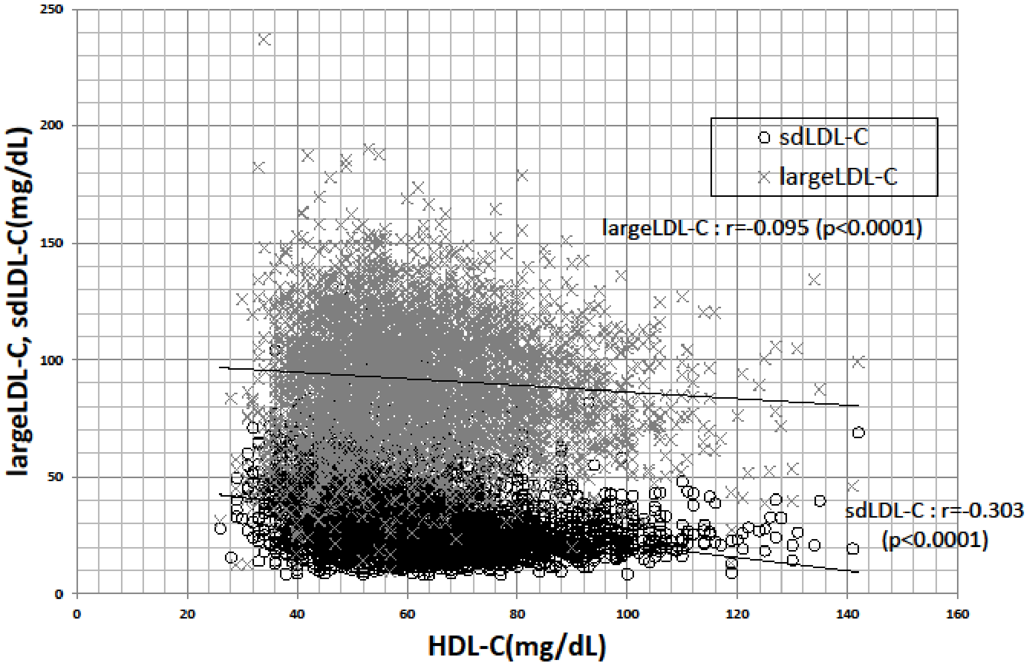

Fig. 3 Correlations between HDL-C and other cholesterols(n=4,388)

Table 1. Characteristics of subjects with or without metabolic syndrome(MetS) and DM status

[MEN]

Parameter	Units	A:Control (n=712)		3:PreMetS (n=544		C:MetS (n=414)		*P* value			D:PreDM (n=300)		E:DM (n=156)		*P* value		
		Median	75%tile	Median	75%tile	Median	75%tile	AvsB	AvsC	BvsC	Median	75%tile	Median	75%tile	AvsD	AvsE	DvsE
Age		46	52	48	55	51	57	<0.001 *	<0.001 *	<0.001 *	51	57	54	59	<0.001 *	<0.001 *	0.006 *
Weight	kg	64.5	70.0	75.7	81.3	77.0	83.6	<0.001 *	<0.001 *	0.009 *	70.7	77.5	71.0	79.8	<0.001 *	<0.001 *	0.734
BMI	kg/cm^2	22.1	23.7	25.5	27.5	26.1	28.4	<0.001 *	<0.001 *	<0.001 *	24.4	26.3	24.9	26.7	<0.001 *	<0.001 *	0.568
waist circumference	cm	80	84.5	90	95	92	97	<0.001 *	<0.001 *	<0.001 *	87	93	88	94	<0.001 *	<0.001 *	0.513
Systolic BP	mmHg	111	118	121	129	129	137	<0.001 *	<0.001 *	<0.001 *	124	133	123	132	<0.001 *	<0.001 *	0.684
Diastolic BP	mmHg	71	77	80	87	87	93	<0.001 *	<0.001 *	<0.001 *	81	90	80	88	<0.001 *	<0.001 *	0.349
FBG	mg/dL	96	100	101	106	112	122	<0.001 *	<0.001 *	<0.001 *	113	117	145	169	<0.001 *	<0.001 *	<0.001 *
HbA1c(NGSP)	%	5.5	5.6	5.6	5.8	5.8	6.3	<0.001 *	<0.001 *	<0.001 *	5.8	6.0	7.2	8.1	<0.001 *	<0.001 *	<0.001 *
Total cholesterol	mg/dL	190	206	211	236	215	236	<0.001 *	<0.001 *	0.131	215	236	204.5	225.5	<0.001 *	<0.001 *	0.026
Triglycerides	mg/dL	80.5	107	134	187	168	228.3	<0.001 *	<0.001 *	<0.001 *	127	176	128.5	174.3	<0.001 *	<0.001 *	0.704
HDL-C	mg/dL	61	71	51	60	49	56	<0.001 *	<0.001 *	0.012	54	66	50.5	59	<0.001 *	<0.001 *	0.001 *
LDL-C	mg/dL	109	124	135	155	134	156.8	<0.001 *	<0.001 *	0.610	132	152	130	152	<0.001 *	<0.001 *	0.556
sdLDL-C	mg/dL	23.0	28.0	39.0	49.8	46.0	60.0	<0.001 *	<0.001 *	<0.001 *	37.1	51.0	39.1	54.2	<0.001 *	<0.001 *	0.817
large LDL-C	mg/dL	85.7	95.9	95.0	112.4	91.1	108.0	<0.001 *	<0.001 *	0.008	93.5	109.5	91.1	109.1	<0.001 *	0.002 *	0.416
sdLDL-C / LDL-C	%	20.9	24.8	28.5	37.0	33.4	42.9	<0.001 *	<0.001 *	<0.001 *	28.5	36.9	29.9	39.9	<0.001 *	<0.001 *	0.539
Smoking, Yes	%	31		33		33		NS	NS	NS	30		33		NS	NS	NS
Alcohol, everyday	%	35		37		43					50		35				
	%	45		47		40		NS	NS	NS	36		40		0.001 *	NS	0.002 *
none	%	20		16		17					14		25				

[WOMEN]

Parameter	Units	A:Control (n=915)		B:PreMetS (n=47)		C:MetS (n=28)		*P* value			D:PreDM (n=58)		E:DM (n=32)		*P* value		
		Median	75%tile	Median	75%tile	Median	75%tile	AvsB	AvsC	BvsC	Median	75%tile	Median	75%tile	AvsD	AvsE	DvsE
Age		45	51	51	57	58	62	<0.001 *	<0.001 *	0.054	55	60	58	61	<0.001 *	<0.001 *	0.707
Weight	kg	51.9	56.6	72.3	77.3	67.4	72.9	<0.001 *	<0.001 *	0.121	57.6	63.3	59.8	63.2	<0.001 *	<0.001 *	0.441
BMI	kg/cm^2	20.5	22.4	28.9	30.2	27.1	29.8	<0.001 *	<0.001 *	0.120	24.1	26.2	24.9	26.2	<0.001 *	<0.001 *	0.374
waist circumference	cm	75	80	95	99	94	97	<0.001 *	<0.001 *	0.869	82	89	86	89	<0.001 *	<0.001 *	0.220
Systolic BP	mmHg	107	115	123	139	133	141	<0.001 *	<0.001 *	0.087	122	131	123	128	<0.001 *	<0.001 *	0.820
Diastolic BP	mmHg	66	72	78	89	79	89	<0.001 *	<0.001 *	0.887	75	80	75	78	<0.001 *	<0.001 *	0.752
FBG	mg/dL	92	97	98	103	115	127	<0.001 *	<0.001 *	<0.001 *	113	118	153	175	<0.001 *	<0.001 *	<0.001 *
HbA1c(NGSP)	%	5.4	5.6	5.7	5.9	6.0	6.6	<0.001 *	<0.001 *	0.002 *	5.9	6.0	7.4	7.7	<0.001 *	<0.001 *	<0.001 *
Total cholesterol	mg/dL	196	213	222	239	223.5	249.3	<0.001 *	<0.001 *	0.322	212	235.5	228.5	260	<0.001 *	<0.001 *	0.101
Triglycerides	mg/dL	64	82	121	143	172.5	253.3	<0.001 *	<0.001 *	0.002 *	98	122.5	116.5	152	<0.001 *	<0.001 *	0.092
HDL-C	mg/dL	71	81	59	65	52	61	<0.001 *	<0.001 *	0.116	59	72.5	58.5	66.3	<0.001 *	<0.001 *	0.676
LDL-C	mg/dL	104	120	137	151	134	170.5	<0.001 *	<0.001 *	0.466	130	145	140.5	171.8	<0.001 *	<0.001 *	0.135
sdLDL-C	mg/dL	19.8	23.6	32.6	43.7	48.8	57.4	<0.001 *	<0.001 *	0.001 *	31.3	39.8	41.4	50.9	<0.001 *	<0.001 *	0.049
large LDL-C	mg/dL	83.9	96.3	102.4	115.6	94.0	120.5	<0.001 *	0.009 *	0.308	99.6	112.0	101.4	121.9	<0.001 *	<0.001 *	0.164
sdLDL-C / LDL-C	%	19.2	21.8	23.0	28.0	28.7	40.4	<0.001 *	<0.001 *	0.004 *	22.5	28.5	26.0	32.6	<0.001 *	<0.001 *	0.217
Smoking, Yes	%	9		6		4		NS	NS	NS	9		6		NS	NS	NS
Alcohol, everyday	%	13		11		7					10		6				
	%	46		34		32		NS	NS	NS	45		31		NS	NS	NS
none	%	41		55		61					45		63				

∗ : statistical significance ($P<0.05$, P value is corrected by Bonferoni's method) , smoking and alcohol were caliculated with Chi-square test

Table 2. Characteristics of subjects with or without fatty liver

[MEN]

Parameter	Units	Control (healthy subjects) A:Non-fatty liver (n=664) Median	75%tile	B:Fatty liver (n=108) Median	75%tile	P value AvsB	preMetS C:Non-fatty liver (n=221) Median	75%tile	D:Fatty liver (n=223) Median	75%tile	P value CvsD	MetS C:Non-fatty liver (n=120) Median	75%tile	D:Fatty liver (n=294) Median	75%tile	P value CvsD	preDM E:Non-fatty liver (n=144) Median	75%tile	F:Fatty liver (n=156) Median	75%tile	P value EvsF	DM E:Non-fatty liver (n=61) Median	75%tile	F:Fatty liver (n=95) Median	75%tile	P value EvsF
Age		46	52	46	52	0.810	51	57	47	53	<0.001 *	54	59	51	56	<0.001 *	52	57	51	56	0.085	57	62	52	58	<0.001 *
Weight	kg	63.7	69.5	68.8	75.9	<0.001 *	72.5	77.7	78.0	85.7	<0.001 *	72.7	77.1	78.8	85.9	<0.001 *	66.3	72.2	75.8	82.8	<0.001 *	63.9	69.8	77.1	84.8	<0.001 *
BMI	kg/cm^2	21.8	23.3	24.2	25.8	<0.001 *	24.8	25.8	26.2	28.1	<0.001 *	24.7	25.6	26.8	29.3	<0.001 *	23.1	24.5	26.0	28.4	<0.001 *	22.8	24.5	25.8	27.9	<0.001 *
waist circumference	cm	79	83	85	90	<0.001 *	88	91	91.5	96	<0.001 *	89	92	94	100	<0.001 *	85	87	91	97	<0.001 *	83	88	91	96	<0.001 *
Systolic BP	mmHg	111	118	114	120	0.005 *	121	130	120	129	0.823	131	140	128	136	0.227	123	133	124.5	134	0.708	119	132	126	133	0.062
Diastolic BP	mmHg	71	77	74	78	0.006 *	80	88	80	87	0.855	88	94	87	92	0.129	80	89	82	90	0.343	78	84	82	89	0.035 *
FBG	mg/dL	96	100	97	101	<0.001 *	100	105	101	106	0.034 *	111	118	113	125	0.024 *	113	115	114	118	0.012 *	139	156	150	182	0.004 *
HbA1c(NGSP)	%	5.4	5.6	5.5	5.6	0.256	5.5	5.7	5.6	5.8	<0.001 *	5.8	6.0	5.9	6.3	<0.001 *	5.7	5.9	5.9	6.0	<0.001 *	6.8	7.6	7.4	8.8	<0.001 *
Total cholesterol	mg/dL	191	207	188	203	0.307	208	235	213	237	0.236	207	230	219	238	0.065	214	236	218	236	0.628	202	222	206	234	0.206
Triglycerides	mg/dL	79	106	98	117	<0.001 *	121	165	144	209	<0.001 *	150	194	176	240	<0.001 *	113	142	147	196	<0.001 *	96	128	152	214	<0.001 *
HDL-C	mg/dL	62	71	54	61	<0.001 *	54	62	48	57	<0.001 *	54	64	47	54	<0.001 *	61	72	50	57	<0.001 *	58	68	46	54	<0.001 *
LDL-C	mg/dL	108	123	117	127	0.003 *	130	151	138	158	0.008 *	129	144	139	159	<0.001 *	127	145	136	159	0.002 *	124	141	132	155	0.026 *
sdLDL-C	mg/dL	22.6	27.5	25.4	31.0	0.002 *	37.0	48.2	40.1	50.7	0.005 *	39.2	52.7	48.8	61.5	<0.001 *	34.5	48.9	40.4	55.3	<0.001 *	26.7	44.2	42.8	61.5	<0.001 *
large LDL-C	mg/dL	85.3	95.2	88.1	100.1	0.024 *	92.7	109.6	95.7	113.7	0.218	87.6	101.7	92.6	109.6	0.047 *	92.7	105.1	96.5	113.4	0.100	88.6	109.1	93.2	109.1	0.798
sdLDL-C / LDL-C	%	20.8	24.6	22.3	25.7	0.142	27.3	35.3	29.1	37.8	0.092	32.7	40.4	33.9	44.5	0.099	27.3	34.8	29.8	33.4	0.026 *	24.8	33.9	32.2	44.3	<0.001 *
AST	IU/L	21	25	22	26	0.044 *	22	26	25	31	<0.001 *	22	26	27	34	<0.001 *	22	26	24	31	<0.001 *	21	25	25	34	<0.001 *
ALT	IU/L	20	25	24	34	<0.001 *	24	31	35	49	<0.001 *	23	30	37	53	<0.001 *	22	28	34	49	<0.001 *	21	29	36	57	<0.001 *
γ-GT	IU/L	23	35	26	34	0.010 *	35	59	41	60	0.010 *	43	63	53	90	<0.001 *	30	62	45	75	0.005 *	30	43	53	75	<0.001 *
ALP	IU/L	198	232	206	234	0.354	206	237	206	243	0.440	206	233	210	263	0.002 *	203	232	213	261	0.028 *	213	240	224	256	0.143 *
ChE	IU/L	332	375	364	397	<0.001 *	363	411	388	430	<0.001 *	360	406	393	444	<0.001 *	364	404	386	436	<0.001 *	343	391	389	445	<0.001 *

[WOMEN]

Parameter	Units	Control (healthy subjects) A:Non-fatty liver (n=6948) Median	75%tile	B:Fatty liver (n=61) Median	75%tile	P value AvsB	preMetS C:Non-fatty liver (n=19) Median	75%tile	D:Fatty liver (n=20) Median	75%tile	P value CvsD	MetS C:Non-fatty liver (n=78) Median	75%tile	D:Fatty liver (n=21) Median	75%tile	P value CvsD	preDM E:Non-fatty liver (n=35) Median	75%tile	F:Fatty liver (n=23) Median	75%tile	P value EvsF	DM E:Non-fatty liver (n=10) Median	75%tile	F:Fatty liver (n=22) Median	75%tile	P value EvsF
Age		48	51	46	53	0.093	55	61	50	53	0.188	55	60	56	60	0.030 *	55	61	53	59	0.089	60	65	58	59	0.095
Weight	kg	51.8	56.0	62.3	67.3	<0.001 *	65.0	69.9	75.8	79.4	<0.001 *	63.1	65.9	70.9	76.5	0.030 *	54.4	59.4	63.3	68.2	<0.001 *	52.2	56.8	66.9	66.8	0.003 *
BMI	kg/cm^2	20.4	22.0	25.0	27.3	<0.001 *	26.3	27.4	29.5	31.0	<0.001 *	24.7	27.1	27.8	29.8	0.094	22.1	24.1	26.3	28.8	<0.001 *	21.7	25.1	25.1	27.2	0.027 *
waist circumference	cm	74	79	86	92	<0.001 *	92	95	96	101	0.001 *	95	98	94	97	0.798	79	85	89	95	<0.001 *	82	87	86	91	0.089
Systolic BP	mmHg	107	115	111	120	<0.001 *	123	143	123	137	0.480	141	157	132	140	0.049 *	119	131	127	136	0.809	124	128	122	129	0.903
Diastolic BP	mmHg	65	72	70	75	<0.001 *	78	89	79	89	0.879	88	101	74	84	0.024 *	74	79	75	85	0.309	74	76	77	80	0.427
FBG	mg/dL	92	96	96	101	<0.001 *	96	99	102	107	0.065 *	107	112	119	130	0.012 *	113	117	114	119	0.184	148	183	153	175	0.207
HbA1c(NGSP)	%	5.4	5.6	5.6	5.7	<0.001 *	5.6	5.8	5.8	6.0	0.045 *	5.7	6.2	6.1	6.8	0.242	5.8	6.0	5.9	6.0	0.308	6.8	7.0	7.5	8.3	0.008 *
Total cholesterol	mg/dL	196	213	189	211	0.382	220	248	217	228	0.036 *	224	266	220	248	0.176	210	236	221	242	0.289	217	232	237	269	0.077
Triglycerides	mg/dL	63	80	86	111	<0.001 *	129	146	112	136	0.274	166	290	179	243	0.730	83	111	115	146	0.017 *	73	100	131	191	0.002 *
HDL-C	mg/dL	71	82	63	72	<0.001 *	60	76	55	63	0.077	57	62	52	59	0.490	68	77	55	60	0.009	71	77	58	63	0.061
LDL-C	mg/dL	104	119	111	131	0.016 *	135	154	138	148	0.880	131	171	135	169	0.811	120	139	142	150	0.063	120	127	164	193	0.002 *
sdLDL-C	mg/dL	19.6	23.3	22.2	27.3	0.004 *	30.6	41.4	33.8	45.1	0.801	51.1	57.2	48.8	57.1	0.807	29.2	36.6	35.9	49.9	0.043 *	27.2	33.1	44.6	56.3	0.002 *
large LDL-C	mg/dL	83.8	96.0	86.4	99.1	0.080	102.4	107.4	107.2	116.6	0.442	102.6	46.3	93.8	112.3	0.791	97.2	107.6	104.3	117.6	0.246	89.8	104.0	115.0	124.7	0.017 *
sdLDL-C / LDL-C	%	19.2	21.7	19.8	23.2	0.082	22.5	27.6	24.2	29.4	0.012	29.7	123.1	27.9	36.6	0.570	22.1	26.3	24.5	33.4	0.133	22.7	25.8	28.8	34.4	0.113
AST	IU/L	19	22	19	25	0.167	19	21	24	30	0.017 *	20	26	25	34	0.151	22	27	23	32	0.388	20	21	22	30	0.048 *
ALT	IU/L	15	18	21	31	<0.001 *	18	21	29	42	<0.001 *	20	22	38	48	0.006 *	19	28	23	47	0.011 *	17	20	26	42	<0.001 *
γ-GT	IU/L	14	19	21	29	<0.001 *	19	21	24	43	0.013 *	20	30	42	53	0.046 *	18	27	24	48	0.013 *	18	23	29	38	0.001 *
ALP	IU/L	166	197	189	236	<0.001 *	201	250	197	250	0.897	211	252	226	263	0.710	236	266	229	260	0.874	190	257	230	296	0.080
ChE	IU/L	290	329	327	377	<0.001 *	373	402	386	414	0.783	442	449	417	459	0.821	373	397	380	441	0.781	355	375	410	444	0.104

large LDL-C = LDL-C(mg/dL)-sdLDL-C(mg/dL)   * : statistical significance (P<0.05)   # : 1woman without ChE data ıble 3. Selected percentiles for sdLDL-C distribution of healthy subjects without fatty liver (mg/ı

Gender	Age	n	5th	10th	25th	50th	75th	90th	95th
Men	<30	4	15.7	16.9	20.2	23.2	24.8	26.0	26.3
	30-39	127	12.2	13.9	17.1	20.3	25.4	30.9	33.0
	40-49	274	13.1	14.4	18.5	22.6	27.2	32.0	37.2
	50-59	163	13.3	15.3	19.1	23.3	28.4	35.3	41.6
	60-69	35	15.8	16.7	19.7	25.7	32.1	38.2	44.8
	70≦	1							
	All	604	13.0	14.5	18.3	22.6	27.5	32.9	38.3
Women	<30	4	14.1	15.1	18.0	19.9	20.5	21.3	21.5
	30-39	197	10.8	11.9	14.6	18.0	21.0	25.1	27.3
	40-49	421	12.5	13.6	16.2	19.1	22.3	26.6	29.6
	50-59	201	14.3	15.6	18.5	22.0	25.7	28.8	31.8
	60-69	37	16	17.6	21.8	25.3	28.4	29.7	31.1
	70≦	4	18.2	18.8	20.5	24.6	27.6	27.6	27.6
	All	864	12.2	13.5	16.3	19.6	23.3	27.6	29.9