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Relationship between plasma lipid concentrations and HDL subclasses

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Abstract

Background: It is generally accepted that different high-density lipoprotein (HDL) subclasses have distinct but interrelated metabolic functions. HDL is known to directly influence the atherogenic process and changes in HDL subclasses distribution may be related to the incidence and prevalence of atherosclerosis.
Methods: Apo-AI contents(mg/l) of plasma HDL subclasses were determined by 2-dimensional gel electrophoresis coupled with immunodetection for apo-AI. Four hundred forty-two Chinese adults subjects aged 33 to 78 years were assigned to different groups according to the third Report of NCEP (ATP III) guidelines. The subjects were first divided into 2 groups, normal and high TG, then further classified by plasma TC, HDL-C and LDL-C concentrations. The subjects were also divided into TC desirable and TC high groups.
Results: Apo-A contents of preβ_1-HDL were higher while HDL_{2b} were lower in high TG subjects vs. the corresponding normal TG subjects according to plasma TC and LDL-C concentrations. With the increase of plasma TC concentrations, apo-AI contents of preβ_1-HDL were significantly higher in high TC subgroup vs. TC desirable subgroup in normal TG subjects. With the decrease of HDL-C concentrations, apo-AI contents of HDL_{2b} tended to decrease in normal TG subjects. And, with the increases of LDL-C concentration, in normal TG subjects, apo-AI contents of preβ_1-HDL and HDL_{3b} were significantly higher and those of HDL_{2b} were significantly lower in very high LDL-C subgroup vs. LDL-C optimal subgroup. On the other hand, apo-AI contents of preβ_1-HDL and HDL_{3a} were significantly higher, while HDL_{2a} and HDL_{2b} were significantly lower in high TG and very high TG subgroup vs. normal TG subgroup within either TC desirable or TC high subjects. In a multivariate linear regression model, TG and TC concentrations were all associated independently and positively with high preβ_1-HDL; however, HDL-C were inversely associated with high preβ_1-HDL. And TG and TC concentrations were all associated independently and negetively with low HDL_{2b}, but HDL-C and apo-AI were positively associated with low HDL_{2b}.
Conclusions: With the increase of plasma TG, TC, LDL-C or the decrease of plasma HDL-C concentrations, there was a general shift toward smaller-sized HDL, which, in turn, indicates that reverse cholesterol transport might be weakened and HDL

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maturation might be abnormal. Plasma TG concentration is a more important factor than TC concentration on the changes of HDL subclass distribution. Moreover, when TG is normal and HDL-C decreased, large-size HDL particles tended to decrease.

Keywords: Apo-AI containing HDL subclasses; Triglyceride; Total cholesterol; High-density lipoprotein-cholesterol; Low-density lipoprotein-cholesterol; Two-dimensional gel electrophoresis-immunodetection

1. Introduction

Numerous clinical and epidemiological studies have firmly established an inverse relation between the risk of coronary heart disease (CHD) and the concentration of high-density lipoprotein-cholesterol (HDL-C) [1]. The HDL is responsible for reverse cholesterol transport (RCT). RCT describes the metabolism and an important antiatherogenic function of HDL, namely, the HDL-mediated efflux of cholesterol from cells of the arterial wall and its subsequent delivery to the liver and steroidogenic organs [2–5].

However, HDL has in common a high density (>1.063 g/ml) and a small size (Stoke's diameter 5–17 nm) [6]. HDL particles are composed of outer layer containing free cholesterol, phospholipids, various apolipoproteins, which covers a hydrophobic core consisting primarily of triglycerides and cholesterol esters. The majority of the HDL particles contain apo-AI [6]. Differences in the quantitative and qualitative content of lipids, apolipoproteins, enzymes and lipid transfer proteins result in the presence of various HDL subclasses, which are characterized by differences in shape, density, size, charge and antigenicity [6]. Subclasses of HDL can be separated by zonal [7] or single-spin vertical ultracentifugation [8], heparin-magnesium precipitation [9], nuclear magnetic resonance (NMR) spectroscopy [10], or 1- and 2-dimensional polyacrylamide gel electrophoresis [11–13].

Using agarose gel electrophoresis, HDL can be separated into 2 parts, i.e., preβ- and α-HDL. Pre-β part can be further distinguished by subsequent polyacrylamide gradient gel electrophoresis into preβ$_1$-, preβ$_2$-, preβ$_3$-HDL and α-HDL can be separated into 5 distinct subclasses HDL$_{3c\ 3b\ 3a\ 2a\ 2b}$, according to their increasing particle size [14,15]. Apo-AI, probably the discoid shape preβ$_1$-HDL (the smallest preβ-HDL), binds to the adenosine triphosphate-binding cassette transporter A1 (ABCA1), thus allowing the transfer of free cholesterol and phospholipidids from cells to HDL [16]. Pre-β$_1$-HDL is transformed by the activity of lecithin: cholesterol acyltranstransferase (LCAT), which esterifies the free cholesterol to form α-HDL particles, which can also be formed by diffusion of cholesterol from cell membranes and by interactions with the scavenger receptor B1(SR-B1). With the further participation of LCAT and other specific plasma factors, i.e., hepatic lipase (HL), the cholesteryl ester transfer protein (CETP) and the phospholipids transfer protein (PLTP), cholesteryl ester is concentrated into the center of the lipoprotein molecule, and HDL particle is transformed from nascent discoidal preβ-HDL to mature spherical HDL$_2$. It has been postulated that RCT indeed was the metabolic process that nascent preβ-HDL converted to mature α-HDL, following the route of preβ$_1$-HDL→preβ$_2$-HDL→preβ$_3$-HDL→HDL$_3$→HDL$_2$. Due to the important role of RCT in maintaining the cholesterol homeostasis and anti-antherosclerosis, the metabolic process of HDL and HDL subclasses distribution may directly influence the antherogenic process and change in HDL distribution may be closely related to the incidence and prevalence of atherosclerosis [17–19].

Miida et al. [25] found that the apo-AI contents of preβ$_1$-HDL in patients with hypercholesterolemia were significantly higher than those with normolipidemia. Saidi et al. [26] demonstrated that patients with mixed hyperlipidemia increased concentrations of small-sized HDL particles (HDL$_{3b}$ and HDL$_{3a}$) and decreased concentrations of large-sized HDL particles (HDL$_{2a}$ and HDL$_{2b}$) [27]. We have investigated the plasma HDL subfractions distribution in hyperlipidemic, obese, non-insulin-dependent diabetes mellitus (NIDDM) and CHD subjects by 2-dimensional gel electrophesis associated with immunodection [20–24]. We found that the characteristic of the transformation of HDL subclasses in these patients appeared to be different, whereas there was a general shift toward smaller sized HDL (preβ$_1$-HDL increased while HDL$_{2a}$ and HDL$_{2b}$ decreased), suggesting that RCT

might be weakened and HDL maturation might be abnormal in all subjects. We found that the concentrations of triglyceride (TG) in subjects with hyperlipidemia showed positive correlation with preβ_1-HDL and negative correlation with HDL_{2b} [20]; with the increase of TG and the decrease of HDL-C, the particle of HDL shifted toward smaller size in subjects with CHD [22]. Thus, in this study of patients classified according to ATP-III [26], we compared changes of apo-AI-containing HDL subclasses in various groups in order to provide evidence on the relationship between subclasses and atherosclerosis.

2. Subjects and methods

2.1. Subjects

Study subjects consisted of 442 Chinese adults who were either current or retired staff. 292 subjects were from the Sichuan University and Sichuan Normal University, in Chengdu, Sichuan province, and 150 subjects from the Nanhua University, in Hengyang, Hunan province. There were 139 females and 303 males, aged 33 to 78 years recruited to the study. According to the third Report of NCEP(ATP-III) [28], the subjects were first divided into 2 groups, normal TG (TG<1.69 mmol/l) and high TG (TG≥1.69 mmol/l) groups, and then, were then further classified by plasma TC, HDL-C and LDL-C concentrations. The subjects were also divided into TC desirable (TC<5.17 mmol/l) and TC high group (TC≥5.17 mmol/l).

2.1.1. TC subgroup

200 normal TG subjects were further divided into 3 subgroups: desirable TC (<5.17 mmol/l, n=80), borderline-high TC (5.17–6.18 mmol/l, n=75) and high TC (≥6.21 mmol/l, n=45). Among the high TG subjects, there were 90 desirable TC, 87 borderline-high TC and 65 high TC subjects.

2.1.2. HDL-C group

200 normal TG subjects were divided into 3 subgroups: high HDL-C (≥1.55 mmol/l, n=65), middle HDL-C (1.03–1.52 mmol/l, n=116) and low HDL-C (<1.03 mmol/l, n=19). Among the 242 high TG subjects, there were 33 high HDL-C, 86 middle HDL-C and 23 low HDL-C subjects.

2.1.3. LDL-C group

200 normal TG subjects were divided 3 subgroups: optimal LDL-C (<2.59 mmol/l, n=34), near-optimal LDL-C (2.59–3.34 mmol/l, n=50), borderline-high LDL-C (3.36–4.11 mmol/l, n=67), high LDL-C (4.14–4.89 mmol/l, n=34) and very high LDL-C (≥4.91 mmol/l, n=15). Among the 242 high TG subjects, there were 79 optimal LDL-C, 63 near-optimal LDL-C, 50 borderline-high LDL-C, 33 high LDL-C, and 17 very high LDL-C subjects.

2.1.4. TG group

170 TC desirable subjects were divided into 4 subgroups: normal TG (<1.69 mmol/l, n=80), borderline-high TG (1.69–2.25 mmol/l, n=16) , high TG (2.26–5.64 mmol/l, n=64) and very high TG (≥5.65 mmol/l, n=10). Among the 274 high TC subjects, there were 120 normal TG, 38 borderline-high TG, 97 high TG and 17 very high TG subjects.

2.2. Specimens

Whole blood specimens were drawn after a 12-h overnight fast into EDTA-containing tubes. Plasma was separated within 1–2 h, stored at 4 °C, and used within 24 h for lipid and apolipoprotein analyses. An aliquot of plasma was stored at −70 °C for the determination of HDL subclasses.

2.3. Plasma lipid analyses

Plasma TG, TC and HDL-C were measured by standard techniques. TC and TG were determined with enzymatic kits (Beijng Zhongsheng Biotechnological, Beijing). HDL-C was determined after precipitation of the apolipoprotein (apo)B-containing lipoproteins by phosphotungstate/magnesium chloride [29]. When TG<4.52 mmol/l, LDL-C was calculated using Friedwald formula [30]. When TG≥4.52 mmol/l, LDL-C was determined with enzymatic kits (Beijng Zhongsen).

2.4. HDL subclasses analyses

HDL subclasses distributions were determined with 2-dimensional gel electrophesis associated with immunodection method as described previously [20]. Briefly, 10 μl of plasma was applied to 0.7% agarose

gel in the first dimension. After electrophoretic separation of lipoproteins in agarose gels, they were further separated by electrophoresis in 2–30% non-denaturing polyacrylamide gradient gel in the second dimension. To determine HDL subclasses, western blotting was conducted after electrophesis, using HRP-labeled goat anti-human apo-AI-IgG. The relative concentration of each HDL subclass was calculated as the percentage of plasma apo-AI (%) according to the density of each spot. HDL particle sizes were calibrated using a standard curve that included bovine serum albumin, ferritin and thyroglobulin (Pharmacia). Then the relative percentage concentration of each HDL subclass was multiplied by apo-AI concentrations in sample individuals, respectively. The result was the relative concentration of each HDL subclass of apo-AI (mg/l, apo-AI in the subclasses). The interassay CVs of relative content of preβ_1-HDL, preβ_2-HDL, HDL_{3c}, HDL_{3b}, HDL_{3a}, HDL_{2a} and HDL_{2b} in plasma sample were 9.4%, 9.8%, 4.9%, 6.2%, 7.3%, 11.1% and 7.9%, respectively (n=5).

2.5. Statistical analysis

Data are presented as mean±standard deviation. The between-group differences were evaluated by an analysis of variance, and the relationship between plasma lipid, apolipoproteins (TG, TC, HDL-C, LDL-C, apo-AI, apoB100) and HDL subclasses was analyzed by multivariate linear regression. Statistical analyses were performed using SPSS statistical packages. In all comparisons, a $p<0.01$ was considered statistically significant.

3. Result

3.1. Apo-AI contents of plasma HDL subclasses according to plasma TC concentrations in normal TG and high TG subjects

Two-dimensional gel electrophoresis and immunodetection disclosed HDL subclasses distribution according to plasma TC concentrations in 442 subjects. As shown in Table 1, in normal TG subjects, apo-AI contents of preβ_1-HDL ($p<0.01$) were significantly higher in TC high subgroup vs. TC desirable subgroup. In addition, apo-AI contents of preβ_1-HDL were significantly higher while HDL_{2b} were significantly lower in high TG subjects vs. corresponding normal TG subgroup ($p<0.01$, $p<0.01$, except TC high subgroup).

3.2. Apo-AI contents of plasma HDL subclasses according to plasma HDL-C concentrations in normal TG and high TG subjects

Table 2 shows the apo-AI content of plasma HDL subclasses according to plasma HDL-C concentrations in normal TG and high TG subjects. In normal TG subjects, with the decrease of plasma HDL-C, apo-AI content of preβ_1-HDL had the same concentration; however, we observed that those of HDL_{2b}

Table 1
Apo-AI content of plasma HDL subclasses according to plasma TC concentration in normal TG and high TG subjects (mg/l, $\bar{x}\pm s$)

	Normal TG			High TG		
	TC desirable	TC borderline-high	TC high	TC desirable	TC borderline-high	TC high
n	80	75	45	90	87	65
preβ_1-HDL	79.2±21.9	82.8±26.3	138.7±40.1[a,b]	112.9±43.7[a,c]	121.5±47.9[a,c]	147.5±54.5
preβ_2-HDL	47.0±13.6	60.0±19.1	64.3±24.3	54.9±20.8	63.9±25.4	56.2±18.8
HDL_{3c}	69.2±25.9	76.1±31.2	91.3±39.6	66.7±27.2	74.3±27.6	77.5±31.4
HDL_{3b}	128.8±38.2	150.8±58.7	178.5±57.9	136.7±45.9	141.1±44.5	164.7±59.2
HDL_{3a}	245.2±48.1	287.7±73.8	315.0±92.5	293.7±99.1	305.1±90.6	275.8±71.6
HDL_{2a}	270.6±67.5	290.5±78.1	276.1±77.2	224.3±67.8	232.0±74.4	233.2±65.1
HDL_{2b}	387.7±106.3	385.9±100.3	310.8±103.1	258.5±100.8[a,c]	263.6±91.8[a,c]	258.2±98.3

[a] $p<0.01$.
[b] Compared with TC desirable subgroup within the same group.
[c] Compared with normal TG relevant subgroup.

Table 2
Apo-AI contents of plasma HDL subclasses according to plasma HDL-C concentrations in normal TG and high TG subjects (mg/l, $\bar{x}\pm s$)

	Normal TG			High TG		
	High HDL-C	Normal HDL-C	Low HDL-C	High HDL-C	Normal HDL-C	Low HDL-C
n	65	116	19	33	86	123
preβ_1-HDL	96.4±30.0	105.2±47.4	110.4±43.2	101.4±49.8	119.9±50.6	134.5±47.7
preβ_2-HDL	62.6±21.9	56.7±19.3	53.7±20.6	64.2±18.4	57.1±24.2	60.3±21.9
HDL_{3c}	71.6±29.4	70.2±21.5	77.1±36.9	64.2±23.2	79.1±29.5	68.7±25.6
HDL_{3b}	135.5±51.1	155.7±58.3	160.1±57.3	143.9±48.5	150.4±57.0	143.8±45.6
HDL_{3a}	295.4±87.7	274.6±75.1	323.8±103.5	257.1±65.1	292.4±86.6	308.7±95.2
HDL_{2a}	308.9±89.0	266.1±60.3	267.1±71.6	257.8±68.1	252.4±75.1	213.0±59.1
HDL_{2b}	386.2±117.2	369.1±100.8	296.5±92.2	310.9±108.6	293.5±92.3	231.6±82.9[a,b]

[a] $p<0.01$.
[b] Compared with high HDL-C subgroup within the same group.

tended to decrease and, i.e., those of HDL_{2b} were lower 23% in low HDL-C subgroup than high HDL-C subgroup ($p<0.05$).

In high TG subjects, with the decrease of plasma HDL-C, apo-AI contents of preβ_1-HDL tended to be higher, while the apo-AI contents of HDL_{2b} ($p<0.01$) were significantly lower in low HDL-C subgroup vs. high HDL-C subgroup. Furthermore, apo-AI contents of HDL subclasses had no significantly difference in high TG subjects vs. the corresponding normal TG subgroup.

3.3. Apo-AI contents of plasma HDL subclasses according to plasma LDL-C concentrations in normal TG and high TG subjects

Table 3 shows the apo-AI contents of plasma HDL subclasses according to plasma LDL-C concentrations in normal and high TG subjects. In normal TG subjects, apo-AI contents of preβ_1-HDL ($p<0.01$) and HDL_{3b} ($p<0.01$) were significantly higher in very high LDL-C subgroup vs. LDL-C optimal subgroup, while apo-AI contents of HDL_{2b} ($p<0.01$) were significantly lower in the very high LDL-C subgroup vs. LDL-C optimal subgroup. Compared with corresponding normal TG subgroup, apo-AI contents of preβ_1-HDL had the same high concentrations (except very high LDL-C subgroup), in contrast, those of HDL_{2b} mostly remained the low concentrations in high TG subjects. Herein, apo-AI contents of preβ_1-HDL were significantly higher in LDL-C optimal, near-optimal and borderline-high subgroups ($p<0.01$, $p<0.01$, $p<0.01$), and those of HDL_{2b} were significantly lower in LDL-C optimal and near-optimal subjects ($p<0.01$, $p<0.01$).

3.4. Apo-AI contents of plasma HDL subclasses according to plasma TG concentrations in TC desirable and TC high subjects

Table 4 shows the apo-AI contents of plasma HDL subclasses according to plasma TG concentrations in TC desirable and TC high subjects. In TC desirable subjects, apo-AI contents of preβ_1-HDL ($p<0.01$, $p<0.01$) were significantly higher in high and very high TG subgroups vs. normal TG subgroup. While compared with normal TG subgroup, those of HDL_{2a} in very high TG subgroup ($p<0.01$) and HDL_{2b} in high and very high TG subgroups ($p<0.01$, $p<0.01$) were significantly lower. In TC high subjects, the apo-AI contents of preβ_1-HDL in high and very high TG subgroups ($p<0.01$, $p<0.01$) and HDL_{3a} in very high TG subgroup ($p<0.01$) were significantly higher than normal TG subgroup. In contrast, apo-AI contents of HDL_{2a} in very high TG subgroup ($p<0.01$) and HDL_{2b} in high and very high TG subgroups ($p<0.01$, $p<0.01$) were significantly lower than normal TG subgroup.

4. Multivariate analysis

To obtain a better understanding of the determinants of HDL subfraction distribution, we performed stepwise multivariate regression analyses with apo-AI contents of preβ_1-HDL (log-transformed) and apo-AI

Table 3
Apo-AI content of plasma HDL subclasses according to plasma LDL-C concentration in normal TG and high TG subjects (mg/l, $\bar{x}\pm s$)

LDL-C	Normal TG					High TG				
	Optional	Near optimal	Borderline high	High	Very high	Optional	Near optimal	Borderline high	High	Very high
n	34	50	67	34	15	79	63	50	33	17
preβ$_1$-HDL	77.6±26.1	79.1±18.8	85.0±25.1	123.2±44.7	147.8±37.2[a,b]	129.6±51.6[a,c]	120.5±51.0[a,c]	116.2±44.3[a,c]	121.7±50.8	169.9±34.0
preβ$_2$-HDL	56.1±22.2	50.7±12.6	59.6±18.9	73.5±30.7	56.7±11.3	59.5±23.7	62.9±25.7	55.3±18.0	57.3±19.5	50.4±20.0
HDL$_{3c}$	74.8±28.0	68.1±25.2	75.5±31.7	86.5±32.1	98.7±46.1	69.8±28.2	72.2±25.9	69.3±25.0	84.4±29.3	104.9±43.0
HDL$_{3b}$	139.3±37.0	133.7±55.8	148.4±58.7	169.4±52.3	194.4±56.9[a,b]	140.2±44.1	148.8±48.2	144.0±50.2	166.2±60.7	177.1±99.7
HDL$_{3a}$	278.2±73.5	266.3±85.1	278.8±64.7	316.4±112.8	336.4±92.4	301.1±91.1	312.8±95.2	275.7±75.3	278.3±82.9	294.3±43.3
HDL$_{2a}$	272.0±81.2	270.3±49.3	296.9±77.8	259.9±86.3	264.7±65.2	223.5±67.5	239.5±76.8	244.1±57.8	213.8±66.5	242.6±77.5
HDL$_{2b}$	386.7±111.5	378.6±102.4	368.7±94.8	326.1±90.2	280.3±81.3[a,b]	274.2±86.3[a,c]	277.8±101.5[a,c]	280.4±98.4	272.7±97.6	252.7±32.3

[a] $p<0.01$.
[b] Compared with optimal LDL-C subgroup within the same group.
[c] Compared with normal TG relevant subgroup.

contents of HDL$_{2b}$ as dependent variables. The small size particles preβ$_1$-HDL was significantly and independently predicted by TG (standardized regression coefficient [SRC] 0.318, $p<0.001$), TC (SRC 0.461, $p<0.001$) and HDL-C (SRC −0.129, $p<0.001$). The adjusted r^2 for this model was 0.323, i.e., it explained about 32% of the variables in preβ$_1$-HDL. The other variables were not related to preβ$_1$-HDL and were removed form the final model. In similar analyses with HDL$_{2b}$ as the dependent variable, TG (SRC −0.27, $p<0.001$), TC (SRC −0.233, $p<0.001$), HDL-C (SRC 0.138, $p=0.001$) and apo-AI (SRC 0.424, $p<0.001$). The adjusted r^2 for this model was 0.422.

5. Discussion

HDL-C appears to influence the course and development of atherosclerosis. However, it has been reported that the changes of HDL-C were not always associated with atherosclerosis. The HDL subclasses are increasingly regarded as an important factor affecting CHD. According to Cheung et al. [31], HDL subclasses distribution was more correlated with CHD than plasma low HDL-C concentrations. Atger et al. [17] considered that the change of plasma HDL subclasses may be an important marker for susceptibility to development of CHD. Moreover, the physiologic basis of HDL subclasses has not been elucidated. It is necessary to disclose the changes of the distribution and content of HDL subclasses with the change of plasma TG, TC, HDL-C and LDL-C concentrations.

The apo-AI contents of preβ$_1$-HDL were higher and those of HDL$_{2b}$ were lower in high TG subjects vs. corresponding normal TG subjects (Tables 1 and 3), which indicated that plasma TG concentrations had an important effect on the distribution and content of HDL subclasses. Most studies have established enhanced HL activity and impaired LCAT and LPL activity with the increase in triglycerides [32]. LCAT may catalyze unesterified cholesterol to cholesterol ester and promote the conversion of preβ$_1$-HDL and HDL$_3$ to HDL$_2$. Therefore, impeded plasma LCAT activity must lead to the increase of small-sized HDL particles. LPL plays an important role in hydrolyzing TG transported in chylomicrons (CM) and VLDL

Table 4
Apo-AI contents of plasma HDL subclasses according to plasma TG concentration in TC desirable and TC high subjects (mg/l, $\bar{x}\pm s$)

	TC desirable				TC high			
	Normal TG	Borderline-high TG	High TG	Very high TG	Normal TG	Borderline-high TG	High TG	Very high TG
n	80	16	64	10	120	38	97	17
preβ_1-HDL	79.2±21.9	82.3±21.3	119.8±40.9[a,b]	157.8±53.7[a,b]	87.8±40.1	100.5±43.7	134.6±45.3[a,b]	183.8±55.1[a,b]
preβ_2-HDL	47.0±13.6	52.9±17.9	56.6±21.8	57.0±25.8	55.7±18.1	61.8±21.2	63.9±24.2	58.8±21.2
HDL_{3c}	69.2±25.9	59.4±20.9	68.0±28.6	72.9±26.1	71.1±20.1	72.7±32.3	75.8±27.1	78.0±34.8
HDL_{3b}	128.8±38.2	134.6±47.4	136.8±46.9	175.4±45.2	142.2±50.9	149.9±59.4	151.9±53.7	167.1±44.6
HDL_{3a}	245.2±48.1	259.1±64.2	309.9±100.5	311.1±91.3	255.3±70.9	268.8±61.9	300.0±86.5	338.6±74.1[a,b]
HDL_{2a}	270.6±67.5	250.5±55.2	221.9±68.7	162.6±62.1[a,b]	285.4±77.5	244.6±86.4	227.2±62.7	175.3±40.4[a,b]
HDL_{2b}	387.7±106.3	378.7±79.3	233.5±85.6[a,b]	186.7±54.9[a,b]	354.7±107.0	321.7±78.3	226.6±75.8[a,b]	172.2±57.9[a,b]

[a] $p<0.01$.
[b] Compared with normal TG subgroup within the same group.

particles. When catabolized by LPL, CM and VLDL release triglyceride, cholesterol, phospholipids, apo-AI and $apoC_s$. Subsequent binding of these products to HDL_3 results in formation of HDL_2 particles. HL promotes the conversion of HDL_2 to HDL_3, and furthermore, excess surface phospholipid and apo-AI dissociated from HDL_2, which may generate much of small-sized preβ_1-HDL.

Our study found that with the increase of plasma TC concentrations, apo-AI contents of small-sized particles (especially preβ_1-HDL) increased. Our data were in good concordance with those of Miida et al. [25], who reported that apo-AI contents of preβ_1-HDL in patients with hypercholesterolemia were clearly higher than those with normolipidemia. Mechanisms for these differences may be partially explained by enhanced CETP activities [25]. Concentration of TC showed positive correlation with CETP activities [33]. CETP mediates exchange of core lipids between VLDL-TG, LDL-TG and HDL-CE, resulting in TG-enrichment of HDL. As the excess TG is hydrolyzed by HL, whose activity is enhanced by the increase of TC, the HDL particle size shifted smaller and the concentrations of the large HDL_{2b} subclasses were reduced. Furthermore, the apo-AI contents of HDL_{2b} and HDL_{2a} were significantly lower in high TG subjects vs. normal TG subjects, while those of HDL_{2b} and HDL_{2a} did not change with plasma TC concentrations in both normal TG and high TG subjects, suggesting that plasma TG concentrations may be more significant to influence the component of HDL subclasses than plasma TC concentrations.

The results of the relationship between plasma HDL-C concentrations and HDL subclasses distribution showed that only the apo-AI contents of HDL_{2b} decreased in high TG subjects (Table 2). Most studies have demonstrated increased CETP activity and decreased PLTP activity in low HDL-C subjects. CETP may be responsible for the formation of small-sized HDL particles; in contrast, PLTP favors the formation of larger-sized HDL particles. Moreover, low concentrations of HDL-C concentrations have been attributed to decrease LPL and HL ratio, which resulted in an increased fractional catabolic rate of larger-sized HDL particles. It has been shown that cholesterol efflux is well correlated with plasma HDL-C concentrations. Asztalos et al. [34] considered that altered HDL subclasses in low HDL-C subjects were prone to CHD by decreased reverse cholesterol transport. It must be noted that the apo-AI contents of preβ_1-HDL had no marked change in low HDL-C subjects within normal TG, while those of HDL_{2b} were lower by 23% in low HDL-C subgroup vs. high HDL-C subgroup ($p=0.023$). Therefore, the concentrations of plasma HDL-C must be corrected in time even in subjects with normal TG concentration, preventing the incidence of atherosclerosis and CHD.

The results of the relationship between plasma LDL-C concentrations and HDL subclasses distribution showed that in normal TG subjects, apo-AI contents of small-sized particles (especially preβ_1-HDL and HDL_{3b}) gradually increased, while large-sized HDL_{2b} gradually decreased. In high TG subjects, apo-AI contents of HDL subclasses (except

very high LDL-C subgroup) showed no changes (Table 3). The apo-AI content of preβ_1-HDL (except in high and very high LDL-C subgroups), and HDL_{2b} (except in borderline-high, high and very high LDL-C subgroups) considerably increased and decreased in high TG subjects vs. normal TG subjects, suggesting, in normal TG subjects, increased LDL-C concentrations accompanied with an increase of small-sized HDL particles and a reduction of large-sized HDL particles; however, in high TG subjects, distribution of HDL subclasses had no significant changes with increased LDL-C concentrations. To summarize, plasma TG and LDL-C concentrations may not have a cooperative effect on the changes of HDL subclass distribution.

Our study found that with the increase of plasma TG concentrations, apo-AI contents of preβ_1-HDL gradually increased ,while HDL_{2a} and HDL_{2b} gradually decreased in TC desirable and TC high subjects (Table 3). Syvanne [35] investigated that the distribution of HDL subclasses was determined by gradient gel electrophoresis (GGE) in 150 NIDDM and CHD subjects, and found that HDL subclasses distributions were not significantly different among groups. In contrast, dividing the whole study population quartiles of plasma TG concentrations showed that high TG concentrations were significant with low HDL_{2b} and high HDL_{3b} contents. In a multivariate liner regression model, HL activity and serum insuline and TG concentrations were all associated independently and inversely with low HDL_{2b} [35]. Therefore, plasma-increased TG concentrations favor the reduction of large-sized HDL particles (HDL_{2a} and HDL_{2b}) and the generation of small-sized HDL particles (preβ_1-HDL).

In addition, stepwise multivariate regression analyses were used to analyze the smallest HDL particles, i.e., preβ_1-HDL as well as the biggest ones, HDL_{2b}. As shown in Table 5, TG and TC concentrations were all associated independently and positively with high preβ_1-HDL; however, HDL-C was inversely associated with high preβ_1-HDL. And TG and TC concentrations were all associated independently and negatively with low HDL_{2b}, but HDL-C and apo-AI were positively associated with low HDL_{2b}.The result is accordance with previous reports [20,35]. In similar multivariate regression analyses of other HDL subclasses (data not shown), TG or TC had a positive relation to the 4 small subclasses preβ_2-HDL, HDL_{3c}, HDL_{3b} and HDL_{3c}, and TG negatively correlates with large subclass HDL_{2a}.

To summarize, with the increase of plasma TG, TC, LDL-C concentrations, or the decrease of plasma HDL-C concentrations, there was a general shift toward smaller-sized HDL, which, in turn, indicates that reverse cholesterol transport might be weakened and HDL maturation might be abnormal. Besides, plasma TG concentration is a more important factor than TC concentration to effect on the changes of HDL subclass distribution. Moreover, when TG is normal and HDL-C decreases, large-sized HDL particles tend to decrease.

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Table 5
Apo-AI contents of preβ_1-HDL (logged) and HDL_{2b} coefficients with plasma lipids and apolipoproteins

		Unstandardized coefficients		Standardized coefficients	t	p
		β	Standard error	β		
preβ_1-HDL (logged)	TG	0.033	0.005	0.318	6.505	0.000
	TC	0.085	0.015	0.461	5.486	0.000
	HDL-C	−0.058	0.023	−0.129	−2.577	0.010
HDL_{2b}	TG	−17.614	2.822	−0.270	−6.241	0.000
	TC	−26.977	4.517	−0.233	−5.972	0.000
	HDL-C	39.355	12.304	0.138	3.198	0.001
	apo-AI	0.237	0.022	0.424	10.816	0.000

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