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Relationship between total cholesterol/high-density lipoprotein cholesterol ratio, triglyceride/high-density lipoprotein cholesterol ratio, and high-density lipoprotein subclasses

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Abstract

Alterations in plasma lipid levels can influence the composition, content, and distribution of plasma lipoprotein subclasses that affect atherosclerosis risk. This study evaluated the relationship between plasma total cholesterol (TC)/high-density lipoprotein cholesterol (HDL-C) ratio, triglyceride (TG)/HDL-C ratio, and HDL subclass distribution. The apolipoprotein A-I contents of plasma HDL subclasses were quantitated by 2-dimensional gel electrophoresis coupled with immunodetection in 442 Chinese subjects. The particle size of HDL shifted toward smaller size with the elevation of TC/HDL-C and TG/HDL-C ratios. The ratio of large-sized HDL_{2b} to small-sized preβ_1-HDL (HDL_{2b}/preβ_1-HDL) was about 4.7 in the subjects with TC/HDL-C of 3.3 or lower and TG/HDL-C of 2.5 or lower, whereas it was only approximately 1.1 in subjects with TC/HDL-C greater than 6 and TG/HDL-C greater than 5. Pearson correlation analysis revealed that the TC/HDL-C ratio was positively correlated with preβ_1-HDL and HDL_{3a} but negatively correlated with HDL_{2a} and HDL_{2b}, whereas the TC/HDL-C ratio was only inversely correlated with HDL_{2b}.The TC/HDL-C and TG/HDL-C ratios together may be a good indicator of HDL subclass distribution. When these 2 ratios increased simultaneously, the trend toward smaller HDL size was obvious, which, in turn, indicated that the maturation of HDL might be impeded and the reverse cholesterol transport might be weakened. In addition, the TG/HDL-C ratio might be a more powerful factor to influence the distribution of HDL subclasses.

1. Introduction

The inverse relationship between plasma high-density lipoprotein cholesterol (HDL-C) and the incidence and prevalence of coronary heart disease (CHD) has been demonstrated in a number of epidemiologic studies [1-4]. The anti-atherogenic effect of HDL has been mainly attributed to the role that it plays in the reverse cholesterol transport (RCT) [5]. High-density lipoprotein particles are extremely heterogeneous containing several subclasses, which are characterized by differences in shape, density, size, charge, and antigenicity [6]. With the use of 2-dimensional gel electrophoresis and subsequent immunoblotting method, HDL can be subdivided into large-sized (HDL_{2a} and HDL_{2b}) and small-sized subclasses (preβ_1-HDL, HDL_{3c}, HDL_{3b}, and HDL_{3a}) and preβ_2-HDL [7,8].

It is generally accepted that different HDL subclasses have distinct but interrelated physiologic functions, and the effective flux of cholesterol through RCT clearly requires coordinated metabolic regulation of HDL subclasses [9,10]. Small lipid-poor particles, preβ_1-HDL, are an initial acceptor of cellular cholesterol. Upon accumulation of cholesterol, these particles are then converted to large spherical HDL particles by the action of cholesterol acyltransferase (LCAT) and are remodeled further by the action of plasma enzymes and transfer proteins such as lipoprotein lipase (LPL), hepatic triglyceride lipase (HTGL), cholesteryl ester transfer protein (CETP), etc. It has been

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postulated that nascent preβ_1-HDL is transformed into mature HDL_2, following the route of preβ-HDL → HDL_3 → HDL_2 [11,12].

In recent years, it was considered that changes in HDL subclass distribution were more closely correlated with atherosclerosis than low plasma HDL-C level [13]. Some studies have reported that small-sized HDL subclasses contribute to increased risk of CHD, whereas large-sized HDL subclasses are associated with decreased risk [14,15]. Asztalos et al [16] found that large α-1 HDL (HDL_{2b}) was most significantly associated with CHD prevalence. Moreover, their study demonstrated that each milligram per decaliter increase in α-1 HDL level decreased the odds of CHD by 26% in a model including all established CHD risk factors. However, epidemiologic data taken as a whole signify that a 1% increase in HDL-C level results in approximately 1% to 2% reduction in CHD risk [17].

The third Adult Treatment Panel guidelines of the US National Cholesterol Education Program (ATP-III) [17] recommend a full fasting lipoprotein profile, including triglyceride (TG), total cholesterol (TC), HDL-C, and low-density lipoprotein cholesterol (LDL-C). Although the guidelines only provide for evaluation of individual lipid fractions, the application of ratios such as TC/HDL-C and TG/HDL-C may offer a refined risk assessment by simultaneously considering both anti-atherogenic and atherogenic lipid parameters. Thus, in this study, using 2-dimensional gel electrophoresis and immunodetection method, we evaluated the alterations in HDL subclass distribution according to the changes in TC/HDL-C and TG/HDL-C ratios, which might provide additional information about the potential role of HDL subclasses in the risk for CHD.

2. Methods

2.1. Subjects

Five hundred and twenty-eight Chinese subjects aged 33 to 78 years (378 were from West China University of Medical Science, Sichuan University and Sichuan Normal University, in Chengdu, Sichuan Province, PR China; 150 were from Nan Hua University, in Hengyang, Hunan Province, PR China) were recruited to participate in a study examining plasma lipid and apolipoprotein concentrations. The study protocol was approved by an ethics committee, and all subjects gave informed consent. Exclusion criteria included the following: (1) presence of nephrosis, diabetes mellitus, hypothyroidism, hepatic impairment; (2) presence of major cardiovascular event (myocardial infarction, severe or unstable angina pectoris, surgery), stroke; (3) taking lipid-altering medications in the previous 1 month; (4) consuming alcohol, taking any medication, and smoking cigarettes in the previous 1 week before the study. After applying the exclusions, 442 normolipidemic and primary hyperlipidemic subjects were included in our present study.

According to the ATP-III, 3 ranges of TC are defined: less than 200, 200-240, and 240 or greater; similarly, 3 ranges of TG are defined: less than 150,150-200, and 200 or greater. In addition, low HDL-C levels are designated as less than 40 mg/dL and high levels as 60 mg/dL or greater. We can therefore make use of the TC/HDL-C ratio of 3.3 (200/60) and 6 (240/40) as the cutpoints. In recent years, it has been reported that risk for cardiac events is significantly higher when the TC/HDL-C ratio is greater than 5 [18]; hence, we inserted an additional TC/HDL-C group ranging from 3.3 to 5. The subjects were divided into 4 groups: TC/HDL-C ≤ 3.3 (n = 69), 3.3 < TC/HDL-C ≤ 5 (n = 191), 5 < TC/HDL-C ≤ 6 (n = 83), and TC/HDL-C > 6 (n = 99) group. Likewise, TG/HDL-C ratios of 2.5 (150/60) and 5 (200/40) were used as the cutpoints. The subjects were divided into 3 groups: TG/HDL-C ≤ 2.5 (n = 167), 2.5 < TG/HDL-C ≤ 5 (n = 120), and TG/HDL-C > 5 group (n = 155).

2.2. Specimens

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

2.3. Plasma lipid and apolipoprotein analyses

Plasma TG, TC, and HDL-C were measured by standard technique. Total cholesterol and TG were determined with enzymatic kits (Beijing Zhongsheng Biotechnological, Beijing, PR China). High-density lipoprotein cholesterol was determined after precipitation of the apolipoprotein (apo) B–containing lipoproteins by phosphotungstate/magnesium chloride [19]. Low-density lipoprotein cholesterol was calculated using the Friedwald formula (TG< 4.52 mmol/L) [20]. When plasma TG was 4.52 mmol/L or higher, LDL-C was determined after the precipitation method with polyvinylsulfate (enzymatic kits). Plasma apoA-I, apoB100, apoC-II, apoC-III, and apoE were determined by radial immunodiffusion methods [21] using kits developed at the Apolipoprotein Research Laboratory, West China Medical Center, Sichuan University [22].

2.4. High-density lipoprotein cholesterol subclass analyses

ApoA-I–containing HDL subclasses were measured by nondenaturing 2-dimensional gel electrophoresis associated with immunodetection method as described previously [12]. Briefly, 10 μL of plasma was first separated by charge on 0.7% agarose gel into preβ and preα mobility particles. In the second dimension, the 2 fractions of HDL were further separated according to size by 2% to 30% nondenaturing polyacrylamide gradient gel electrophoresis. To determine the HDL subclasses, Western blotting was conducted after electrophoresis using horseradish peroxidase–labeled goat antihuman apoA-I immunoglobulin G. The calculation of apoA-I percentage was based on the

Table 1
Concentrations of plasma lipids, lipoproteins, and apolipoproteins among subjects categorized by TC/HDL-C and TG/HDL-C ratios

	TC/HDL-C				TG/HDL-C		
	≤3.3 (n = 69)	3.3-5 (n = 191)	5-6 (n = 83)	>6 (n = 99)	≤2.5 (n = 167)	2.5-5 (n = 120)	>5 (n = 155)
BMI (kg/m^2)	21.6 ± 2.5	23.3 ± 2.8	24.3 ± 2.6†	24.4 ± 2.9†	22.3 ± 2.6	23.8 ± 2.8†	24.6 ± 2.7†
TC (mg/dL)	190.1 ± 33.1	208.7 ± 34.0†	219.2 ± 35.8‡	230.9 ± 39.6‡	202.3 ± 34.6	218.2 ± 37.1‡	217.4 ± 36.2‡
TG (mg/dL)	97.3 ± 31.4	152.2 ± 46.5‡	226.0 ± 74.3‡	374.7 ± 82.5‡	91.8 ± 25.3	186.6 ± 56.9‡	349.2 ± 77.5‡
HDL-C (mg/dL)	59.1 ± 10.7	49.5 ± 9.4†	37.0 ± 6.1‡	33.0 ± 6.5‡	59.2 ± 16.9	46.1 ± 9.8‡	33.2 ± 6.5‡
TC/HDL-C	3.0 ± 0.4	4.2 ± 0.5‡	5.8 ± 0.8‡	7.0 ± 1.2‡	3.4 ± 0.7	4.7 ± 0.7‡	6.6 ± 1.0‡
TG/ HDL-C	1.6 ± 0.4	3.1 ± 0.6‡	6.0 ± 1.3‡	11.4 ± 3.2‡	1.5 ± 0.4	4.5 ± 0.9‡	10.4 ± 3.5‡
LDL-C (mg/dL)	114.3 ± 20.8	128.6 ± 24.7†	131.2 ± 25.1†	147.9 ± 31.5‡	118.5 ± 30.7	139.9 ± 38.1‡	135.6 ± 37.2‡
ApoA-I (mg/L)	1339.1 ± 221.1	1250.4 ± 180.3†	1190.2 ± 186.1‡	1178.8 ± 173.5‡	1327.4 ± 207.7	1201.7 ± 184.2‡	1173.4 ± 192.6‡
ApoB100 (mg/L)	718.2 ± 176.3	867.6 ± 179.2‡	964.6 ± 205.0‡	1066.9 ± 207.6‡	779.8 ± 178.1	919.0 ± 186.7‡	1033.5 ± 203.1‡
ApoC-II (mg/L)	42.1 ± 13.5	55.6 ± 14.0†	84.3 ± 20.2‡	101.7 ± 30.5‡	43.7 ± 14.0	61.7 ± 15.4‡	101.2 ± 30.3‡
ApoC-III (mg/L)	110.4 ± 30.8	130.4 ± 39.3†	166.8 ± 42.6‡	235.9 ± 61.8‡	111.4 ± 34.1	145.2 ± 48.3‡	217.3 ± 60.2‡
ApoE (mg/L)	41.6 ± 12.7	47.4 ± 14.0	58.2 ± 17.3‡	75.2 ± 25.1‡	43.4 ± 12.7	50.2 ± 16.6†	68.7 ± 20.5‡

Values are expressed as mean ± SD.
BMI indicates body mass index.
†$P < .05$, ‡$P < .01$, compared with the TC/HDL-C ≤ 3.3 subgroup within the TC/HDL-C group or compared with the TG/HDL-C ≤ 2.5 subgroup within the TG/HDL-C group.

density of electrophoretic spots, and HDL particle sizes were calibrated using a standard curve that included bovine serum albumin, ferritin, and thyroglobulin (Pharmacia Uppsala, Uppsala, Sweden). Then, apoA-I concentrations of the HDL subclasses were calculated by multiplying the percentage of each subclass by the plasma total apoA-I concentration. The interassay coefficient of variations of the relative content of preβ_1-HDL, preβ_2-HDL, HDL_{3c}, HDL_{3b}, HDL_{3a}, HDL_{2a}, and HDL_{2b} in the plasma sample was 9.4%, 9.8%, 4.9%, 6.2%, 7.3%, 11.1%, and 7.9%, respectively (n = 5).

2.5. Statistical analysis

All statistical analyses were performed using the statistical package SPSS version 11.0 (SPSS, Chicago, IL). Data are presented as the mean ± SD. Newman-Keuls post hoc test was used for comparisons between groups when 1-way analysis of variance (ANOVA) is statistically significant. Two-way ANOVA was performed to evaluate the interactive effects of the TC/HDL-C and TG/HDL-C ratio on the HDL subclasses. Pearson coefficient was calculated to study correlation. Differences were considered significant at $P < .05$.

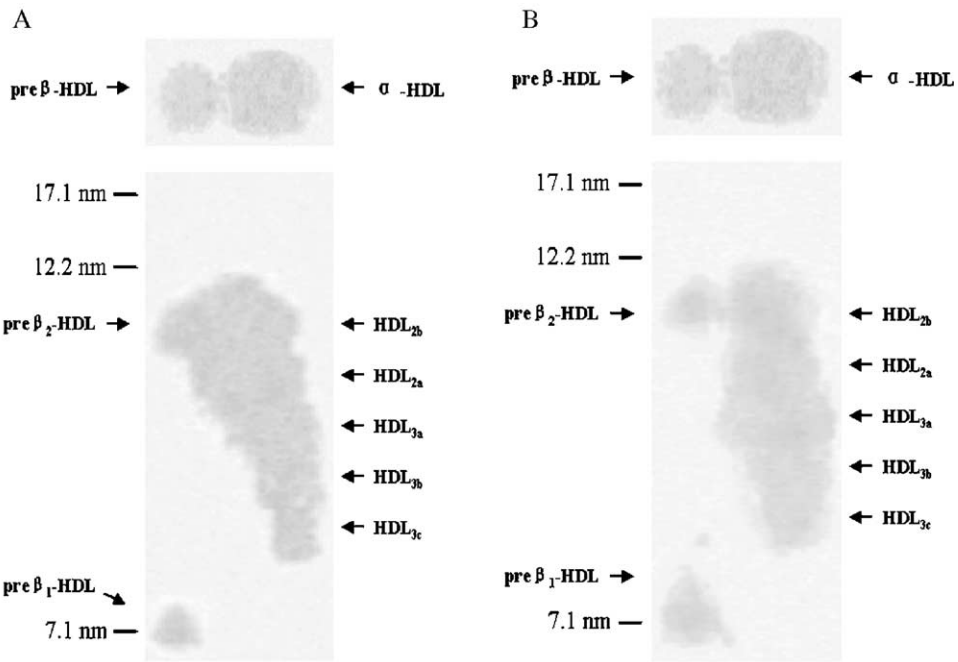

Fig. 1. Electrophoretic comparisons of apoA-I–containing HDL subclasses. High-density lipoprotein subclasses were separated by nondenaturing 2-dimensional gel electrophoresis and immunodetection with a goat antihuman apoA-I–IgG labeled with horseradish peroxidase. A; Normolipidemic subjects. B, Hyperlipidemic subjects.

Table 2
The apoA-I contents of HDL subclasses among subjects categorized by TC/HDL-C and TG/HDL-C ratios

	TC/HDL-C				TG/HDL-C		
	≤3.3 (n = 69)	3.3-5 (n = 191)	5-6 (n = 83)	>6 (n = 99)	≤2.5 (n = 167)	2.5-5 (n = 120)	>5 (n = 155)
Preβ_1-HDL	81.9 ± 19.7	100.9 ± 21.3†	124.7 ± 30.8‡	139.6 ± 33.2‡	83.0 ± 26.5	105.6 ± 25.3†	139.7 ± 32.1‡
Preβ_2-HDL	58.3 ± 14.1	59.6 ± 14.7	60.2 ± 16.1	59.8 ± 15.6	58.5 ± 13.9	57.9 ± 14.4	59.9 ± 16.3
HDL_{3c}	71.2 ± 21.1	74.3 ± 23.6	74.2 ± 23.8	72.0 ± 22.3	72.4 ± 22.4	74.6 ± 23.1	71.8 ± 22.5
HDL_{3b}	143.4 ± 32.3	147.2 ± 32.9	152.0 ± 34.6	147.8 ± 33.4	153.7 ± 34.2	142.3 ± 31.8	147.3 ± 31.4
HDL_{3a}	277.0 ± 64.6	277.7 ± 68.2	305.8 ± 73.4	313.1 ± 71.5†	280.7 ± 68.3	268.1 ± 59.5	315.8 ± 86.2‡
HDL_{2a}	292.4 ± 54.3	264.1 ± 49.7†	232.8 ± 46.5‡	213.8 ± 42.3‡	286.5 ± 53.4	250.8 ± 49.7‡	214.2 ± 43.0‡
HDL_{2b}	407.2 ± 81.6	327.0 ± 78.1‡	264.4 ± 60.3‡	227.1 ± 52.8‡	371.4 ± 78.5	317.9 ± 67.3‡	226.8 ± 50.7‡

Values are expressed as mean ± SD (apoA-I, mg/L).
†$P < .05$, ‡$P < .01$, compared with the TC/HDL-C ≤ 3.3 subgroup within the TC/HDL-C group or compared with the TG/HDL-C ≤ 2.5 subgroup within the TG/HDL-C group.

3. Results

3.1. Concentrations of plasma lipids, lipoproteins, and apolipoproteins according to TC/HDL-C and TG/HDL-C ratios

Table 1 shows that in both TC/HDL-C and TG/HDL-C groups, with the increase of these ratios, body mass index, concentrations of TC, TG, LDL-C, apoB100, apoC-II, apoC-III, and apoE increased significantly, whereas HDL-C and apoA-I decreased significantly.

3.2. Apolipoprotein A-I contents of HDL subclasses according to TC/HDL-C and TG/HDL-C ratios

Fig. 1 shows the distributions of HDL subclasses for representative hyperlipidemic and normolipidemic subjects. The result revealed that in the hyperlipidemic subjects, small-sized preβ_1-HDL spot was larger, whereas large-sized HDL_{2b} spot was smaller in comparison with the normolipidemic subjects. Furthermore, quantification of the HDL subclasses confirmed this observation. As shown in Table 2, not only in the TC/HDL-C group but also in the TG/HDL-C group were the plasma levels of the small-sized preβ_1-HDL and HDL_{3a} significantly higher, whereas those of the large-sized HDL_{2a} and HDL_{2b} were significantly lower with the elevation of these ratios.

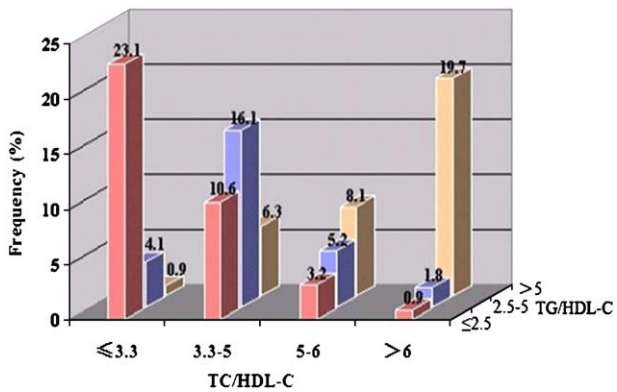

Fig. 2. The frequency distribution characteristics of all subjects according to the TC/HDL-C ratio and the TG/HDL-C ratio.

3.3. Characteristics of HDL subclass contents and frequency distributions according to TC/HDL-C along with TG/HDL-C ratios

As shown in Fig. 2, there were 2 high-frequency distribution groups, which were designated as the low-ratio group (when TC/HDL-C ≤ 3.3 along with TG/HDL-C ≤ 2.5) and high-ratio group (when TC/HDL-C > 6 along with TG/HDL-C > 5). There were 102 (23.1%) subjects in the low-ratio group and 87 (19.7%) subjects in the high-ratio group.

Table 3 shows that, compared to the low-ratio group, preβ_1-HDL and HDL_{3a} ($P < .001$) increased significantly, whereas HDL_{2a} and HDL_{2b} ($P < .001$) decreased significantly in the high-ratio group. Of note, in the low-ratio group, the mature large-sized HDL subclasses (HDL_{2a} and HDL_{2b}) occupied approximately 53.4% and the ratio of HDL_{2b} to preβ_1-HDL (HDL_{2b}/preβ_1-HDL) was about 4.7. However, in the high-ratio group, the percentage of large-sized HDL subclasses was only approximately 31.8% and HDL_{2b}/preβ_1-HDL ratio was about 1.1.

3.4. The correlation analysis between lipids, lipoproteins, and apoA-I contents of HDL subclasses

Pearson correlation analysis revealed that plasma TG level and the TG/HDL-C ratio were positively correlated with preβ_1-HDL and HDL_{3a} but negatively correlated with HDL_{2a} and HDL_{2b}. The correlation between the TG/HDL-C ratio and preβ_1-HDL was 0.562, whereas that between the TG/HDL-C ratio and HDL_{2b} was 0.583. The ratio of

Table 3
The apoA-I contents of HDL subclasses in low-ratio and high-ratio groups

	Low-ratio group (n = 102)	High-ratio group (n = 87)
Preβ_1-HDL	82.1 ± 21.3	155.3 ± 45.8*
Preβ_2-HDL	56.9 ± 16.5	59.2 ± 17.4
HDL_{3c}	73.6 ± 21.4	73.8 ± 22.6
HDL_{3b}	135.7 ± 43.2	158.6 ± 48.3
HDL_{3a}	243.6 ± 66.3	336.5 ± 84.8*
HDL_{2a}	283.4 ± 69.2	190.6 ± 60.1*
HDL_{2b}	389.8 ± 97.2	175.5 ± 52.6*

Values are expressed as mean ± SD (apoA-I, mg/L).
Low-ratio group: TC/HDL-C ≤ 3.3 along with TG/HDL-C ≤ 2.5.
High-ratio group: TC/HDL-C > 6 along with TG/HDL-C > 5.
* $P < .001$ compared with the low-ratio group.

Table 4
Correlation coefficients between plasma lipids, lipoproteins, and the apoA-I contents of HDL subclasses in all study subjects

	Preβ_1-HDL	Preβ_2-HDL	HDL_{3c}	HDL_{3b}	HDL_{3a}	HDL_{2a}	HDL_{2b}
TG	0.406**	0.090	0.105	0.112	0.309**	−0.325*	−0.434**
TC	0.152	0.084	0.078	0.080	0.153	−0.080	−0.147
LDL-C	0.133	0.118	0.109	0.095	−0.073	0.073	−0.056
HDL-C	−0.175	0.099	0.156	0.115	0.176	0.181*	0.372**
TG/HDL-C	0.562**	−0.149	−0.128	0.135	0.256*	−0.401**	−0.583**
TC/HDL-C	0.206	−0.104	−0.096	0.083	−0.041	−0.190	−0.328*

* $P < .05$.
** $P < .01$.

TC/HDL-C was inversely correlated with HDL_{2b}. In contrast, plasma HDL-C level was positively correlated with HDL_{2a} and HDL_{2b} (Table 4). Furthermore, the 2-way ANOVA result suggested that there were significant interaction effects of the TC/HDL-C ratio and the TG/HDL-C ratio on the preβ_1-HDL, HDL_{2a}, and HDL_{2b} (Table 5).

3.5. The influence of change in the TC/HDL-C ratio or the TG/HDL-C ratio on the apoA-I contents of preβ_1-HDL and HDL2b

As shown in Fig. 3, in the TG/HDL-C ≤ 2.5 group, regardless of whether the TC/HDL-C ratios increased or not, both the preβ_1-HDL and HDL_{2b} were almost kept at constant levels. However, in each same TC/HDL-C ratio group, preβ_1-HDL increased significantly, whereas HDL_{2b} decreased significantly with the increase in the TG/HDL-C ratios.

4. Discussion

Different HDL subclasses exert distinct but interrelated metabolic functions, and the anti-atherogenic effects of HDL reflect the functional biologic properties of HDL subclasses rather than absolute plasma levels of HDL-C [23]. The ratio of TC/HDL-C has been used in numerous epidemiologic studies to identify high atherosclerosis risk for individuals and a high ratio may be a good indicator of abnormal TC metabolism [4]. Much evidence suggested that TG-rich lipoproteins were atherogenic. However, it is difficult to assess the independent risk conferred by TG, because the elevated TG levels are often accompanied by other lipid metabolic disturbance, including reduced levels of HDL-C [24]. Thus, the ratio of TG/HDL-C is likely to be the result of metabolic interactions, which may confer greater risk than the isolated factor in either. In the present study, we evaluated the relationship between the ratios of TC/HDL-C, TG/HDL-C, and the alteration of HDL subclasses.

Our results showed that, with the elevation of these ratios, HDL particles shifted toward smaller sizes. The variations of HDL subclass distributions are likely related to the several lipoprotein-modifying plasma enzymes: LPL, LCAT, CETP, and HTGL, etc.

Several studies have shown that enhanced HTGL and impaired LCAT and LPL activities are consistent with higher plasma TG levels [25-27]. Enhanced HTGL activities promote the conversion of HDL_2 to HDL_3; furthermore, excess surface phospholipid and apoA-I dissociate from HDL_2, which may generate much of the small-sized preβ_1-HDL. The LCAT may catalyze unesterified TC to cholesteryl ester (CE) and promote the conversion of preβ_1-HDL and HDL_3 to HDL_2. Lipoprotein lipase plays an important role in hydrolyzing TG transported in chylomicrons and very low density lipoprotein (VLDL) particles. When catabolized by LPL, chylomicrons and VLDL release TG, TC, phospholipids, apoA-I, and $apoC_s$. Subsequent binding of these products to HDL_3 results in formation of HDL_2 particles. Impeded plasma LPL activity must lead to the reduction of HDL_2. Some data suggested that increased plasma TC might contribute to the enhancement of CETP activities, which also favored the reduction of HDL_{2b} particles and the generation of HDL_3 [28]. In addition, it was demonstrated that LCAT activities were also decreased with low plasma HDL-C level [25]. Hence, with the increase of TC/HDL-C and TG/HDL-C ratios, small-sized preβ_1-HDL tended to increase, whereas large-sized HDL_{2a} and HDL_{2b} tended to decrease.

Many data demonstrated that both a decreased concentration of the large-sized HDL_{2b} particles and an increased concentration of the small-sized preβ_1-HDL particles were highly and significantly correlated with the risk of CHD [13,16,29]. Accumulation of small preβ_1-HDL may be a result of inefficient conversion of preβ_1-HDL to preβ_2-HDL or the esterification of cholesterol. Thus, higher level of preβ_1-HDL would have a negative impact on the anti-atherogenic potential of HDL [10,30]. Large cholesterol-rich HDL_{2b} particles may be important in determining the direction of the flow of CE. When the HDL_{2b} particles are sufficient, most HDL-CE is directed to the liver by the

Table 5
P values calculated by 2-way ANOVA for HDL subclasses

	Factor A (TC/HDL-C)	Factor B (TG/HDL-C)	Factor interaction (TC/HDL-C × TG/HDL-C)
Preβ_1-HDL	.000	.000	.032
Preβ_2-HDL	.698	.627	.301
HDL_{3c}	.883	.877	.203
HDL_{3b}	.290	.177	.695
HDL_{3a}	.440	.196	.434
HDL_{2a}	.202	.000	.038
HDL_{2b}	.017	.001	.021

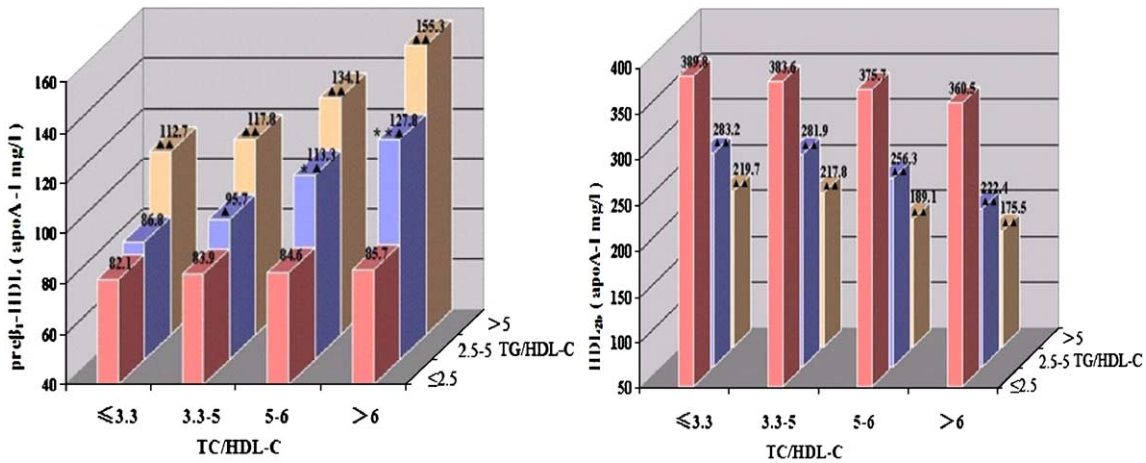

Fig. 3. The apoA-I contents of preβ_1-HDL and HDL_{2b} according to the TC/HDL-C ratio together with the TG/HDL-C ratio. *$P < .05$, **$P < .01$, compared with the TC/HDL-C ≤ 3.3 subgroup within the same TG/HDL-C ratio group. ▲$P < .05$, ▲▲$P < .01$, compared with the TG/HDL-C ≤ 2.5 subgroup within the same TC/HDL-C ratio group.

selective uptake of CE by HDL receptors [29]. However, in the absence of HDL_{2b} particles, HDL-CE is transferred to VLDL and LDL by the action of CETP, resulting in an increase of CE in potentially atherogenic particles [29].

In the present study, we surprisingly found that the apoA-I contents of each HDL subclass in subjects with the ratio of TC/HDL-C greater than 6 were much similar to those in subjects with the ratio of TG/HDL-C greater than 5 (eg, preβ_1-HDL, 139.6 ± 33.2 vs 139.7 ± 32.1; HDL_{2a}, 213.8 ± 42.3 vs 214.2 ± 43.0; HDL_{2b}, 227.1 ± 52.8 vs 226.8 ± 50.7). Why were the HDL subclass distributions almost coincident when they were assessed in different groups of subjects classed according to either TC/HDL-C ratio or TG/HDL-C ratio? Presently, it is unclear and should be further studied. But this result suggests that using ATP-III guidelines to determine the cutpoints for both ratios is reasonable. Subjects with a ratio of TC/HDL-C greater than 5 had a higher incidence of CHD than those with low TC/HDL-C ratios [18]. It is proposed that persons with a ratio of TC/HDL-C greater than 6 should be given lipid-lowering drug treatment [31]. Of note, the TG/HDL-C > 5 group consisted of 155 subjects; however, only 99 of them were in the TC/HDL-C > 6 group, whereas the other 45 subjects were in the 5 < TC/HDL-C ≤ 6 group and 11 subjects in the 3.3 < TC/HDL-C ≤ 5 group. The result suggested that, although additional 56 subjects were in the ratio of TC/HDL-C < 6, the characteristic of the distribution in HDL subclasses was in accordance with the subjects of TG/HDL-C > 5. Hence, the TG/HDL-C ratio might be more sensitive to reflect the alteration of HDL subclass distribution than the TC/HDL-C ratio. It is well known that the most common hyperlipidemia for the Chinese population was characterized by elevated TG levels (ie, hypertriglyceridemia [HTG]), which was much more prevalent than high TC. Our previous study showed that HTG accounted for about 61% of total hyperlipidemia [8]. Liu [32] suggested that HTG in China was induced by high-carbohydrate diets of the populations, which was different from standard Western high-fat diets. High-carbohydrate diets may result in increased concentration of plasma glucose and, thus, high-insulin levels. Hyperinsulinemia stimulates the production and secretion of TG and VLDL, which lead to HTG. Thus, it seemed that predicting the risk of CHD depending only on the high TC/HDL-C ratio might be imperfect especially for the subjects with increased TG level.

Characteristic of HDL subclass distribution for the low-ratio group (when TC/HDL-C ≤ 3.3 along with TG/HDL-C ≤ 2.5) in this study was in accordance with that for the normolipidemic subject in our previous study [8,33-35], and the percentage of small-sized HDL subclasses was low relative to that of the large-sized HDL subclasses generally. Data obtained in the present study suggest that, compared to the low-ratio group, small-sized preβ_1-HDL increased significantly, whereas large-sized HDL_{2b} decreased significantly ($P < .001$), which resulted in an amazing reduction of HDL_{2b}/preβ_1-HDL ratio (1.1 vs 4.7) and the percentage of large-sized HDL subclasses (31.8% vs 53.4%) in the high-ratio group (when TC/HDL-C > 6 along with TG/HDL-C > 5). It revealed that the HDL subclass distribution might be reversed, characterized by the large-sized HDL subclasses decreasing and the small-sized HDL subclasses increasing extremely for the subjects with both high TC/HDL-C and high TG/HDL-C ratios. We think subjects with the high ratios of TC/HDL-C and TG/HDL-C might have increased risk of CHD, because the RCT might be weakened and the potential anti-atherogenic functions of HDL might be impaired seriously among these subjects. Hence, it is important to consider TG/HDL-C and TC/HDL-C ratios simultaneously while assessing the CHD risk.

Our study also showed that in the low TG/HDL-C ratio group (TG/HDL-C ≤ 2.5), subjects with high TC/HDL-C ratios had relative constant levels of preβ_1-HDL and HDL_{2b}

compared with subjects with low TC/HDL-C ratios. However, in each same TC/HDL-C ratio group, preβ_1-HDL increased significantly, whereas HDL_{2b} decreased significantly with the elevation of the TG/HDL-C ratio. These results suggest that increase in the TC/HDL-C ratio alone did not influence the distributions of HDL subclasses significantly when the TG/HDL-C ratio was low (TG/HDL-C $\leq$ 2.5) and the TG/HDL-C ratio might be a more powerful factor to influence the distribution of HDL subclasses than the TC/HDL-C ratio. The correlation analysis also revealed that the TG/HDL-C ratio showed a better correlation with preβ_1-HDL and HDL_{2b}. Gaziano et al [36] considered that the TG/HDL-C ratio was an important marker of abnormal TG metabolism, which might provide valuable additional information about the atherogenic potential of a lipid profile. Jeppesen et al [37] demonstrated that a high TG/HDL-C ratio was a powerful predictor of morbidity and mortality of CHD. Our previous study [33,34] also suggested that elevated TG levels favored the reduction of large-sized HDL particles and the generation of small-sized HDL particles.

In summary, considering the relative ease of measuring TC/HDL-C and TG/HDL-C ratios, as opposed to measuring HDL subclasses, these 2 ratios together may be a good indicator of HDL subclass distribution (and, thus, cardiovascular disease risk). When these 2 ratios increased simultaneously, the trend toward smaller HDL size was obvious and the proportion between the mature large-sized HDL_{2b} and the nascent small-sized preβ_1-HDL was reversed, which, in turn, indicated that the maturation of HDL might be impeded and the RCT might be weakened. In addition, the TG/HDL-C ratio might be a more powerful factor to influence the distribution of HDL subclasses.

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