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Alterations of HDL Subclasses in Different Lipid Levels of Men and Women

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Abstract: With the increase of plasma TG level, both in men and women, apoA-Iconcentrations of preβ_1-HDL and HDL_{3a} were significantly higher while HDL_{2a} and HDL_{2b} were significantly lower. Similarly, with the increase of plasma LDL-C level, apoA-Iconcentrations of preβ_1-HDL, HDL_{3c}(in men) and HDL_{3b} were significantly higher while apoA-Iconcentrations of HDL_{2b} were significantly lower. And with the decrease of plasma HDL-C, apoA-Iconcentrations of preβ_1-HDL, $HDL_{3C}$and HDL_{3b} were significantly higher while apoA-Iconcentrations of HDL_{2a} and HDL_{2b} were significantly lower. In addition, apoA-Iconcentrations of preβ_1-HDL was significantly lower in women TG <1.7 mmol/L and TG 1.7～2.5 mmol/L vs. men groups. And apoA-Iconcentrations of HDL_{2b} in women LDL-C <2.6 mmol/L, LDL 2.6～3.3 mmol/L and HDL-C 1.0～1.6 mmol/L, HDL-C <1.0 mmol/L groups were significantly higher than men groups. In all subjects, with the increase of TG, LDL-C and decrease of HDL-C, the particle size of HDL shifted towards smaller sizes, which, in turn, indicates that the maturation of HDL may be impeded in those subjects with abnormal lipids profile. In women HDL particles are bigger than the men, which potentially result in the gender differences in CHD risk factors and susceptibility to atherosclerosis.

Keywords: apoA-Icontaining HDL subclasses; Triglyceride; Low density lipoprotein-cholesterol; High density lipoprotein-cholesterol; Atherosclerosis; Gender; Two-dimensional gel electrophoresis-immunodetection

INTRODUCTION

It has been firmly established that low the level of HDL-C was an important cardiovascular risk factor[1]. HDL has anti-atherogenic action which is probably related to the reverse cholesterol transport(RCT)[2,3]. RCT is the major physiological pathway by which peripheral cell cholesterol is returned to the liver for metabolic conversion and excretion, it plays an important role in maintaining cholesterol homeostasis and preventing atheroselerosis development in the body[4,5]. Furthermore, Pre-menopausal women have lower rates of coronary heart disease than comparably men, while the incidence of CHD in women rises promptly after surgical or natural menopausal and soon equals the incidence in men[6,7] And, there is significantly sexual difference of plasma lipids, especially subclasses of plasma HDL.

However, HDL has in common a high density (> 1.063g/mL) and a small size (Stoke's diameter 5 to 17 nm) [8]. HDL particles are composed of an 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 apoA-I [8]. 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 [8]. Subclasses of HDL can be separated by zonal [9] or single-spin vertical ultracentifugation [10], heparin-magnesium precipitation [11], nuclear magnetic resonance (NMR) spectroscopy [12], or one- and two-dimensional polyacrylamide gel electrophoresis [13-15].

Using agarose gel electrophoresis, HDL can be separated into two parts, i.e., pre-β and α-HDL. Pre-β part can be further distinguished by subsequent polyacrylamide gradient gel electrophoresis into pre-β_1, pre-β_2HDL and α-HDL can be separated into five distinct subclasses $HDL_{3c\ 3b\ 3a\ 2a\ 2b}$, according to their increasing particle size[16,17]. The diameter(nm) of HDL subclasses are: pre-β_1HDL(5.80±0.12), pre-β_2HDL(11.15±0.21), HDL_{3c} (7.22±0.16), HDL_{3b}(8.06±0.18), HDL_{3a}(8.46±0.25), HDL_{2a}(9.68±0.27), HDL_{2b}(11.55±0.20)[18,19]. The approximate diameter ranges shown above, are those previously determined by calibration using purified subfractions analysed by polyacrylamide gradient gel electrophoresis[12].

ApoA-I, probably the pre-β_1-HDL(the smallest preβ-HDL), which binds to the adenosine triphosphate-binding cassette transporter A1 (ABCA1), thus allow the transfer of free cholesterol and phospholipidids from cells to HDL[20]. Pre-β_1-HDL is transformed by the activity of lecithin: cholesterol acyltranstransferase (LCAT), which esterifies the free cholesterol to form α-HDL particles. α-HDL particles 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 triglyceride lipase (HTGL), endothelial lipase (EL), the cholesteryl ester

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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 at least 2 routes: ABCA1→apoA-I→preβHDL→HDL_3→HDL_2 and SR-B1→HDL_3→HDL_2. The interconversion was also found between HDL_2 and HDL_3: HDL_2→HDL_3. 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 changes in HDL subclasses distribution may be closely related to the incidence and prevalence of atherosclerosis[21-23].

Our laboratory had investigated the apoA-I contents of HDL subclasses distribution in Chinese Population, hyperlipidemic, endogenous hypertriglyceridemia by two-dimensional gel electrophoresis associated with immunodetection method[15,24-26]. We found that the characteristic of the transformation of HDL subclasses in hyperlipidemic and hypertriglyceridemic subjects seemed to be different, whereas, there was a general shift toward smaller sized HDL, suggesting that RCT might be weakened and the maturation of HDL might be abnormal in all those subjects. In relationship between concentrations of lipids and HDL subclasses, we found that plasma concentrations of TG and LDL-C showed positive correlation with the concentrations of small-sized HDL and TG showed negative correlation with that of large-sized HDL. But it was reversed for HDL-C[15,24,25]. Partially according to ATP- [27] classification of plasma lipids and sex, our present study investigated the relationship between TG, HDL-C and LDL-C levels and HDL subclasses in the men and women. The results may be helpful to understand the relationship between atherosclerosis and HDL subclasses.

Subjects and methods

1.1 Subjects

The subjects, consisted of 442 Chinese adults being either current or retired staff, aged 33 to 78 years (54.7±8.2), were recruited to participate in a study examining plasma lipid and apolipoprotein concentrations. 292 subjects were from the Sichuan University and Sichuan Normal University, in Chengdu, Sichuan province, PR China. In which, women were 107 and men were 185. 150 subjects were from the Nanhua University, in Hengyang, Hunan province, PR China. In which, women were 32 and men were 118. In this study, normolipidemic subjects (TG< 2.21 mmol/L and TC <6.21 mmol/L) were free of medication and free of heart attack in 1 week, and who had no history of alcohol abuse and smoking. All subjects have been free of administration of lipid-lowering drugs in the previous 1 month, and the women have been free of administration of hormone replacement therapy or oral contraceptives. According to gender and the third Report of NCEP, Expert panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults(ATP-)[27], the subjects were classified by plasma TG, HDL-C and LDL-C concentrations in men and women respectively. The study was approved by the institutional ethics committee on human, and all subjects gave informed consent.

TG group: TG<1.7 mmol/L group(include 128 men and 67 women), TG (1.7~2.5 mmol/L) group(include 65 men and 24 women) and TG≥2.5mmol/L group(include 110 men and 48 women).

LDL-C group: LDL-C<2.6 mmol/L group(include 71 men and 42 women), LDL-C (2.6~3.3 mmol/L) group(include 68 men and 35 women)and LDL-C>3.3 mmol/L group(include 164 men and 62 women).

HDL-C group: HDL-C>1.6mmol/L group(include 70 men and 28 women), HDL-C (1.0~1.6 mmol/L) group(include 132 men and 70 women) and HDL-C< 1.0 mmol/L group(include 101 men and 41 women).

1.2 Specimens

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

1.3 Plasma lipid and apolipoprotein analyses

Plasma TG, TC and HDL-C were measured by standard techniques. TC and TG were determined with enzymatic kits(Beijng Zhongsen Biotechnological Corporation, Beijing). HDL-C was determined after precipitation of the apolipoprotein(apo)B-containing lipoproteins by phosphotungstate/ magnesium chloride[28]. When TG < 4.52mmol/L, LDL-C was calculated using Friedwald formula[29]. When TG≥4.52mmol/L, LDL-C was determined with enzymatic kits(Beijng Zhongsen Biotechnological Corporation, Beijing). Plasma apoA-I, B100, C , C and E were determined by radial immunodiffusion methods[30] using kits developed at the Apolipoprotein Research Laboratory, West China medical Center, Sichuan University[31].

1.4 HDL subclasses analyses

HDL subclasses distributions were determined by two-dimensional gel electrophesis associated with immunodection method as described previously[15,24-26]. In brief, 10μl of plasma was applied to 0.7% agarose gel in the first dimension. After electrophoretic seperation of lipoproteins in agarose gels, they were further separated by electrophoresis in 2-30% nondenaturing polyacrylamide gradient gel in the second dimension. To determine HDL subclasses, western blotting was conducted after electrophesis, using HRP-labeled goat anti-human apoA-I-IgG. The relative concentration of each HDL subclass was

calculated as the percentage of plasma apoA-I (%) 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 content of each HDL subclass was multiplied by apoA-Iconcentrations in sample individuals respectively. The result was the relative concentration of each HDL subclass of apoA-I (mg/L, apoA-Iin the subclasses). The variation coefficients of relative concentration of pre-β_1HDL, pre-β_2HDL, 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).

1.5 Statistical analysis

Data are presented as mean±standard deviation. The between-group differences were evaluated by an analysis of variance(ANOVA) among the groups. Statistical analyses were performed using SPSS statistical packages. In all comparisons, a $p<0.05$ was considered statistically significant.

RESULT

1. ApoA-Icontents of plasma HDL subclasses according to plasma TG levels in men and women.

Table 1:ApoA-Iconcentrations of plasma HDL subclasses according to plasma TG levels in men and women (mg/L, $\bar{x}$ ±s)

	Men			Women		
TG(mmol/L)	<1.7	1.7～2.5	>2.5	<1.7	1.7～2.5	>2.5
n	128	65	110	67	24	48
pre β 1-HDL	89.1 ± 28.2	102.4 ± 42.9	137.4 ± 49.1[b,c]	71.6 ± 20.6[a,e]	84.7 ± 33.6[a,e]	136.6± 42.1[b,d]
pre β 2-HDL	54.5 ± 18.1	58.7 ± 23.9	59.0 ± 21.6	50.0 ± 14.9	60.0 ± 18.1	60.6± 22.5
HDL_{3c}	76.8 ± 31.7	72.2 ± 27.7	72.5 ± 27.8	75.3 ± 32.6	71.2 ± 37.2	71.0± 27.4
HDL_{3b}	145.1 ± 43.0	150.7 ± 53.8	152.6 ± 49.4	123.3 ± 36.3	142.8 ± 55.4	147.8± 51.9
HDL_{3a}	256.4 ± 86.6	286.4 ± 87.0	309.1 ± 88.7[b,c]	234.3 ± 65.0	277.9 ± 69.8	306.1± 91.7[a,d]
HDL_{2a}	273.4 ± 70.2	248.2 ± 77.1[a,c]	218.6 ± 64.4[b,c]	291.4 ± 81.0	241.9 ± 80.7[b,d]	229.5± 64.0[b,d]
HDL_{2b}	363.7 ± 111.8	296.6 ± 96.9[b,c]	229.7 ± 91.1[b,c]	375.3 ± 99.9	331.1 ± 80.6[a,d]	248.4± 82.6[b,d]

[a] $p<0.05$
[b] $p<0.01$
[c] compared with TG<1.7 group in men
[d] compared with TG<1.7 group in women
[e] compared with corresponding men group

As shown in Table 1, both in men and women, with the increase of plasma TG level, apoA-Icontents of preβ_1-HDL and HDL_{3a} in TG>2.5 group were significantly higher while HDL_{2a} and HDL_{2b} were significantly lower in TG 1.7～2.5 and TG>2.5 groups vs. TG<1.7 groups.

In addition, apoA-Icontents of preβ_1-HDL was significantly lower in women TG <1.7 and TG 1.7～2.5 groups than men.

2. ApoA-Icontents of plasma HDL subclasses according to plasma LDL-C levels in men and women.

Table 2:ApoA-Iconcentrations of plasma HDL subclasses according to plasma LDL-C levels in men and women (mg/L, $\bar{x}$ ±s)

	Men			Women		
LDL-C(mmol/L)	<2.6	2.6～3.3	>3.3	<2.6	2.6～3.3	>3.3
n	71	68	164	42	35	62
pre β 1-HDL	105.6 ± 42.7	103.3 ± 35.1	116.9 ± 37.9[a,c]	100.1 ±38.7	100.1 ± 34.5	112.1 ± 36.6[a,d]
pre β 2-HDL	58.9 ± 18.7	57.9 ± 17.8	60.3 ± 18.9	57.8± 17.6	56.7 ± 17.5	58.2± 18.3
HDL_{3c}	71.8 ± 24.1	70.5 ± 21.7	81.9 ± 26.7[b,c]	70.7± 23.0	70.2 ± 21.4	78.1± 25.4
HDL_{3b}	140.6 ± 42.0	142.9 ± 42.5	160.5 ± 49.9[a,c]	139.0 ±41.7	140.8 ± 41.3	156.8 ± 48.4[a,d]
HDL_{3a}	297.3 ± 87.3	294.1 ± 93.7	289.4 ± 81.2	289.0 ±85.4	288.8 ± 92.8	287.6 ±79.5
HDL_{2a}	249.5 ± 70.4	240.4 ± 65.7	245.1 ± 67.9	291.5 ±79.8	278.1 ± 71.3	277.5 ±68.3
HDL_{2b}	302.4 ± 100.0	289.3 ± 94.3[a,c]	271.4 ± 88.4[b,c]	356.3 ± 103.3[b,e]	322.3 ± 97.8[a,e]	297.5 ± 93.8[b,d]

[a] $p<0.05$
[b] $p<0.01$
[c] compared with LDL-C<2.6 group in men
[d] compared with LDL-C<2.6 group in women
[e] compared with corresponding men group

As shown in Table 2, both in men and women, with the increase of plasma LDL-C level, apoA-Icontents of preβ_1-HDL, HDL_{3b} and HDL_{3c} (in men) in LDL-C >3.3 group were significantly higher while apoA-Icontents of HDL_{2b} were significantly lower in LDL-C 2.6～3.3 or/and LDL-C >3.3 groups compared with LDL-C <2.6 group.

In addition, apoA-Icontents of HDL_{2b} in women LDL-C <2.6 and LDL 2.6 ～ 3.3 groups were significantly higher than men.

3. ApoA-Icontents of plasma HDL subclasses according to plasma HDL-C levels in men and women.

Table 3:ApoA-Iconcentrations of plasma HDL subclasses according to plasma HDL-C levels in men and women (mg/L, $\bar{x}$ ±s)

	Men			Women		
HDL-C(mmol/L)	>1.6	1.0～1.6	<1.0	>1.6	1.0～1.6	<1.0
n	70	132	101	28	70	41
pre β 1-HDL	105.9 ±37.4	100.9 ±38.8	127.3 ± 41.3[a,c]	100.6 ±38.1	100.3 ±31.3	126.6 ± 39.9[a,d]
pre β 2-HDL	63.6± 21.1	58.1± 19.9	58.9± 20.5	61.2± 21.2	54.6± 15.9	58.6± 17.7
HDL_{3c}	66.7± 21.9	75.6± 22.4[b,c]	81.8± 25.4[b,c]	61.8± 17.4	73.3± 25.9	76.6± 21.2
HDL_{3b}	150.5 ±50.9	148.0 ±45.8	167.2 ± 46.3[b,c]	146.3 ±39.0	147.1 ±40.9	160.3 ± 53.6[a,d]
HDL_{3a}	296.9 ±85.8	279.8 ±86.5	302.3 ±90.5	264.0 ±81.6	286.7 ±69.2	309.9 ±98.5
HDL_{2a}	293.8 ±82.8	254.3 ± 67.5[a,c]	220.9 ± 64.3[b,c]	309.9 ±98.6	271.6 ± 66.6[b,d]	220.2 ± 64.3[b,d]
HDL_{2b}	370.4 ± 129.0	326.7 ± 106.0[b,c]	230.4 ± 94.4[b,c]	370.6 ±93.3	356.1 ± 100.2[a,d,b,c]	294.8 ± 75.4[b,d,b,c]

[a] $p<0.05$
[b] $p<0.01$
[c] compared with HDL-C>1.6 group in men
[d] compared with HDL-C>1.6 group in women
[e] compared with corresponding men group

As shown in Table 3, both in men and women, with the decrease of plasma HDL-C, apoA-Icontents of preβ$_1$-HDL, HDL_{3C} and HDL_{3b} were significantly higher while apoA-Icontents of HDL_{2a} and HDL_{2b} were significantly lower in HDL-C 1.0～1.6 or/and HDL-C <1.0 groups than HDL-C >1.6 group.

In addition, apoA-Icontents of HDL_{2b} in women HDL-C 1.0～1.6 and HDL-C <1.0 groups were significantly higher than men.

DISCUSSION

Numerous clinical and epidemilogical studies have firmly established an inverse relationship between the risk of CHD and the concentration of high density lipoprotein-cholesterol, In recent years, it is considered that HDL subclasses distribution was more correlated with CHD than low plasma HDL-C levels. Miida et al.[32] found that the apoA-Icontents of preβ$_1$ –HDL in patients with hypercholesterolemia were significantly higher than those with normolipidemia. Saidi et al.[33] demonstrated that patients with mixed hyperlipidemia increased contents of small-sized HDL particles(HDL_{3b} and HDL_{3a}) and decreased contents of large-sized HDL particles(HDL_{2a} and HDL_{2b}). Our previous studies in atheroselerosis relevant diseases[15,24-26] also found that the particle size of HDL in above subjects shifted toward smaller sizes, and indicated that the maturation of HDL might be abnormal in all those subjects. Furthermore, the effect of sex can influence plasma lipids, especially subclasses of plasma HDL. There are not only men/women differences in lipid and lipoprotein concentrations, but also the sizes of lipoprotein particles or distributions of lipoprotein subclasses. Numerous clinical and epidemiological studies have showed that there is a sex difference in CHD risk. In this study, our present investigated the relationship between the plasma TG, LDL-C and HDL-C levels and HDL subclasses, partially according to ATP-□ classification of plasma lipid in men and women.

1. Effects of plasma TG levels on the distributions of HDL subclasses.

Our study found that, both in men and women, apoA-Icontents of preβ$_1$-HDL and HDL_{3a} were significantly higher while HDL_{2a} and HDL_{2b} were significantly lower in higher level of TG than lower TG group. Most studies have revealed enhanced HTGL activity [34] but impaired LCAT [35] and lipoprotein lipase (LPL) activity [33] with the increase of plasma TG levels. HTGL promotes HDL_2 converting to HDL_3, furthermore, excess surface phospholipid and apo-A□ dissociated from HDL_2, which may generate much of small -sized preβ$_1$-HDL. LCAT may catalyze unesterified cholesterol to cholesterol ester and promote preβ$_1$-HDL and HDL_3 converting to HDL_2.Theorefore, impeded plasma LCAT activity must lead to the increase of small-sized HDL particles. LPL plays an important role in hydrolyzing TG of chylomicrons(CM) and VLDL particles. CM and VLDL can be catabolized by LPL and release triglyceride, cholesterol, phospholipid, apoA-I, etc. Subsequently, binding of these products to HDL_3 results the formation of HDL_2 particles[23]. Impeded plasma LPL activity must lead to the reduction of HDL_2. Syvanne[36] 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 distribution was not significantly different among groups. In contrast, dividing the whole study population quartiles of plasma TG concentrations showed that high TG levels were significantly connected between low HDL_{2b} and high HDL_{3a} contents. In a multivariate liner regression model, HTGL activity and serum insuline and TG concentrations were associated independly and inversely with low HDL_{2b}. Therefore, plasma elevated TG levels favor the reduction of large-sized HDL particles(HDL_{2a} and HDL_{2b})and the generation of small-sized HDL particles(preβ$_1$-HDL and HDL_3).

It is interesting that apoA-Icontents of preβ$_1$-HDL was significantly lower in women than men in corresponding TG levels. Different plasma HTGL activities may be responsible for the variation of HDL subclasses distribution in men and women. HTGL is sex-steroid sensitive, and its activity is increase by androgens and decrease by oestrogens[37]. Although recent studies have shown genetic variation in the HTGL gene (*LIPC*) promoter is important to HTGL

activity, the effect of the HTGL gene promoter polymorphism on HTGL activity is similar in men and women [36]. It was reported that men had approximate 1.5-fold higher activity of HTGL than women[38]. Most studies have demonstrated that androgens promote mRNA expression of HTGL, whereas oestrogens inhibit mRNA expression of HTGL, which contributes to higher HTGL activity in men[36-39]. Thus, men had higher small-sized preβ$_1$-HDL than women.

2. Effects of plasma LDL-C levels on the distributions of HDL subclasses.

A number of earlier studies, including clinical, pathological, genetic and animal studies, have established the robust relationship between plasma LDL-C levels and occurrence and frequency of CHD as well as atherosclerosis. LDL-C, as being a major cause of CHD, becomes a primary target of therapy. Therefore, we investigated association between the distributions of HDL subclasses and the variance of plasma LDL-C levels. We found that HDL particles tended to smaller with the increase of LDL-C level. As Table 2 shown, the concentrations of TC were significantly higher with increase of LDL-C level in men and women. It was known that CETP could be increased when the concentration of plasma TC is increased. CETP can transfer CE of HDL_2 to CM, VLDL and LDL, and transfer TG of VLDL and LDL to HDL_2[32,40]. And TG in HDL_2 is hydrolyzed by HTGL and EL, releasing apoA□and phosphrolipid[41]. Consequently, large-sized HDL_2 was converted into small-sized HDL_3, which results in decrease of HDL_2 and accumulation of HDL_3.

According to LDL-C levels, we observed the gender differences that concentrations of TG and ratios of TG/HDL-C in LDL-C <2.6 group and LDL-C 2.6～3.3 group were significantly lower than men. We already mentioned above that reduction of HDL particles size, which was caused by increased TG levels. It resulted in that women HDL particle size are larger than men, which was agreement with the study of Zhengling et al.[42].

3. Effects of plasma HDL-C levels on the distributions of HDL subclasses.

The result revealed that both in men and women apoA-Icontents of preβ$_1$-HDL, HDL_{3c} and HDL_{3b} significantly higher while those of HDL_{2b} and HDL_{2a} significantly lower with the decrease of plasma HDL-C level. The alteration of HDL subclasses probably relates to the decrease of LCAT activity at the low HDL-C and low apoA-I [43]. Concentrations of apoA-Iwere significantly lower with decrease of HDL-C levels. The major function of LCAT is catalysis of HDL cholesterol ester formation from lecithin cholesterol, which involves the mature process of preβ-HDL→HDL_3→HDL_2[40].Therefore the decrease of LCAT activities induce significant increase of small-sized preβ$_1$-HDL contents following with significant decrease of large-sized HDL_2 contents. Moreover, there is a positive correlation between plasma HDL-C level and PLTP activity[44,45], which means there will be a imped PLTP activity accompanied with the decrease of HDL-C level. PLTP has the ability of transferring phospholipids from the surface of CM and VLDL to HDL_3. Decrease of PLTP activity induces decrease of phospholipids of HDL_3 , and the decrease of the substrates of LCAT results in weakening of process of HDL_3→HDL_2. Consequently, apoA-Icontent of HDL_{3b} increases with the decrease of HDL-C levels[46]. Especially in men we observed that apoA-Icontents of preβ$_1$-HDL, HDL_{3c} and HDL_{3b} were significantly higher, while those of HDL_{2a} and HDL_{2b} were significantly lower with decrease of plasma HDL-C levels.

According to HDL-C levels, apoA-Icontents of HDL_{2b} in women HDL-C 1.0～1.6 and HDL-C <1.0 groups were significantly higher than men, which may be ascribed to the gender differences of sex hormones. It has been reported that women have higher production rate of apoA-I, the major HDL apoprotein, than do men[47, 48]. Previous reports also showed that HDL-C and Lp A-□(large HDL particles) levels increased after estrogen replacement in dyslipidemic postmenopausal women[46]. High HDL-C and high apoA-Icause increase of LCAT activity, and the increase of the substrates of LCAT results in higher contents of HDL_2 in women. We believed that the gender difference of HDL particles sizes according to HDL-C levels was probably one of the reasons in the gender differences of CHD risk factors.

As shown in Table 1, 2 and 3, both in men and women, with the increase of plasma TG, LDL-C and decrease of HDL-C level, preβ$_2$ HDL did not change significantly. Preβ$_2$ HDL is the discoidal HDL. The smallest preβ$_1$ HDL pick up free cholesterol efficiently from endothelial cell membrane, then come into being preβ$_2$HDL. Preβ$_1$HDL→preβ$_2$HDL. Preβ$_2$ HDL bonding with 2 apoA-I, LCAT. And LCAT which esterifies the free cholesterol can be quickly transferred preβ-HDL to HDL_3. It is concluded that preβ$_2$HDL becomes a minor and invariable component.

To summary, with the increase of TG, LDL-C and decrease of HDL-C in all subjects, the particle size of HDL shifted towards smaller size, which, in turn, indicates that the maturation of HDL may be impeded in those subjects with abnormal lipids profile. In women HDL particles are bigger than men, which potentially results in the gender differences in CHD risk factors and susceptibility to atherosclerosis.

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