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Studies on the Distribution of Plasma Lipid Profiles and Body Fatness According to Apo E Polymorphism in Normolipidemic Korean Women

Myoung-Sook Lee

Dept. of Food and Nutrition, Sungshin Women's University, Seoul 136-742, Korea

Abstract

Apo E polymorphism(e2, e3, e4) was among the first reported genetic polymorphism that explained part of the normal variation in plasma cholesterol concentrations. Both alleles E2 and E4 are significantly more frequent in patients with mixed forms of hyperlipidemia and contribute on the observed differences in CHD risk among different populations. Effects of apo E polymorphism on the distribution of plasma lipid profiles were studied in 89 normolipidemic healthy females, aged 19 up to 22 years. The relative frequencies of E3/3 was 0.787, E3/2 was 0.101, E3/4 allele was 0.112, and no E2/2, E2/4 and E4/4 were found. Weight, height and %LBM were elevated in E2 than those in E3 & E4. No differences in the blood pressure among apo E isomers were found, otherwise the pulsation was higher in E4 than that in the others. There were no differences in plasma total-, total HDL-, HDL_3-, HDL_2 cholesterol, apo B-100 and apo A-I. However, phenotype means rank E3/2>E3/3>E3/4 in average TG levels($p<0.0001$) significantly, and rank E3/4>E3/3>E3/2 in LDL cholesterol levels. These results were related to the correlation between atherogenic indices(AI) such as LDL/HDL, (TC-HDL)/HDL, HDL_3/HDL_2. The ratio of HDL_3 & HDL_2 was significantly increased in E2 & E4 than that in E3($P=0.043$). LCAT activity was not different between E2 and E3 but was highly increased in E4 ($p<0.0001$ among apo E isomers), but CETP was not different. Since the negative correlation between LCAT and CETP in apo E2($r=-0.491$) was stronger than that in apo E3, E2 allele impacts the clearance of plasma apo E mediated lipoproteins. In conclusion firstly, E4 mediated alteration through LDL or E receptors results in lower TG or higher β-lipoprotein levels and E2 shows reciprocal effects of E4, respectively. Second, E4 allele was more atherogenic than E2 allele because the higher levels of AI such as HDL_3/HDL_2 were criticized.

Key words: apo E polymorphism, E3 or E4 or E2 allele, cholesterol, triglyceride, atherogenic index, LDL B/E or E receptors, LCAT, CETP

INTRODUCTION

Apo E is the important structural constituent of several plasma lipoproteins and remnant particles such as VLDL, VLDL remnants, chylomicrons, chylomicron remnants and HDL_1 or HDLc. Therefore, apo E serves as the ligand for both the LDL(B/E) and apo E receptors on plasma membranes of the liver cell, and mediates uptake of lipoprotein which contains apo E(1-3). Moreover, apo E has a key role in the function of HDL on reverse cholesterol transport system and may accelerate the turnover of this system because apo E facilitates the acquisition of cholesterol by HDL and apo E itself actively accepts cholesterol from cholesterol loaded cells(4,5).

The common isoforms of arginine-riched apolipoprotein E(E2, E3 and E4) are encoded by three alleles which exist in humans and give up to six apo E phenotypes in plasma. Apo E4(cys 112--arg) and apo E2 (arg 158---cys) are derived by point mutation from the parent E3 isoform during human evolution but occur with various frequencies among different ethnic, cultural and geographical groups (6,7). Apo E2 is impaired by a low rate of metabolism compared to apo E3(3-5). Due to delayed catabolism of both chylomicron and VLDL remnants and the low rate of apo E-mediated conversion of VLDL to LDL, TG-riched lipoproteins can be accumulated in palsma. Finally, the low rate of transport of remnant particles and decreased rate of LDL formation lead to up-regulation of LDL receptors. Therefore, these apo E2-mediated alteration result in higher plasma concentrations of triglyceride or remnant particles and in lower levels of LDL(5,6). Most of results are consistant with apo E4, the effect opposite to that of apo E2. Chylomicron and VLDL remnants will be converted to LDL at a higher rate, with delivery of cholesterol and triglycerides to liver. Also, VLDL will be converted to LDL at a higher rate, resulting in a higher LDL production rate. The higher rate of conversion of VLDL to LDL will lead to down-regulation of LDL receptor. This

will result in higher LDL and lower remnant particles(2, 5,6,8).

Moreover, alleles E2 and E4 are significantly more frequent in patients with mixed forms of hyperlipidemia. Over 20% of these patients have one phenotypes 4/4, 4/2 or 2/2 compared to 5.3% in the general population(5,9). Most commonly, type III hyperlipoproteinemia occurs in association with the E2/2 phenotype. Nonetheless, apo E from hyper-, normo-, or hypocholesterolemic subjects with E2/2 has equally defective binding to the apo B/E LDL receptor. Since 8% of the variation in the concentration of the LDL cholesterol in serum may be associated with apo E gene locus, there is a high correlation between the apo E phenotypes and increased risk of myocardial infarction.

In the world review of relative allele frequencies, south American Indians, Chinese, Japanese and Caucasians populations are estimated(10-14). Each of three alleles are presented in all of the population groups with the exception of South American Indians(6). The relative frequency of E2 is the lowest in Japan wheras that of E4 is the lowest in Chinese(15). A much higher frequency of E4 allele occurs in Finnish populaiton whose risk of CHD(coronary heart disease) is quite high compared to Japanese(16). It seems that the modest differences in allele frequencies will explain the difference in prevalence of CHD between high and low risk populations(17-20).

The net transfer of cholesteryl ester among lipoprotein during the reverse cholesterol transport(RCT) system is a vital step in normal cholesterol homeostasis(21,22). The three processes contribute to RCT system, LCAT reaction, lipid transfer between different lipoprotein by cholesteryl ester transfer protein(CETP) or TG transfer protien(TGTP) and lipoprotein lipase hydolysis of TG and phospholipid(PL) on peripheral tissue or hepatic lipase on the liver. The esterification of free cholesterol(FC) from peripheral cells by LCAT acting on high density lipoprotien(HDL_3 fraction) is 75~80% of total esteriofication of FC in body(21-23). LCAT stimulated by HDL apolipoprotein, apo A-I and to a lesser extent by apo C-I, apo E and apo A-IV(24). Following esterification, the net transfer of HDL derived CE delivered into TG-rich lipoproteins with CETP to the liver via apo E or LDL B/E receptor because liver is the only organ of cholesterol elimination(25). Since LCAT activity increases the ratio of CE from HDL to TG in HDL, the net transfer of CE from HDL to VLDL via CETP is also regulated by LCAT with a feedback mechanism. Therefore, LCAT and CETP activities are an important regulator in RCT system and factors to control the LCAT and CETP activities are also important such as apolipoprotein. In the case of apo E, apo E and CETP are highly correlated in the reverse cholesterol transport system because the redistribution of apo E caused by dietary fat has been attributed to the transfer of apo E from HDL to the lower lipoproteins(26). Also, other evidence is that both apo E and CETP concentration are increased following cholesterol feeding in normal and hyperlipidemic subjects. The efffects of CETP in RCT system may be secondary to changes in apo E synthesis or RCT metabolism, synergistically.

Therefore, the aim of this study was to investigate the effects of different apo E isoforms on the plasma distribution of lipid profiles including all types of cholesterol, TG, β-lipoprotein in 89 females. The two major objectives of this study were the following: 1) to report, firstly, the relative frequencies of apo E2, E3, E4 alleles in Korean population with modification of the isoelectricfocusing method of apo E phenotyping. 2) to evaluate the variation of plasma cholesterols and apolipoproteins according to apo E isoforms and to test the effects of apo E polymorphism on lipid metabolism especially on reverse cholesterol transport mechanism.

MATERIALS AND METHODS

Research design

Subjects were recruited from Sungshin Women's University during September to October, 1996. Among volunteers, 89 healthy females, aged 19 up to 26 years, were screened on the basis of information from a health questionnaire on drug, alcohol, smoking, coffee & exercise, and physical examination for anthropometic parameters. Subjects were not known history of metabolic diseases and had not taken any medication, specially thyroids or steroids, that affects plasma lipid levels. After the consent form was recieved from subjects, anthropometric parameters and body composition were measured and blood was collected in 0.1% Na EDTA after 12 hours of fasting. The menstual stage was checked in order to keep the same stage for drawing blood. Plasma was separated promptly at 4°C by cetrifugation at 3000rpm and were frozon at -70°C for the blood analysis as followings: cholesterol, total HDL-, HDL_3-, HDL_2- and LDL cholesterol TG, apo E phenotyping, apo A-I, apo B-100,

LCAT and CETP activities: (plasma for LCAT and CETP analysis should be used freshly).

Apo E phenotyping

Apo E phenotypes were separated by the isoelectric-focusing(IEF) electrophoresis using urea polyacrylamide gel. This method was modified by Drs. Kamboh and Ferrell, University of Pittsburgh, using a different method of sample preparation with dithiothreitol(DTT) solution (27-29). The IEF gel with 5% PAGE-3M urea gel was placed on an IEF machine and prefocused for 20min at 1000 volts and 150mA. After the gel was run at 1500V, 150mA, 10 W for 30min or 1hr, sample wicks were removed and IEF was continued for 2 or 2½ hours. IEF gel were transferred overnight to a nitrocellulose membrane with blotting paper. Immunoblotting was performed by steps of blocking with 5% nonfat milk and incubation of 1st(Atlantic apo E antigoat) and 2nd antibody(Atlantic rabbit antigoat antibody Ap). Boric acid solution with β-naphyl phosphate, Fast Blue BB salt and magnesium was used for staining until the bands were dark enough.

Anthropometric parameters

Weight(kg) and height(m) were measured at the period of bleeding days and BMI(body mass index) was calculated by the weight/height(30). Fat% was measured by the Bioelectrical Impedence Assay(BIA) using the analyzer GIF-891(Gil-Woo Trading Company) which BI equation derived from Japanese(Tanaka's study) can be applied for the estimation of Koreans, not for Caucasian(31). Blood pressure and pulsation were measured by the automatic blood pressure monitor and pulsimeter.

Total-, free-, esterified, total HDL-, HDL_3-, HDL_2-, LDL cholesterol

Total and free cholesterol were analyzed by enzymatic procedures modified from the total cholesterol procedure (32). Ester form of cholesterol were determined by differences. Using the spectrophometer, total and free cholesterol were determined by reading the solutions at 500nm after incubation at 37°C for 5 minutes. Total HDL cholesterols were measured after precipitation of VLDL and LDL cholesterol with dextransulfate-1.0mol/L Mg solution(33). HDL_3 cholesterol was determined after HDL_2 particles were removed by precipitation with dextran sulfate-3.0mol/L Mg solution. HDL_2 particles were calculated by substraction of total HDL and HDL_3 cholesterol. LDL cholesterol was calculated by Friedewarld's equation: LDL cholesterol=total choleserol- total HDL cholesterol- 0.2 ×TG(34).

Total triglycerides

Total TG were analyzed by enzymatic method using TG kits from Sigma Chemical Co.(#339)(35). Using the spectrophotometer, total TG were measured at 540 nm for 5 minutes at 37°C.

Plasma β-lipoprotein

After the precipitation of β-lipoprotein with the Heparin-Na^+ solution, the levels of cholesterol in plasma β-lipoprotein were analyzed by the modified enzymatic procedure with the spectrophotometer at 505nm(National Chemicals #15110, Japan). Using the standard solution of β-lipoprotein(900mg/dl) including cholesterol(300 mg/dl), β-lipoprotein was calculated by the proportion of cholesterol in plasma β-lipoprotein reciprocally.

Apo A-I and apo B-100

Plasma total apo A-I and B-100 was determined by radial immunodiffusion assay(RIA) methods(Tago, Inc, Burlington, Ca). Data were adjusted to g/L in plasma from standard unit of mg/dl(36).

Atherogenic indices(AI)

The ratio of LDL/HDL and (TC-HDL)/HDL are usually generalized in hospital as an atherogenic indices. In our study, the ratio of HDL_3/HDL_2 and concentration of β- lipoprotein were added to explain the abnormal lipid metabolism. If these AI is significantly different in apo E isoforms, these AI will be criticized because apo E mutant in lipid metabolism are very clear.

LCAT and CETP analysis

[^{3}H] cholesterol esterificaton(LCAT activity) and transfer(CETP) activity were modified by the methods of Stokke and Norum(37) and Channon et al.(38). [^{3}H] cholesterol-albumin emulsion solution was prepared before the enzymatic activity assay by mixing the dissolved 20 ul of stoke [1α,2α,(n)-[^{3}H] cholesterol(code TRK 330. Amerisham Co. Arlington Heights, IL) into 0.3ml acetone(1uCi= 2.333×106dpm) and 3ml of albumin solution(50mg bovine albumin in 1ml of Tris buffer: pH 7.4) 500ul of whole plasma and 50ul of [^{3}H]-cholesterol albumin(0.3UCi: 6.7×

105dpm) were mixed with 1ml Tris buffer(pH 7.4). This mixture was preincubated at 36.5°C for 1hr with 200ul of 10mmol/DTNB(5,5'-dithiobio [2-nitrobenzoic acid]), LCAT inhibitor, to allow equibrium of [^{3}H] cholesterol with endogeneous FC. After, preincubation, incubation was performed for 3hrs with mercaptoethanol to inhibit DTNB. LCAT reaction was stoppped by putting into the ice. After lipid of plasma should be extracted by Folch method (39) and the radioactivity of FC and CE was fractioned by thin layer chromatography(TLC). Each band of FC and CE was scraped from the TLC plates into 7ml LS cocktail(Scintiverse BOA, Fisher Co.) and were counted by LS counter. FC(Sigma) and CE(cholesterol oleate: Sigma) were used as standard of TLC. LCAT activity was calculated as a product of fractional esterification rate(FER : %/h) and unesterified cholesterol concentration(umol cholesterol esterified/ml plasma per hr) as follows :

$$LCAT=1/t\times[FC]\times\frac{dpm[CE]}{dpm[FC]+dpm[CE]}$$

where, t=incubation time(3hrs)
[FC]=plasma content of FC at time 0hr(μmol/L)
dpm[FC]=radioactivity of FC in lipid extracts
dpm[CE]=radioactivity of CE in lipid extracts

CETP activity was determined by precipitation of V-LDL and LDL from aliquots of incubated plasma by adding dextran sulfate-1.0mol/l Mg^{++} solution. After the supernatant containing HDL was removed by centrifugation at 2700rpm for 30min at 4°C, 100ul aliquots were taken for the radioactivity [dpm HDL] in the fomula. Lipids were extracted from the precipitate(VLDL plus LDL) by the Folch method(39) and CE and FC were separated by TLC as described above. CETP activity(umol/CE transferred/l plasma per hr) was calculated by the formular shown in which the 5.66 was the dilution factor for HDL. CETP activity was also adjusted to constant FC(100mg/dl plasma) to correct for the fact that FC concentration different among subjects. The total dpm was adjusted for correction of the 8.8% decomposition of radioactivity FC.

$$CETP=1/t\times[FC]\times\frac{dpm[CE]}{\text{total dpm}}\times\frac{(\text{total dpm}-5.67\ \text{dpm HDL})}{(dpm[\text{pre FC}]+dpm[\text{pre CE}])}$$

where, t=incubation time(3hrs)
[FC]=plasma content of FC at time 0hr(umol/L)
dpm[pre FC]=radioactivity of FC of precipitates
dpm[CE]=radioactivity of CE of precipitates
total dpm=radioactivity in 50μl aliquots of incubation
dpm HDL=radioactivity in 100μl supernatant containig HDL(5.67 is a dilution factor)

Data analysis

After normality test by univariate probability using the all subjects and each different apo E isoforms, student t-test, correlation and regression analyses for the comparison, one-way analysis of variance and multiple regression for the differences among the apo E alleles were used for the data analysis.

RESULTS AND DISCUSSION

Relative frequencies of apo E phenotypes

Among the 89 healthy females, 70 were apo E3/3 (r.f.= 0.787), 9 were E3/2 (r.f.=0.101), 10 were E3/4 (r.f.=0.067), no E2/2, E2/4 and E4/4 were found. Immunolocalized IEF patterns of these three different apo E phenotypes of subjects in this study were shown in Fig. 1. Since we have only three phenotypes, six major bands of apo E phenotypes were shown diagramatically in Fig. 2. The major bands are labeled from ⓐ to ⓗ in order of increasing IEF points. Except for five bands of E3/2 types, all heterozygotic phenotypes consist of six major bands. Urea gel for the phenotyping of apo E polymorphism is the most

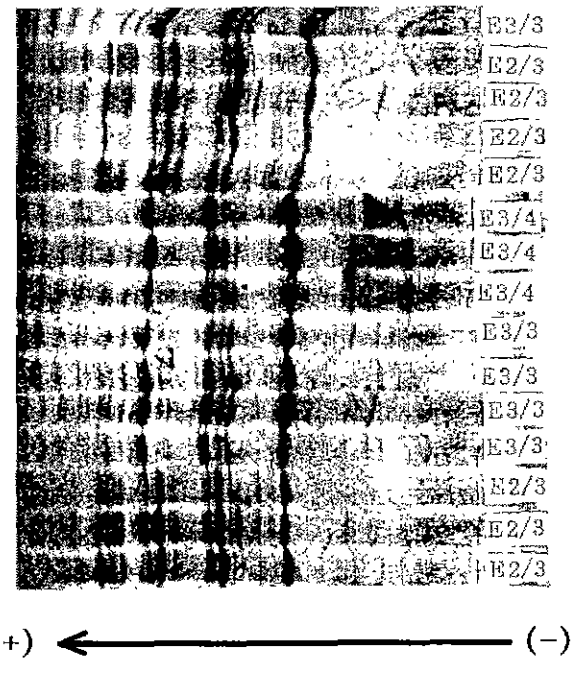

Fig. 1. The binding patterns of three different apo E phenotypes in this study.

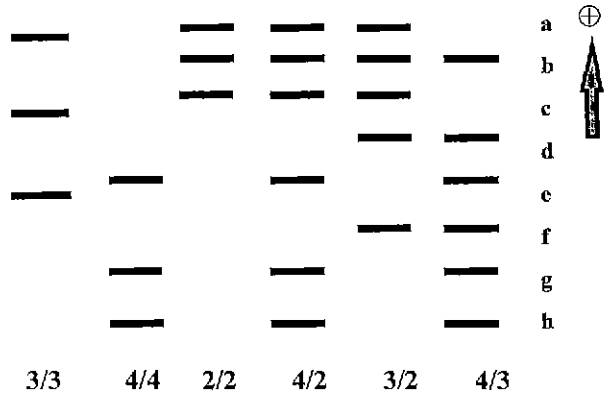

Fig. 2. Major banding patterns of 6 apo E phenotypes.

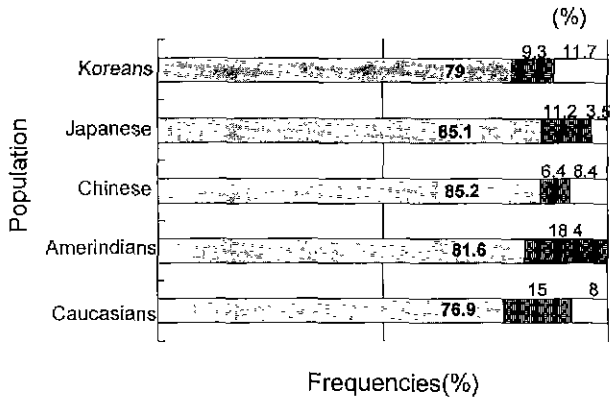

Fig. 3. The relative frequencies of the common apo E alleles for the research I plus research II study.
□ E3 allele, ▦ E4 allele, □ E2 allele

useful technique to identify of rare apo E alleles so far. Moreover, this IEF method has known to have several advantages : a) simple, rapid and reproducible mono-, dimensional IEF as compared to other methods, b) ultra-centrifugation for the separaton of VLDL was not necessary, c) very small amounts of plasma were needed(28,29).

The relative frequencies of common alleles for gene locus of apo E3, E4, and E2 allele 0.848, 0.087, and 0.067, respectively. Comparing the others for apo E polymorphism, the relative frequencies of E2 and E4 alleles were lower than those found from Caucasians such as following studies for Asians. The relative frequency of apo E2 allele was 0.05 in the Chinese(7) and 0.023 in Japanese (12). Sing and Davingnon(14) reported that the relative frequency of apo E2 was 0.07 in Caucasians. The results of relative frequencies of E2 and E4 alleles in Koreans in this study(Research II, 1997) and Research I(1996) explaining that risk of the CHD is relatively low compared to Caucasians (Fig. 3). Even though the sample size for this study was not large enough to represent the population study for Koreans, the relative frequency of apo E2 allele found in these subjects was slightly higher than that for Japanese and the frequency of E2 was slightly higher than that for Chinese.

Anthropometric parameters and body composition

BMI, Triceps and Fat % were not different among the apo E alleles(Table 1). However, weight and height were slightly higher in E2 allele than those in E3 and E4 even BMI was not different. Even though % fat was not different among apo E isoforms, 70% of E2 allele was in the range of 21 to 25 in BMI compared to the 50% of E3 and E4 in that area. Also, 57% of E2 allele were in the range of 26 to 30% of the total fat% even 33%, 22% of E3 and E4 alleles were belonged to(Fig. 4). These mean that the people had a E2 allele which is heavier, higher, fatter than those of E3 and E4 alleles even the statistic significance was not found. The relationship between a fat % and apo E polymorphism could not be concluded because of no comparing data, but the abnormality of plasma lipid metabolism in a apo E mutant condition might be accelerated with a higher percentage of fat in the body, espe-

Table 1. Mean(±SD) values of circumference, skinfold thickness & body composition in the different apo E isoforms

Variables	E3 allele(n=70)	E4 allele(n=10)	E2 allele(n=9)	P-value(E isoforms)
Height(cm)	159.53±4.81[a]	160.62±5.1[b]	163.63±4.86[c]	0.050*
Weight(kg)	53.03±5.59[a]	54.90±9.66[b]	59.94±6.78[c]	0.009*
BMI	20.78±1.98[a]	21.43±3.32[a]	22.35±1.88	0.104
Triceps(mm)	20.98±5.77	18.44±4.69	23.48±4.17	0.146
Subcapular(mm)	13.07±3.05[b]	11.72±3.37[a]	15.36±3.47[c]	0.041*
%Fat(%)	23.09±3.05	23.21±5.07	23.93±2.76	0.781
%LBM(%)	40.65±3.92[a]	41.80±4.82[b]	44.66±5.14[c]	0.025*
%Water(%)	29.70±2.87[a]	30.54±3.53[b]	32.63±3.76[c]	0.025*

*Significance among the apo E3, E4 & E2 alleles at $p<0.05$
[a,b,c]Values within the apo E_3, E_4 & E_2 with different superscripts are significantly at $p<0.05$

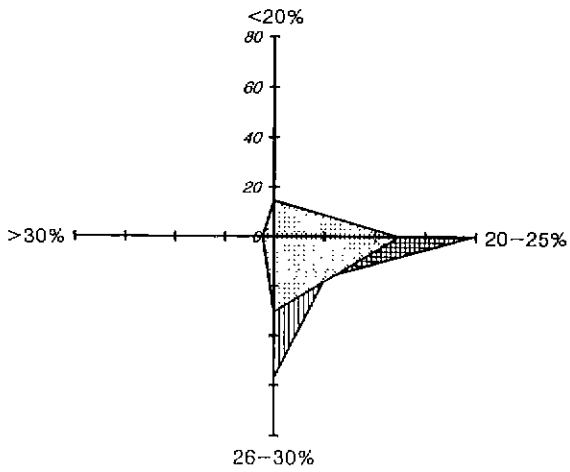

Fig. 4. Body fat % according to apo E alleles.
▦ apo E3/4, ▥ apo E3/2, □ apo E3/3

cially subcapular area(Table 1).

Blood pressure and pulsation

Table 2 presented blood pressure and pulsation in apo E alleles. Systolic and diastolic blood pressure were not different among apo E isoforms. The pulsation was increased by E4 allele with significance of $p<0.05$. These results were consistent with elevated total body fat % in E2 allele. Although the absolute body fat corresponding to significant risk factor for high blood pressure was not known, excess upper-body fat has been asssociated with elevated blood pressure, serum lipid. and lipoprotein fractions(31,32). The reason for the increased body fat and blood pressure in E2 allele compared to E3 or E4 might be due to delayed TG removal from plasma.

Lipid profiles between apo E3 and E2 isoforms

The mean plasma total-, HDL-, TG-riched lipoprotein-, TG, apo A-I and apo B-100 as a lipid profiles in subjects with apo E alleles were shown in Table 3. Total-, HDL, LDL cholesterol were not different among the apo E alleles in this study. Sing and Davingnon(14) reported that the total cholesterol levels of E2/2, E3/2, E3/3, E4/2, E4/3, E4/4 phenotypes were 136, 161, 174, 178, 184, and 180mg/dl, respectively, in populations of Caucasians. Taushiya et al.(12) reported the mean total cholesterol levels of E2/2, E3/2, E3/3, E4/2. E4/3, E4/4 phenotypes were 142, 164, 179, 214, 178, 188mg/dl, respectively, in Japanese population. Sing and Davingnon(14) agreed to the phenotypes mean rank as E2/2<E3/2<E3/3<E4/3 for the average total cholesterol of populations. These mean that plasma cholesterol levels were lower in E2 and higher in E4 than that in E3 allele.

Table 2. Mean(±SD) vaules of systolic blood pressure(SBP), diastolic blood pressure(DBP), and pulsation according to apo E isoforms

Variables	E3 allele(n=70)	E4 allele(n=10)	E2 allele(n=9)	P-value(E isoforms)
SBP(mmHg)	110.01±13.09	115.30±11.17	112.00±10.4	0.450*
DBP(mmHg)	69.93± 8.69	74.2 ±10.04	74.33±10.36	0.183
Pulsation	84.3±12.68[b]	90.8 ±13.26[c]	75.89±17.28[a]	0.050*

[a,b,c]Values within the apo E3, E4 and E2 alleles with different superscripts are significantly at $p<0.05$
*Significance among the apo E3, E4 & E2 alleles at $p<0.05$

Table 3. Mean(±SD) vaules of plasma lipid profiles(mmol/l) & apolipoproteins(mg/dl) in the different apo E alleles

Variables	E3 allele(n=70)	E4 allele(n=10)	E2 allele(n=9)	P-value(E isoforms)
Cholesterol(mmol/L)				
Total-	4.72±0.85	4.99± 0.80	4.53±0.84	0.481
Total HDL- [1]	1.42±0.46	1.40± 0.48	1.38±0.34	0.956
LDL- [2]	3.03±0.83	3.20± 0.83	2.67±0.87	0.364
Total griglyceride	0.86±0.27[a]	1.11± 0.41[b]	1.31±0.68[c]	0.000*
Apolipoprotein(mg/dl)				
Apo A-I	184.92±36.15	191 2 ±40.81	186.22±26.86	0.874
Apo B-100	76.42±16.27	86.9 ±23.75	80.19±28.42	0.239

*Significance among the apo E isoforms at $p<0.01$
[1)]Analyzed by precipitation methods
[2)]Calculated by Frieldwarld equation

The concentration of LDL cholesterol were slightly higher in E4(3.2±0.83mmol/L) and lower in E2(2.67±0.87mmol/L) compared to E3(3.03±0.83mmol/L). Lenzen(40) found that the alleles E2 and E4 had major effects on LDL cholesterol. The same stepwise decreasing gradient in plasma LDL cholesterol, going from E4/3 to E3/3 to E3/2 to E2/2, occurred both in patients with coronary disease and myocardial infraction, and in the normal group. Utermann(5,19) and Eto(13) agreed there was a increasing effect of apo E4 allele on LDL cholesterol as was suggested by this study. These results were consisted to the plasma TG levels, respectively.

The plasma TG levels were significantly higher in the apo E2 allele(1.31±0.68mmol/L) than in the apo E3 allele (0.86±0.44mmol/L) in this study. Otherwise the plasma TG levels in E4 allele(1.61±0.41mmol/L) were significantly lower than that in E2 allele. This result showed the same tendency as reports of Sing and Davingnon(14) and Utermann(19) but, was not same from the report of Lenzen that TG did not vary with apo E phenotypes(40). Since the apo E2 allele had a binding default of LDL receptor and accumulation of TG rich lipoproteins, the higher level of TG in apo E2 allele was affected from clearance of VLDL plus LDL lipoproteins as expected. Moreover, the higher catabolism of plasma TG riched lipoproteins in apo E4 was associated with the lower plasma level of TG. Secondly, higher rate of LDL formation leads to down-regulation of LDL receptors. Therefore, these E4-mediated alteration resulted in lower level of TG and higher level of LDL. Most of results from E4 was opposite to that of E2 allele(1,19). Our data are well accorded with the above results.

Atherogenic indices(AI)

The ratio of LDL/HDL, (TC-HDL)/HDL and the concentration of β-lipoprotein as atherogenic risk factors were not different among apo E isoforms. However, it was interested in that the results of lipid profile in apo E alleles altered the pattern of HDL fraction. Total HDL cholesterol was not different among apo E alleles. However, apo E2 allele subjects had lower levels of total HDL and HDL_2 cholesterol and significantly higher levels of HDL_3 cholesterol than those in apo E3 and E4 allele subjects. Lenzen(40) mentioned that the mean plasma concentrations of total HDL cholesterol did not vary significantly within the apo E phenotypes. Though many researchers did not expect a difference between apo E phenotypes in the levels of HDL_3 and HDL_2 cholesterol, this relationship was examined in this study and previous studies such as Lee's(4,41-4). In this study, apo E2 allele subjects had lower levels of HDL_2 and significantly higher levels of HDL_3 cholesterol than those in apo E3 allele. However, plasma cholesterol levels in HDL fraction was not affected by apo E4 allele. Consistantly, higher ratio of HDL_3 vs HDL_2 cholesterol in apo E2 allele compared to that in apo E3 or E4 suggested the higher risk of CHD. Therefore, AI such as (TC-HDL)/ HDL and HDL_3/HDL_2 were significantly increased in E2 allele than those in E4 allele(Table 4). These results are related to the common reports for apo E polymorphism to be mentioned that all people with type III hyperlipidemia have E2/2, otherwise, fewer than 5% of E2/2 have the type III. Even though E2/2 was not found in this study, it is important with higher levels of AI in E2 allele than that in E3 and E4(43,45,46). This study suggested important two things for apo E polymorphism research. First, the E2 allele is a stronger risk factor of CHD than in E4 allele due to the lower levels of HDL_2 cholesterol. Second, the HDL_3 and HDL_2 fraction should be considered in parts of lipid metabolism study as the influencing factor. In Fig. 5, mean differences of apo E mutant in lipid profiles and AI compared to apo E3. Plasma TG levels and the ratio of HDL_3/HDL_2 were significantly higher than those normal apo E3.

Table 4. Mean(±SD) vaules of plasma distribution of atherogenic indices(AI) in the different apo E alleles

(mmol/L)

Variables	E3 allele(n=70)	E4 allele(n=10)	E2 allele(n=9)	P-value(E isoforms)
LDL/HDL[1)]	2.13±0.25	2.28±0.31	1.91±0.51	0.547
(TC-HDL)/HDL[1)]	2.98±1.42	3.47±2.37	2.85±1.26	0.604
HDL3/HDL2[1)]	1.42±0.59[a]	1.80±0.34[b]	2.18±0.63	0.043*
β-Lipoprotein(mg/dl)	476.1±246.1	514.9±319.5	362.1±152.9	0.363

[1)]Calculated by the ratio of the each cholesterol

*Significance among the apo E isoforms at $p<0.05$

[a,b,c] Values within the apo E3, E4 and E2 alleles with different superscripts are significantly at $p<0.05$

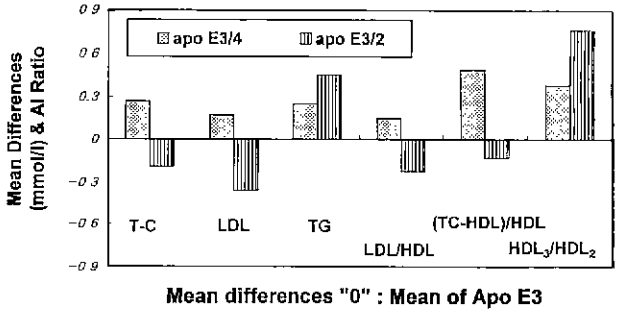

Fig. 5. Mean differences of apo E mutants in lipid profiles & atherogenic indices(AI).

LCAT and CETP activies

LCAT activity was significantly different among the apo E isoforms($p<0.0001$) with higher levels of apo E4 allele because the total free cholesterol was higher in apo E4 allele significantly than in others(Fig. 6, 7). However, CETP was not different even though there are evidneces that apo E mutation tended to increase CETP activity more than normal condition. The changes in CETP activity are related to changes in plasma VDL plus LDL cholesterol, which increased in apo E mutation, especially E2 allele(21-25). In Fig. 6, esterified [^{3}H]-cholesterol in VLDL plus LDL from the total free choelsterol was significantly higher in apo E2 allele than in apo E4 and E3 alleles. This result induced to the stronger negative correlation between LCAT and CETP in apo E2($r=-0.491$) than that in apo E3. That was explained by the E2 allele impacts on the clearance of apo E mediated lipoproteins(47).

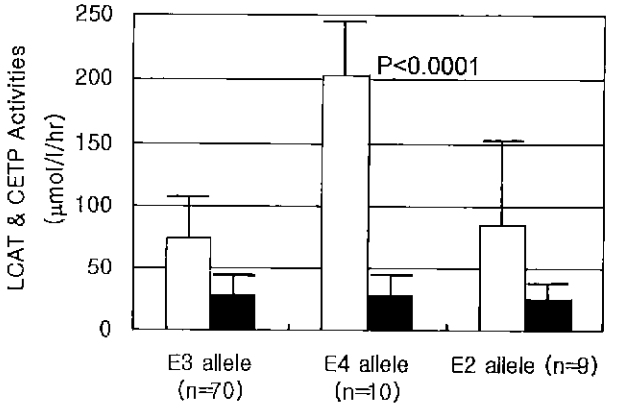

Fig. 6. Mean(±SD) values for LCAT & CETP activities in the different apo E alleles(μmol/L/hr).
□ LCAT, ■ CETP

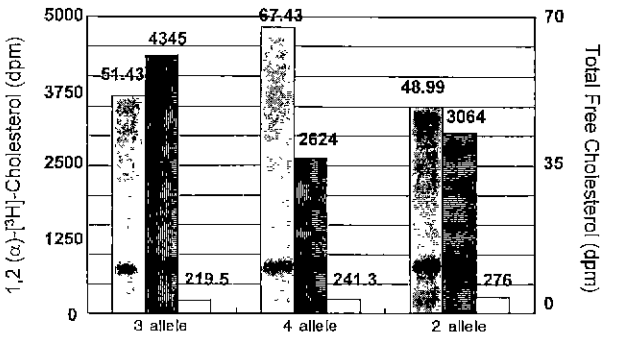

Fig. 7. The concentration of esterified [^{3}H]-cholesterol in HDL & VLDL+LDL from the total free cholesterol.
□ total free cholestrol, ▦ CE(dpm) in HDL
□ CE(dpm) in VLDL-LDL

CONCLUSION

The effects of apo E allele on the distribution of plasma lipid profiles with artherogenic indexes were investigated in 89 normolipidemic female subjects. The summary of results are as followings :

1) The relative frequencies of common alleles for gene locus of apo E in this study were that E3/3 was 0.787, E3/2 was 0.101, E3/4 allele was 0.112, and no E2/2, E4/4 and E2/4 were found. Comparing the others for apo E polymorphism, the relative frequency of E2 and E4 alleles were lower in Asian than those in study for Caucasians.

2) SBP and DBP were slightly elevated in E2 allele more than those in E3 and E4. The pulsation was also significantly increased by E2 allele with excess body fat % in E2 allele. Weight, height, subscapular fat and % LBM were elevated in E2 than those in E3 & E4.

3) There are no differences in plasma levels of total-, total HDL-, LDL cholesterol , apo A-I and apo B-100 among the apo E alleles. However, the plasma TG levels were significantly higher in the apo E2 allele than in the apo E3 allele, otherwise, the plasma TG levels in E4 allele was significantly lower than that in E3 allele. Morever, apo E2 allele subjects had lower levels of total HDL and HDL_2 cholesterol and significantly higher levels of HDL_3 cholesterol than those in apo E3 and E4 allele subjects.

4) Atherogenic indices(AI) such as HDL_3/HDL_2 significantly increase in E2 allele than those in others even though LDL/HDL, (TC-HDL)/HDL and β-lipoprotein, which were usually generalized for AI, were not changed by apo E mutation.

5) LCAT activity was significantly different among the apo E isoforms($p<0.0001$) with higher levels of apo E4 allele because the total free cholesterol was higher in apo E4 allele significantly than in others. CETP activity was not different. However, the esterified [^{3}H]-cholesterol in VLDL plus LDL from the total free choelsterol was significantly higher in apo E2 allele than in apo E4 and E3

alleles. This result induced to the stronger negative correlation between LCAT and CETP in apo E2(r=-0.491) than that in apo E3. That was explained by the E2 allele impacts on the clearance of apo E mediated lipoproteins.

In conclusion, a) Apo E4-mediated alteration through LDL B/E receptors or E receptors in cholesterol metabolism results in lower plasma TG or remnant particles and in higher levels of LDL cholesterol. b) Apo E2 allele shows reciprocal effects of E4 on the plasma lipid metabolism, respectively. c) Apo E2 allele was more atherogenic than apo E4 because the higher levels of HDL_3/HDL_2 ratio as a AI were criticized. Therefore, HDL_3 and HDL_2 fraction should be considered in parts of lipid profiles as the influencing factor. d) Apo E2 allele impacts the clearance of apo E mediated lipoprotein in reverse cholesterol transport system by the high retention of CE in VLDL plus LDL. That's why the clearance of the liver through apo E and LDL(B/E) receptor was inhibited.

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