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The relationship of sterol regulatory element–binding protein cleavage–activation protein and apolipoprotein E gene polymorphisms with metabolic changes during weight reduction

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Abstract

Sterol regulatory element–binding protein cleavage–activating protein (SCAP) and apolipoprotein E (apo E) regulate cellular and plasma lipid metabolism. Therefore, variations in the corresponding genes might influence weight reduction and obesity-associated metabolic changes. We investigated the relationships of SCAP (Ile796Val) and apo E polymorphisms on metabolic changes during weight reduction by using a 12-week very low-energy diet. Body composition, serum lipids, plasma glucose, and insulin were assessed in 78 healthy premenopausal women (initial body mass index, 34 ± 4 kg/m^2; age, 40 ± 4 years) before and after the intervention. The SCAP genotype groups did not differ in the responses of any parameters measured during weight reduction. Apo E did not differentiate the weight loss, but the changes in total and low-density lipoprotein cholesterol for the genotype groups apo E ε2/3, ε3/3, as well as ε3/4 and ε4/4 combined were −0.94 ± 0.56 and −0.59 ± 0.32, −0.71 ± 0.49 and −0.49 ± 0.45, and −0.55 ± 0.47 and −0.37 ± 0.39 mmol/L, respectively ($P < .05$ for both). In conclusion, neither the SCAP Ile796Val nor the apo E polymorphism was associated with weight loss in obese premenopausal women. However, the apo E—but not SCAP genotype—seems to be one of the modifying factors for serum cholesterol concentrations during very low-energy diet in obese premenopausal women.

1. Introduction

Obesity is associated with hypertension and diabetes, and it is an independent risk factor for coronary heart disease [1]. Reduction of body weight decreases the concentrations of serum total and low-density lipoprotein cholesterol (LDL-C) [2,3]. We chose polymorphisms in 2 lipid metabolism modulators—a sterol regulatory element–binding protein (SREBP) cleavage–activating protein (SCAP) and apolipoprotein E (apo E) [4,5]—to study the effects of genes on the responses in LDL-C and total cholesterol during weight reduction.

SCAP is an important regulator of SREBP [6,7], which activates genes encoding, for instance, enzymes of cholesterol and fatty acid biosynthesis [8,9]. A single base alteration, A to G, of the SCAP gene has been identified, and it results in the substitution of isoleucine (Ile) with valine (Val), leading to 3 genotypes [10]. To our knowledge, the importance of this polymorphism to lipid metabolism during weight reduction has not been investigated earlier.

Apo E influences the metabolism of all lipoproteins [5]. The apo E ε4 allele has been found to increase the risk of premature atherosclerosis [11] by causing high total

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Table 1
Main characteristics of the subjects according to SCAP polymorphism before the weight reduction phase and the changes in these characteristics during the weight reduction

Mean	Before weight reduction				Changes during weight reduction			
	I/I (n = 28)	I/V (n = 36)	V/V (n = 14)	P^a	I/I (n = 28)	I/V (n = 36)	V/V (n = 14)	P^b
Weight (kg)	90.6 ± 10.8	93.4 ± 9.0	92.9 ± 9.2	.51	−13.0 ± 3.9	−13.1 ± 3.4	−13.5 ± 2.7	.91
Fat proportion (%)	42 ± 5	45 ± 4	44 ± 3	.04	−4 ± 4	−4 ± 3	−4 ± 3	.97
Fat mass (kg)	38.7 ± 8.4	42.3 ± 6.7	41.2 ± 6.5	.16	−8.5 ± 3.6	−9.0 ± 3.5	−9.2 ± 2.1	.80
Waist circumference (cm)	99 ± 8	105 ± 8.0	103 ± 12	.03	−11 ± 5	−12 ± 4	−13 ± 7	.56
Body mass index (kg/m^2)	33.3 ± 3.8	34.5 ± 3.2	34.6 ± 3.6	.34	−4.9 ± 1.5	−4.9 ± 1.4	−5.0 ± .8	.93
Systolic blood pressure (mm Hg)	118 ± 9	120 ± 12	119 ± 9	.76	−6 ± 10	−3 ± 9	−3 ± 10	.44
Diastolic blood pressure (mm Hg)	77 ± 8	80 ± 7	79 ± 7	.34	−6 ± 7	−6 ± 7	−7 ± 6	.79
Glucose (mmol/L)	5.12 ± 0.69	5.19 ± 0.39	5.05 ± 0.24	.64	−0.29 ± 0.44	−0.20 ± 0.40	−0.22 ± 0.21	.22
Insulin (mU/L)	10.49 ± 4.30	11.46 ± 4.12	11.09 ± 5.68	.69	−3.88 ± 3.73	−4.31 ± 3.68	−5.02 ± 5.35	.46
Total cholesterol (mmol/L)	4.97 ± 0.75	5.20 ± 0.82	4.60 ± 0.85	.07	−0.73 ± 0.50	−0.68 ± 0.51	−0.56 ± 0.46	.65
Triglycerides (mmol/L)	1.20 ± 0.44	1.34 ± 0.42	1.28 ± 0.60	.50	−0.29 ± 0.40	−0.26 ± 0.29	−0.35 ± 0.55	.17
HDL-C (mmol/L)	1.27 ± 0.30	1.19 ± 0.22	1.18 ± 0.20	.38	−0.13 ± 0.20	−0.06 ± 0.13	−0.09 ± 0.13	.33
LDL-C (mmol/L)	3.16 ± 0.71	3.40 ± 0.74	2.85 ± 0.80	.06	−0.47 ± 0.36	−0.51 ± 0.45	−0.31 ± 0.39	.81

Values are expressed as mean ± SD.
[a] P values are from ANOVA.
[b] P values are from ANCOVA.

cholesterol and LDL-C levels [5]. The influence of the apo E genotype on lipid levels during several different dietary interventions has been studied [12], but the data during weight reduction are very limited. We tested whether these 2 polymorphisms affect LDL-C and total cholesterol levels in obese premenopausal women on a very low-energy diet (VLED).

2. Materials and methods

2.1. Subjects

The study protocol has been published earlier [13]. In brief, the subjects participated in a randomized controlled trial investigating the effects of post–weight reduction physical activity on weight maintenance. The volunteers included were clinically healthy premenopausal Finnish women aged 30 to 45 years, with a body mass index (BMI) of 30 to 45 kg/m^2 and who were not taking regular medication (other than hormonal contraceptives). Furthermore, they were not physically active. Suspected binge eaters (symptom scores >20 on the Bulimic Investigatory Test of Edinburgh) were excluded [14]. After screening assessments, including medical history, examination by a physician, and submaximal exercise test, 85 Finnish women (mean age, 40 ± 4 years) were included in the study. Data on 78 women with a completed weight-reduction program and successful genotyping were included in the analysis. Three of the subjects used oral hormonal contraception with both estrogens and progestagens, and 9 had an intrauterine device with levonorgestrel. The study was approved by the Ethical Committee of the UKK Institute for Health Promotion Research. Written informed consent was obtained from the participants as stipulated in the Declaration of Helsinki.

2.2. Weight-reduction phase

The 12-week weight-reduction program included 3 stages: a low-energy diet based on a meal-exchange system for 1 week, followed by a very low-energy diet (VLED) using Nutrilett (energy content, 155 kJ/100 g; Nycomed-Pharma, Oslo, Norway) for weeks 2 to 9, and a low-energy diet for weeks 10 to 12. The subjects met weekly in small groups. A nutritionist supervised all meetings and gave instructions on the diet, weight maintenance, and relapse prevention.

2.3. Anthropometry and body composition

The body density was measured by underwater weighing after full exhalation as described previously [15]. Waist circumference was measured midway between the iliac crest and lowest rib. The mean of 3 readings was used.

2.4. Laboratory measurements

Venous blood samples were obtained in the morning after 12 hours of fasting before and after the weight-reduction phase. Serum and plasma were separated by centrifugation and stored at −70°C until analysis. Serum total cholesterol and triglyceride concentrations were analyzed from frozen samples by enzymatic methods (CHOD-PAP for cholesterol and GPO-PAP for triglycerides; Boehringer Mannheim, Mannhein, Germany). The level of high-density lipoprotein cholesterol (HDL-C) was determined by selective precipitation (dextran sulfate-magnesium chloride), whereas the level of LDL-C was calculated with the Friedewald formula [16]. Plasma insulin determinations were carried out by radioimmunoassay (Phadeseph Insulin, Pharmacia, Uppsala, Sweden). Plasma glucose was assessed by the glucose dehydrogenase method (Merck Diagnostica, Darmstadt, Germany). Automatic analyzers (Hitachi model 717,

Table 2
Main characteristics of the subjects according to apo E genotype before the weight reduction phase and the changes in these characteristics during the weight reduction

Mean	Before weight reduction				Changes during weight reduction			
	ε2/3 (n = 8)	ε3/3 (n = 40)	ε3/4 and ε4/4 (n = 28)	P^a	ε2/3 (n = 8)	ε3/3 (n = 40)	ε3/4 and ε4/4 (n = 28)	P^b
Weight (kg)	87.6 ± 9.7	92.7 ± 10.3	93.3 ± 8.9	.34	−13.4 ± 4.1	−13.0 ± 3.8	−13.2 ± 2.9	.90
Fat proportion (%)	40 ± 6	45 ± 3	44 ± 4	.03	−5 ± 3	−4 ± 3	−4 ± 4	.55
Fat mass (kg)	35.8 ± 8.9	41.7 ± 6.8	41.2 ± 7.7	.12	−9.0 ± 3.4	−8.6 ± 3.2	−9.1 ± 3.5	.85
Waist circumference (cm)	100 ± 12	102 ± 8	104 ± 9	.42	−14 ± 10	−12 ± 5	−12 ± 4	.16
Body mass index (kg/m^2)	32.2 ± 2.6	34.0 ± 3.5	34.6 ± 3.7	.23	−5.0 ± 1.6	−4.8 ± 1.4	−4.9 ± 1.1	.91
Systolic blood pressure (mm Hg)	118 ± 9	119 ± 10	121 ± 12	.72	−4 ± 11	−4 ± 10	−5 ± 9	.99
Diastolic blood pressure (mm Hg)	74 ± 7	79 ± 8	79 ± 7	.21	−2 ± 6	−6 ± 8	−7 ± 5	.48
Glucose (mmol/L)	5.10 ± 0.54	5.05 ± 0.30	5.31 ± 0.68	.11	−0.41 ± 0.49	−0.17 ± 0.29	−0.29 ± 0.47	.14
Insulin (mU/L)	9.60 ± 4.45	10.86 ± 3.74	11.94 ± 5.37	.37	−4.43 ± 4.91	−4.16 ± 3.39	−4.57 ± 4.71	.31
Total cholesterol (mmol/L)	4.66 ± 0.95	5.01 ± 0.73	5.19 ± 0.82	.24	−0.94 ± 0.56	−0.71 ± 0.49	−0.55 ± 0.47	.01
Triglycerides (mmol/L)	1.27 ± 0.28	1.24 ± 0.50	1.35 ± 0.47	.67	−0.44 ± 0.32	−0.24 ± 0.34	−0.31 ± 0.47	.26
HDL-C (mmol/L)	1.26 ± 0.19	1.24 ± 0.27	1.17 ± 0.22	.44	−0.15 ± 0.25	−0.12 ± 0.16	−0.04 ± 0.12	.18
LDL-C (mmol/L)	2.83 ± 0.86	3.21 ± 0.69	3.41 ± 0.71	.12	−0.59 ± 0.32	−0.49 ± 0.45	−0.37 ± 0.39	.02

Values are expressed as mean ± SD.
[a] P values are from ANOVA.
[b] P values are from ANCOVA.

Hitachi, Tokyo, Japan, and Epos 5060, Eppendorf, Darmstadt, Germany) were used for the analyses.

2.5. DNA extraction and genotyping

DNA was prepared from peripheral blood leukocytes with the salting-out method [17]. The polymorphism of isoleucine/valine at codon 796 in exon 16 of the SCAP gene, a 235-base-pair fragment containing an *Msl*I enzyme restriction site, was amplified by polymerase chain reaction (PCR) using the primers 5′-TTGTGCTGCGCGGCCACCTCA-3′ and 5′-AGGAGGAAAGGGCAGCCGCAC-3′ [10]. PCR was performed in a volume of 50 μL. Cycle conditions were 94°C for 4 minutes, then 28 cycles at 94°C for 1 minute, 64°C for 1 minute, and 72°C for 1 minute, with a final extension step of 5 minutes at 72°C in a PTC-225 thermal cycler (MJ Research, Waltham, MA). Dimethyl sulfoxide (10%) was included in the PCR reaction. The amplified DNA fragment was digested for 16 hours with 10 U of *Msl*I (New England Biolabs, Ipswich, MA). After digestion, the fragments were separated by electrophoresis on a 3% agarose gel (SeaKem, Cambrex, Baltimore, MD). Apo E genotypes were determined by PCR after the restriction enzyme digestion [18]. The PCR conditions were denaturation at 95°C, annealing at 62°C, and extension at 72°C for 40 cycles, followed by final extension at 72°C. The fragments were visualized on 5% MetaPhor (FMC Bio-Products, Rockland, ME) agarose gels.

2.6. Statistical analysis

SPSS release 12.0.1 for Windows (SPSS, Chicago, IL) was used for statistical analysis. The χ^2 test was used to confirm that the genotype distributions of alleles were in Hardy-Weinberg equilibrium. At baseline, means of variables among the genotypes were compared with 1-way analysis of variance (ANOVA). The changes in biochemical and blood pressure variables (Tables 1 and 2) after the weight reduction were compared by analysis of covariance (ANCOVA) using baseline levels of the variables studied (one by one), age, baseline body fat mass, change in fat mass during the diet, and the use of contraception (oral or intrauterine device) as covariates. The ε2/2 and ε2/4 genotype groups of apo E were excluded from the analyses because of the low number of subjects (one in each group). The data are expressed as mean ± SD, unless otherwise specified. $P < .05$ was considered significant.

3. Results

3.1. Baseline characteristics

The baseline mean weight was 92.2 ± 9.7 kg, mean BMI, 34.3 ± 3.5 kg/m^2, and mean fat proportion, 43.9% ± 4.2% in all subjects.

SCAP genotype frequencies were I/I = 0.359, I/V = 0.462, and V/V = 0.179. The apo E genotype frequencies were ε2/2 = 0.013, ε2/3 = 0.103, ε2/4 = 0.013, ε3/3 = 0.513, ε3/4 = 0.295, and ε4/4 = 0.064. The genotype distributions adhered to Hardy-Weinberg equilibrium.

The baseline clinical characteristics for the 3 SCAP genotype groups are presented in Table 1. Fat mass and waist circumference varied significantly among the SCAP genotypes. Neither the cholesterol values nor the plasma glucose and insulin levels varied significantly among the SCAP genotype groups. The corresponding values for the apo E polymorphism are given in Table 2. Fat mass differed significantly among the apo E genotype groups in the beginning of the study, whereas the other parameters did not.

3.2. Changes during weight reduction

After the 12-week weight reduction with VLED, the mean decrease in body mass was 13.1 ± 3.4 kg (14.2%).

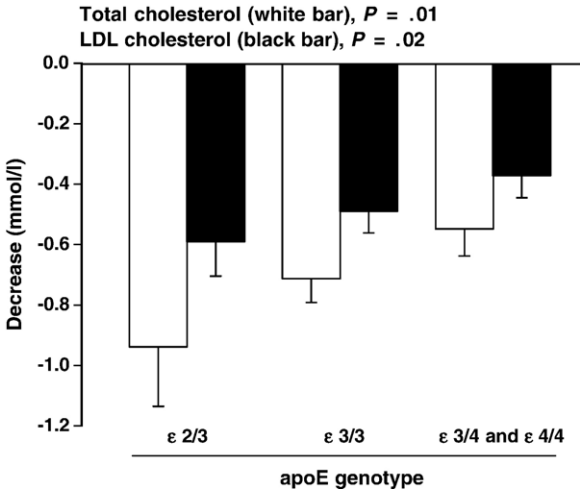

Fig. 1. Decrease (mean ± SEM) in total cholesterol (white bars) and LDL-C (black bars) during weight reduction according to apo E genotype. The *P* values were derived with ANCOVA by using age, baseline levels of the variables studied (one by one), baseline fat mass, change in fat mass during the 12-week VLED, and use of hormonal contraception as covariates.

The fat mass decreased from 44.0% to 39.9%. The overall mean change in BMI was −4.9 kg/m^2. The decrease in body weight, fat mass, and BMI did not differ significantly between the SCAP and apo E genotype groups (Tables 1 and 2). SCAP polymorphism did not show any significant difference in any of the changes in anthropometric and metabolic measurements. During the weight-reduction phase, the levels of total cholesterol and LDL-C decreased more in the ε2/3 genotype group than in the ε3/3 or the combined ε3/4 and ε4/4 groups (Fig. 1). The reduction of total cholesterol was 20.2% in the ε2/3 group, 14.1% in the ε3/3 group, and 10.6% in the combined ε3/4 and ε4/4 group. The corresponding percentages for the reduction of LDL-C were 20.8%, 15.3%, and 10.9%, respectively.

4. Discussion

SCAP is an activator of cellular cholesterol metabolism. It is located in the endoplasmic reticulum, and it stimulates the cleavage of SREBPs, which activate the synthesis and uptake of cholesterol and fatty acids from plasma. As SCAP is a major regulator of cholesterol metabolism, we hypothesized that the common polymorphism Ile796Val of SCAP would modify the changes in lipid metabolism caused by weight reduction. Nevertheless, neither the lipid levels at baseline nor after the weight reduction were affected by the SCAP genotype. The anthropometric changes during VLED were also not influenced by the SCAP polymorphism. It therefore seems that the Ile796Val polymorphism of SCAP is not related to body composition and metabolic changes during weight loss. It is possible, however, that the limited population size of the present study prevents these differences from reaching statistical significance. On the other hand, most of the absolute differences between the groups (Table 1) might not be clinically important even if statistical significance had been reached.

There are conflicting results on the effects of SCAP polymorphism on the response in the lipid levels during treatment with 3-hydroxy-3methylglutaryl coenzyme A (HMG CoA) reductase inhibitors [19,20], suggesting that the polymorphism may be relevant in certain other aspects of lipid metabolism and treatment.

We found significant differences at baseline in fat proportion (but not in fat mass) and waist circumference among the different SCAP genotype groups, in addition to a difference in the baseline fat proportions of the apo E genotypes. However, the differences were relatively minor, and the heterozygotes had the highest values, suggesting a random effect.

Many studies have been devoted to differences in dietary responsiveness among the apo E genotypes [12]. Some studies have found more pronounced lipid changes in apo E ε4 carriers [21], whereas others have reported no such effects [22]. However, knowledge on the influence of this polymorphism during weight loss, particularly using VLED, is scarce. In a study that included 54 obese normolipidemic women, apo E polymorphism did not affect the anthropometric or lipoprotein variables during a 2-month low-energy diet resulting in an average weight loss of 10.1 kg [23]. A study of elderly participants (aged 74 ± 3 years vs 40 ± 4 years in ours) showed an association between apo E ε4 and weight loss in women [24], especially in those with Alzheimer disease. However, the weight loss was not statistically significant in the nondemented subjects carrying the ε4 allele after controlling for diabetes and exercise.

Another study subjected 146 men and women (baseline BMI, 31 ± 3 kg/m^2; age 51 ± 10 years) to a 12-week energy-controlled diet that resulted in an average weight loss of 3.2 kg [25]. The apo E ε4 carriers were hyporesponsive to weight loss with regard to their total cholesterol levels. This finding is supported by our results because the reduction in LDL-C and total cholesterol levels was smallest among the ε4 carriers, who also had the highest baseline levels of LDL-C and cholesterol. Interestingly, it seems that the apo E ε4 allele may have a differential influence on cholesterol levels at a stable body weight and during weight reduction. However, the mechanism underlying this finding remains unclear. Larger scale studies are required to confirm these results.

In conclusion, obese premenopausal women with the ε4 genotype of apo E seem to have a more minor reduction in LDL-C and cholesterol levels during VLED when compared with subjects with no ε4 allele. The Ile796Val polymorphism of SCAP does not differentiate lipid changes during weight loss. Neither the SCAP Ile796Val nor the apo E polymorphism is associated with weight loss in obese premenopausal women.

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References

[1] Poirier P, Giles TD, Bray GA, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association scientific statement on obesity and heart disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Circulation 2006;113:898-918.

[2] Dattilo AM, Kris-Etherton PM. Effects of weight reduction on blood lipids and lipoproteins: a meta-analysis. Am J Clin Nutr 1992;56:320-8.

[3] Yu-Poth S, Zhao G, Etherton T, Naglak M, Jonnalagadda S, Kris-Etherton PM. Effects of the National Cholesterol Education Program's Step I and Step II dietary intervention programs on cardiovascular disease risk factors: a meta-analysis. Am J Clin Nutr 1999;69:632-46.

[4] Weber LW, Boll M, Stampfl A. Maintaining cholesterol homeostasis: sterol regulatory element–binding proteins. World J Gastroenterol 2004;10:3081-7.

[5] Greenow K, Pearce NJ, Ramji DP. The key role of apolipoprotein E in atherosclerosis. J Mol Med 2005;83:329-42.

[6] Meier CA. Regulation of cholesterol synthesis: of SCAP SREBP, CBP and more. Eur J Endocrinol 1997;136:271-2.

[7] Nakajima T, Hamakubo T, Kodama T, Inazawa J, Emi M. Genomic structure and chromosomal mapping of the human sterol regulatory element binding protein (SREBP) cleavage-activating protein (SCAP) gene. J Hum Genet 1999;44:402-7.

[8] Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 1997;89:331-40.

[9] Jia H, Hingorani AD, Sharma P, et al. Association of the G(s)alpha gene with essential hypertension and response to beta-blockade. Hypertension 1999;34:8-14.

[10] Iwaki K, Nakajima T, Ota N, Emi M. A common Ile796Val polymorphism of the human SREBP cleavage-activating protein (SCAP) gene. J Hum Genet 1999;44:421-2.

[11] Ilveskoski E, Perola M, Lehtimäki T, et al. Age-dependent association of apolipoprotein E genotype with coronary and aortic atherosclerosis in middle-aged men: an autopsy study. Circulation 1999;100:608-13.

[12] Rubin J, Berglund L. Apolipoprotein E and diets: a case of gene-nutrient interaction? Curr Opin Lipidol 2002;13:25-32.

[13] Fogelholm M, Kukkonen-Harjula K, Nenonen A, Pasanen M. Effects of walking training on weight maintenance after a very-low-energy diet in premenopausal obese women: a randomized controlled trial. Arch Intern Med 2000;160:2177-84.

[14] Henderson M, Freeman CP. A self-rating scale for bulimia. The "BITE". Br J Psychiatry 1987;150:18-24.

[15] Fogelholm GM, Sievanen HT, van Marken Lichtenbelt WD, Westerterp KR. Assessment of fat-mass loss during weight reduction in obese women. Metabolism 1997;46:968-75.

[16] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.

[17] Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.

[18] Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with *Hha*I. J Lipid Res 1990;31:545-8.

[19] Fan YM, Laaksonen R, Janatuinen T, et al. Effects of pravastatin therapy on serum lipids and coronary reactivity are not associated with SREBP cleavage-activating protein polymorphism in healthy young men. Clin Genet 2001;60:319-21.

[20] Fiegenbaum M, Silveira FR, Van der S and CR, et al. Determinants of variable response to simvastatin treatment: the role of common variants of SCAP SREBF-1a and SREBF-2 genes. Pharmacogenomics J 2005;5:359-64.

[21] Sarkkinen E, Korhonen M, Erkkila A, Ebeling T, Uusitupa M. Effect of apolipoprotein E polymorphism on serum lipid response to the separate modification of dietary fat and dietary cholesterol. Am J Clin Nutr 1998;68:1215-22.

[22] Weggemans RM, Zock PL, Ordovas JM, Pedro-Botet J, Katan MB. Apoprotein E genotype and the response of serum cholesterol to dietary fat, cholesterol and cafestol. Atherosclerosis 2001;154:547-55.

[23] Muls E, Vansant G, Beirinckx AM, Jans I. Dietary fat intake in relation to apo E isoforms and body fat distribution in obese women. Acta Cardiol 1993;48:473-5.

[24] Vanhanen M, Kivipelto M, Koivisto K, et al. APOE-epsilon4 is associated with weight loss in women with AD: a population-based study. Neurology 2001;56:655-9.

[25] Kee F, Young IS, Poirier O, et al. Do polymorphisms of apoB, LPL or apoE affect the hypocholesterolemic response to weight loss? Atherosclerosis 2000;153:119-28.