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The apolipoprotein E polymorphism and the cholesterol-raising effect of coffee

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Abstract

Background: The response of serum cholesterol to diet may be affected by the apolipoprotein E (APOE) $\epsilon 2/\epsilon 3/\epsilon 4$ polymorphism, which also is a significant predictor of variation in the risk of coronary heart disease (CHD) and CHD death. Here, we test the hypothesis that the APOE polymorphism may modulate the cholesterol-raising effect of coffee.

Objective: We determined the effect of a coffee abstention period and a daily intake of 600 mL coffee on serum cholesterol and triglycerides with respect to the APOE polymorphism.

Design: 121 healthy, non-smoking men (22%) and women (78%) aged 29–65 years, took part in a study with four intervention periods: 1 and 3) a coffee free period of three weeks, 2 and 4) 600 mL coffee/day for four weeks.

Results: APOE $\epsilon 2$ positive individuals had significantly lower total cholesterol concentration at baseline (4.68 mmol/L and 5.28 mmol/L, respectively, $p = 0.01$), but the cholesterol-raising effect of coffee was not influenced significantly by APOE allele carrier status.

Conclusions: The APOE $\epsilon 2$ allele is associated with lower serum cholesterol concentration. However, the APOE polymorphism does not seem to influence the cholesterol-raising effect of coffee.

Introduction

Apolipoprotein E (apoE) is a structural component of triglyceride-rich lipoproteins, chylomicrons, very-low-density lipoproteins (VLDL), and high-density-lipoproteins (HDL) [1]. Variation in the APOE gene sequence results in

the 3 common alleles ($\epsilon 2$, $\epsilon 3$ and $\epsilon 4$), which can produce 6 different genotypes ($\epsilon 2/\epsilon 2$, $\epsilon 2/\epsilon 3$, $\epsilon 2/\epsilon 4$, $\epsilon 3/\epsilon 3$, $\epsilon 3/\epsilon 4$ and $\epsilon 4/\epsilon 4$). The $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$ alleles encode three distinct forms of apoE (E2, E3 and E4) and have approximate frequencies of 8%, 77%, and 15%, respectively, in white

populations [2]. ApoE3 seems to be the normal isoform in all known functions, while apoE4 and apoE2 can each be dysfunctional [3,4]. In most populations, individuals with the APOE ε2 allele display lower levels of plasma cholesterol compared with individuals carrying the APOE ε3 allele, whereas individuals with the APOE ε4 allele show higher levels of plasma cholesterol, especially LDL-cholesterol [1,2,5]. Subjects with APOE ε3/ε4 and ε4/ε4 genotypes absorb cholesterol effectively and have higher non-fasting serum triglyceride values than ε4 negative individuals [6,7]. The allelic variation in the APOE gene is shown to be a significant predictor of variation in the risk of coronary heart disease (CHD) and CHD death [2-4,8-10], but the results from an extensive prospective study showed no associations [11]. Both the MONICA Project [12] and the Scandinavian Simvastatin Survival Study [13] suggest an increased risk of CHD for individuals carrying the APOE ε4 allele. The APOE ε4 allele is also considered a strong risk factor for Alzheimer's disease [14-16].

The serum cholesterol-raising effect of coffee is due to the diterpenes kahweol and cafestol [17]. Earlier studies have shown a cholesterol-raising effect mainly of unfiltered coffee, because a major part of the diterpenes is retained by a paper filter [18-20]. A recent trial, however, indicates that filtered coffee has a more pronounced serum cholesterol-raising effect than previously anticipated [21]. This finding was further corroborated in a randomized intervention study, where we demonstrated a considerable cholesterol-raising effect of filtered coffee [22]. In the study two coffee abstention periods were associated with a significant decline in serum cholesterol of 0.22 and 0.36 mmol/L, respectively, while 600 mL filtered coffee a day

during two different periods increased serum cholesterol by 0.25 and 0.15 mmol/L, respectively. Here, we test the hypothesis that these effects might be modulated by the APOE ε2/ε3/ε4 polymorphism.

Subjects and methods

Trial design

The study was organised as a prospective, controlled study with four consecutive trial periods. The first and third periods were 3 weeks of total coffee abstention. The second and fourth period consisted of 4 weeks with the subjects consuming 600 mL filter brewed coffee/day.

The main outcome or effect variable was total serum cholesterol and the effect was assessed as the difference between the measurements at the beginning and the end of the coffee free periods (coffee abstention) and the difference between measurements at the beginning and at the end of the four weeks of coffee consumption (Figure 1). Trial duration of 3-4 weeks has previously been shown to be sufficient to get an effect of coffee on serum cholesterol [21,23].

Subjects and procedure

Participants were recruited by advertising in Gothenburg's major newspaper. Inclusion criteria were age-range 30-65 years, free from clinically recognized chronic diseases, such as cardiovascular diseases, cancer, renal disorders, liver disease and diabetes mellitus. The participants were required to be free from anti-epileptic or cholesterol lowering drugs, and had been using coffee on a regular basis for at least five years and were currently non-smokers (at least for the last six months).

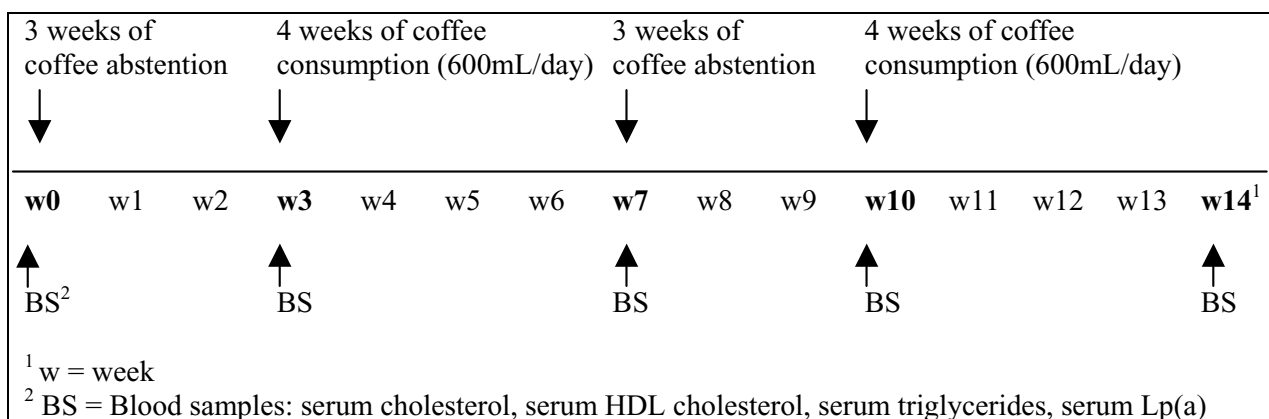


Figure 1
Study design

During the coffee drinking periods the participants were instructed to drink about 600 mL filter brewed coffee/day (4 cups), according to standardised measures. The coffee was provided to guarantee that they were all exposed to the same brand and quality of filter brewed coffee. All participants also got the same kind of standardised coffee filter and measuring spoon.

The coffee filters used were of the brand Euro-Shopper, made by Indupa N.V., Zaventem, Belgium. Divergence from the 4 cups was reported. The participants were allowed to drink tea and other caffeine containing beverages during the coffee-free periods.

Effect variables

Non-fasting blood samples were drawn at inclusion and at three, seven, ten and fourteen weeks after start of the study. Prior to analysis, prepared serum was stored at -70°C.

The blood samples were analysed for blood lipids (total cholesterol, HDL cholesterol, triglycerides, lipoprotein(a) (Lp(a)) and urate in serum. Serum cholesterol and triglycerides were determined by an enzymatic procedure on a Hitachi 917 analyzer. HDL cholesterol was determined enzymatically after precipitation of VLDL, LDL and chylomicrons by α -cyclodextrinsulphate and dextranulphate. Determination of Lp(a) was done by the method Tint Elize Lp(a) of Biopool International. Serum urate was analysed by Hitachi 917 autoanalyser. Body Mass Index (BMI; kg/m²) was recorded once during the study. Blood pressure was recorded by manual device and EKG and heart rate were recorded at all five visits.

The dietary habits were assessed by dietary frequency questionnaires at the beginning of the study. A follow-up survey with special emphasis on changes in food habits during the four different periods was undertaken. The dietary questionnaire was based upon a Norwegian version, which has been used in a number of previous studies [24].

Genotype Analysis

APOE genotypes were determined by solid-phase minisequencing as previously described by Blennow et al [25].

Statistical methods

All analyses were performed using the SAS® software version 8. Signed rank test was used to test differences in the groups. Wilcoxon rank sum test was used to test differences at baseline and differences between the groups. The mean was used as location measure and measures of variation were described in terms of standard deviation. P-values < 0.05 were considered statistically significant.

Table 1: APOE genotype and allele frequency, n = 121

	n	%
$\epsilon 2/\epsilon 2$	2	1.7
$\epsilon 2/\epsilon 3$	9	7.4
$\epsilon 2/\epsilon 4$	2	1.7
$\epsilon 3/\epsilon 3$	69	57.0
$\epsilon 3/\epsilon 4$	36	29.8
$\epsilon 4/\epsilon 4$	3	2.5
$\epsilon 2$	15	6.2
$\epsilon 3$	183	75.6
$\epsilon 4$	44	18.2

Table 2: Serum cholesterol concentration (mmol/L) at baseline and after two 3-week periods of coffee abstinence (week 0 – 3 and week 7 – 10) for APOE $\epsilon 2$ -positive (n = 13) and APOE $\epsilon 2$ -negative (n = 108) individuals

	APOE $\epsilon 2$ -positive n = 13	APOE $\epsilon 2$ -negative n = 107/103 ^a	p
<i>First trial period</i>			
week 0	4.68 (0.80)	5.28 (0.93)	0.01 ^b
week 3	4.49 (0.71)	5.05 (0.90)	
diff week 0–3	-0.18 (0.24)	-0.23 (0.55)	0.30 ^c
p (diff 0–3)	0.02 ^d	<0.0001 ^d	
<i>Second trial period</i>			
week 7	4.52 (0.71)	5.34 (0.93)	
week 10	4.34 (0.64)	4.95 (0.89)	
week 7–10	-0.18 (0.41)	-0.39 (0.55)	0.08 ^c
p (diff 7–10)	0.13	<0.0001 ^d	

^a 107 participants in the first trial period and 103 participants in the second trial period

^b Significant difference between APOE $\epsilon 2$ -positive and APOE $\epsilon 2$ -negative at baseline, Wilcoxon rank sum test

^c No significant difference between APOE $\epsilon 2$ -positive and APOE $\epsilon 2$ -negative in differences between week 0–3 or week 7–10, Wilcoxon rank sum test

^d Significant difference between week 0–3 for the APOE $\epsilon 2$ -positive group and between week 0–3 and week 7–10 for the APOE $\epsilon 2$ -negative group, Signed rank test

Results

A total of 156 persons responded to the advertisement and of these 124 fulfilled the criteria and were able to take part. Three persons decided to withdraw during the study, leaving a total of 121 participants. One person was not able to take part during the first two periods and five persons were not able to take part in the last two periods, which resulted in 120 participants in the first trial period and 116 in the subsequent trial period.

Table 3: Serum cholesterol concentration (mmol/L) after two 4-week periods of coffee consumption (week 3 – 7 and week 10 – 14) for APOE ϵ 2-positive (n = 13) and APOE ϵ 2-negative (n = 108) individuals

	APOE ϵ 2-positive n = 13	APOE ϵ 2-negative n = 107/103 ^a	p
<i>First trial period</i>			
week 3	4.49 (0.71)	5.05 (0.90)	
week 7	4.52 (0.71)	5.34 (0.93)	
diff week 3–7	0.03 (0.57)	0.29 (0.57)	0.09 ^b
p (diff 3–7)	0.70	<0.0001 ^c	
<i>Second trial period</i>			
week 10	4.34 (0.64)	4.95 (0.89)	
week 14	4.54 (0.84)	5.09 (0.85)	
diff week 10–14	0.20 (0.47)	0.14 (0.59)	0.37 ^b
p (diff 10–14)	0.15	0.009 ^c	

^a 107 participants in the first trial period and 103 participants in the second trial period

^b No significant difference between ϵ 2-positive and ϵ 2-negative in differences between week 3–7 or week 10–14, Wilcoxon rank sum test

^c Significant difference between week 0–3 and week 7–10 for the APOE ϵ 2-negative group, Signed rank test

Table 4: Baseline characteristics for APOE ϵ 2-positive (n = 13) and APOE ϵ 2-negative (n = 108) individuals

	APOE ϵ 2-positive n = 13	APOE ϵ 2-negative n = 108	p
Sex (% women)	77	79	ns ^a
Age (years)	46.6	48.7	0.44 ^b
BMI (kg/m ²)	25.7	25.8	0.87 ^b
Coffee consumption (cups/day)	4.3	3.7	0.12 ^b

^a No significant difference between APOE ϵ 2-positive and APOE ϵ 2-negative at baseline, Chi square test

^b No significant difference between APOE ϵ 2-positive and APOE ϵ 2-negative at baseline, Wilcoxon rank sum test

Genotype frequencies

The APOE allele frequencies were 6.1% for the ϵ 2 allele, 75.6% for the ϵ 3 allele and 18.2% for the ϵ 4 allele. This distribution agrees well with those reported in other populations in northern Europe [2,3]. Genotype and allele frequencies for the APOE polymorphism are given in Table 1.

Serum cholesterol concentrations according to genotype and coffee exposure

Individual APOE genotypes (six subgroups, Table 1) did not influence baseline values or coffee-induced changes in

serum cholesterol, serum HDL cholesterol, serum triglycerides or serum Lp(a), possibly due to a small sample size (data not shown). ϵ 4-positive individuals had similar serum cholesterol levels and coffee-induced changes in cholesterol concentration as ϵ 4-negative individuals (data not shown). However, when grouping ϵ 2-positive individuals it was revealed that these displayed significantly lower cholesterol at baseline (Table 2). There was a significant difference in cholesterol decrease between week 0 and 3 for both groups. There was no difference between the two groups regarding the cholesterol decrease in the first coffee abstinence period but there was a significant difference in cholesterol decrease in the second coffee abstinence period, where ϵ 2-negative individuals displayed a larger decrease in cholesterol (Table 2).

Coffee consumption resulted in a significant cholesterol increase in the ϵ 2-negative group in both trial periods (w 3–7 and w 10–14), but not in the ϵ 2-positive group (Table 3). There were no differences between the ϵ 2-positive and the ϵ 2-negative group according to baseline characteristics as sex, age, body mass index (BMI) and coffee consumption prior to the study (Table 4).

Dietary monitoring and compliance

The dietary survey did not reveal any important changes during the four intervention periods [22]. Coffee consumption or non-compliance was reported by six persons during the first coffee abstinence period (mean 1.8 cups/period), whereas four persons reported coffee consumption in the second coffee abstinence period (mean 0.7 cups/period).

Discussion

Subjects with different APOE genotypes differ in the absorption efficiency of cholesterol from the intestine, in the synthesis rates of cholesterol and bile acids, and in the production of LDL apoprotein B [3,26]. This suggests that the response of serum cholesterol to diet may be affected by the APOE ϵ 2/ ϵ 3/ ϵ 4 polymorphism [27,28]. One previous study examined the effect of purified cafestol on serum lipids in relation to the APOE polymorphism [26] and found that responses of LDL-cholesterol to cafestol were slightly smaller among carriers of the APOE ϵ 4 allele. Here, we investigate for the first time the possible influence of the APOE polymorphism on the cholesterol-raising effect of filtered coffee.

APOE ϵ 4-positive individuals did not differ significantly from ϵ 4-negative individuals with regard to baseline cholesterol concentration or coffee-induced changes in cholesterol concentration. However, we confirm that ϵ 2-positive individuals display significantly lower cholesterol levels at baseline than ϵ 2-negative individuals. A tendency was seen that ϵ 2-positive individuals might be partly pro-

tected from the cholesterol increasing effect of coffee consumption. This was, however, only seen in the first trial period and will require further investigations. In conclusion, the *APOE* $\epsilon 2/\epsilon 3/\epsilon 4$ polymorphism is not a strong modulator of the cholesterol-increasing effect of coffee. Other genes should be discussed and further investigation is needed to see if there is a genetic factor in the cholesterol-raising effect of coffee.

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