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APOE Genotypes, Lipid Profiles, and Associated Clinical Markers in a Finnish Population with Cardiovascular Disease Risk Factors

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Keywords
APOE · Genetic variation · Lipid · Fatty acid · Lifestyle

Abstract

Introduction: The *APOE ε4* allele predisposes to high cholesterol and increases the risk for lifestyle-related diseases such as Alzheimer's disease and cardiovascular diseases (CVDs). The aim of this study was to analyse interrelationships of *APOE* genotypes with lipid metabolism and lifestyle factors in middle-aged Finns among whom the CVD risk factors are common. ***Methods:*** Participants ($n = 211$) were analysed for *APOE ε* genotypes, physiological parameters, and health- and diet-related plasma markers. Lifestyle choices were determined by a questionnaire. ***Results:*** *APOE* genotypes *ε3*/*ε4* and *ε4*/*ε4* (*ε4* group) represented 34.1% of the participants. Genotype *ε3*/*ε3* (*ε3* group) frequency was 54.5%. Carriers of *ε2* (*ε2* group; *ε2*/*ε2*, *ε2*/*ε3* and *ε2*/*ε4*) represented 11.4%; 1.9% were of the genotype *ε2*/*ε4*. LDL and total cholesterol levels were lower ($p < 0.05$) in the *ε2* carriers than in the *ε3* or *ε4* groups, while the *ε3* and *ε4* groups did not differ. Proportions of plasma saturated fatty acids (SFAs) were higher ($p < 0.01$), and omega-6 fatty acids lower ($p = 0.01$) in the *ε2* carriers compared with the *ε4* group. The *ε2* carriers had a higher ($p < 0.05$) percentage of 22:4n-6 and 22:5n-6 and a lower ($p < 0.05$) percentage of 24:5n-3 and 24:6n-3 than individuals without the *ε2* allele. ***Conclusions:*** The plasma fatty-acid profiles in the *ε2* group were characterized by higher SFA and lower omega-6 fatty-acid proportions. Their lower cholesterol values indicated a lower risk for CVD compared with the *ε4* group. A novel finding was that the *ε2* carriers had different proportions of 22:4n-6, 22:5n-6, 24:5n-3, and 24:6n-3 than individuals without the *ε2* allele. The significance of the differences in fatty-acid composition remains to be studied.

Introduction

Cardiovascular diseases (CVDs) and dementias, including Alzheimer's disease (AD), are among the most common causes of morbidity and mortality for adults in Finland [1]. These conditions arise as a complex interplay between various lifestyle factors and contributory risk genes, one of the best-known being the *APOE* gene, which

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encodes apolipoprotein E [2, 3]. This is also reflected onto the phenotype: a healthy diet and a sufficient amount of exercise seem to delay the onset of AD and CVD [4–6].

The APOE protein has been discovered to play a role not only in lipoprotein metabolism but also in neuroprotection, antimicrobial defence, oxidative stress, and inflammation and functions as a potential transcription factor [7, 8]. This protein occurs in three major isoforms, APOE ε2, ε3, and ε4. The concentration of the APOE protein in plasma has been shown to be associated with the *APOE* genotype [9, 10]. The most frequent isoform, ε3, is associated with normal function, whereas the ancestral ε4 predisposes to high cholesterol and an increased risk of AD and CVD. The isoform ε2, on the other hand, seems to be neuroprotective but a susceptibility factor for type III hyperlipoproteinaemia [11]. The *ε4* allele is more common in patients suffering from AD, its prevalence being about 48.8% in AD patients worldwide. In healthy people, its world average prevalence is 13.7% [12], whereas in Finland, the prevalence of the ε*4* allele was 18.5% in a population-based cohort study with a total of 13,275 subjects [13]. There is evidence that increased LDL cholesterol levels increase the risk for the development of AD and CVD [6, 14].

In the present study, carotenoids, retinol, α-tocopherol, phenolic compounds, and plasma fatty-acid composition as well as the APOE protein were analysed to verify the self-reported dietary and lifestyle habits. Plasma fatty acids were analysed because the metabolism of fatty acids, most importantly omega-3 fatty acids, is possibly disturbed in *ε4* carriers when compared to non-carriers [15]. In previous studies, the reported plasma [16], blood [17], or plasma LDL and HDL [18] fatty-acid profiles have been limited. Earlier results have indicated that the *ε4* allele in males is associated with higher plasma EPA concentrations and possibly also higher DPA and DHA [16]. In addition, the ε*4* allele is associated with a higher omega-6/omega-3 ratio and lower ALA and DHA in LDL fractions [18] compared to individuals not carrying that allele. Due to these previous observations, we considered it important to screen a wider palette of fatty acids when trying to find possible differences between *APOE* genotypes.

The aim of this study was to analyse interrelationships of the *APOE* genotypes with lipid metabolism and lifestyle factors (physical activity and diet) among 211 Finnish volunteers. Among the Finns, several CVD risk factors, such as high cholesterol and overweight, are common. In the present study, the palette of measured biochemical indicators was broader and the reported plasma fatty-acid composition was more comprehensive than in previous studies describing the effects of the *APOE* genotype on these markers [16–19]. The participants were categorized into three groups based on their *APOE* genotype: *ε2* group (genotypes *ε2*/*ε2*, *ε2*/*ε3,* and *ε2*/*ε4*), *ε3* group (genotype *ε3*/*ε3*), and *ε4* group (genotypes *ε3*/*ε4* and *ε4*/*ε4*). Because *ε2* has a powerful protective effect [11], in this study, all *ε2* carriers were grouped into the *ε2* group. On the other hand, as the *ε4* allele is associated with an increased risk of AD and CVD [11], genotypes *ε3*/*ε4* and *ε4*/*ε4* were grouped into the *ε4* group.

Materials and Methods

The 211 volunteers were middle-aged Finns recruited from the region of South Ostrobothnia in Finland at the beginning of 2017 (Fig. 1). The inclusion criteria were age between 40 and 60 years, overall good health, body mass index under 35 kg/m^2, blood pressure under 140/90 mm Hg (medication allowed), serum total cholesterol under 7.25 mmol/L (medication allowed), and fasting plasma glucose level under 7 mmol/L. Overall good health as the inclusion criteria was defined as follows: no current or previous diagnosis of chronic conditions, e.g., mental disorders, cancer, and liver or kidney disease. As CVD risk factors are common, medications for blood pressure, type 2 diabetes, hypothyroidism, and high cholesterol were not exclusion criteria.

Recruiting advertisements were placed in local newspapers, on notice boards, and on the internal communication channels of some major employers in Seinäjoki, the principal town in the region. An information letter describing the study protocol and an overview of the *APOE* gene were sent to those interested in participating. Of approximately 300 contacts, 250 persons met the preliminary inclusion criteria and expressed interest in continuing after receiving further information. Of those, 24 did not participate in the compulsory genetics lecture, 10 dropped out after the 2-week reconsideration period, and 5 did not participate in the baseline measurements. The participants were interviewed on their medical history, medication and dietary supplement use, exercise habits, smoking, and alcohol consumption.

Physical activity and diet, including dietary fat quality, consumption of vegetables, fruits and berries, fish, high-fat/high-sugar foods, and alcohol, were determined by a voluntary online questionnaire using the Webropol 2.0 Software (Webropol Ltd., Helsinki, Finland), and physiological measurements were conducted by standard methods as described in Leskinen et al. [20] and online supplementary Methods (for all online suppl. material, see www.karger.com/doi/10.1159/000520864). Briefly, the consumption of vegetables, fruits, and berries was reported as portions per day and sweet and savoury delicacies and fish as times per week. Consumption of alcohol was reported as units of alcohol per week. In the dietary fat quality assessment, a score of 27–33 points equalled a sufficient proportion of unsaturated fats, whereas <15 points equalled mostly saturated fats. Of the 211 participants, 189 completed the questionnaires. The self-reported medication use is provided in online supplementary Table S1.

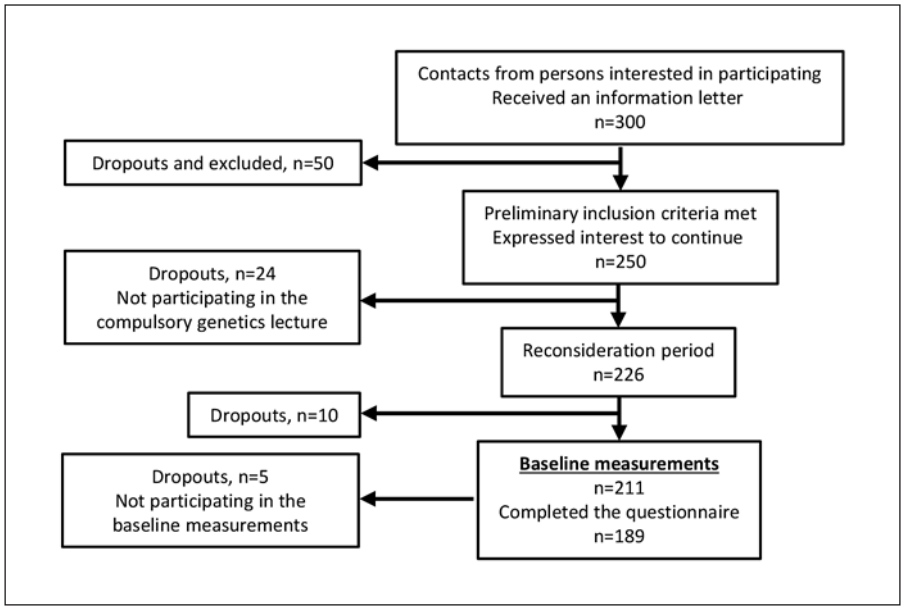

Fig. 1. CONSORT diagram of the study. Participants were healthy Finnish adults (age 40–60 years) recruited for an intervention study.

The subjects were instructed to abstain from alcohol consumption and vigorous physical activity during the preceding day before giving the blood sample. Venous blood samples were collected in the morning (between 7:30 a.m. and 9:30 a.m.) after a 12-h overnight fast. Samples were also collected 30 min and 2 h after a glucose challenge (250 mL of the GlucosePro glucose solution containing 75 g of glucose [Comed Oy, Ylöjärvi, Finland]). The samples were stored at −80°C until analysis.

DNA was extracted from whole blood samples using the DNeasy Blood & Tissue Kits (QIAGEN, Hilden, Germany). Genotyping the variations rs429358 and rs7412, combinations of which form the *APOE* genotypes, was performed according to Hietaranta-Luoma et al. [21]. Serum lipids, complete blood count, glucose tolerance test samples, and general clinical biomarkers were analysed according to standard guidelines using an automatic clinical analyser (cobas 8000; Roche Diagnostics, Risch-Rotkreuz, Switzerland). C-peptide response was used to measure insulin response in the glucose tolerance test. Equimolar amounts of C-peptide and insulin are released when proinsulin is activated to insulin. The half-life of C-peptide is longer than that of insulin which makes it easier to measure from blood samples. The concentration of the APOE protein was analysed using the Human ApoE ELISA kit (Elabscience E-EL-H0470) as instructed by the kit providers, and samples were analysed randomly in duplicates and diluted 1:10.

Carotenoids, retinol, and α-tocopherol were extracted from plasma according to Karppi et al. [22] and analysed by the Agilent 1100 high-performance liquid chromatography instrument equipped with a diode array detector (Agilent, Santa Clara, CA, USA) and Kinetex® EVO C18 as an analytical column (250 × 4.6 mm; 5 µm; Phenomenex, Torrance, CA, USA) at 40°C using a mixture of methanol and ethanol (75:25, vol/vol) as an isocratic eluent at 0.9 mL/min [23]. Carotenoids were detected at 480 nm, retinol at 292 nm, and α-tocopherol at 325 nm, and the UV/Vis spectrum was recorded at 190–600 nm. β-Apo-8′-carotenal, retinyl acetate, and α-tocopherol acetate were used as internal standards. In these analytical conditions, lutein and zeaxanthin were not separated.

The Folin-Ciocalteu method is commonly used for the estimation of phenolic compounds. It measures the reducing capacity of the soluble compounds present and thus is not specific to only phenolic compounds. The plasma concentration of total phenolic compounds was determined using the Folin-Ciocalteu assay. Plasma samples (100 µL) were deproteinized by the addition of 300 µL methanol-ethanol (1:4, vol/vol) [24]. An aliquot of the centrifuged sample was then treated according to Medina [25].

Plasma fatty-acid composition was analysed by extracting plasma (50 µL) with methanol and methyl tert-butyl ether and transesterifying fatty acids to methyl esters using acetyl chloride in methanol (1:9, vol/vol) [26]. Fatty-acid methyl esters were analysed with a gas chromatograph (6890N; Agilent Technologies) equipped with a ZB-FAME column (30 m × 0.25 mm i.d., 0.2 µm film thickness) coupled with a 5-m Zebron Guardian column (Phenomenex) and a flame ionization detector. The injector and detector temperatures were kept at 240°C and 260°C, respectively. Helium was used as a carrier gas operated at a flow rate of 1.2 mL/min and a nominal initial pressure of 114.5 kPa. The column temperature gradient programme was as follows: temperature was held at 100°C for 2 min, increased by 10°C/min to 140°C, increased by 3°C/min to 190°C, increased by 20°C/min to 260°C, and then held for 4 min. The fatty-acid composition as weight percentages was obtained using theoretical response factors [27].

Statistical Analysis

The participants were categorized into three groups based on their *APOE* genotype: *ε2* group (genotypes *ε2*/*ε2*, *ε2*/*ε3*, and *ε2*/*ε4*), *ε3* group (genotype *ε3*/*ε3*), and *ε4* group (genotypes *ε3*/*ε4* and *ε4*/*ε4*). The sample size in this study was justified beforehand by performing appropriate power calculations for total cholesterol [20]. The sample size calculation was conducted for the intervention study [20], in which the main objective was to study whether the *APOE* genotype information affects the participants' motivation for lifestyle change. Briefly, the effect size was set to 0.26 mmol/L, an estimate of residual variance was obtained using 3.3

Table 1. Genotype frequencies and demographic parameters of the study population

Demographic parameter	Total (n = 211)	*ε2* Group[1] (n = 24)	*ε3* Group[1] (n = 115)	*ε4* Group[1] (n = 72)
Mean age, years	51.1	49.9	51.1	51.4
Gender/women, % (n)	82.0 (173)	79.2 (19)	81.7 (94)	83.3 (60)
Educational level: higher education,[2] % (n)	56.1 (106)	37.5 (9)	50.4 (58)	54.2 (39)
Education in health or natural science, % (n)	31.2 (59)	20.8 (5)	30.4 (35)	26.4 (19)
Non-smoking, % (n)	91.0 (192)	87.5 (21)	91.3 (105)	91.7 (66)
Prior health consultation[3], % (n)	30.8 (65)	25.0 (6)	30.4 (35)	33.3 (24)
APOE genotype, % (n)				
ε2/ε2	0.5 (1)	4.2 (1)		
ε2/ε3	9.0 (19)	79.2 (19)		
ε2/ε4	1.9 (4)	16.7 (4)		
ε3/ε3	54.5 (115)		100.0 (115)	
ε3/ε4	30.3 (64)			88.9 (64)
ε4/ε4	3.8 (8)			11.1 (8)

[1] The specific genotypes included in the genotype groups are as follows: *ε2* group, *ε2/ε2*, *ε2/ε3*, and *ε2/ε4*; *ε3* group, *ε3/ε3*; and *ε4* group, *ε3/ε4* and *ε4/ε4*. [2] Academic or University of Applied Sciences. [3] Health consultation regarding cholesterol or blood glucose values

mmol/L as minimum and 7.3 mmol/L as maximum, the approximate standard deviation for total cholesterol was 0.67 mmol/L, and the calculated approximate variance of 0.45 was used. The power calculation yielded powers of about 85%.

The physiological and lifestyle-related markers and plasma fatty-acid profile variables were analysed using a one-way analysis of variance. In these models, the explanatory variable was the 3-level *APOE* group. In addition, a few of the physiological variables were analysed using two-way analysis of variance models, where, in addition to the *APOE* group, gender was included in the model as well as the interaction of the *APOE* group and gender. In cases where a log transformation was applied, the presented estimated means and 95% confidence intervals for the means were transformed back to the original scale. The statistical analyses were conducted using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Physical activity at least twice a week was analysed analogously to previous one-way ANOVA models but using a logistic regression model with a 3-level *APOE* group as the explanatory variable. This model was fitted with the GLIMMIX procedure of SAS 9.4. For background variables, only descriptive statistics and frequency tables were produced. Outliers were detected using studentized residuals, and deviations of more than three units were investigated in more detail as outliers. Significance was defined at $p \leq 0.05$, and probabilities at $0.05 \leq p < 0.10$ were regarded as close to significant. For post hoc comparisons, Tukey-Kramer tests were used.

Results

Altogether 54.5% of the participants were homozygotes for the *APOE ε3* allele (*ε3/ε3*; *ε3* group; Table 1). The *APOE* genotypes *ε3/ε4* and *ε4/ε4* represented 34.1% of the participants (*ε4* group), whereas 11.4% of the participants were carriers of the *ε2* allele (*ε2/ε2*, *ε2/ε3* and *ε2/ε4*; *ε2* group). The frequency of the genotype *ε2/ε4* was 1.9%. More participants (9.7%) in the *ε4* group had cholesterol-lowering medication compared with the *ε3* (4.3%) or *ε2* groups (0%; online suppl. Table S1).

The *ε4* group was physically more active than the other groups, reporting to exercise most often at least three times a week (65%, 75%, and 89% for the *ε2*, *ε3*, and *ε4* groups, respectively; $p = 0.03$; online suppl. Table S2). The prevalence of overweight in *ε4* men was close to significantly lower than in men in the other groups (60%, 75%, and 31% for the *ε2*, *ε3*, and *ε4* groups, respectively; $p = 0.06$). They also had the lowest levels of the visceral fat compared to the *ε2* and *ε3* men (11.8, 11.5, and 8.9 for the *ε2*, *ε3*, and *ε4* groups, respectively; $p = 0.01$). A similar difference was not observed among the women (online suppl. Table S2).

Total cholesterol and LDL cholesterol were lower in the *ε2* group than in the *ε3* and *ε4* groups ($p = 0.018$ and $p = 0.004$, respectively, Fig. 2a, b; online suppl. Table S3). One-third of the *ε2* group had total cholesterol over the recommended level (5.0 mmol/L), whereas in the *ε4* group, two-thirds of the participants had total cholesterol levels higher than 5.0 mmol/L. The composition of plasma fatty acids was partly reflected by the *APOE* grouping (Fig. 2; Table 2). The proportion of palmitic acid (16:0) and total proportion of saturated fatty acids (SFA) was higher ($p = 0.02$ and $p < 0.01$, respectively; Fig. 2c, d) in

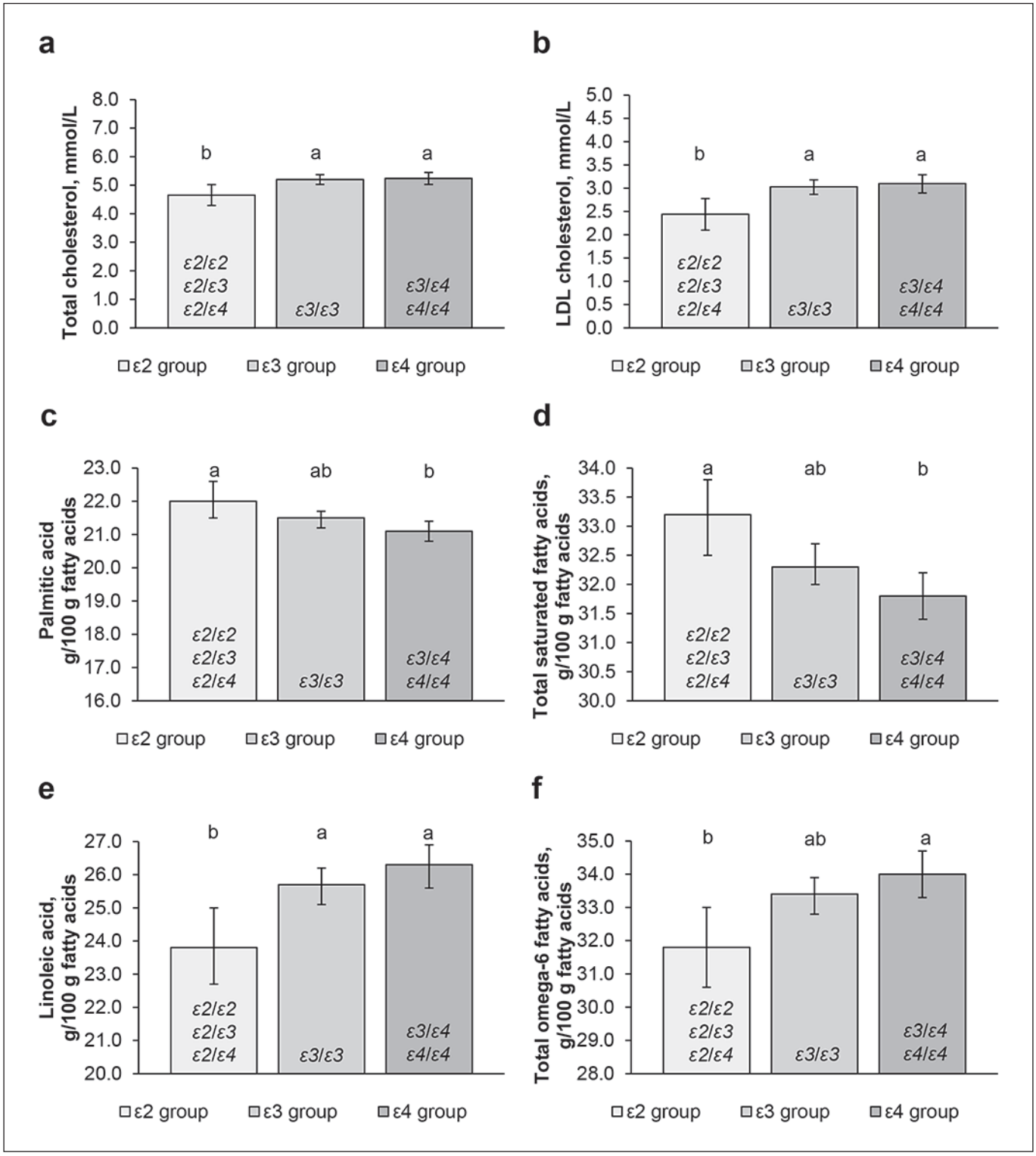

Fig. 2. Cholesterol concentrations and fatty-acid proportions in plasma of the study participants by the *APOE* genotype. Total cholesterol values (**a**), LDL cholesterol values (**b**), the proportion of palmitic acid (**c**), total SFAs (**d**), linoleic acid (**e**), and total omega-6 fatty acids (**f**). Data are presented as mean estimates with 95% confidence intervals. Different letters above the bars (a vs. b) denote significant differences between means ($p < 0.05$). Log transformation was used for palmitic acid, and estimated means and 95% confidence intervals for the means were transformed back to the original scale.

Table 2. Plasma fatty-acid profiles by *APOE* genotype groups

	ε2 Group[1]			*ε3* Group[1]			*ε4* Group[1]			*p* value[2]
	mean	confidence interval		mean	confidence interval		mean	confidence interval		
		lower	upper		lower	upper		lower	upper	
Fatty acid, g/100 g fatty acids										
Total SFA	33.2[a]	32.5	33.8	32.3[a, b]	32.0	32.7	31.8[b]	31.4	32.2	<0.01
12:0[3]	0.10	0.07	0.13	0.08	0.07	0.09	0.08	0.06	0.09	0.24
14:0[3]	1.09	0.95	1.25	1.00	0.94	1.06	0.97	0.90	1.05	0.35
16:0[3]	22.0[a]	21.5	22.6	21.5[a, b]	21.2	21.7	21.1[b]	20.8	21.4	0.02
18:0	7.22	6.99	7.45	7.05	6.94	7.15	6.96	6.83	7.10	0.17
20:0	0.25	0.24	0.27	0.26	0.26	0.27	0.27	0.26	0.27	0.31
Total MUFA	27.3	26.3	28.4	26.3	25.8	26.7	26.1	25.5	26.7	0.13
18:1n-9	21.1	20.2	22.0	20.3	19.9	20.7	20.1	19.6	20.7	0.18
Total PUFA	38.1[b]	36.7	39.4	40.0[a]	39.4	40.6	40.8[a]	40.0	41.5	<0.01
20:3n-9[3]	0.145	0.124	0.169	0.125	0.116	0.134	0.127	0.116	0.139	0.24
Total omega-6	31.8[b]	30.6	33.0	33.4[a, b]	32.8	33.9	34.0[a]	33.3	34.7	0.01
18:2n-6	23.8[b]	22.7	25.0	25.7[a]	25.1	26.2	26.3[a]	25.6	26.9	<0.01
18:3n-6[3]	0.322	0.269	0.385	0.314	0.290	0.341	0.309	0.279	0.343	0.92
20:2n-6[3]	0.176	0.164	0.189	0.172	0.166	0.177	0.182	0.175	0.190	0.08
20:3n-6	1.48	1.36	1.59	1.47	1.41	1.52	1.46	1.39	1.52	0.95
20:4n-6	5.68	5.23	6.13	5.47	5.27	5.68	5.53	5.28	5.79	0.70
22:2n-6	0.030	0.028	0.032	0.032	0.031	0.033	0.032	0.031	0.033	0.18
22:4n-6	0.150[a]	0.139	0.160	0.134[b]	0.129	0.139	0.132[b]	0.126	0.139	0.02
22:5n-6	0.109[a]	0.097	0.121	0.087[b]	0.082	0.091	0.091[b]	0.085	0.097	<0.01
Total omega-3[3]	6.01	5.57	6.47	6.39	6.18	6.62	6.50	6.22	6.79	0.20
18:3n-3[3]	0.837	0.751	0.933	0.840	0.800	0.883	0.899	0.845	0.957	0.22
18:4n-3[3]	0.038	0.031	0.047	0.034	0.031	0.038	0.037	0.033	0.042	0.45
20:3n-3[3]	0.026	0.023	0.030	0.026	0.025	0.028	0.028	0.026	0.031	0.23
20:4n-3[3]	0.108	0.090	0.130	0.111	0.102	0.121	0.111	0.100	0.123	0.97
20:5n-3[3]	0.97	0.80	1.17	1.05	0.96	1.15	1.08	0.97	1.21	0.60
22:5n-3	0.553	0.500	0.605	0.553	0.529	0.577	0.552	0.522	0.583	1.0
22:6n-3[3]	1.90	1.68	2.15	2.00	1.89	2.12	2.07	1.93	2.22	0.46
24:5n-3	0.254[b]	0.239	0.269	0.281[a]	0.274	0.288	0.278[a]	0.270	0.287	0.01
24:6n-3	1.18[b]	1.11	1.25	1.32[a]	1.29	1.35	1.32[a]	1.28	1.36	<0.01
Omega-6/omega-3 ratio	5.37	4.95	5.79	5.32	5.13	5.51	5.29	5.05	5.53	0.94

Values are estimated means and their 95% confidence intervals. MUFA, monounsaturated fatty acids. [1] The specific genotypes included in the genotype groups are as follows: *ε2* group, *ε2*/*ε2*, *ε2*/*ε3*, and *ε2*/*ε4*; *ε3* group, *ε3*/*ε3*; and *ε4* group, *ε3*/*ε4* and *ε4*/*ε4*. [2] Type I error can be taken into account by using Bonferroni correction for the *F* test, when the number of tested variables is 30. To reach $p < 0.05$ with Bonferroni correction, the *p* value for the *F* test should be $p < 0.0017$. This is achieved for 22:5n-6 ($p < 0.0012$) and 24:6n-3 ($p < 0.0007$) but not for other variables. [3] Log transformation was used, and estimated means and confidence intervals for the means were transformed back to the original scale. [a, b] Different superscript letters within a row (a vs. b) denote significant differences between means ($p < 0.05$).

the *ε2* group compared to the *ε4* group. It is also noteworthy that if one outlier participant (the participant had the lowest proportion of 18:0, 5.11 g/100 g fatty acids, in the whole study population) in the *ε2* group was excluded, the proportion of 18:0 would become higher ($p < 0.05$) in the *ε2* group compared to the *ε4* group (data not shown). In contrast, total proportion of polyunsaturated fatty acids (PUFA) was the lowest ($p < 0.01$) in the *ε2* group (38.1, 40.0, and 40.8 g/100 g fatty acids for the *ε2*, *ε3*, and *ε4* groups, respectively; Table 2). Linoleic acid (18:2n-6) was present in lower ($p < 0.01$) proportions in the *ε2* group than in the *ε3* and *ε4* groups (Fig. 2e), but the proportion of total omega-6 fatty acids was lower ($p = 0.01$) in the *ε2* group compared with the *ε4* group (Fig. 2f). In addition, the proportions of the fatty acids 22:4n-6 (0.150, 0.134, and 0.132 g/100 g fatty acids for the *ε2*, *ε3*, and *ε4* groups, respectively) and 22:5n-6 (0.109, 0.087, and 0.091 g/100 g fatty acids for the *ε2*, *ε3*, and *ε4* groups, respectively) were

Table 3. Nutritional markers by *APOE* genotype groups

Marker	*ε2* Group[1]			*ε3* Group[1]			*ε4* Group[1]			*p* value
	mean	confidence interval		mean	confidence interval		mean	confidence interval		
		lower	upper		lower	upper		lower	upper	
Lutein + zeaxanthin, mg/L	0.40	0.34	0.48	0.48	0.44	0.52	0.48	0.44	0.53	0.17
β-Cryptoxanthin[2], mg/L	0.33	0.24	0.43	0.40	0.35	0.45	0.37	0.32	0.44	0.47
Lycopene[2], mg/L	0.16	0.13	0.20	0.19	0.17	0.21	0.19	0.16	0.21	0.50
α-Carotene[2], mg/L	0.16	0.12	0.21	0.16	0.14	0.18	0.15	0.13	0.17	0.64
β-Carotene[2], mg/L	0.48	0.37	0.62	0.52	0.46	0.59	0.53	0.46	0.62	0.78
Retinol, mg/L	1.23	1.08	1.38	1.16	1.09	1.22	1.22	1.14	1.31	0.41
α-Tocopherol, mg/L	13.64	12.54	14.84	14.39	13.84	14.96	14.29	13.60	15.01	0.53
Total phenolics, mg/L	102.5	98.1	107.0	104.3	102.2	106.3	106.0	103.4	108.5	0.36
APOE protein, mg/L	5.7	3.9	8.4	4.5	3.8	5.4	4.2	3.4	5.3	0.40

Values are estimated means and their 95% confidence intervals. [1] The specific genotypes included in the genotype groups are as follow: *ε2* group, *ε2*/*ε2*, *ε2*/*ε3*, and *ε2*/*ε4*; *ε3* group, *ε3*/*ε3*; and *ε4* group, *ε3*/*ε4* and *ε4*/*ε4*. [2] Log transformation was used, and estimated means and confidence intervals for the means were transformed back to the original scale.

the highest ($p = 0.02$ and $p < 0.01$, respectively) in the *ε2* group in comparison with the other groups (Table 2). In contrast, the proportions of fatty acids 24:5n-3 (0.254, 0.281, and 0.278 g/100 g fatty acids for the *ε2*, *ε3*, and *ε4* groups, respectively) and 24:6n-3 (1.18, 1.32, and 1.32 g/100 g fatty acids for the *ε2*, *ε3*, and *ε4* groups, respectively) were the lowest ($p = 0.01$ and $p < 0.01$, respectively) in the *ε2* group in comparison with the other groups (Table 2). No other differences were observed in the proportions of individual omega-3 fatty acids or total omega-3 fatty acids. No other statistically significant differences were observed in physiological parameters, lifestyle factors (online suppl. Table S2), clinical markers in plasma (online suppl. Table S3), fatty-acid proportions in plasma (Table 2), or nutritional biomarkers in plasma (Table 3) between the *APOE* genotype groups.

Discussion

Worldwide, the *APOE ε4* allele frequency varies from 0.052 to 0.407, the lowest frequencies being found in the Mediterranean and certain Asian populations and the highest frequencies in Central Africa, Oceania, Aboriginal Australians, and the Lappish Sámi people [28]. In our study population, the *APOE ε4* allele frequency was 0.201, which was slightly higher than has been previously reported in Finnish populations (0.16–0.185) [13, 29, 30].

In our study population, the total cholesterol and LDL cholesterol were at the same level as reported in the national FinHealth Study 2017 [31]. In the *ε3* and *ε4* groups, the concentrations of total and LDL cholesterol were higher than 5 mmol/L and 3 mmol/L, respectively, which are the estimated safe limits as markers for CVD. The significantly lower total cholesterol values of the *ε2* carriers when compared to the *ε3* and *ε4* carriers are consistent with previous results in other populations and likely due to the diminished cholesterol binding ability of the ε2 protein, resulting in lower lipoprotein uptake [32]. In addition, we did not observe any differences in the self-reported dietary intake and diet composition data between the groups, which could explain the differences in total and LDL cholesterol values.

We did not observe higher total and LDL cholesterol in the *ε4* than in the *ε3* group, which is in line with previous studies referred to in a review by Minihane et al. [32]. This may be due to several possible reasons. First of all, previous health consultation in general health care, typically given to those with high cholesterol, probably has had an effect on the lifestyle, such as exercise or diet, of the *ε4* group. Of all the participants, individuals in the *ε4* group reported the most physical exercise, and the men in the *ε4* group had the lowest visceral fat level, and they tended to be leaner compared to the men in the other groups. In addition, the lower SFA in the *ε4* group in comparison to the *ε2* group could be explained by health awareness in the *ε4* group. Also, a higher proportion of the participants in the *ε4* group had cholesterol-lowering medication compared to the other groups. Oestrogen has been reported to have a protective effect against hyper-

cholesterolaemia [33], and due to the over-representation of women in our participant population, this may have had an effect on the overall values in the *ε4* group. However, contrary to our results, differences in total and LDL cholesterol concentrations between *ε3*/*ε3* and *ε4* carriers have also been reported in earlier studies [17, 32].

It has been shown that the metabolism of fatty acids is disturbed in *ε4* carriers when compared to non-carriers [15]. Contrary to previous results [16], in our study, no differences were observed in plasma EPA, DPA, or DHA proportions between the *ε4* group and the other groups. Similarly, no differences were found between the different *APOE* groups when using the omega-3 index (EPA + DHA; data not shown). This is consistent with the study by Fallaize et al. [17] who found no differences in the omega-3 index of blood fatty acids between the *APOE* genotypes (*ε2* carriers and *ε3*/*ε3* and *ε4* carriers) when total intakes of fat, SFA, monounsaturated fatty acids, PUFA, and omega-3 were taken into account.

In the study by Dang et al. [18], *APOE ε4* carriers had higher omega-6/omega-3 ratio and lower ALA and DHA in LDL fractions compared to non-carriers (*ε3*/*ε3* + *ε3*/*ε2*). In our study, plasma omega-6/omega-3 ratios were not different between the *APOE* groups. However, Dang et al. [18] reported a genotype effect on 16:0 and 18:2n-6 fatty acids in HDL after supplementation with omega-3 PUFA, which aligns with our findings on lower plasma 16:0 proportion and higher 18:2n-6 proportion in the *ε4* group compared with the *ε2* group. The lower proportions of 16:0 in the *ε4* group could be a result of increased β-oxidation in those individuals, which has been suggested in earlier studies [18, 19].

Interestingly, we also observed higher proportions of fatty acids 22:4n-6 and 22:5n-6 and lower proportions of 24:5n-3 and 24:6n-3 in the *ε2* group compared with the *ε3* and *ε4* groups. To the best of our knowledge, no studies reporting associations between the *APOE* genotype and these fatty acids have been published before. The dietary fat quality score, adherence to recommended fish intake (at least twice per week), or consumption of high-fat and -sugar foods were not different between the *APOE* genotype groups, which suggests that quality of dietary fat was not different between groups. No differences were found in the concentration of plasma carotenoids, total phenolic compounds, retinol, or α-tocopherol, which supports the lack of differences in the self-reported nutrition-related lifestyle factors.

The visceral fat level among men in the *ε4* group was lower than in the other groups, and there were higher percentage of individuals in the *ε4* group that had physical activity at least twice a week. In the present study, APOE protein concentrations were not different between the *APOE* genotype groups, although the concentrations of APOE in plasma and cerebrospinal fluid are reported to be lower for *APOE ε4* compared to *ε2* and *ε3* [9, 10]. However, exercise has been shown to preserve APOE expression in mice [34], and a higher physical activity level in the *ε4* group in the present study may have inhibited the decrease in their plasma APOE protein concentrations.

Conclusions

The plasma fatty-acid profiles in the *ε2* group were characterized by higher SFA, especially 16:0, and lower omega-6 fatty acid proportions compared with the *ε4* group. Nevertheless, their cholesterol values were lower compared with the *ε3* and *ε4* groups, indicating a reduced risk for CVD. A novel finding was that the participants in the *ε2* group had higher proportions of plasma 22:4n-6 and 22:5n-6 and lower proportions of plasma 24:5n-3 and 24:6n-3 fatty acids compared with the *ε3* and *ε4* groups. The significance of the differences in fatty-acid composition remains to be studied.

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Statement of Ethics

This study was conducted in accordance with the guidelines of the Declaration of Helsinki. Ethics approval was obtained from the Coordinating Ethics Committee of the Pirkanmaa Hospital District, Finland (12/2016, R16055). After written and oral information of the study and a 2-week reconsideration period, volunteering participants gave a written informed consent.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

H.P., M.S., K.V., R.T., and A.H. designed the research; H.L., M.T., H.K., T.I.-T., H.-L.H.-L., P.M., J.-M.P., K.Å., S.M., and S.R. conducted the research; T.H. performed statistical analysis; H.L., M.T., H.K., T.I.-T., T.H., R.T., and S.R. wrote the first draft of the paper; H.L., T.I.-T., M.S., K.V., R.T., S.R., and A.H. had primary responsibility for the final content; and all the authors read and approved the final manuscript.

Data Availability Statement

Data cannot be shared for confidentiality reasons. Queries about the data should be directed to the corresponding author.

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