The apolipoprotein E gene promoter (-219G/T) polymorphism determines insulin sensitivity in response to dietary fat in healthy young adults.

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Juan Antonio Moreno[a], Francisco Pérez-Jiménez[a], Carmen Marín[a], Pablo Pérez-Martínez[a], Rafael Moreno[a], Purificación Gómez[a], Yolanda Jiménez-Gómez[a], Juan Antonio Paniagua[a], Denis Lairon[b], José López-Miranda[a].

[a] Lipids and Atherosclerosis Research Unit. Hospital Universitario Reina Sofía. Córdoba. Spain. [b] UMR 476-INSERM/ 1260-INRA,University and Central Analytical Laboratory, Ste Marguerite University Hospital, Marseille, France.

Correspondence and reprint requests to: José López-Miranda. Unidad de Lípidos y Arteriosclerosis. Hospital Universitario Reina Sofía. Avda Menéndez Pidal, s/n. 14004 Córdoba, Spain. Phone: 34-957-218250 FAX: 34-957-218250. **E-mail**: md1lomij@uco.es

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ABSTRACT

Insulin sensitivity (IS) is determined by genetic and environmental factors, including diet. The *apoE* gene promoter -219G/T polymorphism is associated with coronary heart disease and increased postprandial triacylglycerol-rich lipoprotein concentration, circumstances related to insulin resistance. Thus, our aim was to determine whether this polymorphism modifies the IS to dietary fat in healthy young adults. 43 volunteers with the apoE3/E3 genotype (8 GG, 25 GT and 10 TT) completed 3 dietary periods, each lasting 4 wk. They first consumed a saturated fatty acid (SFA)-rich diet (38% fat (% of energy in the total diet), 20% SFA (% of energy in the total diet)), and then, in a randomized, crossover design, a carbohydrate (CHO)-rich diet (30% fat, 55% carbohydrate) or a monounsaturated fatty acid (MUFA)-rich diet (38% fat, 22% MUFA). After each diet period, we investigated peripheral IS using the insulin suppression test. The steady-state plasma glucose (SSPG) concentration was lower ($p<0.05$) in GG subjects than in GT and TT individuals, regardless of the diet consumed A significant diet x genotype interaction effect was found for SSPG and plasma non-esterified free fatty acid (NEFA) concentrations. Thus, the shift from the SFA-rich diet to the MUFA- or CHO-rich diets decreased ($p<0.05$) the SSPG and NEFA concentrations in GG and GT subjects, but not in TT volunteers. In conclusion, carriers of the –219T allele have lower IS than GG individuals. Furthermore, only carriers of the -219G allele have an improvement in IS when a MUFA- or a CHO-rich diets are consumed instead of a SFA-rich diet.

Key words: apoE gene promoter (-219G/T) polymorphism, dietary intervention, insulin sensitivity, genetics.

59 Insulin resistance has been associated with type 2 diabetes mellitus (T2DM),
60 obesity, hypertension, coronary heart disease (CHD) and a dyslipidemic profile
61 characterized by a high plasma triacylglycerol and LDL cholesterol plasma
62 concentrations and low HDL cholesterol (1). The genetic background of T2DM and
63 insulin resistance is complex and heterogeneous and is related not only to genes linked
64 to glucose and insulin metabolism, but also to genes seemingly unrelated to
65 carbohydrate metabolism (2,3).

66 Apolipoprotein E (ApoE) plays an important role in lipid metabolism, both
67 promoting efficient uptake of triacylglycerol-rich lipoproteins from the circulation and
68 taking part in the cellular cholesterol efflux and reverse cholesterol transport (4). It has
69 been suggested that the effect of ApoE on LDL cholesterol concentration may require
70 cofactors to activate the receptor-mediated uptake of this lipoprotein, since ApoE is not
71 a structural part of LDL (5). One of these cofactors could be insulin. Thus, previous
72 data suggest that the uptake of ApoE-enriched lipoproteins is doubled or tripled when
73 rat isolated adipocytes are exposed to physiological concentrations of insulin (6), and
74 there is evidence suggesting that the apoE genotypes may modify the effect of insulin
75 on CHD or some CHD risk factors, including BMI, plasma triacylglycerol, and total and
76 LDL cholesterol plasma concentration (7-11). Studies assessing associations between
77 insulin resistance and apoE genotypes have shown contradictory results. Whereas some
78 studies have found that fasting and 2-h post load insulin and glucose concentration were
79 higher in apoE4 subjects, others no reported this relationship (7-12), suggesting that
80 other genetic or environmental factors may be involved in the link between apoE gene
81 and insulin resistance.

82 In accordance with this hypothesis, a polymorphism in the proximal promoter
83 region of the *apoE* gene was described at position -219 G/T (13,14). The –219T allele

84 was associated with decreased transcriptional activity (13) and plasma ApoE
85 concentration (15,16), prolonged and enhanced postprandial lipemic response (16),
86 increased concentration of glucose at 2-hours after an oral glucose tolerance test (17),
87 increased LDL cholesterol concentrations and susceptibility to oxidation in response to
88 a diet rich in saturated fat (18) and increased risk of myocardial infarction (15) and
89 premature CHD (17).

90 Insulin sensitivity (IS) is determined by the interaction between genetic and
91 environmental factors, including diet (19). Thus, our goal was to study whether the
92 presence of the *apoE* gene promoter (-219G/T) polymorphism determines insulin
93 sensitivity in response to changes in the quantity and quality of dietary fat in healthy
94 young adults with the apoE3/E3 genotype.

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109 **SUBJECTS AND METHODS**

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111 **Human Subjects**

112 Since the *apoE* E2/E3/E4 polymorphism strongly determines the plasma ApoE
113 concentration (20) and have been implicated in a variable lipid response to dietary
114 changes (21), we studied the effect of the *apoE* gene promoter (-219G/T) polymorphism
115 in apoE3/E3 subjects. A group of healthy young adults (n=43; 8 GG, 25 GT and 10 TT),
116 including both men (n=22; 4 GG, 13 GT and 5 TT) and women (n=21; 4 GG, 12 GT
117 and 5 TT), were recruited from among students at the University of Cordoba. The
118 subjects were 23.1 ± 1.8 y old (mean ± SD). These subjects had participated in a previous
119 study (22). Informed consent was obtained from all participants. All subjects underwent a
120 comprehensive medical history, physical examination, and clinical chemistry analysis
121 before enrolment. Subjects showed no evidence of any chronic disease (hepatic, renal,
122 thyroid, or cardiac dysfunction) obesity, or unusually high levels of physical activity (e.g.,
123 sports training). None of the subjects had a family history of premature coronary artery
124 disease or had taken medications or vitamin supplements in the 6 mo prior to the study.
125 Physical activity and diet, including alcohol consumption, were recorded in a personal
126 log for 1 wk and the data were used to calculate individual energy requirements. The
127 BMI was 22.8 ± 2.4 kg/m^2 (mean ± SD) at the onset of the study and remained constant
128 throughout the experimental period. Subjects were encouraged to maintain their regular
129 physical activity and lifestyle and were asked to record in a diary any event that could
130 affect the outcome of the study, such as stress, change in smoking habits and alcohol
131 consumption or intake of foods not included in the experiment design. The study
132 protocol was approved by the Human Investigation Review Committee at the Reina
133 Sofia University Hospital.

Diets

The study design included an initial 28-day period during which all subjects consumed a saturated fat (SFA) enriched diet, with 15% protein (% of energy in the total diet), 47% carbohydrate (CHO) and 38% fat (20% SFA (% of energy in the total diet), 12% monounsaturated fatty acid (MUFA) and 6% polyunsaturated fatty acid (PUFA)). After this period, volunteers were randomly assigned to 1 of 2 diet sequences. Thirty subjects consumed a MUFA-rich diet containing 15% protein, 47% CHO and 38% fat (<10% SFA, 6% PUFA, 22% MUFA) for 28 d. This diet was followed for 28 d by consumption of a CHO-rich diet containing 15% protein, 55% CHO and <30% fat (<10% SFA, 6% PUFA, 12% MUFA). The other 29 subjects received the CHO diet before the MUFA diet. The cholesterol intake was constant (<300 mg/d) during the 3 periods. 80% of the MUFA content during MUFA diet was provided by virgin olive oil, which was used for cooking, salad dressing and as a spread. Carbohydrate intake of the CHO diet was based on the consumption of biscuits, jam and bread. Butter and palm oil were used during the SFA dietary period.

The composition of the experimental diets was calculated using the United States Department of Agriculture (23) food tables and Spanish food composition tables for local foodstuffs (24). All meals were prepared in the hospital kitchen and were supervised by a dietitian. Lunch and dinner were eaten in the hospital dining room, whereas breakfast and an afternoon snack were eaten in the medical school cafeteria. Menus (n=14) were prepared with regular solid foods and rotated during the experimental period. Duplicate samples from each menu were collected, homogenized, and stored at –70ºC. Protein, fat, and carbohydrate contents of the diet were analyzed by

159 standard methods (25). Dietary compliance was verified by analyzing the fatty acids in
160 plasma LDL cholesterol esters at the end of each dietary period (26). The study took
161 place from January through March to minimize seasonal effects and academic stress.
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163 **Lipids analysis and biochemical determinations**

164 Venous blood samples for insulin, glucose, lipid and lipoprotein analysis were
165 collected into EDTA-containing (1g/L) tubes from all subjects after a 12-h overnight
166 fast at the beginning of the study and at the end of each dietary period. Plasma was
167 obtained by low speed centrifugation (1500 x g) for 15 min at 4°C within 1h of
168 venipuncture. To reduce interassay variation, plasma was stored at -80°C and analyzed
169 at the end of the study. Plasma total cholesterol and triacylglycerol (TG) concentrations
170 were measured using enzymatic techniques (27,28). HDL cholesterol was measured
171 after precipitation with phosphotungstic acid (29). ApoA-I and ApoB were determined
172 by immunoturbidimetry (30). Plasma ApoE concentration was measured using an
173 immunonephelometric method on a BN ProSpec System with commercial kits (Dade
174 Behring, Deerfield, USA). LDL cholesterol concentration was calculated using the
175 Friedewald formula (31). Non-esterified free fatty acid (NEFA) concentrations were
176 analyzed by an enzymatic colorimetric assay (Boehringer Mannheim, Mannheim,
177 Germany) (32).
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179 **Glucose suppression test.**

180 A modified insulin suppression test was carried out on all the subjects at the end
181 of the dietary period (33,34). The technique used in the present study to quantify insulin
182 sensitivity was the insulin suppression test, a simple and cost-effective test for the
183 measurement of insulin resistance, which has been used increasingly often in recent

184 years. The study began at 08:00, after 12 h of fasting. A continuous infusion of
185 somatostatin (214 nmol/h), insulin (180 pmol·m^{-2}·min^{-1}) and glucose (13.2mmol·m^{-2}·min^{-1}) were infused in the same vein. Somatostatin was used to inhibit endogenous
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187 insulin secretion. Blood was sampled every 30 min for the first 2.5 h, by which time
188 steady-state plasma glucose (SSPG) and steady-state plasma insulin (SSPI)
189 concentrations were achieved. Blood was then sampled at 10 min intervals for the last
190 30 min (at minutes 150, 160, 170 and 180) for measurement of plasma glucose and
191 insulin concentrations. We considered the mean of these 4 values to determine the
192 SSPG and SSPI concentrations. Since SSPI concentrations were similar in all subjects,
193 SSPG concentrations provided a measure of the ability of insulin to promote the
194 disposal of infused glucose. Subjects with high SSPG are relatively more insulin
195 resistant than those with lower SSPG (33).

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197 **DNA amplification and genotyping**

198 Genomic DNA extraction and *apoE* E2/E3/E4 and –219G/T genotypes were
199 determined as previously described (16,18). Digested DNA was separated by
200 electrophoresis on an 8% non-denaturing polyacrylamide gel at 150 V for 2 h. Bands
201 were visualized by silver staining. Samples containing the T allele of –219G/T
202 polymorphism were amplified a second time to verify the genotype.

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204 **Statistical methods**

205 We used the analysis of variance (ANOVA) for repeated measures to test the
206 effects of the *apoE* gene promoter (-219G/T) polymorphism on plasma SSPG, NEFA,
207 fasting glucose, fasting insulin, total cholesterol, LDL cholesterol, HDL cholesterol,
208 trialcylglycerol, ApoE, ApoA-I, and ApoB concentration at the end of each dietary

209 period. ANOVA was used to test the effect of the replacement of SFA-rich diet by
210 MUFA- or CHO-rich diets within a genotype or a gender group. When F-test was
211 significant, Tukey's post-hoc test was used to identify between-group differences. To
212 determine whether plasma NEFA concentration was correlated with plasma SSPG
213 concentration we used the Pearson correlation test. Independent sample *t*-test was made
214 between the two groups that got MUFA then CHO vs. CHO then MUFA diet to test
215 whether the MUFA-CHO differences depended on whether MUFA or CHO was first
216 (order effects). Plasma triacylglycerol and ApoE concentrations were log transformed
217 before statistical analyses. Differences were considered significant at $P<0.05$. Statistical
218 analyses were conducted using the SPSS statistical software, version 9.0 (SPSS Inc,
219 Chicago).
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234 **RESULTS**

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236 Baseline anthropometric characteristics and plasma lipid and apolipoprotein
237 concentration did not differ among the groups as a whole (**Table 1**) or in men or women
238 (not shown). Daily nutrient intakes of the participants are shown in **Table 2**. Plasma
239 LDL cholesterol esters concentrations at the end of each dietary period showed good
240 adherence in the different intervention stages. After the SFA-diet period, palmitic acid
241 in the LDL cholesterol esters (27.2 ± 1.4 %) was greater than after the CHO (18.9 ± 3.9
242 %) and MUFA (15.1 ± 0.4 %) diet periods ($p<0.005$). In addition, after the MUFA-diet
243 period, oleic acid in the cholesterol esters (49.7 ± 4.7 %) was greater than after the CHO
244 (38.5 ± 9.0 %) diet ($p<0.05$), but not after the SFA (45.5 ± 4.4 %) diet period.

245 The *apoE* gene promoter (-219G/T) polymorphism affected the plasma SSPG
246 concentration which was lower ($p=0.045$) in GG (4.85 ± 0.86 mmol/L) subjects than in
247 GT (6.43 ± 0.48 mmol/L) and TT (7.30 ± 0.77 mmol/L) individuals independently of
248 the diet consumed. In addition, a significant diet x genotype interaction effect was found
249 for SSPG and for plasma non-esterified free fatty acid (NEFA) and fasting insulin
250 concentrations. Thus, the shift from the SFA-rich diet to the MUFA- or CHO-rich diets
251 decreased ($p<0.05$) the SSPG concentrations in GG and GT subjects, but not in TT
252 volunteers (**Table 3**). These data indicate that the presence of the -219G allele was
253 associated with significantly increased insulin sensitivity when they consumed a
254 MUFA- or CHO-rich diet. Plasma NEFA and fasting insulin concentrations were lower
255 after the MUFA- and CHO-rich diets than in SFA-rich diet in carriers of the -219G
256 allele, but not TT subjects (**Table 3**). There was no interaction between gender and
257 plasma SSPG or NEFA concentrations by genotype (not shown). Additionally, plasma

258 NEFA concentration was correlated positively with plasma SSPG concentration
259 (r=0.26; p=0.002).
260 There were no significant differences between genotypes for any of the lipid
261 parameters after the three dietary periods (**Table 3**). However, a significant sex by diet x
262 genotype interaction effect was observed on plasma ApoB (p=0.041), LDL cholesterol
263 (p=0.043) and ApoE (p=032) concentrations. Men, but not women, with the TT and GT
264 genotype, had significantly (p<0.05) higher plasma ApoB (0.74±0.12 and 0.74±0.1 vs.
265 0.58±0.08 g/L) and LDL cholesterol concentrations (2.81±0.39 and 2.71±0.41 vs.
266 2.13±0.19 mmol/L) and lower plasma ApoE concentration (0.027±0.006 and
267 0.030±0.006 vs. 0.035±0.007 g/L) than men homozygous for the G allele, after the SFA
268 diet. Thus, in TT and GT men, the decrease in ApoB (-18% and -21% vs. -3%) and
269 LDL cholesterol (-24% and -20% vs, -6%) plasma concentrations was significantly
270 (p<0.05) greater than in GG men when these subjects switched from the SFA diet to the
271 CHO one. Furthermore, in men, the replacement of the SFA diet by the CHO diet
272 induced a greater increase (p<0.05) of plasma ApoE concentration in TT (22%) subjects
273 than in GG (-6%) individuals, but this effect was not observed in women. However,
274 when the SFA diet was compared with the MUFA diet, no significant differences were
275 observed for plasma ApoB, ApoE or LDL cholesterol concentrations between
276 genotypes, either men or women.
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283 **DISCUSSION**

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285 Our findings show that carriers of the –219T allele have a lower insulin
286 sensitivity than GG individuals, independently of the diet consumed. Furthermore, the
287 replacement of a SFA-rich diet by a MUFA- or a CHO-rich diets increases insulin
288 sensitivity in GG and GT subjects, but not in TT volunteers.

289 Experimental animal models have shown that insulin action may be modulated
290 by replacement not only the amount of total fat, but also the type of fat. Studies in
291 humans have shown different results, probably due to the small number of subjects
292 involved in those studies (35). A recent multi-centre trial including a large healthy
293 population has shown that a change from a SFA rich diet to one rich in MUFA
294 improved insulin sensitivity (36). In agreement with these results, our group has shown
295 that shifting from a SFA-rich diet to a MUFA olive oil-rich diet also improved insulin
296 sensitivity in young subjects (22). Both studies were performed under strictly controlled
297 conditions using natural foods, thus increasing the generalization of the findings.

298 Although there are evidences suggesting that the apoE genotypes modulate the
299 effect of insulin on CHD or some CHD risk factors, including BMI, plasma
300 triacylglycerol, and total and LDL cholesterol plasma concentration (7-11); studies
301 assessing associations between apoE gene polymorphism and insulin resistance have
302 shown contradictory results. Whereas some studies have found that fasting and 2-h post
303 load insulin and glucose concentration were higher in apoE4 subjects, others no
304 reported this relationship (7-12), suggesting that other genetic or environmental factors
305 may be involved in the link between apoE gene and insulin resistance.

306 In accordance with this hypothesis, a polymorphism in the proximal promoter
307 region of the *apoE* gene have recently been described at position -219 G/T that produces

308 variations in the transcriptional activity of the gene (13,14) and it is associated with an
309 increased risk of myocardial infarction (15) and premature CHD (17). However, the
310 mechanisms via which the -219T allele enhances atherothrombosis are yet to be
311 elucidated. In a previous study (15), the -219G/T polymorphism did not modify baseline
312 plasma lipid or lipoprotein concentrations, as in our study. The authors speculated that
313 the -219TT genotype could increase the risk of myocardial infarction at a local level by
314 modifying the macrophage *apoE* expression, but experimental data to corroborate this
315 hypothesis do not exist. In a previous study, the -219T allele was associated with lower
316 postprandial ApoE concentration and a higher postprandial response in large and small
317 TRL (16). It has been suggested that the enlarged pool of circulating TRL could also
318 increase plasma fatty acid concentrations by saturating peripheral removal mechanism
319 and thus contribute to establishing an insulin-resistant state (37). This fact could explain
320 our results since carriers of the -219T allele showed a lower insulin sensitivity.
321 Furthermore our results are in agreement with a previous study, where the -219T allele
322 was associated with higher concentration of glucose at 2-hours after an oral glucose
323 tolerance test (17).

324 Our study clearly shows that gender interacts with genotype and diet to
325 determine ApoE concentration. In the liver, apoE expression is regulated by diet
326 (38,39), several hormones like the thyroid hormone, insulin, the growth hormone and
327 estrogens (40). Many of the effects of estrogens arise at the genomic level by the
328 classical molecular mechanism of estrogens binding to nuclear estrogens receptors,
329 ESR1 and ESR2. This is followed by binding of estrogens receptors to consensus
330 estrogens response elements (ERE) in the target gene and leads to altered transactivation
331 of gene expression (41). A recent study reported an allelic dependent regulation of *apoE*
332 gene expression in response to estrogens by the -219G/T polymorphism (42). This

333 phenomenon is related to a differential ESR1 binding to ERE-like sequences in the
334 promoter region. Thus, estrogens diminish the differences in activity between the T and
335 G forms, most probably enhancing the transcriptional activity of the -219T allele. This
336 phenomenon would explain why we observed no significant differences in plasma
337 ApoE concentration in women. Contrary to women, our results show that diet interacts
338 with -219G/T polymorphism to determine ApoE concentration in men. Thus, men
339 carriers of the -219T allele had lower plasma ApoE concentration than did men
340 homozygous for the G allele after the SFA diet. However, the mechanism how *apoE*
341 expression is regulated by saturated fatty acid in men carriers of the -219T allele is not
342 known yet. Bohnet *et al*. (43) have demonstrated that ApoE concentrations in VLDL
343 help to determine VLDL affinity for the ApoE-binding receptors, and probably
344 subsequent variations in plasma LDL cholesterol concentration. It is probable that the
345 lower plasma ApoE concentration observed in men carriers of the T allele after the
346 SFA-rich diet are also associated with lower ApoE-VLDL concentration, thus
347 decreasing clearance by hepatic receptors. This phenomenon could explain the higher
348 LDL cholesterol and ApoB plasma concentration observed in these subjects after SFA-
349 rich diet (18).

350 The current study is the first to examine the association between *apoE* gene
351 promoter (-219G/T) polymorphism and insulin sensitivity to dietary fat. We have
352 observed a significant diet x genotype interaction effect was found for SSPG and for
353 plasma NEFA and fasting insulin concentrations. Thus, the replacement of a SFA-rich
354 diet by a CHO- or a MUFA-rich diets, increases insulin sensitivity in GG and GT
355 individuals, but not in TT subjects. Inappropriate release of NEFA into the circulation is
356 a hallmark of the metabolic syndrome and it is likely both to reduce the sensitivity of
357 glucose metabolism to insulin and to enhance postprandial lipemia. In our study, plasma

358 NEFA concentration also were higher after the SFA-rich diet than in the other two diets
359 in carriers of the –219G allele. This fact could inhibit glucose utilization by peripheral
360 cells (44) and increase gluconeogenesis in the liver (45). Both of these circumstances
361 lead to reduce the effect of peripheral insulin. Currently, the mechanism for the
362 association between the *apoE* gene promoter (-219G/T) polymorphism and diet to
363 determine insulin sensitivity is not known.

364 In conclusion, our findings show that carriers of the –219T allele have a lower
365 insulin sensitivity than GG individuals, independently of the diet consumed.
366 Furthermore, only carriers of the -219G allele have an improvement in insulin
367 sensitivity when a MUFA- or a CHO-rich diets are consumed instead of SFA-rich diet.
368 The present findings in a Spanish population need to be replicated in independent
369 studies to determine whether the presence of the –219G/T polymorphism determines
370 insulin sensitivity and it is truly implicated in insulin resistance in individuals at risk.
371 We can not exclude the possibility that the –219G/T polymorphism is not itself
372 responsible for the observed association with diet to determine insulin sensitivity, but
373 instead it is in linkage disequilibrium with an unknown causative variant in a distal
374 regulatory site or with an unidentified causative polymorphism in a gene different from,
375 but close to, the *apoE* gene.

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383 **ABBREVIATIONS**

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385 Abbreviations used: apo, apolipoprotein; BMI, body mass index; C, cholesterol; CHD,
386 coronary heart disease, CHO, carbohydrate; NEFA, non-esterified free fatty acid;
387 MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated
388 fatty acid; SSPG, steady-state plasma glucose; SSPI, steady-state plasma insulin; TG,
389 triacylglycerol; TRL, triacylglycerol rich lipoprotein, T2DM, type 2 diabetes mellitus.

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TABLE 1. Baseline anthropometric characteristics and plasma lipid and apolipoprotein concentration in healthy young adults with different apoE (-219G/T) genotype.

	GG	**GT**	**TT**
n	8	25	10
Age, y	22.83±1.16	22.15±1.14	22.25±1.50
BMI, kg/m^2	19.85±3.47	21.89±3.12	21.01±2.24
Total cholesterol, mmol/L	3.67±0.62	4.17±0.58	4.10±0.73
LDL-C, mmol/L	2.01±0.41	2.49±0.58	2.43±0.69
HDL-C, mmol/L	1.44±0.31	1.33±0.42	1.35±0.36
Apo A-1, g/L	1.44±0.11	1.48±0.15	1.46±0.20
Apo B, g/L	0.66±0.07	0.74±0.12	0.73±0.14
Apo E, g/L	0.034±0.006	0.033±0.006	0.032±0.007
Triacylglycerol, mmol/L	0.66±0.31	0.73±0.32	0.71±0.25
Fasting glucose, mmol/L	4.84±0.15	5.02±0.23	4.96±0.30
Fasting insulin, pmol/L	94.17±46.60	89.53±29.62	99.37±25.31
Fasting NEFA, mmol/L	0.52±0.26	0.57±0.35	0.55±0.31

Values are mean ± SD.

TABLE 2. Daily intakes of healthy young adults with different apoE (-219G/T) genotype consuming different quantities and types of fat.

	Diet Period		
	SFA	**CHO**	**MUFA**
Protein (% of energy intake)			
Calculated	15	15	15
Analyzed	18.1	17.6	17.5
Fat (% of energy intake)			
Saturated			
Calculated	20	10	10
Analyzed	22.6	9.2	9.2
Monounsaturated			
Calculated	12	12	22
Analyzed	10.1	13.5	24.4
Polyunsaturated			
Calculated	6	6	6
Analyzed	5.0	5.2	4.8
Carbohydrates (% of energy intake)			
Calculated	47	57	47
Analyzed	44.2	54.5	44.1
Complex	27.1	33.3	27.5
Simple	17.1	21.2	16.6
Cholesterol (mg/d)			
Calculated	115	115	115

Analyzed	112	113	117
Fiber (g/d)			
Calculated	30	30	30
Analyzed	25.9	26.1	24.9
Energy (MJ)	10.2	10.2	10.2

SFA, saturated fatty acids-rich diet; CHO, low-fat, high-carbohydrate diet; MUFA, monounsaturated fatty acids-rich diet,

TABLE 3: SSPG and plasma NEFA, glucose, insulin, lipids and apoproteins concentrations in young adults with different apoE (-219G/T) genotype during the insulin suppression test conducted at the end of the SFA, CHO and MUFA diet periods.

Genotype-Diet	n	SSPG mmol/L (%)	NEFA mmol/L (%)	Glucose mmol/L	Insulin pmol/L (%)	Total-C mmol/L	LDL-C mmol/L	HDL-C mmol/L	TG mmol/L	ApoB g/L	ApoA-I g/L	ApoE g/L
GG	8											
SFA		5.78±3.61 [a]	0.64±0.20 [a]	4.78±0.20	94.45±15.73 [a]	3.77±0.37	2.15±0.27	1.33±0.15	0.62±0.14	0.62±0.08	1.48±0.19	0.035±0.007
CHO		4.32±1.94 (-25) [b]	0.24±0.13 (-62) [b]	4.68±0.48	50.52±22.66 (-47) [b]	3.21±0.39	1.72±0.34	1.15±0.20	0.69±0.16	0.56±0.11	1.44±0.16	0.032±0.007
MUFA		4.39±1.99 (-24) [b]	0.19±0.10 (-70) [b]	4.71±0.39	67.19±25.06 (-29) [b]	3.38±0.59	1.72±0.39	1.27±0.28	0.68±0.27	0.56±0.10	1.43±0.14	0.033±0.007
GT	25											
SFA		7.35±3.59[a]	0.62±0.17 [a]	4.89±0.42	91.78±21.85 [a]	4.28±0.50	2.69±0.57	1.26±0.31	0.70±0.29	0.74±0.13	1.49±0.13	0.034±0.008
CHO		6.10±2.33 (-17) [b]	0.34±0.21 (-45) [b]	4.83±0.46	74.53±23.88 (-19) [b]	3.67±3.12	2.18±0.56	1.16±0.27	0.71±0.29	0.64±0.12	1.41±0.18	0.033±0.008
MUFA		5.82±2.22 (-20) [b]	0.36±0.19 (-41) [b]	4.79±0.36	79.79±24.72 (-13) [b]	3.74±0.60	2.23±0.51	1.18±0.30	0.72±0.24	0.65±0.11	1.41±0.14	0.031±0.009
TT	10											
SFA		7.34±2.28	0.64±0.43	4.85±0.34	108.49±48.73	4.29±0.50	2.68±0.55	1.27±0.29	0.71±0.30	0.74±0.11	1.44±0.11	0.031±0.008
CHO		7.26±3.20 (1)	0.53±0.39 (-17)	4.76±0.20	98.27±32.75 (9)	3.63±0.59	2.18±0.60	1.12±0.27	0.68±0.26	0.66±0.14	1.37±0.16	0.033±0.010
MUFA		7.30±3.37 (1)	0.52±0.28 (-19)	4.87±0.43	108.18±41.88 (0.3)	3.78±0.79	2.30±0.70	1.16±0.27	0.67±0.29	0.68±0.15	1.35±0.08	0.029±0.006
P												
Diet		0.032	0.006	0.379	0.001	0.001	0.001	0.001	0.205	0.001	0.001	0.032
Genotype		0.045	0.225	0.725	0.083	0.201	0.075	0.788	0.806	0.093	0.518	0.718
Interaction		0.039	0.017	0.428	0.039	0.133	0.148	0.153	0.175	0.134	0.248	0.117

Values are mean ± SD. Percentage change from SFA diet in parentheses when a significant diet x genotype interaction was observed. Means in a column within a genotype without a common letter differ, $P<0.05$