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Article

Accepted Version

Fallaize, R., Celis-Morales, C., Macready, A. L. ORCID: <https://orcid.org/0000-0003-0368-9336>, Marsaux, C. F. M., Forster, H., O'Donovan, C., Woolhead, C., San-Cristobal, R., Kolossa, S., Hallmann, J., Mavrogianni, C., Surwillo, A., Livingstone, K. M., Moschonis, G., Navas-Carretero, S., Walsh, M. C., Gibney, E. R., Brennan, L., Bouwman, J., Grimaldi, K., Manios, Y., Traczyk, I., Drevon, C. A., Martinez, J. A., Daniel, H., Saris, W. H. M., Gibney, M. J., Mathers, J. C. and Lovegrove, J. A. (2016) The effect of the apolipoprotein E genotype on response to personalized dietary advice intervention: findings from the Food4Me randomized controlled trial. *American Journal of Clinical Nutrition*, 104 (3). pp. 827-836. ISSN 0002-9165 doi: <https://doi.org/10.3945/ajcn.116.135012> Available at <https://centaur.reading.ac.uk/66411/>

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To link to this article DOI: <http://dx.doi.org/10.3945/ajcn.116.135012>

Publisher: American Society for Nutrition

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The American Journal of Clinical Nutrition
Official publication of the American Society for Nutrition

The American Journal of Clinical Nutrition
AJCN/2016/135012
Version 2

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Date Received: 8 Jun 2016

Trial registered: ClinicalTrials.gov Reg No. NCT01530139

Information for Authors: <http://www.ajcn.org/site/misc/ifa.xhtml>

The effect of APOE genotype on response to personalized dietary advice intervention: findings from the Food4Me randomized controlled trial¹⁻²

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Footnotes

¹ Supported by grant 265494 from the European Commission under the Food, Agriculture, Fisheries and Biotechnology Theme of the 7th Framework Programme for Research and Technological Development

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PubMed indexing: Fallaize, Celis-Morales, Macready, Marsaux, Forster, O'Donovan, Woolhead, San-Cristobal, Kolossa, Hallmann, Mavrogianni, Surwillo, Livingstone, Moschonis, Navas-Carretero, Walsh, Gibney, Brennan, Bouwman, Grimaldi, Manios, Traczyk, Drevon, Martinez, Daniel, Saris, Gibney, Mathers and Lovegrove

Key Words: *APOE*, Nutrigenomics, Food4Me, Dietary Fat, Personalized Nutrition

Abbreviations: BCT, behavioral change technique; BMI, body mass index; CHD, coronary heart disease; DBS, dried blood spot; DHA, docosahexanoic acid; EPA, eicosapentanoic acid; GLM, general linear model; LDL-C, low-density lipoprotein cholesterol; MetS, metabolic syndrome; MUFA, monounsaturated fatty acid; PA, physical activity; PN, personalized nutrition; RCT, randomised controlled trial; SFA, saturated fatty acid; TC, total cholesterol; %TE, % total energy

Running Head: APOE response to personalized diet intervention

Clinical trial registration: Clinicaltrials.gov as NCT01530139

Word count (excluding title page and references): 5959

Number of Figures: 1

Number of Tables: 7

1 **ABSTRACT (word count = 299)**

2 **Background:** The *APOE* risk allele ($\epsilon 4$) is associated with higher total cholesterol
3 (TC), amplified response to saturated fatty acid (SFA) reduction and increased CVD.
4 While knowledge of gene 'risk' may enhance dietary change, it is unclear whether $\epsilon 4$
5 carriers would benefit from gene-based personalized nutrition (PN).

6 **Objectives:** The aims of this study were to investigate **interactions between *APOE***
7 **genotype and (a) habitual dietary fat intake and (b) modulations of fat intake** on
8 **metabolic outcomes; (c) determine whether gene-based PN results in greater dietary**
9 **change compared with standard dietary advice (Level 0) and non-gene-based PN**
10 **(Levels 1-2) and (d) assess the impact of knowledge of *APOE* risk (risk: E4+, non-**
11 **risk: E4-) on dietary change following gene-based PN (Level 3).**

12 **Design:** Individuals (n=1466) recruited into the Food4Me pan-European **PN dietary**
13 **intervention** study **were randomized to four treatment arms** and genotyped for *APOE*
14 (rs429358 and rs7412). Diet and dried blood spot TC and omega-3 index were
15 determined at baseline and after 6-months intervention. **Data were analyzed using**
16 **adjusted general linear models.**

17 **Results:** Significantly higher TC concentrations were observed in E4+ participants
18 compared with E4- ($P < 0.05$). Although there were no significant differences in *APOE*
19 response to gene-based PN (E4+ vs. E4-), both groups had a greater reduction in
20 SFA (%TE) intake when compared with Level 0 (E4+, -0.72% vs. -1.95%, $P = 0.035$;
21 E4-, -0.31% vs. -1.68%, $P = 0.029$). Gene-based PN was associated with a smaller
22 reduction in SFA intake compared with non-gene-based PN (Level 2) for E4-
23 participants (-1.68% vs. -2.56%, $P = 0.025$).

24 **Conclusions:** The *APOE* $\epsilon 4$ allele was associated with greater TC. Whilst gene-
25 based PN targeted to *APOE* was more effective in reducing SFA intake than

26 standard dietary advice, there was no difference between *APOE* 'risk' and 'non-risk'
27 groups. Furthermore, disclosure of *APOE* 'non-risk' may have weakened dietary
28 response to PN.

29 INTRODUCTION

30 Coronary heart disease (CHD) is the leading cause of global mortality,
31 accounting for 1 of 5 deaths in Europe (1). Recent estimates suggest that up to 80%
32 of CHD and cerebrovascular disease could be avoided by improving diet and lifestyle
33 (2). While intervention strategies have traditionally used a 'one-size-fits-all' approach
34 to change dietary behaviour, recent evidence suggests that a personalized approach
35 may be more effective (3, 4). Moreover, there has been much interest in the use of
36 genetic information to tailor dietary advice, yet further RCTs are needed to establish
37 the benefit of such advice on sustained dietary changes (5, 6). Of particular interest
38 in relation to CHD risk is the *APOE* genotype.

39 The *APOE* gene is a key regulator of cholesterol and lipid metabolism. *APOE* is
40 polymorphic, with the common *missense* polymorphisms (rs429358 and rs7412)
41 resulting in three alleles, ϵ 2, ϵ 3 and ϵ 4, combining to form 6 haplotypes, E2/E2,
42 E2/E3, E2/E4, E3/E3, E3/E4 and E4/E4. In a sample of 5805 Caucasians, the *APOE*
43 allele frequency for ϵ 2, ϵ 3 and ϵ 4 was 0.08, 0.77 and 0.15 respectively (7). The ϵ 4
44 allele is associated with increased serum total cholesterol (TC), low-density
45 lipoprotein cholesterol (LDL-C) as well as coronary artery disease and mortality (8-
46 12). Estimates of the CHD hazard ratio for E4+ (E3/E4 and E4/E4), compared with
47 E4- (E3/E3), range from 1.06 to 1.42 (8, 9, 11, 13). There is also a growing body of
48 evidence showing that the *APOE* genotype may influence lipid response to dietary
49 fat; data from intervention studies suggest that E4+ participants may be more
50 sensitive to dietary cholesterol, total fat and, in particular, SFA modulation (14, 15).
51 Given their predisposition to CHD, ϵ 4 carriers might benefit from a lower dietary SFA
52 and blood cholesterol (16) and gene-based PN intervention. However, there is a

53 concern that gene-based PN may reduce motivation for dietary change in individuals
54 without 'risky genes' and undermine current healthy eating messages (17).

55 The Food4Me study is a pan-European, 6-month, web-based RCT designed to
56 assess the impact of personalizing dietary advice on change in dietary behaviour.
57 Participants were allocated into one of four intervention groups based on standard
58 guidelines (control), dietary intake (level 1), dietary intake and phenotype (level 2)
59 and dietary intake, phenotype and genotype (level 3). Level 3 participants received
60 feedback on four genes: *MTHFR*, *FADS1*, *TCF7L2*, *FTO* and *APOE*.

61 The aim of the present analysis was to investigate **interactions** between *APOE*
62 genotype and **(a) habitual dietary fat intake and (b) modulations of fat intake on**
63 metabolic outcomes in the Food4Me study, **(c)** assess whether gene-based PN led to
64 greater changes in diet compared with standard dietary advice (control) and non-
65 gene-based PN for E4- and E4+ participants and **(d)** assess the impact of knowledge
66 of *APOE* risk on changes in diet and metabolic outcomes **following** gene-based PN.

67

68 **PARTICIPANTS AND METHODS**

69 The Food4Me Proof-of-Principle (PoP) study is a 6-month randomized
70 controlled dietary **advice** intervention study conducted in 7 European research
71 centers: University College Dublin, Ireland, University of Reading, UK, Maastricht
72 University, the Netherlands, University of Navarra, Spain, Harokopio University,
73 Greece, National Food and Nutrition Institute, Poland, and Technische Universität
74 München, Germany. The study had a parallel design with 4 intervention arms and
75 was conducted via the web to emulate a web-delivered PN service
76 (www.food4me.org) (18). Ethics approval was granted at each center and digital
77 informed consent was obtained prior to participation. The study was registered at

78 clinicaltrials.gov (ref. NCT01530139) and was developed following international
79 regulations and the Helsinki Declaration.

80 **Participants**

81 A total of 1,607 participants (aged ≥ 18 years) were recruited to the Food4Me
82 study, as detailed elsewhere (19). Exclusion criteria were: no or limited access to the
83 Internet, following a medically prescribed diet in the past 3 months, or presence of a
84 condition likely to alter dietary requirements e.g. Crohn's disease, coeliac disease,
85 food allergy/intolerance, pregnancy or lactation.

86 **Study design**

87 A randomization scheme, incorporating both gender and age categories (< 45
88 years and >45 years), was used to allocate participants to one of the four Food4Me
89 intervention groups: Level 0: standard non-personalized dietary and physical activity
90 (PA) advice; Level 1: advice based on dietary intake and PA; Level 2: advice based
91 on dietary intake, PA and phenotype (blood biomarkers) and Level 3: advice based
92 on dietary intake, PA, phenotype and genotype. Detailed recruitment and study
93 procedures are reported elsewhere (19).

94 Interaction with study participants was conducted remotely via the Food4Me
95 website, by e-mail and post, using standardized operating procedures. A study
96 welcome pack was sent to the participants via post containing: a dried blood spot
97 (DBS) collection kit (Vitas Ltd, Oslo, Norway), an Isohelix SK-1 DNA buccal swab kit
98 (LCG Genomics, Hertfordshire, UK), a TracmorD tri-axial accelerometer (Philips
99 Consumer Lifestyle, The Netherlands; <http://www.directlife.philips.com>), measuring
100 tape and standardized instructions for completion of baseline measurements (m0).
101 On the allocated study day and following an 8-hour overnight fast, participants

102 collected DBS and buccal swab samples, and measured their height, weight and
103 waist circumference (WC). Questionnaires to be completed on the same day
104 included the validated Food4Me food frequency questionnaire (20, 21) and the
105 validated Baecke physical activity questionnaire (22-24). Participants repeated these
106 measurements, excluding the buccal cell sample, at 3 (m3) and 6 months (m6). The
107 TracmorD tri-axial accelerometer (25) was worn for the entire duration of the study,
108 and data were uploaded on a bi-weekly basis.

109 **Dietary feedback**

110 Following analysis of data collected at m0 and m3, participants received tailored
111 dietary feedback (in their native language) according to their study allocation group.

112 **The dietary feedback provided was based on a pre-defined set of algorithms**
113 **incorporating dietary, anthropometric, PA, phenotypic and genotypic data where**
114 **appropriate. The system was designed to ensure consistent feedback across centres**
115 **and has since been successfully automatized (26).** *APOE* gene variants were coded
116 as 'risk' (a genetic variation that can be modified by diet, i.e. E3/E4 or E4/E4 (E4+))
117 or 'non-risk' (E2/E2, E2/E3, E3/E3 (E4-)). Alongside the risk result, Level 3
118 participants received the following basic information about the *APOE* genotype: "A
119 *specific variation of this gene is associated with a greater need to maintain healthy*
120 *cholesterol levels. Decreasing saturated fat intake has been associated with an*
121 *improvement in cholesterol and factors relating to cardiovascular health in these*
122 *individuals."* For Level 3 E4+ participants with high dietary SFA intake and/or high
123 blood TC, who were being advised to lower dietary SFA, reference to 'gene risk' was
124 also included in the advice message, i.e. "You have a genetic variation that can
125 benefit by keeping a healthy intake of saturated fat and a normal level of blood
126 cholesterol."

127 **Biochemical analysis**

128 Participants were asked to complete 2 DBS cards each containing 5 blood
129 spots, at m0, m3 and m6 (approximately 150 μ L blood per card). After drying the
130 blood spots at room temperature for 2-4 hours, the cards were placed in a sealed
131 aluminum bag (Whatman Foil Bags, item no. 10534321, Whatman Inc., Sanford, ME)
132 containing a drying sachet (Sorb-it, item no. 10548234, Süd-Chemie, Germany) and
133 posted back to the research center in their country. Researchers subsequently
134 shipped the DBS cards to Vitas (Vitas Ltd, Norway) for analysis of whole blood TC
135 (LC-UV) and omega-3 index [(eicosapentaenoic acid (EPA) + docosahexaenoic acid
136 (DHA)/ total fatty acids) \times 100] (27). Fatty acids were measured using GC-FID.

137 **DNA extraction and genotyping**

138 Participants were instructed to rub the Isohelix SK-1 DNA buccal swab against
139 the inside of their cheek for one minute before returning it to a plastic tube containing
140 an Isohelix Dri-capsule. Upon return to the center, swabs were shipped to LCG
141 Genomics (LCG Genomics, Hertfordshire, UK) for genotypic analysis. Following DNA
142 extraction, KASPTM genotyping assays were used to provide bi-allelic scoring of
143 polymorphisms in the *APOE* gene (rs429358 and rs7412). Hardy-Weinberg
144 equilibrium for multiple alleles was analyzed, no significant deviation was observed
145 for rs7412 (0.91; $P=1.00$) whereas rs429358 displayed linkage disequilibrium (0.005;
146 $P=0.008$).

147 **Statistical analyses**

148 Data are presented as means \pm SEM. Data were checked for normality of
149 distribution and skewed variables were normalised using Log₁₀ (omega-3 index) and
150 square root (TC) transformations. General linear models (GLM), adjusted for center,

151 gender, age and body mass index (BMI), were used to assess differences in baseline
152 anthropometric and biochemical values between genotype groups. Habitual **nutrient**
153 **intake-gene interactions** were assessed using the same GLM model but with the
154 addition of a dietary fat \times genotype interaction term; fat were dichotomised by median
155 intake to assess the impact of the *APOE* genotype on TC and omega-3 index in
156 participants with a similar habitual intake. Post-hoc Bonferroni tests were used to
157 detect specific differences between groups.

158 Interactions between genotype and dietary **fat** on TC and omega-3 index
159 following dietary **advice** intervention were assessed using % change in dietary fat
160 intake, with 0% used as a reference to dichotomize participants (i.e. reduction vs.
161 increase in fat intake), and then using the resulting groups as fixed factors in the
162 GLM. The interaction term genotype \times change in **fat** was then added to the GLM,
163 with the change in biomarker as the response variable and the respective pre-
164 intervention/ baseline biomarker value as a covariate. The model was adjusted for
165 baseline variables, age, gender, center and weight change [post intervention weight
166 (kg) – pre intervention weight (kg)].

167 The impact of knowledge of *APOE* risk (risk: E4+, E3E4 and E4/E4; and non-
168 risk: E4-, E2/E2, E2/E3 and E3/E3) on change in diet and TC and omega-3 index
169 (m6-m0) for Level 3 participants advised to lower their SFA at baseline (with high
170 dietary SFA and/or high blood TC) were assessed using GLM. Models were adjusted
171 for baseline variables, age, gender, center and weight change. To assess whether
172 gene-based PN led to greater changes in diet, TC and omega-3 index (m6-m0) than
173 standard dietary advice (Level 0) and non gene-based PN (Levels 1-2), a contrast
174 analysis was performed. Separate analyses were conducted for E4+ (risk) and E4-
175 (non-risk) with Level 3 as the reference group and Levels 0, 1 and 2 as the

176 comparison groups. As previously, participants with high dietary SFA and/or high
177 blood TC who were advised to lower their SFA at baseline were included and
178 analyses were adjusted for baseline variables, age, gender, center and weight
179 change. Statistical analyses were performed using STATA (version 13.0, StataCorp,
180 TX, USA).

181

182 **RESULTS**

183 **Subject characteristics**

184 A total of 1466 of the 1607 participants randomized into the Food4Me study
185 were genotyped for *APOE* and included in the baseline analysis. Frequency of *APOE*
186 genotype and *APOE* allele according to Food4Me country are presented in **Table 1**.
187 *APOE* E2/E4 participants (n=27) were removed from subsequent analysis due to
188 their low population frequency. Subject characteristics including anthropometry and
189 fasted biomarkers are presented according to *APOE* genotype in **Table 2**. There was
190 no evidence of a genotype-dependant difference in baseline anthropometry, although
191 E4+ participants had higher TC than E4- ($P = 0.040$ for E3/E3 and $P = 0.002$ for E2
192 carriers).

193 **Habitual dietary and genotype effects at baseline**

194 The associations between dietary fat (total fat, SFA, monounsaturated fatty
195 acids (MUFA), polyunsaturated fatty acids (PUFA) and omega-3), *APOE* genotype,
196 dietary fat \times genotype interactions and TC and omega-3 index, are reported in **Table**
197 **3**. Dietary intake was dichotomized at the median (total fat, 35.8%; SFA, 14.0%;
198 MUFA, 13.5%; PUFA, 5.6; omega-3, 0.67%) to determine the effect of specific

199 genotypes in participants with similar habitual dietary fat intakes; presented in **Table**
200 **3** according to genotype group.

201 An independent effect of genotype was observed for dietary **fat** and TC
202 concentrations at baseline (total **fat**, $P= 0.002$; SFA, $P= 0.002$; MUFA, $P= 0.002$;
203 PUFA, $P= 0.003$ and omega-3, $P= 0.004$), with the highest TC concentrations seen in
204 carriers of $\epsilon 4$ allele (E4+). Overall diet effects (SFA, $P= 0.008$; MUFA, $P= 0.025$;
205 PUFA, $P= 0.007$ and omega-3, $P < 0.001$) were observed for omega-3 index, with
206 lower dietary SFA ($11.7\% \pm 0.1$) and higher PUFA ($6.80\% \pm 0.05$) and omega-3
207 ($0.89\% \pm 0.01$) fat intake associated with a higher omega-3 index. Although a
208 significant MUFA \times *APOE* interaction was observed for omega-3 index ($P = 0.025$),
209 no differences between genotype groups and **fat** intakes were observed following
210 post-hoc analyses.

211 **Dietary and genotype effects of intervention (irrespective of group allocation)**

212 The associations between change in dietary **fat** intake (total **fat**, SFA, MUFA,
213 PUFA and omega-3), *APOE* genotype and change in **fat** \times *APOE* interactions on TC
214 and omega-3 index following intervention (m6-m0) are reported in **Table 4**. Dietary
215 intake was split into participants who reduced fat intake and those who increased fat
216 intake. **Mean reductions and increases in dietary fat intakes are presented according**
217 **to genotype group.**

218 There was a significant impact of genotype on change in TC concentrations
219 following dietary **advice** intervention (total **fat**, $P= 0.016$; SFA, $P= 0.025$; MUFA, $P=$
220 0.019 ; PUFA, $P= 0.024$ and omega-3, $P= 0.027$). There were no independent effects
221 of diet on lipid biomarkers following dietary **advice** intervention, although trends were
222 observed for change in PUFA ($P= 0.068$) and omega-3 **fat** intakes ($P= 0.087$) on

223 omega-3 index. A trend was also observed for an omega-3 **fat** intake \times *APOE*
224 interaction on omega-3 index ($P= 0.087$).

225 **Effect of knowledge of *APOE* gene risk on dietary change compared with other** 226 **levels of personalization**

227 The allocation of *APOE* risk according to intervention level is shown in **Figure**
228 **1**. Participants (levels 1-3) advised to lower dietary SFA at baseline were selected for
229 subsequent analysis. The effects of knowledge of *APOE* risk (E4+) in participants
230 advised to reduce SFA intake at baseline on changes in diet, TC and omega-3 index
231 (m6-m0) compared with other levels of personalization are reported in **Table 5 A**
232 significantly greater reduction in total **fat** and SFA (%TE) was observed in E4+
233 participants receiving gene-based PN (Level 3) compared to those in the control
234 group ($P=0.034$ and $P=0.035$ respectively). However, there were no differences in
235 change in diet or biomarkers between personalized intervention groups.

236 The effects of knowledge of *APOE* non-risk (E4-) in participants advised to
237 reduce SFA intake at baseline on changes in diet, TC and omega-3 index (m6-m0)
238 compared with other levels of personalization are reported in **Table 6**. As previously,
239 participants receiving gene-based PN had a significantly greater reduction in dietary
240 SFA (%TE) compared with those in the control group ($P=0.029$). For total **fat** (%FE),
241 a slight increase in intake was observed for the control group (Level 0) compared
242 with a reduction in Level 3 (difference 2.72% TE, $P=0.006$). The opposite was
243 observed for total carbohydrate, which reduced in the control group (Level 0) and
244 increased in Level 3 (difference 2.15 %TE, $P=0.027$).

245 When comparing levels of personalization, a 0.88% greater reduction in SFA
246 (%TE) was observed in E4- participants receiving non-gene-based PN (Level 2; PN
247 based on diet and phenotype) compared with those E4- participants receiving gene-

248 based PN ($P = 0.025$). There were no significant differences between change in total
249 fat, PUFA, MUFA, omega-3, carbohydrate and protein intake, or TC and omega-3
250 index for E4- carriers according to whether they received gene-based or non-gene-
251 based PN (L3 vs. L1-2).

252 **Effect of knowledge of *APOE* genotype on dietary change following gene-** 253 **based personalized advice PN**

254 The effect of knowledge of *APOE* risk (risk: E4+, E3/E4 and E4/E4 and non-
255 risk: E4-, E2/E2, E2/E3 and E3/E3) in participants advised to reduce SFA intake at
256 baseline on changes in diet, TC and omega-3 index (m6-m0) following gene-based
257 PN (L3) are reported in **Table 7**. Approximately 30% of E4- participants receiving
258 gene-based PN were advised to lower their SFA intake at baseline, compared with
259 53% of E4+ carriers (**Figure 1**). Following intervention, there were no significant
260 differences in dietary response or change in biomarker between E4+ and E4-
261 participants.

262

263 **DISCUSSION**

264 Key findings in the present analysis were higher TC concentrations in E4
265 carriers (E4+) and a nutrient intake-gene interaction between *APOE* genotype and
266 MUFA intake for omega-3 index at baseline. Following intervention, gene-based PN
267 resulted in significantly greater reductions in total fat and SFA (%TE) compared with
268 standard dietary advice (control), irrespective of gene risk. For E4- ('non-risk')
269 participants advised to lower SFA intake, gene-based PN resulted in smaller changes
270 in dietary SFA intake at month 6 than non-gene-based PN (Level 2).

271 Although the *APOE* rs429358 distribution was not in Hardy-Weinberg
272 equilibrium, the haplotype frequencies observed in the Food4Me cohort (ϵ 2, 6.5; ϵ 3,
273 79.3; ϵ 4, 14.2) were similar to those reported in previous studies of European
274 populations (28). In contrast to previous observations (29, 30), there was no clear
275 geographical cline in ϵ 4 frequency.

276 DBS TC differed according to *APOE* genotype with significantly higher TC
277 observed in E4+ participants compared with E4-. The difference in TC between E4+
278 and those who were E4-: E3/E3 in the present study (0.15 mmol/L) was similar to
279 previous data (0.16-0.36 mmol/L) in a large meta-analysis of 54,377 participants (31).

280 At baseline, there was a significant nutrient **intake**-gene interaction between
281 total MUFA intake and *APOE* on long-chain omega-3 index, a reliable biomarker of
282 omega-3 status, and dietary omega-3 PUFA, EPA and DHA intake (32, 33).
283 Furthermore, there is a dose-dependent inverse association between omega-3 index
284 and CHD mortality (33), with an index \geq 8% offering the most cardio-protective
285 effects and an index \leq 4% being associated with the greatest risk of CHD mortality
286 (27). Thus, the omega-3 index may be a risk factor for CHD (34). In the Food4Me
287 study, a higher omega-3 index was associated with lower SFA and higher PUFA and
288 dietary omega-3 intake. In a study investigating the determinants of omega-3 index in
289 a Mediterranean population, there were significant associations between EPA and
290 DHA intakes and omega-3 index ($P < 0.001$) and a trend for an inverse association
291 between dietary SFA and omega-3 index ($P = 0.095$) (35).

292 It has been suggested that gene-based dietary information is more
293 understandable and useful than general dietary guidelines (36) and may enhance
294 motivation to change (37). In a 2010 systematic review, a beneficial effect of
295 genome-based risk estimates on dietary behavior was reported (pooled OR for 2

296 RCT 2.24, 95% CI 1.17 to 4.27, $P = 0.01$, $I^2 = 0\%$); but no benefit of genome-based
297 risk estimates on intention to change dietary behavior was observed (5).

298 Furthermore, in a Canadian RCT, knowledge of *ACE* gene risk resulted in a
299 significantly greater reduction in sodium intake compared with non-gene based
300 advice (-287 ± 114 vs. 130 ± 118 mg/day, $P = 0.008$) at 12-month follow-up (38).

301 Change in sodium intake by participants carrying the 'non-risk' *ACE* genotype (-244
302 mg/day) was not significantly different ($P = 0.11$) compared with the control group. In
303 our present study, gene-based PN promoted significantly greater reductions in the
304 intake of total fat and SFA than standard dietary advice (control), for both risk (E4+)
305 and non-risk (E4-) participants advised to lower SFA. However, there were no
306 significant differences in change of diet, TC or omega-3 index between *APOE* risk
307 groups (E4+ and E4-) receiving gene-based PN. In the REVEAL study, which
308 investigated the impact of knowledge of Alzheimer's disease (AD) risk (estimated
309 using *APOE* genotype and family history to generate a numerical risk) on dietary
310 behaviors, E4+ participants were significantly more likely to endorse AD-specific
311 health behavior change than E4- participants at 12 months follow-up (39). A similar
312 result was observed in a study investigating the impact of knowledge of *FTO*
313 genotype on readiness to control weight; whereby individuals with higher 'risk' (AA or
314 AT) displayed greater willingness to change than those with lower risk (TT) ($P =$
315 0.051) (40).

316 Whilst there was no additional benefit of gene-based PN for E4+ participants in
317 the Food4Me study, knowledge of 'non-risk' (E4-) resulted in a lower reduction in
318 SFA intake at 6 months compared with E4- participants receiving non-gene-based
319 PN (Level 2) who were not informed of their *APOE* risk (-1.68% vs. -2.56%).

320 Providing 'no-risk' genotypic results may reduce motivation to follow dietary advice

321 (41). A potential reason for the lack of response in Food4Me E4 carriers is the
322 absence of a specific behavior change technique (BCT) involving information on the
323 consequences of a specific behavior related to genotype. A key BCT in the CALO-RE
324 taxonomy (a 40-item taxonomy to improve PA and healthy eating behaviors) is to
325 “provide information of the consequences of the behavior to the individual”. In the
326 context of *APOE* genotype, a consequence of carrying the $\epsilon 4$ allele would be
327 increased CVD risk (31) and the corresponding risk-reducing behavior would be
328 lowering SFA intake. In the present study, *APOE* risk information conveyed to
329 participants was framed positively viz : “you have a genetic variation that can benefit
330 by keeping a healthy intake of saturated fat and a normal level of blood cholesterol.”
331 The lack of an explicit link to an adverse consequence of E4+ status, e.g. higher
332 CVD risk, may have reduced the efficacy of this advice. In the REVEAL study,
333 participants were informed that the E4 allele was associated with an increased risk of
334 Alzheimer’s disease prior to gene disclosure (39). Whilst genotypic testing for
335 polygenic disease risk may result in a fatalistic attitude (37), information on
336 consequences of personal characteristics (e.g. genotype) and fear arousal can be
337 useful aids in enhancing behavior change (42). In a meta-analysis of fear arousal
338 techniques, stronger fear messages promoted greater intention and behavior change
339 in public health campaigns, provided that the threat was perceived to be severe,
340 personally relevant, and that the individual could take specific action to mitigate their
341 risk (43). In a Finnish RCT, knowledge of personal *APOE* risk resulted in greater
342 short-term improvements in dietary quality, WC and serum triacylglycerol, when
343 participants were informed of the link between dietary fat, cholesterol and CVD risk in
344 an oral communication session (44). Furthermore, E4+ individuals significantly

345 improved fat quality at 6-months ($P < 0.01$), whereas there was no difference in fat
346 quality in the E4- or control groups (44).

347 A limitation of internet-delivered PN (as used in our Food4Me study) is the
348 reduced opportunity to employ BCT in response to verbal and non-verbal cues (e.g.
349 body-language, facial expressions). Recent focus group data also revealed a lack of
350 understanding amongst consumers of the use of genetic information to tailor dietary
351 advice, and opinions regarding gene-based PN were mostly negative (45). **Given that**
352 **understanding and 'knowledge' of specific gene-based PN advice was not evaluated**
353 **in the Food4Me study, it is not possible to ascertain if this contributed to the lack of**
354 **effect observed.** The Food4Me study was designed to assess the impact of three
355 levels of personalization on dietary change and was not specifically targeted to the
356 *APOE* genotype. Furthermore, although participants were informed that they had a
357 'risky' gene variant that would benefit from dietary change, advice was not stratified
358 according to specific genotype groups (e.g. differing advice for E2/E3 and E3/E3).
359 Strengths of this study include using the internet to assess and deliver dietary advice,
360 prospective genotyping, a larger sample size than reported previously (39, 44, 46),
361 the measurement of actual dietary change, as distinct from intention to change, and
362 the availability of relevant blood-based biomarkers of **fat status** (obtained from
363 unsupervised sampling). As such, the Food4Me study provides robust evidence of
364 the impact of knowledge of *APOE* risk on adherence to dietary advice.

365

366 **CONCLUSION**

367 *APOE* status was significantly associated with TC at baseline with highest
368 concentrations in E4+ participants. Whilst gene-based PN targeted to *APOE* was
369 more effective in reducing SFA intake than standard dietary advice, there was no

370 added benefit of knowledge of *APOE* 'risk' on dietary change. **Furthermore, it**
371 **appears that disclosure of genotypic 'non-risk' status may have weakened the** dietary
372 response to PN. Future research should explore ways in which this detrimental
373 response to gene-based PN can be mitigated.

374

375 **ACKNOWLEDGEMENTS**

376 The authors' responsibilities were as follows: MG, JCM, JAL, ERG, LB, WHMS, HD,
377 CAD, JAM, YM and IT, contributed to the research design. RF, ALM, CCM, CFMM,
378 HF, SNC, SK, LT, AS, MCW and JCM conducted the intervention. RF and CCM
379 performed the statistical analysis for the manuscript. RF and JAL drafted the paper.
380 All authors contributed to a critical review of the manuscript during the writing
381 process. All authors approved the final version to be published. CAD is a founder,
382 board member, stockowner and a consultant for Vitas Ltd. No other authors declare a
383 conflict of interest. This work was funded by the EU FP7 Food4Me (KBBE.2010.2.3-
384 02, Project no. 265494).

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TABLE 1. Frequency of *APOE* genotype and *APOE* allele by Food4Me center (n=1466)

	All	Ireland	UK	The Netherlands	Germany	Poland	Spain	Greece
Genotype (n, %)								
E2/E2	6 (0.4)	1 (0.5)	0 (0.0)	3 (1.4)	0 (0.0)	2 (1.0)	0 (0.0)	0 (0.0)
E2/E3	152 (10.4)	14 (6.5)	22 (10.6)	28 (12.7)	21 (10.2)	29 (14.4)	22 (10.4)	16 (7.7)
E2/E4	27 (1.8)	3 (1.4)	6 (2.9)	3 (1.4)	7 (3.4)	4 (2.0)	1 (0.5)	3 (1.4)
E3/E3	922 (62.9)	133 (62.1)	132 (64.1)	124 (56.4)	125 (61.0)	125 (62.1)	139 (65.6)	144 (69.2)
E3/E4	330 (22.5)	57 (26.6)	43 (20.8)	58 (26.4)	48 (23.4)	38 (18.9)	46 (21.7)	40 (19.2)
E4/E4	29 (2.0)	6 (2.8)	3 (1.5)	4 (1.8)	4 (2.0)	3 (1.5)	4 (1.9)	5 (2.4)
Total	1466 (100)	214 (100)	206 (100)	220 (100)	205 (100)	201 (100)	212 (100)	208 (100)
E2 carriers ¹	158 (10.8)	15 (7.0)	22 (10.7)	31 (14.1)	21 (10.2)	31 (15.4)	22 (10.4)	16 (7.7)
E4 carriers ¹	359 (24.5)	63 (29.4)	46 (22.3)	62 (28.2)	52 (25.4)	41 (20.4)	50 (23.6)	45 (21.6)
Allele frequency (%)								
ε2	6.5	4.4	6.5	8.4	6.8	8.9	5.4	4.6
ε3	79.3	78.7	76.2	75.9	77.8	76.0	81.6	82.7
ε4	14.2	16.8	17.4	15.7	15.3	15.1	13.0	12.7

¹Genotype groups combined; E2 carriers represent E2/E2 and E2/E3, E4 carriers represent E4/E3 and E4/E4

TABLE 2. Anthropometric characteristics and fasted blood biomarkers by *APOE* genotype in European adults in the Food4Me study¹

	<i>APOE</i> genotype ¹				<i>P</i> ²
	All (n=1439)	E4-		E4+	
		E2 carriers (n=158)	E3/E3 (n=922)	E4 carriers (n=359)	
Gender ratio (M/F)	611/846				
Age (y)	40 ± 0.4	40 ± 1	40 ± 0.4	40 ± 0.7	0.630
BMI (kg/m ²)	25.5 ± 0.13	25.7 ± 0.4	25.4 ± 0.2	25.5 ± 0.3	0.704
Weight (kg)	74.6 ± 0.44	76.8 ± 1.4	74.3 ± 0.5	75.4 ± 0.8	0.608
Waist circumference (m)	0.86 ± 0.004	0.87 ± 0.01	0.86 ± 0.005	0.85 ± 0.01	0.693
Height (m)	1.71 ± 0.003	1.73 ± 0.01	1.71 ± 0.003	1.72 ± 0.005	0.252
Cholesterol (mmol/L)	4.59 ± 0.03	4.42 ± 0.08 ^a	4.55 ± 0.03 ^a	4.70 ± 0.05 ^b	0.002
Omega 3 index	5.68 ± 0.03	5.81 ± 0.10	5.66 ± 0.04	5.74 ± 0.06	0.341

¹ Data are means ± SEM

² Data were analyzed by GLM with adjustment for age, gender, center and BMI. Where *P* for genotype < 0.05, a Bonferroni post-hoc test was applied to determine between-group effects. Superscript letters ^a and ^b denote significant differences between genotype groups, *P* < 0.05.

TABLE 3. Effect of *APOE* genotype and dietary fat intake (total and fat classes)¹ on metabolic markers measured in dried blood spots at baseline in the Food4Me intervention study²

	E4-				E4+		Diet	<i>P</i> ³	Diet x Genotype
	E2 carriers (n=158)		E3/E3 (n=922)		E4 carriers (n=359)				
	Low Intake	High Intake	Low Intake	High Intake	Low Intake	High Intake			
Total fat	(n=80)	(n=78)	(n=452)	(n=470)	(n=188)	(n=171)			
Total fat (%TE)	31.7 ± 0.4	39.9 ± 0.4	31.3 ± 0.2	40.6 ± 0.2	31.3 ± 0.3	40.6 ± 0.3			
Cholesterol (mmol/L)	4.37 ± 0.11	4.48 ± 0.11	4.45 ± 0.04	4.64 ± 0.04	4.66 ± 0.07	4.73 ± 0.07	0.251	0.002	0.435
Omega-3 index	5.81 ± 0.10	5.81 ± 0.13	5.66 ± 0.06	5.64 ± 0.06	5.79 ± 0.09	5.68 ± 0.09	0.989	0.344	0.456
SFA	(n=77)	(n=81)	(n=456)	(n=466)	(n=187)	(n=172)			
SFA (%TE)	11.7 ± 0.2	16.7 ± 0.2	11.7 ± 0.1	16.7 ± 0.1	11.6 ± 0.1	16.4 ± 0.1			
Cholesterol (mmol/L)	4.40 ± 0.11	4.44 ± 0.11	4.49 ± 0.04	4.61 ± 0.04	4.66 ± 0.07	4.73 ± 0.07	0.413	0.002	0.789
Omega-3 index	5.86 ± 0.14	5.76 ± 0.13	5.72 ± 0.06	5.58 ± 0.06	5.88 ± 0.09	5.57 ± 0.09	0.008	0.343	0.573
MUFA	(n=84)	(n=74)	(n=451)	(n=471)	(n=185)	(n=174)			
MUFA (%TE)	11.7 ± 0.2	15.5 ± 0.2	11.4 ± 0.1	16.1 ± 0.1	11.5 ± 0.1	16.1 ± 0.2			
Cholesterol (mmol/L)	4.40 ± 0.10	4.45 ± 0.11	4.49 ± 0.04	4.60 ± 0.04	4.98 ± 0.07	4.80 ± 0.07	0.078	0.002	0.470
Omega-3 index	5.67 ± 0.13	5.97 ± 0.14	5.71 ± 0.06	5.60 ± 0.06	5.86 ± 0.09	5.60 ± 0.09	0.025	0.280	0.025
PUFA	(n=86)	(n=72)	(n=460)	(n=462)	(n=174)	(n=185)			
PUFA (%TE)	4.7 ± 0.1	6.8 ± 0.1	4.6 ± 0.1	6.8 ± 0.1	4.7 ± 0.1	6.7 ± 0.1			
Cholesterol (mmol/L)	4.38 ± 0.10	4.47 ± 0.11	4.51 ± 0.04	4.59 ± 0.04	4.69 ± 0.07	4.69 ± 0.07	0.445	0.003	0.614
Omega-3 index	5.65 ± 0.13	6.00 ± 0.14	5.52 ± 0.06	5.77 ± 0.06	5.62 ± 0.09	5.84 ± 0.09	0.007	0.291	0.803
Omega-3	(n=80)	(n=78)	(n=485)	(n=437)	(n=155)	(n=204)			
Omega-3 (%TE)	0.55 ± 0.01	0.90 ± 0.03	0.55 ± 0.01	0.89 ± 0.01	0.55 ± 0.01	0.89 ± 0.02			
Cholesterol (mmol/L)	4.43 ± 0.11	4.41 ± 0.11	4.50 ± 0.04	4.61 ± 0.05	4.64 ± 0.08	4.74 ± 0.07	0.068	0.004	0.820
Omega-3 index	5.50 ± 0.13	6.12 ± 0.08	5.34 ± 0.05	5.99 ± 0.06	5.30 ± 0.09	6.07 ± 0.08	<0.001	0.546	0.463

¹ Intakes of fat were dichotomised at the median: total fat, 35.8% (low intake, 31.4% \pm 0.1; high intake 40.5% \pm 0.1); SFA, 14.0% (low intake, 11.7% \pm 0.1; high intake 16.6% \pm 0.1); MUFA, 13.5% (low intake, 11.5% \pm 0.1; high intake 16.0% \pm 0.1); PUFA, 5.6% (low intake, 4.67% \pm 0.02; high intake 6.80% \pm 0.05); omega-3, 0.67% (low intake, 0.55% \pm 0.01; high intake 0.89% \pm 0.01)

² Genotype groups combined; E2 carriers represent E2/E2 and E2/E3, E4 carriers represent E4/3 and E4/E4; %TE, % total energy; low intake, less than median fat intake; high intake, greater than median fat intake; data are mean \pm SEM

³ Data were analysed by GLM with adjustment for centre, gender, age and BMI. Where *P* for diet x genotype < 0.05, a Bonferroni post-hoc test was applied to determine between-group effects (significant differences were not detected post-hoc)

TABLE 4. Effect of APOE genotype and change in dietary fat intake (total and fat classes)¹ on changes in metabolic markers measured in dried blood spots between baseline and month 6 for participants in the Food4Me intervention study²

	E4-				E4+		Diet	P ³	
	E2 carriers (n=132)		E3/E3 (n=794)		E4 carriers (n=315)				
	Decreased Intake	Increased Intake	Decreased Intake	Increased Intake	Decreased Intake	Increased Intake			
Total fat	(n=72)	(n=60)	(n=424)	(n=370)	(n=178)	(n=137)			
Total fat (%TE)	-4.49 ± 0.42	3.90 ± 0.41	-4.91 ± 0.19	3.93 ± 0.18	-4.76 ± 0.29	4.16 ± 0.34			
Cholesterol (mmol/L)	-0.26 ± 0.12	-0.24 ± 0.13	-0.18 ± 0.05	-0.21 ± 0.05	-0.26 ± 0.08	-0.03 ± 0.09	0.527	0.016	0.313
Omega-3 index	0.24 ± 0.15	-0.08 ± 0.16	0.26 ± 0.06	0.25 ± 0.06	0.40 ± 0.09	0.15 ± 0.11	0.808	0.136	0.384
SFA	(n=86)	(n=46)	(n=484)	(n=310)	(n=206)	(n=109)			
SFA (%TE)	-2.56 ± 0.21	2.01 ± 0.23	-2.68 ± 0.10	1.75 ± 0.08	-2.48 ± 0.14	2.13 ± 0.19			
Cholesterol (mmol/L)	-0.32 ± 0.11	-0.14 ± 0.14	-0.21 ± 0.05	-0.17 ± 0.06	-0.18 ± 0.07	-0.11 ± 0.10	0.982	0.025	0.941
Omega-3 index	0.24 ± 0.14	-0.14 ± 0.17	0.33 ± 0.06	0.14 ± 0.07	0.39 ± 0.09	0.10 ± 0.12	0.986	0.069	0.377
MUFA	(n=64)	(n=68)	(n=397)	(n=397)	(n=165)	(n=150)			
MUFA (%TE)	-1.88 ± 0.18	1.65 ± 0.17	-2.10 ± 0.10	2.00 ± 0.10	-2.19 ± 0.15	2.13 ± 0.17			
Cholesterol (mmol/L)	-0.29 ± 0.13	-0.21 ± 0.12	-0.21 ± 0.05	-0.19 ± 0.05	-0.29 ± 0.08	-0.01 ± 0.08	0.392	0.019	0.583
Omega-3 index	0.25 ± 0.15	-0.04 ± 0.15	0.23 ± 0.06	0.28 ± 0.06	0.36 ± 0.10	0.21 ± 0.10	0.547	0.309	0.373
PUFA	(n=58)	(n=74)	(n=357)	(n=437)	(n=153)	(n=162)			
PUFA (%TE)	-0.83 ± 0.10	1.12 ± 0.11	-1.06 ± 0.06	1.13 ± 0.06	-0.93 ± 0.07	1.13 ± 0.09			
Cholesterol (mmol/L)	-0.28 ± 0.13	-0.23 ± 0.12	-0.12 ± 0.05	-0.26 ± 0.05	-0.23 ± 0.08	-0.09 ± 0.08	0.611	0.024	0.148
Omega-3 index	-0.004 ± 0.16	0.18 ± 0.14	0.18 ± 0.07	0.32 ± 0.06	0.41 ± 0.10	0.17 ± 0.10	0.068	0.467	0.303
Omega-3	(n=53)	(n=79)	(n=294)	(n=500)	(n=129)	(n=186)			

Omega-3 (%TE)	-0.12 ± 0.02	0.18 ± 0.02	-0.14 ± 0.01	0.22 ± 0.02	-0.13 ± 0.01	0.15 ± 0.03			
Cholesterol (mmol/L)	-0.15 ± 0.14	-0.32 ± 0.11	-0.23 ± 0.06	-0.18 ± 0.05	-0.18 ± 0.09	-0.14 ± 0.08	0.738	0.027	0.738
Omega-3 index	0.02 ± 0.17	0.14 ± 0.14	0.02 ± 0.07	0.39 ± 0.06	0.24 ± 0.11	0.32 ± 0.09	0.087	0.412	0.087

¹ 0% change in fat intake used as a reference to dichotomize participants i.e. comparison of reduction vs. increase in fat intake; total fat (decrease, -4.82% ± 0.15; increase 3.98% ± 0.15), SFA (decrease, -2.62% ± 0.08; increase 1.84% ± 0.08), MUFA (decrease, -2.10% ± 0.07; increase 1.99% ± 0.08), PUFA (decrease, -1.00% ± 0.04; increase 1.13% ± 0.04), omega-3 (decrease, -0.14% ± 0.01; increase 0.22% ± 0.02)

² Genotype groups combined; E2 carriers represent E2/E2 and E2/E3, E4 carriers represent E4/3 and E4/E4; %TE, % total energy; increased intake, greater than 0% change in fat intake; decreased intake, less than 0% change in fat intake; data are mean change ± SEM (m6 - m0)

³ Data were analysed by GLM with adjustment for baseline values, centre, gender, age and change in weight (m6 - m0).

TABLE 5. Effect of knowledge of *APOE* risk (E4+) on change in dietary intake between baseline and month 6 for participants in the Food4Me intervention study¹

	Control	Personalized intervention arms			<i>P</i> ²		
	Level 0 (L0) <i>APOE</i> risk (n=77)	Level 1 (L1) <i>APOE</i> risk (n=47)	Level 2 (L2) <i>APOE</i> risk (n=35)	Level 3 (L3) <i>APOE</i> risk (n=40)	L3 vs. Control (L0)	L3 vs. L1	L3 vs. L2
Total fat (%TE)	0.37 ± 0.65	-3.03 ± 0.79	-1.63 ± 1.00	-3.07 ± 0.86	0.034	0.970	0.317
SFA (%TE)	-0.72 ± 0.35	-2.53 ± 0.37	-1.58 ± 0.56	-1.95 ± 0.45	0.035	0.335	0.537
MUFA (%TE)	0.37 ± 0.32	-0.71 ± 0.35	-0.41 ± 0.42	-1.05 ± 0.36	0.073	0.467	0.303
PUFA (%TE)	-0.04 ± 0.13	0.20 ± 0.19	0.30 ± 0.23	0.01 ± 0.23	0.718	0.965	0.720
Omega-3 (%TE)	0.04 ± 0.03	0.08 ± 0.03	0.08 ± 0.03	0.08 ± 0.03	0.899	0.900	0.990
Carbohydrate (%TE)	-0.89 ± 0.76	1.89 ± 0.85	0.11 ± 0.98	1.55 ± 0.92	0.127	0.945	0.130
Protein (%TE)	0.38 ± 0.43	0.40 ± 0.43	0.49 ± 0.49	1.37 ± 0.40	0.392	0.245	0.226
BMI (kg/m ²)	-0.25 ± 0.13	-0.35 ± 0.15	-0.04 ± 0.19	-0.44 ± 0.18	0.231	0.590	0.086
Cholesterol (mmol/L)	-0.32 ± 0.11	-0.04 ± 0.16	-0.39 ± 0.15	-0.19 ± 0.16	0.240	0.663	0.228
Omega-3 index	-0.04 ± 0.11	0.29 ± 0.16	0.38 ± 0.16	0.14 ± 0.16	0.545	0.610	0.240

¹ E4-, E2/E2, E2/E3 and E3/E3; E4+, E3/E4 and E4/E4; %TE, % total energy; data are mean change ± SEM (m6 - m0)

² Data were analysed by GLM with adjustment for baseline values, centre, gender, age and change in weight (m6 - m0).

TABLE 6. Effect of knowledge of *APOE* non-risk (E4-) on change in dietary intake between baseline and month 6 for participants in the Food4Me intervention study¹

	Control	Personalized intervention arms			P²		
	Level 0 (L0) <i>APOE</i> non-risk (n=225)	Level 1 (L1) <i>APOE</i> non-risk (n=145)	Level 2 (L2) <i>APOE</i> non-risk (n=119)	Level 3 (L3) <i>APOE</i> non-risk (n=72)	L3 vs. Control (L0)	L3 vs. L1	L3 vs. L2
Total fat (%TE)	0.31 ± 0.37	-2.63 ± 0.47	-3.42 ± 0.51	-2.41 ± 0.66	0.006	0.280	0.381
SFA (%TE)	-0.31 ± 0.20	-1.88 ± 0.25	-2.56 ± 0.27	-1.68 ± 0.35	0.029	0.119	0.025
MUFA (%TE)	0.32 ± 0.17	-0.75 ± 0.22	-0.87 ± 0.24	-0.64 ± 0.31	0.012	0.382	0.601
PUFA (%TE)	0.25 ± 0.11	-0.01 ± 0.14	0.04 ± 0.15	-0.18 ± 0.19	0.053	0.273	0.119
Omega-3 (%TE)	0.13 ± 0.03	0.02 ± 0.04	0.05 ± 0.05	0.06 ± 0.06	0.278	0.442	0.903
Carbohydrate (%TE)	-1.22 ± 0.45	1.65 ± 0.55	1.92 ± 0.61	0.93 ± 0.79	0.027	0.211	0.558
Protein (%TE)	0.85 ± 0.21	0.77 ± 0.26	0.80 ± 0.28	1.17 ± 0.36	0.997	0.346	0.634
BMI (kg/m ²)	-0.28 ± 0.08	-0.44 ± 0.09	-0.41 ± 0.10	-0.51 ± 0.13	0.970	0.711	0.364
Cholesterol (mmol/L)	-0.27 ± 0.07	-0.22 ± 0.08	-0.39 ± 0.09	-0.41 ± 0.12	0.855	0.959	0.560
Omega-3 index	0.27 ± 0.07	0.11 ± 0.09	0.26 ± 0.09	0.18 ± 0.12	0.536	0.700	0.464

¹ E4-, E2/E2, E2/E3 and E3/E3; E4+, E3/E4 and E4/E4; %TE, % total energy; data are mean change ± SEM (m6 - m0)

² Data were analysed by GLM with adjustment for baseline values, centre, gender, age and change in weight (m6 - m0).

TABLE 7. Effect of knowledge of *APOE* genotype on change in dietary intake between baseline and month 6 for participants receiving gene-based personalized nutrition (Level 3) in the Food4Me intervention study¹

	Level 3 (L3)		<i>P</i> ²
	<i>APOE</i> non-risk (E4-) (n=72)	<i>APOE</i> risk (E4+) (n=40)	
Total fat (%TE)	-2.41 ± 0.64	-3.07 ± 0.86	0.433
SFA (%TE)	-1.68 ± 0.33	-1.95 ± 0.45	0.348
MUFA (%TE)	-0.64 ± 0.28	-1.05 ± 0.36	0.307
PUFA (%TE)	-0.18 ± 0.17	0.01 ± 0.23	0.223
Omega-3 (%TE)	0.06 ± 0.02	0.08 ± 0.03	0.392
Carbohydrate (%TE)	0.93 ± 0.68	1.55 ± 0.92	0.421
Protein (%TE)	1.17 ± 0.30	1.37 ± 0.40	0.502
BMI (kg/m ²)	-0.51 ± 0.13	-0.44 ± 0.18	0.229
Cholesterol (mmol/L)	-0.41 ± 0.12	-0.19 ± 0.16	0.203
Omega-3 index	0.18 ± 0.12	0.14 ± 0.16	0.777

¹ E4-, E2/E2, E2/E3 and E3/E3; E4+, E3/E4 and E4/E4; %TE, % total energy; data are mean change ± SEM (m6 - m0)

² Data were analysed by GLM with adjustment for baseline values, centre, gender, age and change in weight (m6 - m0).

Figure 1: Consort diagram of participants randomized into the Food4Me Proof of Principle Study * Total number of participants reporting one or more exclusion criteria. **Parentheses** indicate the percentage of each group who received advice to reduce SFA intake at month 0.

