The effect of the apolipoprotein E genotype on response to personalized dietary advice intervention: findings from the Food4Me randomized controlled trial

Article

Accepted Version

The American Journal of Clinical Nutrition
AJCN/2016/135012
Version 2
The effect of APOE genotype on response to personalized dietary
intervention: findings from the Food4Me randomized controlled trial

Corresponding Author: *Julie A Lovegrove*
Additional Authors: *Rosalind Fallaize* , *Carlos Celis-Morales* , *Anna L
Macready* , *Cyril FM Marsaux* , *Hannah Forster* , Clare O'Donovan,
Clara Woodhead, Rodrigo San-Cristobal, Silvia Kolossa, Jacqueline
Hallmann, Christina Mavrogianni, Agnieszka Surwillo, Katherine M
Livingstone, George Moschonis, Santiago Navas-Carretero, Marianne
C Walsh, Eileen R Gibney, Lorraine Brennan, Jildau Bouwman, Keith
Grimaldi, Yannis Manios, Iwona Traczyk, Christian A Drevon, J Alfredo
Martinez, Hannelore Daniel, Wim HM Saris, *Michael J Gibney* , John C
Mathers

Date Received: 8 Jun 2016

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

The effect of *APO*E genotype on response to personalized dietary advice intervention: findings from the Food4Me randomized controlled trial [1-2]

Rosalind Fallaize[3], Carlos Celis-Morales[4], Anna L. Macready[3], Cyril F.M. Marsaux[5], Hannah Forster[6], Clare O'Donovan[6], Clara Woolhead[6], Rodrigo San-Cristobal[7], Silvia Kolossa[8], Jacqueline Hallmann[8], Christina Mavrogianni[9], Agnieszka Surwillo[10], Katherine M. Livingstone[4], George Moschonis[9], Santiago Navas-Carretero[7], Marianne C. Walsh[6], Eileen R. Gibney[6], Lorraine Brennan[6], Jildau Bouwman[11], Keith Grimaldi[12], Yannis Manios[9], Iwona Traczyk[10], Christian A. Drevon[13], J. Alfredo Martinez[7], Hannelore Daniel[8], Wim H.M. Saris[5], Michael J. Gibney[6], John C. Mathers and Julie A. Lovegrove[3], on behalf of the Food4Me Study

Footnotes

Author affiliations

[3] Hugh Sinclair Unit of Human Nutrition and Institute for Cardiovascular and Metabolic Research, University of Reading, Reading; [4]Human Nutrition Research Centre, Institute of Cellular Medicine, Newcastle University, Newcastle Upon Tyne, UK; [5]Department of Human Biology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre +, Maastricht, The

Netherlands; [6]UCD Institute of Food and Health, University College Dublin, Belfield, Dublin 4, Ireland; [7]Department of Nutrition, Food Science and Physiology, University of Navarra, Pamplona, IdiSNA, Instituto de Investigación Sanitaria de Navarra, Pamplona and CIBERobn, Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Madrid Spain; [8]ZIEL Research Center of Nutrition and Food Sciences, Biochemistry Unit, Technische Universität München, München, Germany; [9]Department of Nutrition and Dietetics, Harokopio University, Athens, Greece; [10]National Food & Nutrition Institute (IZZ), Warsaw, Poland; [11]Microbiology and Systems Biology Group, TNO, Utrechtseweg 48, 3704 HE Zeist, The Netherlands; [12]Eurogenetica Ltd. 7 Salisbury Road, Burnham-on-Sea, UK, [13]Department of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Oslo, Norway.

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 (ε4) 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 ε4

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* ε4 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, ε2, ε3 and ε4, 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 ε2, ε3 and ε4 was 0.08, 0.77 and 0.15 respectively (7). The ε4

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, ε4 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.

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.

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.*"

Biochemical analysis

127

128   Participants were asked to complete 2 DBS cards each containing 5 blood

129   spots, at m0, m3 and m6 (approximately150 µL 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) × 100] (27). Fatty acids were measured using GC-FID.

DNA extraction and genotyping

137

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, KASP^{TM} 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).

Statistical analyses

147

148   Data are presented as means ± SEM. Data were checked for normality of

149   distribution and skewed variables were normalised using Log_{10} (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 × 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 × 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 × 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 ε4 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% ± 0.1) and higher PUFA (6.80% ± 0.05) and omega-3

207   (0.89% ± 0.01) fat intake associated with a higher omega-3 index. Although a

208   significant MUFA × *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 × *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 × *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 sigificantly 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 (ε2, 6.5; ε3,

273   79.3; ε4, 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 ε4 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 ≥ 8% offering the most cardio-protective

285   effects and an index ≤ 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 ε4 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

REFERENCES

1.   Nichols M, Townsend N, Scarborough P, Rayner M. Cardiovascular disease in Europe 2014: epidemiological update. Eur Heart J 2014;35(42):2950-9.

2.   Alwan A, Armstrong T, Bettcher D, Branca F, Chisholm D, Ezzati M, Garfield R, MacLean D, Mathers C, Mendis S, et al. Global status report on noncommunicable diseases 2010. Geneva: World Health Organization, 2011.

3.   Kreuter MW, Wray RJ. Tailored and targeted health communication: strategies for enhancing information relevance. Am J Health Behav 2003;27(Supplement 3):S227-S32.

4.   Celis-Morales C, Lara J, Mathers JC. Personalising nutritional guidance for more effective behaviour change. Proc Nutr Soc 2014:1-9.

5.   Marteau TM, French DP, Griffin SJ, Prevost AT, Sutton S, Watkinson C, Attwood S, Hollands GJ. Effects of communicating DNA‐based disease risk estimates on risk‐reducing behaviours. The Cochrane Library 2010. doi: 10.1002/14651858.CD007275.pub2.

6.   San-Cristobal R, Milagro FI, Martínez JA. Future challenges and present ethical considerations in the use of personalized nutrition based on genetic advice. Journal of the Academy of Nutrition and Dietetics 2013;113(11):1447-54.

7.   Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Arterioscler Thromb Vasc Biol 1988;8(1):1-21. doi: 10.1161/01.ATV.8.1.1

8.   Song Y, Stampfer MJ, Liu S. Meta-analysis: apolipoprotein E genotypes and risk for coronary heart disease. Ann Intern Med 2004;141(2):137-47. doi: 10.7326/0003-4819-141-2-200407200-00013

9.   Bennet AM, Di Angelantonio E, Ye Z, Wensley F, Dahlin A, Ahlbom A, Keavney B, Collins R, Wiman B, de Faire U, et al. Association of apolipoprotein E genotypes with lipid levels and coronary risk. JAMA 2007;298(11):1300-11. doi: 10.1001/jama.298.11.1300

10.   Waterworth DM, Ricketts SL, Song K, Chen L, Zhao JH, Ripatti S, Aulchenko YS, Zhang W, Yuan X, Lim N, et al. Genetic variants influencing circulating lipid levels and risk of coronary artery disease. Arterioscler Thromb Vasc Biol 2010;30(11):2264-76. doi: 10.1161/ATVBAHA.109.201020

11.   Wilson PW, Schaefer EJ, Larson MG, Ordovas JM. Apolipoprotein E alleles
        and risk of coronary disease. A meta-analysis. Arterioscler Thromb Vasc Biol
        1996;16(10):1250-5. doi: 10.1161/01.ATV.16.10.1250

12.   Povel CM, Boer JM, Imholz S, Dollé ME, Feskens EJ. Genetic variants in lipid
        metabolism are independently associated with multiple features of the
        metabolic syndrome. Lipids Health Dis 2011;10:118. doi: 10.1186/1476-511X-
        10-118

13.   Ward H, Mitrou PN, Bowman R, Luben R, Wareham NJ, Khaw K-T, Bingham
        S. APOE genotype, lipids, and coronary heart disease risk: a prospective
        population study. Arch Intern Med 2009;169(15):1424-9. doi:
        10.1001/archinternmed.2009.234

14.   Masson LF, McNeill G, Avenell A. Genetic variation and the lipid response to
        dietary intervention: a systematic review. Am J Clin Nutr 2003;77(5):1098-111.

15.   Carvalho-Wells AL, Jackson KG, Lockyer S, Lovegrove JA, Minihane AM.
        APOE genotype influences triglyceride and C-reactive protein responses to
        altered dietary fat intake in UK adults. Am J Clin Nutr 2012;96(6):1447-53. doi:
        10.3945/ajcn.112.043240

16.   Ordovas JM, Lopez-Miranda J, Mata P, Perez-Jimenez F, Lichtenstein AH,
        Schaefer EJ. Gene-diet interaction in determining plasma lipid response to
        dietary intervention. Atherosclerosis 1995;118:S11-S27.

17.   Lovegrove JA, Gitau R. Personalized nutrition for the prevention of
        cardiovascular disease: a future perspective. Journal of Human Nutrition and
        Dietetics 2008;21:306-16.

18.   Food4Me. Internet: http://www.food4me.org (accessed March 2016).

19. Celis-Morales C, Livingstone KM, Marsaux CF, Forster H, O'Donovan CB, Woolhead C, Macready AL, Fallaize R, Navas-Carretero S, San-Cristobal R. Design and baseline characteristics of the Food4Me study: a web-based randomised controlled trial of personalised nutrition in seven European countries. Genes Nutr 2015;10(1):1-13. doi: 10.1007/s12263-014-0450-2

20. Forster H, Fallaize R, Gallagher C, O'Donovan CB, Woolhead C, Walsh MC, Macready AL, Lovegrove JA, Mathers JC, Gibney MJ. Online Dietary Intake Estimation: The Food4Me Food Frequency Questionnaire. J Med Internet Res 2014;16(6). doi: 10.2196/jmir.3105

21. Fallaize R, Forster H, Macready AL, Walsh MC, Mathers JC, Brennan L, Gibney ER, Gibney MJ, Lovegrove JA. Online dietary intake estimation: reproducibility and validity of the Food4Me Food Frequency Questionnaire against a 4-day weighed food record. J Med Internet Res 2014;16(8). doi: 10.2196/jmir.3355

22. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. The American journal of clinical nutrition 1982;36(5):936-42.

23. Montoye HJ, Kemper HC, Saris WH, Washburn RA. Measuring physical activity and energy expenditure: Human Kinetics Champaign, IL, 1996.

24. Philippaerts RM, Westerterp KR, Lefevre J. Doubly labelled water validation of three physical activity questionnaires. Int J Sports Med 1999;20(5):284-9.

25. Bonomi AG, Plasqui G, Goris AH, Westerterp KR. Estimation of Free‐Living Energy Expenditure Using a Novel Activity Monitor Designed to Minimize Obtrusiveness. Obesity 2010;18(9):1845-51. doi: 10.1038/oby.2010.34

26.   Forster H, Walsh MC, O'Donovan CB, Woolhead C, McGirr C, O'Riordan R, Celis-Morales C, Fallaize R, Macready AL, Marsaux CF, et al. A Dietary Feedback System for the Delivery of Consistent Personalized Dietary Advice in the Web-Based Multicenter Food4Me Study. J Med Internet Res 2016;18(6):e150. doi: 10.2196/jmir.5620

27.   Harris WS, von Schacky C. The Omega-3 Index: a new risk factor for death from coronary heart disease? Prev Med 2004;39(1):212-20. doi: 10.1016/j.ypmed.2004.02.030

28.   Schiele F, De Bacquer D, Vincent-Viry M, Beisiegel U, Ehnholm C, Evans A, Kafatos A, Martins MC, Sans S, Sass C. Apolipoprotein E serum concentration and polymorphism in six European countries: the ApoEurope Project. Atherosclerosis 2000;152(2):475-88. doi: 10.1016/S0021-9150(99)00501-8

29.   Eichner JE, Dunn ST, Perveen G, Thompson DM, Stewart KE, Stroehla BC. Apolipoprotein E polymorphism and cardiovascular disease: a HuGE review. Am J Epidemiol 2002;155(6):487-95. doi: 10.1093/aje/155.6.487

30.   Tiret L, de Knijff P, Menzel H-J, Ehnholm C, Nicaud V, Havekes LM. ApoE polymorphism and predisposition to coronary heart disease in youths of different European populations. The EARS Study. European Atherosclerosis Research Study. Arterioscler Thromb Vasc Biol 1994;14(10):1617-24. doi: 10.1161/01.ATV.14.10.1617

31.   Khan TA, Shah T, Prieto D, Zhang W, Price J, Fowkes GR, Cooper J, Talmud PJ, Humphries SE, Sundstrom J. Apolipoprotein E genotype, cardiovascular biomarkers and risk of stroke: Systematic review and meta-analysis of 14 015

stroke cases and pooled analysis of primary biomarker data from up to 60 883

individuals. Int J Epidemiol 2013;42(2):475-92. doi: 10.1093/ije/dyt034

32.   Andersen LF, Solvoll K, Drevon CA. Very-long-chain n-3 fatty acids as

biomarkers for intake of fish and n-3 fatty acid concentrates. The American

journal of clinical nutrition 1996;64(3):305-11.

33.   Harris WS. The omega-3 index as a risk factor for coronary heart disease. Am

J Clin Nutr 2008;87(6):1997S-2002S.

34.   von Schacky C. Omega-3 index and cardiovascular health. Nutrients

2014;6(2):799-814. doi: 10.3390/nu6020799

35.   Sala-Vila A, Harris WS, Cofán M, Pérez-Heras AM, Pintó X, Lamuela-

Raventós RM, Covas M-I, Estruch R, Ros E. Determinants of the omega-3

index in a Mediterranean population at increased risk for CHD. Br J Nutr

2011;106(03):425-31. doi: 10.1017/S0007114511000171

36.   Nielsen DE, El-Sohemy A. A randomized trial of genetic information for

personalized nutrition. Genes Nutr 2012:1-8. doi: 10.1007/s12263-012-0290-x

37.   Joost HG, Gibney MJ, Cashman KD, Görman U, Hesketh JE, Mueller M, van

Ommen B, Williams CM, Mathers JC. Personalised nutrition: status and

perspectives. Br J Nutr 2007;98(1):26-31. doi: 10.1017/S0007114507685195

38.   Nielsen DE, El-Sohemy A. Disclosure of genetic information and change in

dietary intake: a randomized controlled trial. PloS one 2014;9(11):e112665.

doi: 10.1371/journal.pone.0112665

39.   Chao S, Roberts JS, Marteau TM, Silliman R, Cupples LA, Green RC. Health

behavior changes after genetic risk assessment for Alzheimer disease: The

REVEAL Study. Alzheimer Dis Assoc Disord 2008;22(1):94. doi:

10.1097/WAD.0b013e31815a9dcc

40.   Meisel SF, Wardle J. Responses to FTO genetic test feedback for obesity in a

        sample of overweight adults: a qualitative analysis. Genes Nutr 2014;9(1):1-4.

41.   Hunter DJ, Khoury MJ, Drazen JM. Letting the Genome out of the Bottle —

        Will We Get Our Wish. N Engl J Med 2008;358:105-7. doi:

        10.1056/NEJMp0708162

42.   Wilson BJ. Designing media messages about health and nutrition: what

        strategies are most effective? J Nutr Educ Behav 2007;39(2):S13-S9. doi:

        10.1016/j.jneb.2006.09.001

43.   Witte K, Allen M. A meta-analysis of fear appeals: Implications for effective

        public health campaigns. Health Educ Behav 2000;27(5):591-615. doi:

        10.1177/109019810002700506

44.   Hietaranta-Luoma HL, Tahvonen R, Iso-Touru T, Puolijoki H, Hopia A. An

        Intervention Study of Individual, apoE Genotype-Based Dietary and Physical-

        Activity Advice: Impact on Health Behavior. J Nutrigenet Nutrigenomics

        2014;7(3):161-74. doi: 10.1159/000371743

45.   Berezowska A, Fischer ARH, Ronteltap A, Kuznesof S, Macready AL, Fallaize

        R, van Trijp HCM. Understanding consumer evaluations of personalised

        nutrition services in terms of the privacy calculus: a qualitative study. Public

        Health Genomics 2014;17:127-40. doi: 10.1159/000358851

46.   Hietaranta-Luoma HL, Åkerman K, Tahvonen R, Puolijoki H, Hopia A. Using

        Individual, ApoE Genotype-Based Dietary and Physical Activity Advice to

        Promote Healthy Lifestyles in Finland—Impacts on Cardiovascular Risk

        Markers. Open Journal of Preventive Medicine 2015;5(05):206. doi:

        10.4236/ojpm.2015.55024

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

[1]Genotype 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 study[1]

| | | *APOE* genotype[1] | | | |
| | | E4- | | E4+ | |
	All (n=1439)	E2 carriers (n=158)	E3/E3 (n=922)	E4 carriers (n=359)	*P*[2]
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 study[2]

| | E4- | | | | E4+ | | | P[3] | |
| | E2 carriers (n=158) | | E3/E3 (n=922) | | E4 carriers (n=359) | | | | |
	Low Intake	High Intake	Low Intake	High Intake	Low Intake	High Intake	Diet	Genotype	Diet × Genotype
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% ± 0.1; high intake 40.5% ± 0.1); SFA, 14.0% (low intake, 11.7% ± 0.1; high intake 16.6% ± 0.1); MUFA, 13.5% (low intake, 11.5% ± 0.1; high intake 16.0% ± 0.1); PUFA, 5.6% (low intake, 4.67% ± 0.02; high intake 6.80% ± 0.05); omega-3, 0.67% (low intake, 0.55% ± 0.01; high intake 0.89% ± 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 ± 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 study[2]

| | E4- | | | | E4+ | | 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	Diet	Genotype	Diet × Genotype
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 study[1]

	Control	Personalized intervention arms			P^2		
	Level 0 (L0) *APOE* risk (n=77)	Level 1 (L1) *APOE* risk (n=47)	Level 2 (L2) *APOE* risk (n=35)	Level 3 (L3) *APOE* 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 study[1]

	Control	Personalized intervention arms			P[2]		
	Level 0 (L0) *APOE* non-risk (n=225)	Level 1 (L1) *APOE* non-risk (n=145)	Level 2 (L2) *APOE* non-risk (n=119)	Level 3 (L3) *APOE* 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 ± 014	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 study[1]

	Level 3 (L3)		P^2
	APOE non-risk (E4-) (n=72)	*APOE* 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.

Enrollment

Participants who registered online for the Food4Me Study
n=5562

Excluded, n=3995
- Incomplete screening questionnaires, n=646
- Meets exclusion criteria, n=1284*
- Consent not given, n=274
- Incomplete/under-reported food frequency questionnaire, n=634
- Sample size reached n=1157

Participants randomized into one of the 4 arms on the intervention
n=1607

Allocation

Level 0 "Control" (Standard dietary guidelines)
n=387

Level 1 (PN based on diet)
n=414

Level 4 (PN based on diet and phenotype)
n=404

Level 3 (PN based on diet, phenotype and genotype)
n=402

Dropouts
n=75 (19%)

Dropouts
n=102 (25%)

Dropouts
n=81 (20%)

Dropouts
n=81 (21%)

Intervention

Level 0 completers
n=312

Level 1 completers
n=312

Level 2 completers
n=325

Level 3 completers
n=321

Excluded, n=11
- E2/E4, n=10
- Missing genotype, n=1

Excluded, n=5
- E2/E4, n=4
- Missing genotype, n=1

Excluded, n=6
- E2/E4, n=3
- Missing genotype, n=3

Excluded, n=8
- E2/E4, n=6
- Missing genotype, n=2

Analysis

APOE E4+ n=77

APOE E4- n=225

APOE E4+ n=84

APOE E4- n=223

APOE E4+ n=79

APOE E4- n=240

APOE E4+ n=75

APOE E4- n=238

APOE E4+ Received advice (month 0):
- to reduce SFA intake, n=47 (56%)

APOE E4- Received advice (month 0):
- to reduce SFA intake, n=145 (65%)

APOE E4+ Received advice (month 0):
- to reduce SFA intake, n=35 (44%)

APOE E4- Received advice (month 0):
- to reduce SFA intake, n=119 (50%)

APOE E4+ Received advice (month 0):
- to reduce SFA intake, n=40 (53%)

APOE E4- Received advice (month 0):
- to reduce SFA intake, n=72 (30%)