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

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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

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ABSTRACT (word count = 299)

Background: The *APOE* risk allele (ε4) is associated with higher total cholesterol (TC), amplified response to saturated fatty acid (SFA) reduction and increased CVD. While knowledge of gene 'risk' may enhance dietary change, it is unclear whether ε4 carriers would benefit from gene-based personalized nutrition (PN).

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

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

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

Conclusions: The *APOE* ε4 allele was associated with greater TC. Whilst gene-based PN targeted to *APOE* was more effective in reducing SFA intake than

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

INTRODUCTION

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

The *APOE* gene is a key regulator of cholesterol and lipid metabolism. *APOE* is polymorphic, with the common *missense* polymorphisms (rs429358 and rs7412) resulting in three alleles, ε2, ε3 and ε4, combining to form 6 haplotypes, E2/E2, E2/E3, E2/E4, E3/E3, E3/E4 and E4/E4. In a sample of 5805 Caucasians, the *APOE* allele frequency for ε2, ε3 and ε4 was 0.08, 0.77 and 0.15 respectively (7). The ε4 allele is associated with increased serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) as well as coronary artery disease and mortality (8-12). Estimates of the CHD hazard ratio for E4+ (E3/E4 and E4/E4), compared with E4- (E3/E3), range from 1.06 to 1.42 (8, 9, 11, 13). There is also a growing body of evidence showing that the *APOE* genotype may influence lipid response to dietary fat; data from intervention studies suggest that E4+ participants may be more sensitive to dietary cholesterol, total fat and, in particular, SFA modulation (14, 15). Given their predisposition to CHD, ε4 carriers might benefit from a lower dietary SFA and blood cholesterol (16) and gene-based PN intervention. However, there is a

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

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

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

PARTICIPANTS AND METHODS

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

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

Participants

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

Study design

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

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

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

Dietary feedback

Following analysis of data collected at m0 and m3, participants received tailored dietary feedback (in their native language) according to their study allocation group. The dietary feedback provided was based on a pre-defined set of algorithms incorporating dietary, anthropometric, PA, phenotypic and genotypic data where appropriate. The system was designed to ensure consistent feedback across centres and has since been successfully automatized (26). *APOE* gene variants were coded as 'risk' (a genetic variation that can be modified by diet, i.e. E3/E4 or E4/E4 (E4+)) or 'non-risk' (E2/E2, E2/E3, E3/E3 (E4-)). Alongside the risk result, Level 3 participants received the following basic information about the *APOE* genotype: "*A specific variation of this gene is associated with a greater need to maintain healthy cholesterol levels. Decreasing saturated fat intake has been associated with an improvement in cholesterol and factors relating to cardiovascular health in these individuals.*" For Level 3 E4+ participants with high dietary SFA intake and/or high blood TC, who were being advised to lower dietary SFA, reference to 'gene risk' was also included in the advice message, i.e. "*You have a genetic variation that can benefit by keeping a healthy intake of saturated fat and a normal level of blood cholesterol.*"

Biochemical analysis

Participants were asked to complete 2 DBS cards each containing 5 blood spots, at m0, m3 and m6 (approximately150 µL blood per card). After drying the blood spots at room temperature for 2-4 hours, the cards were placed in a sealed aluminum bag (Whatman Foil Bags, item no. 10534321, Whatman Inc., Sanford, ME) containing a drying sachet (Sorb-it, item no. 10548234, Süd-Chemie, Germany) and posted back to the research center in their country. Researchers subsequently shipped the DBS cards to Vitas (Vitas Ltd, Norway) for analysis of whole blood TC (LC-UV) and omega-3 index [(eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA)/ total fatty acids) × 100] (27). Fatty acids were measured using GC-FID.

DNA extraction and genotyping

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

Statistical analyses

Data are presented as means ± SEM. Data were checked for normality of distribution and skewed variables were normalised using Log_{10} (omega-3 index) and square root (TC) transformations. General linear models (GLM), adjusted for center,

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

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

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

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

RESULTS

Subject characteristics

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

Habitual dietary and genotype effects at baseline

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

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

An independent effect of genotype was observed for dietary fat and TC concentrations at baseline (total fat, *P*= 0.002; SFA, *P*= 0.002; MUFA, *P*= 0.002; PUFA, *P*= 0.003 and omega-3, *P*= 0.004), with the highest TC concentrations seen in carriers of ε4 allele (E4+). Overall diet effects (SFA, *P*= 0.008; MUFA, *P*= 0.025; PUFA, *P*= 0.007 and omega-3, *P*< 0.001) were observed for omega-3 index, with lower dietary SFA (11.7% ± 0.1) and higher PUFA (6.80% ± 0.05) and omega-3 (0.89% ± 0.01) fat intake associated with a higher omega-3 index. Although a significant MUFA × *APOE* interaction was observed for omega-3 index (*P* = 0.025), no differences between genotype groups and fat intakes were observed following post-hoc analyses.

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

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

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

omega-3 index. A trend was also observed for an omega-3 fat intake × *APOE* interaction on omega-3 index (*P*= 0.087).

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

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

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

When comparing levels of personalization, a 0.88% greater reduction in SFA (%TE) was observed in E4- participants receiving non-gene-based PN (Level 2; PN 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).

Although the *APOE* rs429358 distribution was not in Hardy-Weinberg equilibrium, the haplotype frequencies observed in the Food4Me cohort (ε2, 6.5; ε3, 79.3; ε4, 14.2) were similar to those reported in previous studies of European populations (28). In contrast to previous observations (29, 30), there was no clear geographical cline in ε4 frequency.

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

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

It has been suggested that gene-based dietary information is more understandable and useful than general dietary guidelines (36) and may enhance motivation to change (37). In a 2010 systematic review, a beneficial effect of 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

(41). A potential reason for the lack of response in Food4Me E4 carriers is the absence of a specific behavior change technique (BCT) involving information on the consequences of a specific behavior related to genotype. A key BCT in the CALO-RE taxonomy (a 40-item taxonomy to improve PA and healthy eating behaviors) is to "provide information of the consequences of the behavior to the individual". In the context of *APOE* genotype, a consequence of carrying the ε4 allele would be increased CVD risk (31) and the corresponding risk-reducing behavior would be lowering SFA intake. In the present study, *APOE* risk information conveyed to participants was framed positively viz : "you have a genetic variation that can benefit by keeping a healthy intake of saturated fat and a normal level of blood cholesterol." The lack of an explicit link to an adverse consequence of E4+ status, e.g. higher CVD risk, may have reduced the efficacy of this advice. In the REVEAL study, participants were informed that the E4 allele was associated with an increased risk of Alzheimer's disease prior to gene disclosure (39). Whilst genotypic testing for polygenic disease risk may result in a fatalistic attitude (37), information on consequences of personal characteristics (e.g. genotype) and fear arousal can be useful aids in enhancing behavior change (42). In a meta-analysis of fear arousal techniques, stronger fear messages promoted greater intention and behavior change in public health campaigns, provided that the threat was perceived to be severe, personally relevant, and that the individual could take specific action to mitigate their risk (43). In a Finnish RCT, knowledge of personal *APOE* risk resulted in greater short-term improvements in dietary quality, WC and serum triacylglycerol, when participants were informed of the link between dietary fat, cholesterol and CVD risk in 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

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

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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

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

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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.

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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+				
	E2 carriers (n=158)		E3/E3 (n=922)		E4 carriers (n=359)		*P*[3]		
	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

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[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)

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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+				
	E2 carriers (n=132)		E3/E3 (n=794)		E4 carriers (n=315)		*P*[3]		
	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)			

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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).

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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).

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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).

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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).

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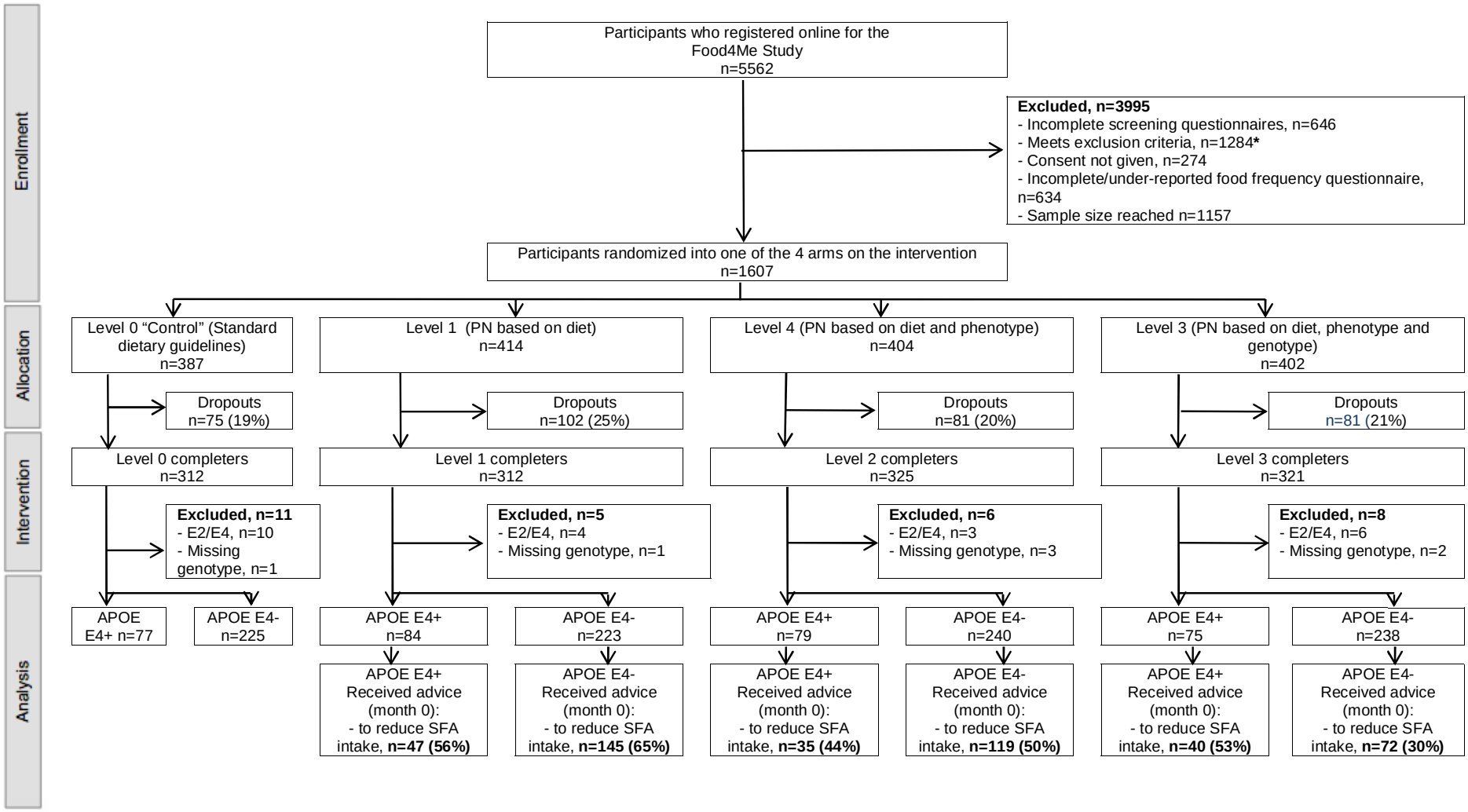
Figure 1: Consort diagram of participants randomized into the Food4Me Proof of Principle Study * Total number of participants reporting one or more exclusion criteria. Parentheses indicate the percentage of each group who received advice to reduce SFA intake at month 0.


Enrollment
Allocation
Intervention
Analysis
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
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%)
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
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%)