



Title	Phenotypic factors influencing the variation in response of circulating cholesterol level to personalised dietary advice in the Food4me study
Authors(s)	Kirwan, Laura, Walsh, Marianne C., Celis-Morales, Carlos, O'Donovan, Clare, Woolhead, Clara, Forster, Hannah, Gibney, Michael J., Gibney, Eileen R., Brennan, Lorraine, et al.
Publication date	2016-12
Publication information	Kirwan, Laura, Marianne C. Walsh, Carlos Celis-Morales, Clare O'Donovan, Clara Woolhead, Hannah Forster, Michael J. Gibney, Eileen R. Gibney, Lorraine Brennan, and et al. "Phenotypic Factors Influencing the Variation in Response of Circulating Cholesterol Level to Personalised Dietary Advice in the Food4me Study." Cambridge University Press, December 2016. https://doi.org/10.1017/S0007114516004256 .
Publisher	Cambridge University Press
Item record/more information	http://hdl.handle.net/10197/8319
Publisher's statement	This article has been accepted for publication and will appear in a revised form, subsequent to peer review and/or editorial input by Cambridge University Press, in British Journal of Nutrition.
Publisher's version (DOI)	10.1017/S0007114516004256

Downloaded 2026-05-01 23:38:08

The UCD community has made this article openly available. Please share how this access benefits you. Your story matters! (@ucd_oa)



© Some rights reserved. For more information

1 **Phenotypic factors influencing the variation in response of circulating cholesterol level**
2 **to personalised dietary advice in the Food4me study.**

3

4 Laura Kirwan¹, Marianne C Walsh¹, Carlos Celis-Morales², Cyril F. M. Marsaux³, Katherine
5 M Livingstone², Santiago Navas-Carretero^{4,5}, Rosalind Fallaize⁶, Clare O'Donovan¹, Clara
6 Woolhead¹, Hannah Forster¹, Silvia Kolossa⁷, Hannelore Daniel⁷, George Moschonis⁸,
7 Yannis Manios⁸, Agnieszka Surwillo⁹, Magdalena Godlewska⁹, Iwona Traczyk⁹, Christian A.
8 Drevon¹⁰, Mike J. Gibney¹, Julie A. Lovegrove⁶, J. Alfredo Martinez^{4,5}, Wim H. M. Saris³,
9 John C. Mathers², Eileen R Gibney¹ and Lorraine Brennan¹ on behalf of the Food4me study

10 ¹UCD Institute of Food and Health, UCD School of Agriculture and Food Science,
11 University College Dublin, Belfield, Dublin 4, Republic of Ireland

12 ²Human Nutrition Research Centre, Institute of Cellular Medicine, Newcastle University,
13 Newcastle Upon Tyne, UK

14 ³Department of Human Biology, NUTRIM School of Nutrition and Translational Research in
15 Metabolism, Maastricht University Medical Centre + (MUMC+), Maastricht, The
16 Netherlands

17 ⁴Department of Nutrition, Food Science and Physiology, Centre for Nutrition Research,
18 University of Navarra, Pamplona

19 ⁵CIBER Fisiopatología de la Obesidad y Nutrición (CIBERObn), Instituto de Salud Carlos III,
20 Madrid, Spain

21 ⁶Hugh Sinclair Unit of Human Nutrition and Institute for Cardiovascular and Metabolic
22 Research, University of Reading, Reading, UK

23 ⁷ZIEL Research Center of Nutrition and Food Sciences, Biochemistry Unit, Technische
24 Universität München, München, Germany

25 ⁸Department of Nutrition and Dietetics, Harokopio University, Athens, Greece

26 ⁹National Food & Nutrition Institute (IZZ), Warsaw, Poland

27 ¹⁰Department of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine,
28 University of Oslo, Oslo, Norway

29

30 Corresponding author: Lorraine Brennan, University College Dublin, Belfield, Dublin 4,
31 Ireland. email: lorraine.brennan@ucd.ie, phone: +353 1 7162811

32 Keywords: Personalised nutrition, cholesterol, responders, phenotype, fatty acid profile

33

34 Short title: Cholesterol response to dietary advice

35

36 ABSTRACT

37 Individual response to dietary interventions can be highly variable. The phenotypic
38 characteristics of those who will respond positively to personalised dietary advice are largely
39 unknown. The objective of this study was to compare the phenotypic profiles of differential
40 responders to personalised dietary intervention, with a focus on total circulating cholesterol.
41 Subjects from the Food4Me multi-centre study were classified as responders or non-
42 responders to dietary advice based on the change in cholesterol level from baseline to month
43 6, with lower and upper quartiles defined as the responder and non-responder groups,
44 respectively. There were no significant differences between the demographic and
45 anthropometric profiles of the groups. Furthermore, with the exception of alcohol, there was
46 no significant difference in reported dietary intake, at baseline. However, there were marked
47 differences in baseline fatty acid profiles. The responder group had significantly higher
48 levels of stearic acid (18:0, $p=0.034$) and lower levels of palmitic acid (16:0, $p=0.009$). Total
49 monounsaturated fatty acids ($p=0.016$) and total polyunsaturated fatty acids ($p=0.008$) also
50 differed between the groups. In a stepwise logistic regression model, age, baseline total
51 cholesterol, glucose, five fatty acids and alcohol intake were selected as factors that
52 successfully discriminated responders from non-responders, with sensitivity of 82% and
53 specificity of 83%. The successful delivery of personalised dietary advice may depend on our
54 ability to identify phenotypes that are responsive. The results demonstrate the potential use of
55 metabolic profiles in identifying response to an intervention and could play an important role
56 in the development of precision nutrition.

57

58

59

60 INTRODUCTION

61 At a population level, generic dietary advice is provided using a ‘one-size-fits-all’ approach
62 based on requirements for population groups ⁽¹⁾, which ignores inter-individual differences ,
63 and therefore nutrient requirements. In addition, individuals' responses to dietary
64 interventions can be highly variable ^(2; 3; 4). Demographic characteristics such as sex and age,
65 and factors such as adiposity, physical activity, metabolic profile, and genetic factors
66 contribute to this variation ⁽⁵⁾. This phenomenon is well recognised in the medical field with a
67 current emphasis on precision medicine ⁽⁶⁾. Considering the reported variation in response to
68 dietary interventions there is now an emerging recognition that this should be considered in
69 development of personalised or precision nutrition. ^(7; 8). Personalised nutrition, or dietary
70 advice that has been tailored to an individual, offers the possibility of improving health and
71 reducing risk of diet-related diseases ⁽⁹⁾. Many studies suggest that tailored dietary advice is
72 more effective than generic advice, promoting greater improvements in dietary behaviours
73 and related health outcomes such as body weight ^(9; 10). A recent meta-analysis reported that
74 personalised interventions were more effective than non-personalised advice, with
75 participants receiving the personalised intervention reducing body weight by 1.8 kg more on
76 average than those receiving the non-personalised advice ⁽⁹⁾. However, these studies have not
77 taken individual variability into account and in the longterm the effectiveness of the
78 personalised dietary advice will depend on the ability to tailor advice taking into account
79 knowledge about an individual’s potential response to the intervention ⁽¹¹⁾.

80 The concept of using metabolic profiles to identify responders to dietary interventions is
81 relatively new (Brennan, 2015). However, a number of examples exist in the literature
82 demonstrating the potential of such an approach. O’Sullivan *et al.* (2011) used k-means
83 cluster analysis to identify responders and non-responders to a vitamin D intervention ⁽¹²⁾.
84 van Bochove *et al.* (2012) applied k-means clustering to lipoprotein profiles and identified
85 three clusters, two of which responded positively to fenofibrate ⁽¹³⁾, while Elnenaei *et al.*
86 (2011) identified responders and non-responders to vitamin D and Ca supplementation, based
87 on a baseline metabolomic profile ⁽¹⁴⁾. Metabolomic and transcriptomic profiles have also
88 been used to discriminate between responders and non-responders to an n-3 PUFA
89 supplementation ⁽¹⁵⁾. The objective of this study is to investigate differences in the phenotype
90 and in particular blood lipids of responders and non-responders to personalised nutrition, with
91 a specific focus on changes in circulating cholesterol levels. Using data from the Food4Me
92 personalised dietary intervention study, individuals with borderline high baseline total

93 cholesterol (> 5 mmol/L) were examined for factors that predict their response to the
94 intervention.

95

96 MATERIALS AND METHODS

97 Subjects were participants in the Food4Me study, a 6-month, web-based randomised control
98 trial conducted in 7 European countries. The aim of the study was to determine whether
99 providing personalised dietary advice leads to improvements in dietary intakes and health
100 outcomes relative to population-based public health messages. The 1,607 adult subjects were
101 randomly assigned to one of four intervention treatment groups – level 0 (standard
102 nonpersonalised dietary and physical activity guidelines), level 1 (personalised advice based
103 on current diet and physical activity), level 2 (personalised advice based on current diet,
104 physical activity and phenotype) and level 3 (personalised advice based on current diet,
105 physical activity, phenotype and genotype)⁽¹⁶⁾. The control group received conventional,
106 non-personalised advice and so are not considered for this analysis. The study protocol is
107 detailed in Celis-Morales *et al.*⁽¹⁶⁾.

108

109 All data were collected remotely following standardized operating procedures. At baseline,
110 participants received study kits by post containing all necessary materials to perform
111 measurements at home. Printed instructions were included and demonstration videos were
112 available on the Food4Me website (<http://www.food4me.org>). Following measurements at
113 baseline and 3 months, participants received a personalised report. The personalised feedback
114 provided was based on a predefined set of algorithms, including anthropometric, physical
115 activity (Levels 1-3), phenotypic (Levels 2 and 3), and genotypic (Level 3 only) data⁽¹⁶⁾.

116 **Demographic characteristics**

117 The measurement of characteristics including age, country and sex and have been described
118 elsewhere⁽¹⁶⁾. Having excluded the control group and those with normal total cholesterol
119 levels at baseline (total cholesterol < 5 mmol/L), there were 151 males and 162 females, with
120 a mean age of 46.8 years from 7 European countries, Germany (n=67), Greece (n=48),
121 Ireland (n=39), Netherlands (n=54), Poland (n=30), Spain (n=43) and the United Kingdom
122 (n=32). Subjects were classified as responders and non-responders based on the change in
123 blood cholesterol from baseline to month 6. To achieve this the subjects were firstly stratified

124 into quartiles based on cholesterol response. Two of the groups, the lower and upper
125 quartiles, were defined as the responders and non-responders, respectively. This resulted in
126 n=78 responders and n=79 non-responders.

127

128

129 **Anthropometric measurements**

130 Body weight, height and waist circumference were self-measured and self-reported by
131 participants via the Internet, as described previously ⁽¹⁶⁾. They were provided with clear
132 instructions in text and video format to facilitate accurate measurements and a validation
133 study demonstrated the reliability of these internet-based self-reported anthropometric data
134 ⁽¹⁷⁾. Waist circumference was measured at the midpoint between the lower rib and the iliac
135 crest using the same tape measure. Physical Activity was self-reported using the Baecke
136 questionnaire online ^(18; 19) based on physical activity during the last month. Physical activity
137 level scores (PAL) were calculated at baseline and month 6, according to the questionnaire
138 protocol.

139 **Dietary intake measurements**

140 Habitual dietary intake was quantified using an online food frequency questionnaire (FFQ)
141 including food items frequently consumed in each of the 7 recruitment sites. The Food4me
142 FFQ has been compared to a paper based FFQ ⁽²⁰⁾ and 4-day weighed food record ⁽²¹⁾ for both
143 food group and nutrient intakes. Bland Altman analysis showed good agreement between the
144 on-line and paper-based FFQ for both the nutrient and food group level. Cross-classification
145 into exact plus adjacent quartiles ranged from 77 % to 97% at the nutrient level and 77% to
146 99% at the food group level. For comparison with the weighed food record the mean cross-
147 classification into exact agreement plus adjacent was 80% and 78% for nutrient and food
148 groups respectively. Importantly the energy intake estimated by the FFQ was in agreement
149 with the weighed food record. Overall, indicating that overall the on-line FFQ was a suitable
150 tool for assessing dietary intake.

151 **Fatty acid and carotenoid profiles**

152 Finger-prick blood samples were collected by participants using a test kit provided by Vitas
153 Ltd, Oslo, Norway, as described previously ⁽²²⁾. Each participant filled two Dry Blood Spot

154 cards (equivalent to five drops of blood or 150 μ L of blood per card) at each collection time
155 point. The samples were sent to Vitas (Vitas Ltd, Norway) for measurements of total
156 cholesterol, carotenoids, and 32 fatty acids (FA). The n-3 fatty acid index was calculated as
157 the sum of eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3). The Δ 5
158 desaturase index (D5D) and Δ 6 desaturase index (D6D) are calculated based on key enzymes
159 in the metabolism of polyunsaturated fatty acids. The D5D was calculated as the ratio of
160 arachidonic acid (20:4n-6) to dihomo γ linoleic acid (20:3n-6) the D6D was calculated as the
161 ratio of dihomo γ linoleic acid (20:3n-6) to linoleic acid (18:2n-6).

162

163 ETHICS

164 This study was conducted according to the guidelines laid down in the Declaration of
165 Helsinki. The Research Ethics Committees at each participating centre granted ethical
166 approval for the study ⁽¹⁶⁾.

167

168 STATISTICAL ANALYSIS

169 The baseline demographic and phenotypic characteristics of the responders and non-
170 responders were compared using generalised linear models. Models were fitted using the
171 GLM (for continuous variables) and GENMOD (for categorical variables) procedures in SAS
172 9.3 (SAS Institute, Cary NC). To account for multiple comparisons, False Discovery Rate
173 (FDR) adjusted p-values are presented for fatty acid profile data.

174 To assess whether baseline demographic or phenotypic characteristics can discriminate
175 between responders and non-responders, a stepwise logistic regression procedure was applied
176 in four stages. Firstly, only anthropometric characteristics were included (Model 1). Then
177 baseline cholesterol was added to the model (Model 2). Thirdly, dietary intake data were
178 added to the analysis (Model 3) and lastly, all demographic, anthropometric, dietary intake
179 and biochemical characteristics were included (Model 4). At each stage, the stepwise
180 procedure selected the characteristics that best discriminated between the two groups.
181 Variables were tested using a bootstrapping approach to correct for overoptimism in model
182 fitting. The ability of the models to classify responders and non-responders was assessed
183 using area under the ROC curves. ROC comparisons were performed by using a contrast
184 matrix to take differences of the areas under the empirical ROC curves.

185

186 **RESULTS**

187 **Characteristics of responders and non-responders**

188 Demographic characteristics did not differ significantly between the responder and non-
189 responder groups by country ($\chi_6^2=5.0$, $p=0.544$, **Table 1**), sex ($\chi_1^2=0.16$, $p=0.693$, Table 1)
190 or age ($p=0.082$, Table 1). There was also little difference between the responder and non-
191 responder groups for the anthropometric characteristics measured at baseline (Table 1).

192 During the intervention period, both groups significantly reduced BMI, weight and waist
193 circumference, with both groups exhibiting similar effect sizes (Table 1). The responders
194 significantly increased their blood omega-3 index, whereas the non-responders did not (mean
195 change $\Delta = 0.31$ versus 0.14 , $p<0.001$).

196 At baseline, the responders and non-responders had similar dietary intakes of most food
197 groups, with the exception of alcohol (**Table 2**) for which the responders had lower intake
198 (170g day^{-1} versus 258g day^{-1} , $p=0.035$). Post-intervention, the responders reported reduced
199 intake of dairy ($\Delta= -59\text{ g day}^{-1}$, Table 2) and both responders and non-responders reported
200 significantly reduced red meat intake ($\Delta= -31$ and -28 g day^{-1} respectively).

201 The percentage of participants receiving dietary advice for specific target nutrients was
202 broadly similar (**Table S1**). The most common nutrient targeted at baseline was salt (73% of
203 responders and 59% of non-responders). There was no difference in the percentage of
204 responders and non-responders receiving a dietary message specifically targeted at
205 cholesterol (24% versus 23%, $p=0.816$), although a greater number of non-responders
206 received a message to increase physical activity (56% of responders versus 73% of non-
207 responders, $p=0.027$). While the responders had a significant reduction in cholesterol, there
208 was no significant change in physical activity during the intervention period for either group.

209 At baseline, the responders had higher total cholesterol level than the non-responders (6.09
210 mmol/L versus 5.54 mmol/L , $p<0.001$, Table 1). The fatty acid profiles differed between the
211 responders and non-responders at baseline (**Table 3**). There was no difference between the
212 groups for total saturated fatty acids (SFA, $p=0.203$), but the responders had lower palmitic
213 acid ($16:0$, $p=0.009$). At baseline, the responders had significantly lower total
214 monounsaturated fatty acids (MUFA, $p=0.016$), and in particular lower palmitoleic acid
215 ($16:1n-7$, $p=0.012$) and cis-vaccenic acid ($18:1n-7$, $p=0.001$). At baseline, the responders had

216 higher total PUFA ($p=0.008$), in particular linoleic acid ($18:2n-6$, $p=0.011$), eicosadienoic
217 acid ($20:2n-6$, $p=0.006$) and docosapentaenoic acid (DPA $22:5n-3$, $p=0.014$). At baseline
218 both groups had similar carotenoids profiles (**Table 4**).

219

220 **Discriminating between responders and non-responders**

221 When the stepwise logistic regression model was applied using demographic and
222 anthropometric data, age and weight were selected as being important factors in
223 discriminating responders from non-responders (Model 1, **Table 5**). The classification
224 accuracy (as measured by the area under the ROC curve, **Figure 1**) was 0.61, indicating that
225 the demographic and anthropometric data do not provide sufficient discriminatory power. As
226 expected, the classification accuracy improved when the model was adjusted for baseline
227 cholesterol, (Model 2 area under curve=0.76, Table 5, Figure 1). Including dietary intake data
228 (Model 3) did not improve the discriminatory power, with none of the food groups being
229 selected when tested in the stepwise model. When the additional biochemical data were
230 added to the model (Model 4), the key variables selected were baseline levels of cholesterol,
231 glucose, stearic acid, DPA, and eicosenoic acid, each with significant positive coefficients
232 and EPA and trans fatty acids, with significant negative coefficients. Alcohol intake also had
233 a significant negative coefficient in this model that included the biochemical variables. The
234 coefficients of the final logistic regression discriminant model are detailed in **Table 6**.
235 Increases in the variables with positive or negative coefficients were associated with
236 increased or decreased probability of being a responder, respectively. The additional
237 biochemical data significantly improved the classification accuracy (Model 4 area under
238 curve=0.90, Table 5, Figure 1), with increases in the true positive rate (sensitivity) resulting
239 in only a small trade-off with the false positive rate (1- sensitivity). For example, to achieve
240 a sensitivity of 80% for Model 3, the false positive rate is only 10%. This compares with 67%
241 for Model 1 and 44% for Model 2 (Figure 1). Furthermore, it is also worth noting that
242 intervention group was not selected as a discriminant variable indicating that it did not
243 contribute to classification as a responder or non-responder.

244

245 **DISCUSSION**

246 Identification of sub phenotypes that respond differently to dietary interventions has the
247 possibility to significantly enhance delivery of personalised nutrition. In the current study a
248 baseline phenotype characterised by age, alcohol intake, and levels of stearic acid, DPA,
249 EPA, eicosenoic acid and trans fatty acids, was identified which could discriminate
250 responders and non-responders in 90% of cases. Discriminant analysis has previously been
251 used in dietary intervention studies to test whether metabolic profiles may be used to identify
252 responders and non-responders. In a choline-depletion study, analysis of the baseline
253 metabolomics profile predicted which participants developed liver dysfunction when
254 deprived of dietary choline⁽²³⁾. Mutch *et al.* 2007 classified responders and non-responders to
255 dietary intervention using linear discriminant analysis on a gene expression snapshot⁽²⁴⁾.
256 Here we used a stepwise logistic regression model to select the individual factors that best
257 classified the probability of being a responder. Incorporation of such information into dietary
258 advice strategies has the potential to significantly enhance the success of interventions.

259

260 Wide inter-individual variation has been observed in the response of total, LDL and HDL
261 cholesterol to dietary change^(25; 26; 27) with little alterations in blood cholesterol for some
262 participants despite significant changes in dietary fatty acid pattern and cholesterol intake⁽²⁸⁾.
263 This means that while the population response to a diet can be estimated, the responsiveness
264 of a single individual will have as a result of dietary change is difficult to determine⁽²⁹⁾.
265 Lefevre *et al.*⁽³⁰⁾ observed that variability in the change of serum was related to
266 anthropometric measurements including BMI, waist circumference and body fat percentage.
267 Furthermore, there is a large body of evidence to support the genetic influence on response of
268 plasma cholesterol to dietary interventions^(25; 31; 32; 33). The present study determined a profile
269 which was responsive to dietary advice in terms of lowering cholesterol levels. Overall, this
270 work in conjunction with the literature provides compelling evidence that individual variation
271 and response to interventions needs to be incorporated into dietary advice strategies.

272

273 The most marked differences between the responder and non-responder phenotypes were
274 found in their baseline fatty acid profiles. The responders had a lower mean percentage of
275 *trans* fatty acids at baseline. TFAs have been found to increase LDL- and decrease HDL-
276 cholesterol levels⁽³⁴⁾. While the responders and non-responders did not differ in their total
277 percentage of SFA, contributions of different SFAs differed. The responders had lower

278 palmitic acid (16:0) and higher stearic acid (18:0) than the non-responders. A review
279 comparing the risk factors for stearic acid with other saturated fatty acids ⁽³⁵⁾ reported that
280 diets high in stearic acid have favourable effects on LDL cholesterol compared with palmitic
281 acid. However, it has also been reported that stearic acid itself has no cholesterol-enhancing
282 effect in clinically very well controlled exchange of single fatty acids, whereas palmitic,
283 myristic and lauric acids have strong cholesterol-raising effects ⁽³⁶⁾.

284

285 The responder group had lower total MUFA, in particular palmitoleic acid (16:1n-7) and cis-
286 vaccenic acid (18:1n-7). A meta-analysis investigating the effects of MUFA on
287 cardiovascular and diabetic risk factors observed no consistent evidence for a relationship
288 between MUFA and total cholesterol ^(36;37). The PUFA profiles differed between the
289 responders and non-responders, with a more marked difference in the n-6 PUFAs. The
290 responders had higher levels of linoleic acid (18:2n-6) and eicosadienoic acid (20:2n-6)
291 compared to the non-responders at baseline. Linoleic acid, the primary n-6 PUFA, has been
292 shown to have a cholesterol lowering effect ^(36;38) and a recent meta-analysis reported a lower
293 risk of coronary heart disease events and deaths with increasing linoleic acid intake ⁽³⁹⁾.
294 While the total n-3 PUFAs did not differ between the two groups, the responders had a higher
295 percentage of DPA (22:5n-3). Higher levels of DPA in human blood have been shown to be
296 correlated with lower cholesterol ⁽⁴⁰⁾. Overall, the data supports the growing evidence that
297 fatty acids patterns as opposed to single individual fatty acids are important in determining
298 health. Moreover, it supports the importance of adequate intake of PUFAs.

299

300 The demographic profiles of the responders and non-responders did not differ, and at
301 baseline, the groups also had similar anthropometric characteristics. Dietary intake at baseline
302 was similar across the two groups, with only alcohol intake differing. As this was a study of
303 the effects of personalised nutrition the dietary advice given to the participants differed
304 between individuals. However, for all the participants, the percentage of subjects receiving
305 dietary advice for specific target nutrients was generally similar. The strengths of this study
306 were that it was a multi-country group with multiple time points allowing analysis of change
307 in response to the intervention. Furthermore, the participants are well phenotyped. A
308 limitation of the study is the unique study design involving personalised nutrition advice
309 which makes replication and prospective analysis in an independent cohort difficult.

310

311 An objective of this study was to investigate whether the different types of data were useful
312 in classifying whether an individual will respond to the dietary intervention. Our study has
313 shown that baseline phenotypic data provided more classification power than anthropometric
314 or dietary intake data in classifying responsiveness to personalised dietary advice. While the
315 work identified particular predictive characteristics, it was not our aim to establish causative
316 relationships between the variables. Our study has shown that, in principle, we can predict, *a*
317 *priori* whether an individual's health status will improve in response to the consumption of a
318 given food/diet. This strengthens the evidence base for the concept that intervention and
319 dietary advice can be personalised with more confidence. Future work should examine the
320 optimal method for incorporation of such data into dietary advice and should pave the way
321 for precision nutrition.

322 ACKNOWLEDGEMENTS

323 This project was supported by the European Commission under the Food, Agriculture,
324 Fisheries and Biotechnology Theme of the 7th Framework Programme for Research and
325 Technological Development, grant number 265494. The authors' contributions are as
326 follows: L.K. L.B., E.R.G. and M.C.W. derived the research question for this manuscript,
327 drafted the manuscript and conducted statistical analysis; J. C. M. was the study director of
328 the proof-of-principle study of Food4Me; H.D., I.T., C.A.D., M.G., J.A. L., Y.M., J.A.M. and
329 W.H.M.S. contributed to the design of the proof-of-principle study and were principle
330 investigators for their respective research centre; L.B., R.F., H.F., E.R.G., M.G., S.K.,
331 K.M.L., C.F.M.M., C.C.-M., G.M., S. N.-C., C. B.O.'D., A.S., M.C.W. and C.W. contributed
332 to the study design and execution at the research centres. All authors read and approved the
333 final version of the manuscript. C.A.D. is a founder, stock owner, board member and
334 consultant for Vitas Ltd, Oslo, Norway. The other authors have no potential financial or
335 personal conflicts of interest to declare.

336

337

338

339

340 FIGURE LEGENDS

341 Figure 1. ROC curves illustrating the performance of models M1, M2 and M4 at
342 discriminating responders from non-responders. The selected variables in M3 were identical
343 to M2 and so it has not been included. The diagonal reference line represents random
344 discrimination, with points above the line indicating discrimination ability.

345

346

347 REFERENCES

- 348 1. de Roos B (2013) Personalised nutrition: ready for practice? *Proceedings of the Nutrition*
349 *Society* **72**, 48-52.
- 350 2. van Ommen B, Keijer J, Kleemann R *et al.* (2008) The challenges for molecular nutrition
351 research 2: quantification of the nutritional phenotype. *Genes & nutrition* **3**, 51-59.
- 352 3. Konstantinidou V, Ruiz LAD, Ordovás JM (2014) Personalized Nutrition and
353 Cardiovascular Disease Prevention: From Framingham to PREDIMED. *Advances in*
354 *Nutrition: An International Review Journal* **5**, 368S-371S.
- 355 4. Zeevi D, Korem T, Zmora N *et al.* (2015) Personalized Nutrition by Prediction of
356 Glycemic Responses. *Cell* **163**, 1079-1094.
- 357 5. Lampe JW, Navarro SL, Hullar MA *et al.* (2013) Inter-individual differences in response
358 to dietary intervention: integrating omics platforms towards personalised dietary
359 recommendations. *The Proceedings of the Nutrition Society* **72**, 207-218.
- 360 6. Schork NJ (2015) Personalized medicine: Time for one-person trials. *Nature* **520**, 609-611.
- 361 7. Brennan L (2015) Metabotyping: moving towards personalised nutrition. In *Metabolomics*
362 *as a Tool in Nutrition Research*, pp. 137-144: Woodhead publishing series in food science,
363 technology and nutrition.
- 364 8. Kaput J, Morine M (2012) Discovery-based nutritional systems biology: developing N-of-
365 1 nutrigenomic research. *International journal for vitamin and nutrition research*
366 *Internationale Zeitschrift für Vitamin- und Ernährungsforschung Journal international de*
367 *vitaminologie et de nutrition* **82**, 333-341.
- 368 9. Celis-Morales C, Lara J, Mathers JC (2015) Personalising nutritional guidance for more
369 effective behaviour change. *Proceedings of the Nutrition Society* **74**, 130-138.
- 370 10. Curtis PJ, Adamson AJ, Mathers JC (2012) Effects on nutrient intake of a family-based
371 intervention to promote increased consumption of low-fat starchy foods through education,
372 cooking skills and personalised goal setting: the Family Food and Health Project. *British*
373 *Journal of Nutrition* **107**, 1833-1844.
- 374 11. Ryan NM, O'Donovan CB, Forster H *et al.* (2015) New tools for personalised nutrition:
375 The Food4Me project. *Nutrition Bulletin* **40**, 134-139.
- 376 12. O'Sullivan A, Gibney MJ, Connor AO *et al.* (2011) Biochemical and metabolomic
377 phenotyping in the identification of a vitamin D responsive metabotype for markers of the
378 metabolic syndrome. *Molecular nutrition & food research* **55**, 679-690.

- 379 13. van Bochove K, van Schalkwijk DB, Parnell LD *et al.* (2012) Clustering by plasma
380 lipoprotein profile reveals two distinct subgroups with positive lipid response to fenofibrate
381 therapy. *PloS one* **7**, e38072.
- 382 14. Elnenaï MO, Chandra R, Mangion T *et al.* (2011) Genomic and metabolomic patterns
383 segregate with responses to calcium and vitamin D supplementation. *The British journal of*
384 *nutrition* **105**, 71-79.
- 385 15. Rudkowska I, Paradis AM, Thifault E *et al.* (2013) Differences in metabolomic and
386 transcriptomic profiles between responders and non-responders to an n-3 polyunsaturated
387 fatty acids (PUFAs) supplementation. *Genes & nutrition* **8**, 411-423.
- 388 16. Celis-Morales C, Livingstone KM, Marsaux CF *et al.* (2015) Design and baseline
389 characteristics of the Food4Me study: a web-based randomised controlled trial of
390 personalised nutrition in seven European countries. *Genes & nutrition* **10**, 450.
- 391 17. Celis-Morales C, Livingstone KM, Woolhead C *et al.* (2015) How reliable is internet-
392 based self-reported identity, socio-demographic and obesity measures in European adults?
393 *Genes & nutrition* **10**, 1-10.
- 394 18. Baecke JA, Burema J, Frijters JE (1982) A short questionnaire for the measurement of
395 habitual physical activity in epidemiological studies. *The American journal of clinical*
396 *nutrition* **36**, 936-942.
- 397 19. Marsaux CFM, Celis-Morales C, Livingstone KM *et al.* (2016) Changes in Physical
398 Activity Following a Genetic-Based Internet-Delivered Personalized Intervention:
399 Randomized Controlled Trial (Food4Me). *Journal of medical Internet research* **18**, e30.
- 400 20. Forster H, Fallaize R, Gallagher C *et al.* (2014) Online dietary intake estimation: the
401 Food4Me food frequency questionnaire. *Journal of medical Internet research* **16**, e150.
- 402 21. Fallaize R, Forster H, Macready AL *et al.* (2014) Online dietary intake estimation:
403 reproducibility and validity of the Food4Me food frequency questionnaire against a 4-day
404 weighed food record. *Journal of medical Internet research* **16**, e190.
- 405 22. Hoeller U, Baur M, Roos FF *et al.* (2016) Application of dried blood spots to determine
406 vitamin D status in a large nutritional study with unsupervised sampling: the Food4Me
407 project. *British Journal of Nutrition* **115**, 202-211.
- 408 23. Sha W, da Costa KA, Fischer LM *et al.* (2010) Metabolomic profiling can predict which
409 humans will develop liver dysfunction when deprived of dietary choline. *FASEB journal :*
410 *official publication of the Federation of American Societies for Experimental Biology* **24**,
411 2962-2975.

- 412 24. Mutch DM, Temanni MR, Henegar C *et al.* (2007) Adipose gene expression prior to
413 weight loss can differentiate and weakly predict dietary responders. *PloS one* **2**, e1344.
- 414 25. Masson LF, McNeill G, Avenell A (2003) Genetic variation and the lipid response to
415 dietary intervention: a systematic review. *The American journal of clinical nutrition* **77**,
416 1098-1111.
- 417 26. Beynen AC, Katan MB, Van Zutphen LF (1987) Hypo- and hyperresponders: individual
418 differences in the response of serum cholesterol concentration to changes in diet. *Advances in*
419 *lipid research* **22**, 115-171.
- 420 27. Jacobs DR, Anderson JT, Hannan P *et al.* (1983) Variability in individual serum
421 cholesterol response to change in diet. *Arteriosclerosis, Thrombosis, and Vascular Biology* **3**,
422 349-356.
- 423 28. Cox C, Mann J, Sutherland W *et al.* (1995) Individual variation in plasma cholesterol
424 response to dietary saturated fat. *BMJ (Clinical research ed)* **311**, 1260-1264.
- 425 29. Denke MA, Adams-Huet B, Nguyen AT (2000) Individual cholesterol variation in
426 response to a margarine- or butter-based diet: A study in families. *JAMA* **284**, 2740-2747.
- 427 30. Lefevre M, Champagne CM, Tulley RT *et al.* (2005) Individual variability in
428 cardiovascular disease risk factor responses to low-fat and low-saturated-fat diets in men:
429 body mass index, adiposity, and insulin resistance predict changes in LDL cholesterol. *The*
430 *American journal of clinical nutrition* **82**, 957-963; quiz 1145-1146.
- 431 31. Qi Q, Durst R, Schwarzfuchs D *et al.* (2015) CETP genotype and changes in lipid levels
432 in response to weight-loss diet intervention in the POUNDS LOST and DIRECT randomized
433 trials. *Journal of lipid research* **56**, 713-721.
- 434 32. Asztalos B, Lefevre M, Wong L *et al.* (2000) Differential response to low-fat diet
435 between low and normal HDL-cholesterol subjects. *Journal of lipid research* **41**, 321-328.
- 436 33. Wallace AJ, Mann JI, Sutherland WH *et al.* (2000) Variants in the cholesterol ester
437 transfer protein and lipoprotein lipase genes are predictors of plasma cholesterol response to
438 dietary change. *Atherosclerosis* **152**, 327-336.
- 439 34. Hunter JE (2014) Health and nutrition update on trans fatty acids. *Lipid Technology* **26**,
440 199-201.
- 441 35. Hunter JE, Zhang J, Kris-Etherton PM (2010) Cardiovascular disease risk of dietary
442 stearic acid compared with trans, other saturated, and unsaturated fatty acids: a systematic
443 review. *The American journal of clinical nutrition* **91**, 46-63.

- 444 36. Müller H, Kirkhus B, Pedersen JI Serum cholesterol predictive equations with special
445 emphasis on Trans and saturated fatty acids. An analysis from designed controlled studies.
446 *Lipids* **36**, 783-791.
- 447 37. Schwingshackl L, Hoffmann G (2012) Monounsaturated Fatty Acids and Risk of
448 Cardiovascular Disease: Synopsis of the Evidence Available from Systematic Reviews and
449 Meta-Analyses. *Nutrients* **4**, 1989-2007.
- 450 38. Harris WS, Mozaffarian D, Rimm E *et al.* (2009) Omega-6 Fatty Acids and Risk for
451 Cardiovascular Disease: A Science Advisory From the American Heart Association Nutrition
452 Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on
453 Cardiovascular Nursing; and Council on Epidemiology and Prevention. *Circulation* **119**, 902-
454 907.
- 455 39. Farvid MS, Ding M, Pan A *et al.* (2014) Dietary Linoleic Acid and Risk of Coronary
456 Heart Disease: A Systematic Review and Meta-Analysis of Prospective Cohort Studies.
457 *Circulation* **130**, 1568-1578.
- 458 40. Byelashov OA, Sinclair AJ, Kaur G (2015) Dietary sources, current intakes, and
459 nutritional role of omega-3 docosapentaenoic acid. *Lipid Technology* **27**, 79-82.
- 460

Table 1. Demographic and phenotypic profiles of responders and non-responders

		Responder		Non-responder		Responder vs Non- responder					
		n	%	n	%	Chi-sq	p-value				
Sex	Total	78		79							
	Male	40	51.20%	43	55.13%						
	Female	38	48.70%	36	46.15%	0.16	0.693				
Country	Germany	19	24.40%	23	29.11%						
	Greece	10	12.80%	11	13.92%						
	Ireland	11	14.10%	7	8.86%						
	Netherlands	16	20.50%	13	16.46%						
	Poland	4	5.10%	8	10.13%						
	Spain	11	14.10%	14	17.72%						
	United Kingdom	7	9.00%	3	3.80%	5.0	0.544				
		Baseline		Δ		Baseline		Δ		p-value for difference	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Baseline	Δ
Age (years)		45.1	1.35			48.2	1.15			0.082	
Height (m)		1.72	0.01			1.74	0.01			0.262	
Weight (kg)		78.1	1.66	-1.7	0.39	82.6	1.79	-1.3	0.37	0.065	0.429
BMI (kg/m ²)		26.4	0.52	-0.6	0.14	27.5	0.56	-0.4	0.13	0.17	0.495
Waist circumference (m)		0.9	0.015	-0.02	0.005	0.93	0.015	-0.02	0.005	0.091	0.764
Physical activity level		1.54	0.012	0.03	0.01	1.53	0.012	0.027	0.01	0.687	0.908
Total Cholesterol (mmol/L)		6.09	0.091	-2.01	0.072	5.54	0.063	0.47	0.06	<.001	<0.001
Glucose (mmol/L)		4.13	0.08	-0.82	0.113	3.88	0.111	-0.23	0.111	0.934	0.259
Omega-3 index		5.68	0.127	0.31	0.096	5.69	0.13	0.14	0.109	0.068	<0.001

Measurements at baseline and mean change (Δ) between baseline and month 6 are presented as mean ± standard error. P-values were obtained from generalised linear models including the responder group as a factor. Bolded p-values are significant at the 5% level.

1 Table 2. Baseline dietary intake (g day⁻¹) and change from baseline to month 6 for responders and non-responders

	Responders				Non-responders				P-value for difference	
	Baseline		Δ		Baseline		Δ		Baseline	Δ
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM		
Fruit	445	45	6	33	387	32	37	31	0.289	0.487
Vegetables	234	22	-6	22	229	14	4	15	0.851	0.71
Whole grains	169	18	6	15	127	14	22	12	0.064	0.413
Oily fish	23	3	9	6	21	3	4	3	0.691	0.42
Red meat	95	11	-31	10	85	7	-28	5	0.424	0.763
Dairy	337	30	-59	27	286	28	-16	24	0.214	0.247
Nuts	7	2	0	1	6	1	1	1	0.694	0.638
Alcohol	170	23	-22	25	258	34	-16	35	0.035	0.892

2 Dietary intake at baseline and mean change (Δ) between baseline and month 6 are presented as mean ± standard error. Bolded mean changes are
 3 significant at the 5% level. P-values were obtained from generalised linear models including responder group as a factor. Bolded p-values are
 4 significant at the 5% level.

5

6 Table 3. Mean % of blood total fatty acid at baseline for responders and non-responders and mean change from baseline to month 6

			Responders				Non-responders				P-value for difference			
			Baseline		Δ		Baseline		Δ		Baseline		Δ	
			Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	p-value	FDR	p-value	FDR
		Trans fatty acids	0.79	0.027	0.04	0.035	0.9	0.028	-0.06	0.039	0.007	0.040	0.059	0.295
SFA	(14:0)	Myristic	0.78	0.042	-0.07	0.046	0.85	0.048	-0.02	0.044	0.281	0.351	0.398	0.807
SFA	(15:0)	Pentadecyclic	0.21	0.006	0	0.005	0.2	0.006	0.01	0.006	0.166	0.239	0.046	0.295
SFA	(16:0)	Palmitic	22.89	0.157	-0.04	0.188	23.63	0.229	-0.46	0.36	0.009	0.040	0.308	0.807
SFA	(17:0)	Margaric	0.32	0.005	-0.01	0.006	0.31	0.008	-0.01	0.009	0.799	0.799	0.617	0.807
SFA	(18:0)	Stearic	12.81	0.118	0.67	0.156	12.44	0.129	0.68	0.275	0.034	0.076	0.978	0.978
SFA	(20:0)	Arachidic	0.2	0.007	0.15	0.032	0.19	0.007	0.17	0.027	0.639	0.710	0.621	0.807
MUFA	(16:1n-7)	Palmitoleic	1.26	0.056	-0.08	0.041	1.49	0.072	-0.02	0.059	0.012	0.040	0.436	0.807
MUFA	(18:1n-9)	Oleic n9	19.21	0.278	-0.34	0.245	19.9	0.241	-0.84	0.328	0.063	0.126	0.225	0.807
MUFA	(18:1n-7)	Cis-vaccenic	1.34	0.021	0.22	0.046	1.48	0.039	0.03	0.056	0.001	0.020	0.01	0.200
MUFA	(20:1)	Eicosenoic	0.26	0.006	-0.01	0.006	0.25	0.007	-0.01	0.008	0.167	0.239	0.686	0.807
n-3 PUFA	(18:3n-3)	□-linolenic ALA	0.33	0.013	0	0.017	0.34	0.018	0	0.018	0.528	0.621	0.943	0.978
n-3 PUFA	(20:5n-3)	Eicosapentaenoic EPA	0.73	0.045	0.04	0.038	0.82	0.061	0.07	0.056	0.208	0.277	0.661	0.807
n-3 PUFA	(22:5n-3)	Docosapentaenoic DPA	1.41	0.039	0.03	0.027	1.28	0.035	0.01	0.036	0.014	0.040	0.67	0.807

n-3 PUFA	(22:6n-3)	Docosahexaenoic DHA	2.96	0.1	0.31	0.069	3.01	0.095	0.09	0.076	0.696	0.733	0.041	0.295
n-6 PUFA	(18:2n-6)	Linoleic	19.92	0.259	-0.6	0.221	18.96	0.266	-0.61	0.311	0.011	0.040	0.969	0.978
n-6 PUFA	(18:3n-6)	γ linolenic GLA	0.2	0.01	0.01	0.012	0.23	0.014	0	0.012	0.101	0.182	0.669	0.807
n-6 PUFA	(20:2n-6)	Eicosadienoic	0.22	0.004	0	0.003	0.21	0.004	0	0.004	0.006	0.040	0.431	0.807
n-6 PUFA	(20:3n-6)	Dihomo γ linolenic DGLA	1.58	0.036	-0.07	0.029	1.46	0.037	-0.04	0.033	0.024	0.060	0.467	0.807
n-6 PUFA	(20:4n-6)	Arachidonic ARA	8.66	0.152	0.08	0.156	8.32	0.146	-0.15	0.184	0.109	0.182	0.351	0.807
Desaturase index D5D		ARA/ DGLA	5.7	0.17	0.35	0.12	5.93	0.07	0.12	0.17	0.338		0.102	
Desaturase index D6D		DGLA/ Linoleic	0.08	0.002	0	0.002	0.08	0.001	0.002	0.002	0.442		0.393	
		SFA	37.2	0.22	0.7	0.294	37.63	0.255	0.38	0.594	0.203		0.639	
		MUFA	22.07	0.299	-0.21	0.256	23.07	0.282	-0.83	0.372	0.016		0.166	
		PUFA	36	0.338	-0.21	0.361	34.66	0.365	-0.63	0.565	0.008		0.533	
		PUFA n-3	5.43	0.156	0.37	0.2	5.46	0.155	0.23	0.2	0.872		0.419	
		PUFA n-6	30.58	0.315	-0.57	0.317	29.18	0.313	-0.43	0.317	0.002		0.747	
		n-3 / n-6	0.18	0.006	-0.017	0.004	0.19	0.006	0.009	0.004	0.181		0.221	

7 Fatty acid percentage at baseline and mean change (Δ) between baseline and month 6 are presented as mean \pm standard error. P-values were obtained from generalised linear models including responder group as a
8 factor. FDR adjusted p-values control for false discovery rate. Bolded p-values are significant at the FDR 5% level. The $\Delta 5$ desaturase (D5D) was calculated as the ratio of arachidonic acid (20:4n-6) to dihomo γ linoleic
9 acid (20:3n-6). The $\Delta 6$ desaturase (D6D) was calculated as the ratio of dihomo γ linoleic acid (20:3n-6) to linoleic acid (18:2n-6).

Table 4. Mean blood carotenoid levels ($\mu\text{mol/L}$) for responders and non-responders at baseline

	Responders				Non-responders				P-value for difference	
	Baseline		Δ		Baseline		Δ		Baseline	Δ
	Mean	SE	Mean	SE	Mean	SE	Mean	SE		
Lutein	0.23	0.013	-0.03	0.012	0.25	0.014	-0.03	0.013	0.328	0.74
Zeaxanthin	0.06	0.004	-0.02	0.004	0.05	0.003	-0.01	0.003	0.525	0.282
β Cryptoxanthin	0.24	0.028	-0.08	0.028	0.19	0.018	0.01	0.017	0.098	0.022
α Carotene	0.14	0.014	0.01	0.018	0.11	0.011	0.01	0.008	0.146	0.448
β Carotene	0.45	0.035	0.05	0.034	0.4	0.035	0.02	0.021	0.276	0.098
Lycopene	0.55	0.027	-0.07	0.026	0.54	0.033	0.01	0.038	0.863	0.225
Total Carotenoids	1.67	0.078	-0.21	0.072	1.54	0.08	0.03	0.071	0.263	0.082

Carotenoid levels at baseline and mean change (Δ) between baseline and month 6 are presented as mean \pm standard error. P-values were obtained from generalised linear models containing responder group as a factor. Bolded p-values are significant at the 5% level.

Table 5. Examining the ability to classify responders and non-responders.

	Area	SE	p-value ^a	Asymptotic 95% Confidence Interval	p-value ^b
M1: Anthropometric data only	0.61	0.045	0.014	0.53 – 0.70	
M2: M1 plus baseline cholesterol	0.76	0.037	<0.001	0.69 – 0.836	0.0007
M3: M2 plus dietary intake data	0.76	0.037	<0.001	0.69 – 0.836	0.999
M4: M3 plus biochemical data	0.90	0.026	<0.001	0.85 – 0.95	0.0003

Area under the ROC curve (AUC). The area measures the accuracy, or discrimination ability, to classify responders and non-responders.

Area under the curve is presented as area \pm standard errors.

^a Null hypothesis: true area = 0.5

^b P-value for comparison of C-statistic versus previous model

Table 6. List of discriminating parameters.

		Estimate	Standard Error	t-value	p-value
	Constant	30.56	6.347	23.17	0.001
	Baseline cholesterol	2.95	0.583	25.55	0.001
	Baseline glucose	1.02	0.354	8.34	0.10
	Age	-0.06	0.0232	6.67	0.016
SFA	(18:0) Stearic acid	0.62	0.253	6.03	0.025
MUFA	(20:1) Eicosenoic acid	13.53	5.16	6.88	0.007
n-3 PUFA	(22:5n-3) Docosapentaenoic acid (DPA)	4.51	1.04	18.76	0.001
n-6 PUFA	(20:5n-3) Eicosapentaenoic acid (EPA)	-2.73	0.717	14.53	0.001
	Trans Fatty acids	-3.03	1.054	8.27	0.010
	Alcohol intake	0.0033	0.0011	8.25	0.042

Stepwise logistic regression discriminant analysis. Estimates are on the logit scale. This is the final model selected using stepwise selection procedure including all demographic, anthropometric, dietary intake, fatty acids and carotenoids as potential predictors. The logistic regression model estimates the probability of being a responder. A positive coefficient for an independent variable implies an increased probability of being a responder with increasing values of the variable.

Figures

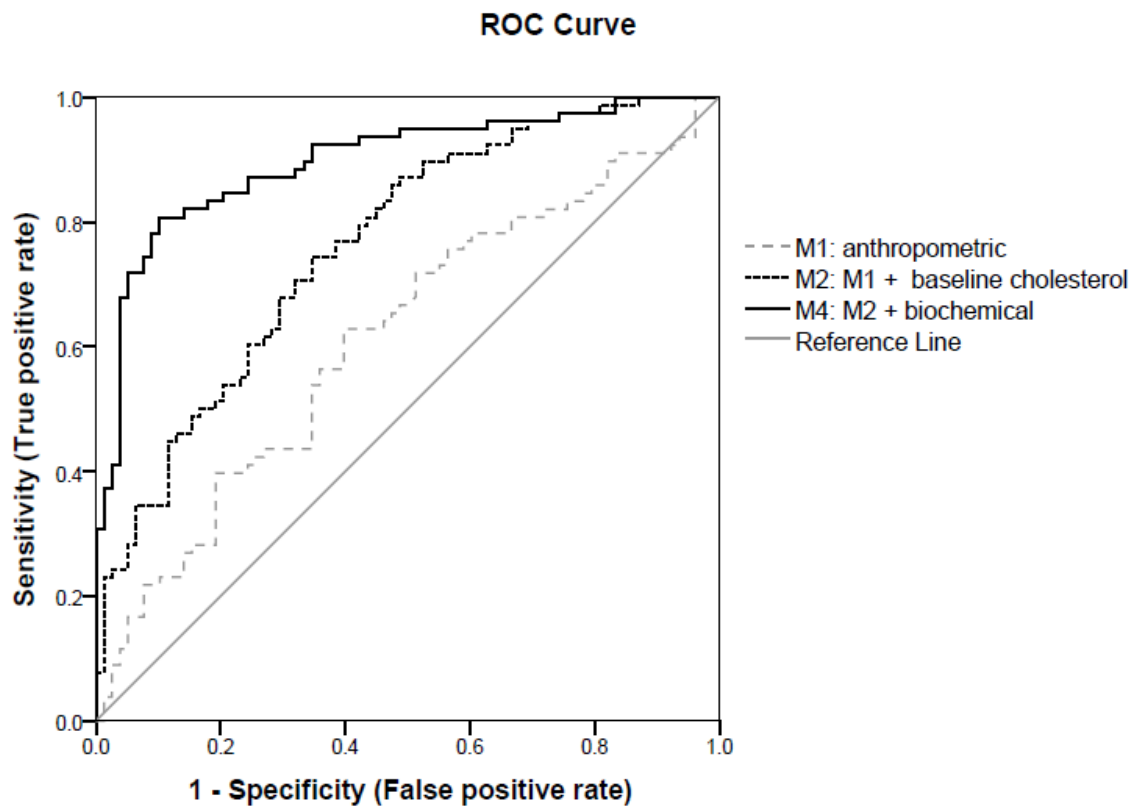


Figure 1. ROC curves illustrating the performance of models M1, M2 and M4 at discriminating responders from non-responders. The selected variables in M3 were identical to M2 and so it has not been included. The diagonal reference line represents random discrimination, with points above the line indicating discrimination ability.

Supplementary Material

Table S1. Percentage of participants receiving dietary advice for specific target nutrients at baseline

Message	Responders	Non-responders	p-value *
Nutrient			
Salt	73%	59%	0.073
Saturated fat	37%	33%	0.575
Fibre	32%	29%	0.690
Carotenoids	28%	30%	0.765
Folate	26%	27%	0.893
Cholesterol	24%	23%	0.816
Unsaturated fat	21%	19%	0.810
Omega 3	18%	23%	0.453
Reduce total fat	14%	11%	0.611
Increase calcium	6%	16%	0.056
Body weight			
Increase PAL	56%	73%	0.027
Bodyweight & cholesterol	55%	65%	0.229
Reduce BMI	31%	43%	0.113
Reduce waist circumference	18%	34%	0.022

* P-values were obtained from logistic regression models including responder group as a factor. Bolded p-values are significant at the 5% level