

Profile of European adults interested in internet-based personalized nutrition: The Food4Me Study

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59

1 **Abstract (words count= 250)**

2 **Purpose**

3 Personalised intervention may have greater potential for reducing the global burden of non-
4 communicable diseases and for promoting better health and wellbeing across the life-span
5 than the conventional “one size fits all” approach. However, the characteristics of
6 individuals interested in personalised nutrition (PN) are unclear. Therefore, the aim of this
7 study was to describe the characteristics of European adults interested in taking part in an
8 internet-based PN study.

9

10 **Methods**

11 Individuals from seven European countries (UK, Ireland, Germany, the Netherlands, Spain,
12 Greece and Poland) were invited to participate in the study via the Food4Me website
13 (<http://www.food4me.org>). Two screening questionnaires were used to collect data on
14 socio-demographic, anthropometric and health characteristics as well as dietary intakes.

15

16 **Results**

17 A total of 5662 individuals expressed an interest in the study (mean age 40 ± 12.7 ; range 15-
18 87 years). Of these 64.6% were female and 96.9% were Caucasian. Overall, 12.9% were
19 smokers and 46.8% reported the presence of a clinically diagnosed disease. Furthermore,
20 46.9% were overweight or obese and 34.9% were sedentary during leisure time. Assessment
21 of dietary intakes showed that 54.3% of individuals reported consuming at least 5 portions

22 of fruit and vegetables per day, 45.9% consumed more than 3 servings of wholegrains and
23 37.2% limited their salt intake to less than 5.75g per day.

24

25 **Conclusions**

26 Our data indicate that individuals volunteering to participate in an internet-based PN study
27 are broadly representative of the European adult population, most of whom had adequate
28 nutrient intakes but who could benefit from improved dietary choices and greater physical
29 activity. Future use of internet-based PN approaches is thus relevant to a wide target
30 audience.

31

32 **Trial registration** – Clinicaltrials.gov NCT01530139

33 (<http://clinicaltrials.gov/show/NCT01530139>)

34 **Key Words** – Personalised nutrition, European profile, tailored intervention, internet-based,
35 randomized controlled trial.

36 Introduction

37 Non-communicable diseases (NCD), are the leading cause of death and are responsible for
38 36 million global deaths annually [1]. With modifiable risk factors estimated to account for
39 over 80% of premature deaths from CVD and cerebrovascular disease [2], lifestyle-based
40 interventions, including diet and physical activity, have been identified as an effective
41 strategy for minimising the burden of NCD [3]. However, realising this potential will require
42 the development, testing and implementation of much more effective behaviour change
43 interventions than are used conventionally [4-6]. To achieve such changes, interventions will
44 need to move from a conventional “one size fits all” approach to more predictive,
45 personalised, preventive and participatory interventions [7]. The concept of personalised
46 nutrition (PN) has been developed based on emerging understanding of the interactions
47 between diet, phenotype and genes on health [8]. In contrast with conventional ‘one-size
48 fits all’ approaches to dietary intervention, PN aims to provide advice on an individual (or
49 group) basis that is tailored to specific needs based on knowledge of current diet and
50 phenotypic and/or genotypic information. However, public acceptability will be a key
51 prerequisite for the successful implementation of PN [9]. A survey of 6000 individuals across
52 eight European countries found that 27% of individuals were willing to undertake genetic
53 testing for the purpose of PN [10]. The internet offers substantial opportunities for cost-
54 effective implementation of PN intervention strategies with the potential for scalability and
55 reach [6]. With an estimated 85% of the European population now using the internet[11],
56 knowledge of the characteristics of individuals who would be interested in receiving PN
57 advice via the internet would be valuable for planning future lifestyle-based interventions
58 aiming to reduce health inequalities and to improve overall public health.

The Food4Me Proof of Principle (PoP) Study is an internet-based randomized controlled trial conducted across seven European countries designed to compare the effects of different levels of PN on dietary behaviour and other health-related outcomes [12]. The present paper describes the characteristics of individuals interested in internet-based PN advice who were screened for inclusion in the Food4Me PoP Study.

Materials and methods

The present paper outlines responses to the screening questionnaires provided by individuals who indicated an interest in participating in the Food4Me PoP randomized controlled trial. The protocol for the Food4Me PoP Study has been published elsewhere [12].

Participant recruitment

Recruitment was conducted between July 2013 and February 2014 across seven European countries, via the internet, to emulate an internet-based PN service. Participants indicated their interest in joining the study by voluntarily registering their details on the Food4Me website (<http://www.food4me.org/>), which was set up for the purposes of the study (see Online Resource 1, Figure S1). The Food4Me PoP recruitment sites were as follows: University College Dublin (Ireland); Maastricht University (the Netherlands); University of Navarra (Spain); Harokopio University (Greece); University of Reading (United Kingdom; UK); National Food and Nutrition Institute (Poland); Technische Universität München (Germany).

Local and national advertising of the study via the internet, radio, posters, e-flyers, social media and word of mouth were used to aid recruitment (see Online Resource 1, Figure S2).

Screening Questionnaires

Once participants registered their details on the Food4Me website and consented to take part in the study, they were assigned a unique username and password and asked to complete two online screening questionnaires.

First Screening Questionnaire

The first screening questionnaire contained nine items on one screen. Individuals were asked to provide their age and sex, as well as information on internet access, pregnancy, food intolerances and allergies, since these data were used as exclusion criteria for the later randomized controlled trial (RCT).

Second Screening Questionnaire

Participants eligible for inclusion in the RCT completed a second online questionnaire. The primary purpose of this questionnaire was to collect detailed socio-demographic, health, anthropometric and dietary data. Following completion of this questionnaire, participants were asked to complete a screening food frequency questionnaire (FFQ) to estimate habitual dietary intake. The online Food4Me FFQ included 157 food items consumed frequently in each of the seven recruitment countries and intakes of foods and nutrients were computed in real time using a food composition database. The FFQ and food composition database were developed and validated specifically for the Food4Me PoP study

[13,14]. In the present analysis, dietary intakes of foods and food groups were assessed against six dietary recommendations: eat at least 5 portions of fruit and vegetables every day; eat at least 3 portions of wholegrain products every day; eat at least 1 portion of oily fish per week; eat less than 3 portions of red meat and processed meat per week; consume less than 5.75g/day of salt and consume less than 10% energy from sugars.

Anthropometric measurements and physical activity

Body weight and height were self-measured and self-reported by participants via the internet. Occupational and non-occupational physical activity were self-reported via the internet prior to completion of the FFQ. Participants were asked to categorise their occupational physical activity as light (e.g. administrative and managerial), moderate (e.g. sales worker) or heavy (e.g. equipment operator) and their non-occupational physical activity as sedentary (little walking/cycling/exercise), moderately active (intense exercise lasting 20-45 minutes at least twice per week) or very active (intense exercise lasting at least an hour per day).

Ethical approval and participant consent

The Research Ethics Committees at each University or Research Centre delivering the intervention granted ethical approval for the study. The Food4Me trial was registered as a Randomized Clinical Trial (NCT01530139) at Clinicaltrials.gov. All participants who expressed an interest in the study were asked to sign online consent forms at two stages in the screening process: prior to submitting any details and prior to the screening FFQ. These consent forms were automatically directed to the local study investigators to be counter-signed and archived. All Ethical Committees accepted an online informed consent

procedure, except for The Netherlands and Germany whose ethics committees requested an additional written informed consent form for participants who registered to participate in the study. In the latter countries, hard copy consent forms were sent by post to the respective recruitment centres. Personal information from respondents was stored on a secure, password-protected server.

Statistical analysis

Data were analysed using Stata (version 13; StataCorp., College Station, TX, USA). Results from descriptive analyses are presented as means and SD for continuous variables or as percentages for categorical variables. Chi squared tests and multinomial regression analyses were used to test for significant differences across categorical variables. For multinomial comparisons across countries, the overall average was used as the reference group. ANOVA and Fisher-Hayter pairwise comparisons were used for continuous variables. Results were deemed significant at $P < 0.05$.

Results

Participant characteristics at first screening

A total of 5562 individuals registered their name and contact details on the Food4Me website (<http://www.food4me.org/>) and a total of 5442 individuals completed the first screening questionnaire (Tables 1 and 2). The completion rate for this questionnaire was 88.6% with 120 Dutch participants choosing to not proceed to the first screening

questionnaire. Of the individuals who consented to participate in the study, 64.6% were female and 64.0 % were below 45 years of age.

A total of 1631 individuals were ineligible for the subsequent RCT based on the first screening questionnaire. This was due mainly to having a food allergy or intolerance and/or not completing the second screening questionnaire (Figure 1). Reported food allergies and intolerances were more common among females than males (Table 1). Inter-country differences for the prevalence of therapeutic diets, food allergies and intolerances are presented in Table 2. The most common means of recruitment to the study was through magazines and newspaper articles, followed by word of mouth, but this varied by country and age group. Social media were responsible for recruiting more than three times as many individuals under, than over, the age of 45 years (Table 1).

Participant characteristics at second screening

Characteristics of the 3811 subjects who completed the second screening questionnaire are summarised in Tables 3 and 4. The completion rate for this questionnaire was 68.5% with 1751 individuals choosing to not proceed to the second screening questionnaire. The profile of these participants was similar to that of the whole cohort who expressed an initial interest in the Food4Me study: 62.4% were female and 62.8% were younger than 45 years of age. The percentage of females at this screening stage was more comparable across countries (range 56.6- 73.8%) than at the initial screening (range 48.7 -77.3%). We observed that 96.9% of the participants were Caucasian.

171 Obesity prevalence and reported health status

172 Nearly half (46.9%) of participants were classified as overweight or obese but this
 173 proportion varied considerably by sex, age and country (Table 3 and 4). As summarised in
 174 Tables 5 and 6, nearly half (44.6%) of individuals reported that they were on medication:
 175 33.2% on prescribed and 11.5% non-prescribed medication. Prescribed and non-prescribed
 176 medication use was higher in females than males (38% vs. 25% and 13.1% vs. 8.7%
 177 respectively) and higher in individuals over the age of 45 years (44.8% vs. 26.3% and 14.5%
 178 vs. 9.6% respectively; see Online Resource 1 Table S1). Prescribed medication use was
 179 higher in Germany (38.5%) and The Netherlands (47.4%) and lower in Spain (28.4%),
 180 compared with overall, whereas non-prescribed medication use was higher in Poland
 181 (17.9%) and Germany (16.3%), compared with overall (see Online Resource 1 Table S2). In
 182 addition, 47.3% of individuals indicated that they were suffering from one or more clinically
 183 diagnosed diseases. Overall, 19.9% of individuals reported having an allergy, with the
 184 highest prevalence in Spain (26.9%) and lowest in Ireland (13.3%). Furthermore, 9.3% of
 185 individuals reported high blood pressure which was more common in males than in females
 186 (12.6% vs. 7.3%), and among individuals over, than under, the age of 45 years (18.9% vs.
 187 3.6%; see Online Resource 1 Table S1). The prevalence of type I or type II diabetes was only
 188 0.9 %, but was higher in individuals over, than under, the age of 45 years (1.8% vs. 0.4%). On
 189 average, 12.9% of individuals were current smokers and smoking prevalence was more than
 190 five times higher in Greece than in the UK (see Online Resource 1 Table S1).

191

192 Reasons for interest in the Food4Me PoP Study

Nearly three quarters of the individuals (75.4%) indicated an interest in the Food4Me study because they were interested in PN, while 80.7% were interested in learning about what foods were best for them (Table 4). These results varied little by sex but slightly more individuals under, than over, the age of 45 years were interested in PN (Table 3). Just over half of individuals (50.6%) indicated that their reason for registering with the study was due to a desire to lose (48.8%) or, much less commonly, gain (1.9%) weight. When asked if their interest was due to concerns for their health and well-being, up to 87.5% of the participants selected this option (Table 4). The proportion of individuals interested in health and well-being did not vary much by sex but was slightly higher in individuals over, than under, the age of 45 years (Table 3).

Dietary intake and physical activity characteristics

A total of 2764 individuals provided complete data on dietary intake and PA at screening. The completion rate for this questionnaire was 77.3%, with 811 individuals choosing not to complete the screening FFQ after providing a second consent. Comparisons of screenees' dietary intakes with current dietary recommendations in Europe that were used in this study are presented in the Online Resource 1 Table S3, Figure S3-S6. Regarding fruit and vegetables intake 54.3% of individuals reported consuming at least five portions per day and the mean intake of the cohort (651.4g, SD 488.6) was greater than the WHO/FAO recommended minimum of 400g per day [15]. Just under half of participants (45.9%) consumed at least three portions of wholegrains per day. A third of participants (36.3%) consumed more than one portion of oily fish per week. Two thirds (66.2%) of individuals consumed less than three portions (450g) of red or processed meat weekly. Furthermore,

only 37.2% of individuals consumed less than 5.75g of salt per day (mean 7.56 g, SD 4.88). Overall, only 2.1% of participants consumed less than 10% energy from sugars. Nearly three quarters (72.9 %) of individuals reported being in light/sedentary occupations, whereas only 34.9% of individuals were sedentary during leisure time (Table 3 and 4).

Discussion

Main findings

The present paper characterised the 5562 individuals who registered interest in participating in the Food4Me PoP PN intervention. Our main findings are that the European individuals interested in participating in an online PN study were not restricted to one specific group of individuals. Potential volunteers in the Food4Me PoP Study were broadly representative of the European adult population, most of whom had adequate nutrient intakes but could benefit from improved dietary choices and more physical activity to reduce their risk of common non-communicable diseases [16].

Comparison with other studies

The Food4Me PoP study is the first pan-European internet-based PN intervention study to collect detailed characteristics of individuals who would be interested in using such a service. However, a recent study across six European countries indicated that individuals over 65 years of age would be more interested in undertaking a genetic test for the purpose of PN than adults aged 25 to 34 years (55% vs. 28.5%) [10]. Our findings identified that interest in PN was a strong motivator for participating in the study, and that this was

comparable across ages, sexes and countries. Stewart-Knox *et al.* [10] found that slightly more (2.2%) females than males would be interested in having a genetic test done for the purposes of PN. Our findings confirm these results and suggest that females are more interested in participating in nutrition interventions [17], including those delivered via the internet [18]. Furthermore, we observed that females were more likely than men to be interested in participating in this study because of a desire to lose weight.

The percentage of overweight adults in different European countries ranges between 30% and 70% [19] and the prevalence of obesity in Europe is between 4% and 36.5%, with higher prevalence in Central, Eastern, and Southern Europe than in Western and Northern Europe [20]. Recent estimates from the Organisation for Economic Co-operation and Development suggest that the average prevalence of obesity among EU adults is 16.6% [21], which is similar to the prevalence of obesity in individuals who registered to participate in the present study (15.7%).

There is strong evidence in support of an inverse relationship between PA and CVD risk [22]. Despite this, according to the WHO, 69% of European adults fail to achieve at least half an hour of moderate-intensity PA on most days of the week [23]. Our data confirm these trends in the work place, with only 27.1% of individuals being moderately active at work, but suggest that during leisure time, 65.1% of individuals undertake intense exercise lasting at least 20-45 minutes at least twice per week.

Our results suggest that less than half of screenees adhered to most of the major European food-based dietary recommendations. This is consistent with the most recently available EU-wide data which show that mean intakes of fruit and vegetables in Germany (371g/day), Ireland (355g/day), the Netherlands (359g/day) and the UK (343g/day) [24] are less than the recommended 400g/day (equivalent to 5 portions). Although we found that mean fruit and

vegetable intake was greater than 400g, it should be noted that these estimates of fruit and vegetable intakes were obtained using a 157 item FFQ [14] and there is evidence that FFQs may over-estimate dietary intakes [25], especially when they include larger numbers of food items [26]. Importantly, the FFQ used in the present study was validated against a four-day weighed record [13,14]. Recent country-specific Nutrition, Physical Activity and Obesity reports by the WHO suggest that, with the exception of Greece (data unavailable), mean salt intake is in excess of 5.75g per day across all six countries that provided participants for the present study[16]. These data are in line with our findings, where reported mean salt intakes ranged between 6g in Greece and 8.3g in The Netherlands. The current UK and WHO draft recommendations to limit sugars intake to less than 10% energy [27,28] were met by very few of our participants and only one individual out of the 5562 screenees would meet the recent proposal to limit sugars intake to less than 5% energy. In addition, the reported mean percentage energy from sugars in our study (21.4%) is comparable with the recent estimate of 19.1% for the UK from the National Diet and Nutrition Survey [29]. Overall, our observations suggest that the dietary inadequacies of the individuals interested in PN in the present study are comparable with those of the wider European population.

Individuals with ill-health, or with a food allergy or intolerance, may benefit from PN [10].

We found that 12.4 % of the screenees declared a food allergy or intolerance. This prevalence is comparable with a recent finding that, among European adults, 11.5 % self-reported the presence of a food allergy (cow's milk, egg, wheat, soy, peanut, tree nuts, fish and shell-fish) [30]. Furthermore, a large proportion of our participants reported being on medication (44.6%) or suffering from a disease (47.3%). These results are in line with data from Stewart-Knox *et al.* [10], which showed that interest in having a genetic test performed for the purposes of PN is higher in individuals with central obesity (38.4 %) and high blood

pressure (38.1 %), than individuals with no signs of the metabolic syndrome (22.1 %). These findings confirm that those interested in PN include those for whom an effective dietary and/or physical activity intervention is likely to improve their health.

Strengths and limitations

The Food4Me PoP Study data were collected from a relatively large number of European adults, with a wide age range, who demonstrated their interest in PN by registering to join the Food4Me PoP Study. The two stage consenting process enabled the capture of data on individuals who were interested in a PN service but were not necessarily eligible to be included in the subsequent RCT. These characteristics included demographic information, adiposity, habitual physical activity, disease status, prevalence of food allergies and intolerances and dietary intake. A potential limitation of the study is that our data were obtained by self-report via the internet, which may have introduced measurement error. However, there is no reason to believe that such data are more likely to be mis-reported than data collected by conventional face-to-face interview or by paper-based questionnaires [31]. The validity of internet-based, self-reported anthropometric data is high [32] and this been confirmed in the present study (Celis-Morales C *et al.*, paper submitted).

Implications for health professionals

Improving diet and lifestyle behaviours is a key element in national and international strategies for reducing the risk of NCDs and improving overall health across the life-span. However, realising this potential will require the development, testing and implementation of much more predictive, personalised, preventive and participatory interventions to achieve effective behavioural changes. Moreover, using the internet as a delivery method is

311 likely to be an important route to scalable and sustainable interventions [6]. Characterising
312 those individuals who are interested in PN and its delivery via the internet will be valuable
313 information for the future design and implementation of PN interventions aiming to
314 improve health and to reduce health inequalities. The present results suggest that those
315 who registered to participate in this internet-based PN study were broadly representative of
316 the European adult population in terms of demographic, anthropometric and health
317 characteristics. Our findings provide strong evidence for the use of internet-based PN for
318 engaging individuals who would benefit from improved lifestyle behaviours and a reduction
319 in risk of obesity and NCDs.

Abbreviations

Cardiovascular disease (CVD); Food frequency questionnaire (FFQ); Non-communicable diseases (NCD); Personalized nutrition (PN); Proof of Principle (PoP); Randomized controlled trial (RCT)

Competing interests

None of the authors had a personal or financial conflict of interest.

Authors' contributions

The authors' responsibilities were as follows: YM, IT, CAD, ERG, LB, JAL, JAM, WHMS, HD, MG and JCM contributed to the research design. JCM was the Proof of Principle study leader. CCM, CFMM, HF, CBO, CW, AM, RF, SNC, RSC, SK, LT, CPL, MG, AS, MCW, ERG, LB and JCM contributed to the developing the Standardised Operating Procedure for the study. CCM, SNC, RSC, CW, CBO, HF, CFMM, AM, RF, SK, LT, CPL, MG, AS, MCW and JCM conducted the intervention. CCM, CFMM and WHMS contributed to physical activity measurements. KML and CCM drafted the paper and performed the statistical analysis for the manuscript and are joint first authors. All authors contributed to a critical review of the manuscript during the writing process. All authors approved the final version to be published.

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Table 1 Characteristics of individuals by sex and age; data obtained from the first screening questionnaire¹

| | Sex | | P | Age | | P |
|------------------------------|-------------|-------------|--------|------------|-------------|--------|
| | Male | Female | | <45 y | ≥45 y | |
| Total (n) | 1971 | 3591 | - | 3484 | 1956 | - |
| Sex - female (%) | - | - | - | 67.5 | 35.5 | <0.001 |
| Age (years) ² | 41.6 (13.1) | 39.2 (12.4) | <0.001 | 32.1 (7.0) | 54.2 (6.98) | 0.001 |
| Age range (years) | 15-87 | 15-76 | - | 15-44 | 45-87 | - |
| Pregnant (%) | - | 5.0 | - | 5.1 | 0.2 | <0.001 |
| Therapeutic diet (%) | 6.4 | 6.7 | 0.609 | 6.4 | 7.0 | 0.434 |
| Food allergy/intolerance (%) | 8.3 | 14.5 | <0.001 | 12.8 | 11.7 | 0.239 |
| Internet access (%) | 99.5 | 99.4 | 0.642 | 99.7 | 99.0 | 0.002 |
| Heard about Food4Me | | | | | | |
| Word of Mouth | 30.0 | 30.4 | 0.762 | 35.9 | 20.7 | <0.001 |
| Internet Search | 8.5 | 8.2 | 0.671 | 9.4 | 6.6 | 0.001 |
| Food4Me Website | 2.5 | 1.9 | 0.188 | 2.0 | 2.4 | 0.349 |
| Social Media | 3.4 | 5.5 | 0.001 | 6.5 | 1.9 | <0.001 |
| Magazine/Newspaper | 50.0 | 45.8 | 0.005 | 41.4 | 57.2 | <0.001 |
| TV/radio advert | 1.9 | 2.1 | 0.758 | 1.4 | 3.1 | <0.001 |
| Poster/leaflet | 2.2 | 2.5 | 0.477 | 1.8 | 3.3 | 0.001 |
| Other | 9.5 | 10.2 | 0.397 | 9.6 | 10.6 | 0.228 |

¹Chi squared tests and ANOVA were used to test for significant differences across categorical and continuous variables respectively.

²Values are means ± SDs

Table 2 Characteristics of individuals by country; data obtained from the first screening questionnaire¹

| | All | Country | | | | | | |
|----------------------------------|----------------|----------------|-----------------|-----------------|-----------------|----------------|-----------------|-----------------|
| | | UK | IRE | GER | NED | ESP | POL | GRE |
| Total (n) | 5562 | 599 | 586 | 788 | 721 | 1839 | 458 | 571 |
| Sex - female (%) | 64.6 | 70.6** | 65.0 | 70.6** | 48.7*** | 61.9* | 77.3*** | 68.0 |
| Age (years) ² | 40.0 (12.7) | 37.2 (9.6)* | 38.0 (12.4)* | 44.5 (13.9)* | 49.3 (13.9)* | 38.4 (9.6)* | 36.0 (12.6)* | 37.7 (11.5)* |
| Age range (years) | 15-87 | 17-87 | 15-72 | 15-80 | 18-79 | 18-78 | 17-73 | 18-70 |
| Age categories | | | | | | | | |
| <45 years (%) | 64.0 | 70.3** | 68.3* | 44.5*** | 45.0*** | 76.1*** | 71.0** | 70.6** |
| ≥45 years (%) | 36.0 | 29.7** | 31.7* | 55.5*** | 55.0*** | 23.9*** | 29.0** | 29.4** |
| Pregnant (%) | 3.3 | 2.7 | 2.9 | 2.3 | 0.3*** | 4.4* | 4.2*** | 4.9 |
| Therapeutic diet (%) | 6.6 | 3.7** | 6.0 | 2.4*** | 4.3* | 3.7*** | 5.7 | 9.6** |
| Food allergy/ Intolerance (%) | 12.4 | 15.7* | 12.5 | 17.1*** | 12.8 | 10.1** | 12.5 | 9.3* |
| Internet access (%) | 99.5 | 100.0 | 99.7 | 99.5 | 99.8 | 99.7 | 99.6 | 97.2*** |
| Heard about Food4Me (%) | | | | | | | | |
| Word of Mouth | 30.3 | 41.8*** | 43.5*** | 15.9*** | 15.8*** | 17.3*** | 67.5*** | 59.7*** |
| Internet Search | 8.3 | 15.4*** | 10.1 | 2.9*** | 1.7*** | 10.6** | 12.0** | 4.9** |
| Food4Me Website | 2.1 | 4.1** | 1.3 | 1.0* | 3.7* | 1.0** | 4.4** | 2.6 |
| Social Media | 4.8 | 8.8*** | 1.6* | 1.7*** | 1.5*** | 5.2 | 3.1 | 10.5*** |
| Magazine/ Newspaper | 47.3 | 7.3*** | 3.6*** | 73.5*** | 76.8*** | 68.8*** | 5.7*** | 4.6*** |
| TV/radio advert | 2.0 | 0.6* | 24.8*** | 0.4** | 1.3 | 0.7*** | 0.2* | 0.0 |
| Poster/leaflet | 2.4 | 5.4*** | 8.5*** | 0.5** | 2.2 | 0.0 | 4.8** | 4.7** |
| Other | 9.9 | 26.6*** | 12.1 | 11.2 | 5.5*** | 3.9*** | 9.2 | 16.5*** |

¹Multinomial regression analyses were used to test for significant differences across categorical variables. For multinomial comparisons across countries, the overall average was used as the reference group. ANOVA and Fisher-Hayter pairwise comparisons were used for continuous variables. Results were deemed significant at * P<0.05, ** P<0.01 and *** P<0.001.

²Values are means ± SDs

Table 3 Characteristics of individuals by sex and age group; data obtained from the second screening questionnaire¹

| | Sex | | P | Age | | P |
|---------------------------------------|-------------|-------------|--------|-------------|-------------|--------|
| | Male | Female | | <45 | ≥45 | |
| Total (n) | 1432 | 2379 | - | 2395 | 1416 | - |
| Sex - female (%) | - | - | - | 63.6 | 60.4 | 0.005 |
| Age (years) ² | 41.6 (13.1) | 39.4 (12.7) | <0.001 | 31.9 (7.06) | 54.2 (7.04) | <0.001 |
| Age range (years) | 18-80 | 17-76 | - | 17-44 | 45-80 | - |
| Ethnicity (%) | | | | | | |
| Caucasian | 96.6 | 97.1 | 0.410 | 96.3 | 97.8 | 0.011 |
| Asians-Chinese | 0.5 | 0.7 | 0.478 | 0.8 | 0.2 | 0.016 |
| Black | 0.1 | 0.2 | 0.622 | 0.1 | 0.3 | 0.273 |
| Mixed | 1.5 | 1.4 | 0.841 | 1.5 | 1.3 | 0.558 |
| Other | 1.3 | 0.7 | 0.040 | 1.2 | 0.4 | 0.014 |
| Anthropometrics | | | | | | |
| Height (m) ² | 1.8 (0.1) | 1.7 (0.1) | <0.001 | 1.7 (0.1) | 1.7 (0.1) | 0.7592 |
| Weight (kg) ² | 85.2 (15.0) | 68.5 (14.2) | <0.001 | 73.0 (16.6) | 77.7 (16.1) | <0.001 |
| BMI (kgm ⁻²) ² | 25.0 (4.9) | 26.5 (4.9) | <0.001 | 26.7 (4.5) | 24.9 (5.1) | <0.001 |
| BMI Classification (%) | | | | | | |
| Under weight | 0.5 | 3.2 | <0.001 | 2.5 | 1.6 | 0.071 |
| Normal Weight | 40.5 | 57.2 | <0.001 | 56.8 | 41.0 | <0.001 |
| Overweight | 41.2 | 25.3 | <0.001 | 27.6 | 37.3 | <0.001 |
| Obese | 17.8 | 14.4 | 0.006 | 13.1 | 20.1 | <0.001 |
| Physical Activity (%) ³ | | | | | | |
| Occupational | | | | | | |
| Light | 73.9 | 72.3 | 0.382 | 69.3 | 78.9 | <0.001 |
| Moderate | 22.1 | 26.7 | 0.007 | 28.8 | 18.7 | <0.001 |
| Heavy | 4.0 | 0.1 | <0.001 | 1.9 | 2.4 | 0.375 |
| Non-Occupational | | | | | | |
| Sedentary | 28.2 | 38.9 | <0.001 | 35.8 | 33.6 | 0.235 |
| Moderately active | 54.5 | 51.9 | 0.182 | 50.9 | 56.1 | 0.008 |
| Active | 17.3 | 9.2 | <0.001 | 13.3 | 10.3 | 0.020 |
| Reason for interest (%) | | | | | | |
| Personalised nutrition | 76.4 | 73.6 | 0.051 | 76.9 | 72.7 | 0.004 |
| Knowing what foods are best | 82.9 | 79.3 | 0.026 | 81.8 | 78.8 | 0.007 |
| Losing weight | 42.6 | 52.5 | <0.001 | 46.4 | 52.9 | <0.001 |
| Gaining weight | 3.3 | 1.1 | <0.001 | 2.5 | 0.9 | <0.001 |
| Concerns for health | 88.0 | 87.2 | 0.465 | 88.9 | 85.1 | 0.001 |

¹Chi squared tests and ANOVA were used to test for significant differences across categorical and continuous variables respectively.

²Values are means ± SDs

³Physical activity was estimated from the food frequency questionnaire in 2763 individuals

⁶Compared with less than 1 hour

Table 4 Characteristics of individuals by country; data obtained from the second screening questionnaire¹

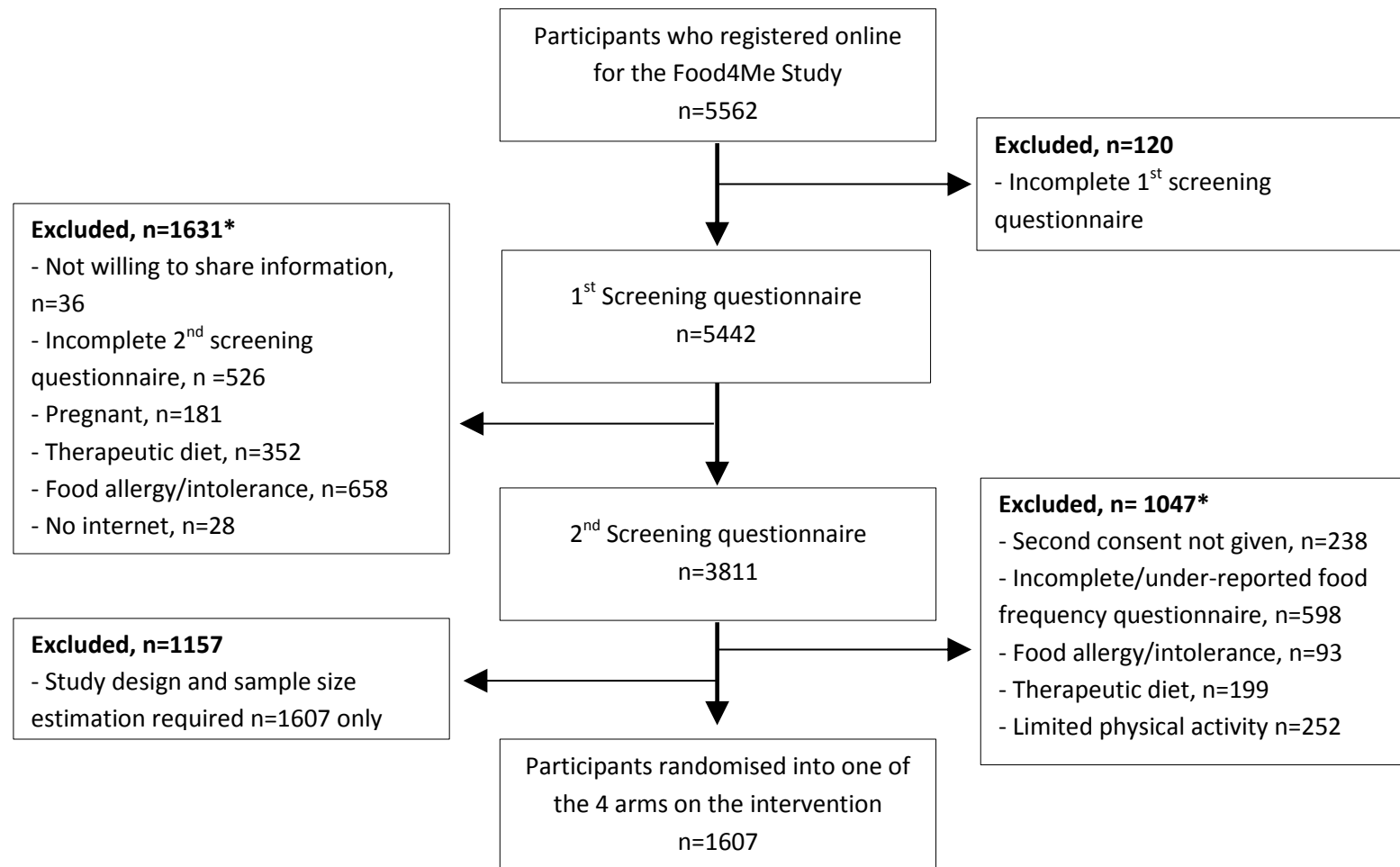
| | All | Country | | | | | | |
|--------------------------------------|-------------|----------------|----------------|----------------|-----------------|----------------|----------------|----------------|
| | | UK | IRE | GER | NED | ESP | POL | GRE |
| Total (n) | 3811 | 413 | 405 | 535 | 511 | 1206 | 340 | 401 |
| Sex - female (%) | 62.4 | 66.8 | 62.7 | 66.4 | 56.6* | 57.2** | 73.8*** | 65.8 |
| Age (years) | 40.2 (12.9) | 37.0 (13.3) | 37.9 (12.4) | 44.9 (13.9) | 49.2 (14.2) | 38.3 (9.47) | 36.3 (12.8) | 37.4 (11.6) |
| Age range (years) ² | 17-80 | 18-72 | 18-72 | 17-80 | 18-79 | 18-70 | 17-73 | 18-70 |
| Age categories (%) | | | | | | | | |
| <45 years | 62.8 | 70.5** | 67.7 | 44.5*** | 31.5*** | 76.0*** | 69.7* | 69.3* |
| ≥45 years | 37.2 | 29.5** | 32.3 | 55.5*** | 68.5*** | 24.0*** | 30.3* | 30.7* |
| Ethnicity (%) | | | | | | | | |
| Caucasian | 96.9 | 89.6*** | 97.5 | 96.8 | 96.5 | 97.8 | 100 | 99.0* |
| Asians-Chinese | 0.6 | 3.2*** | 1.0 | 0.2 | 0.8 | 0.1 | 0.0 | 0.0 |
| Blacks | 0.2 | 0.5 | 0.0 | 0.4 | 0.2 | 0.1 | 0.0 | 0.3 |
| Mixed | 1.4 | 3.2** | 1.2 | 1.7 | 1.4 | 1.7 | 0.0 | 0.0 |
| Other | 0.9 | 3.6*** | 0.3 | 0.9 | 1.2 | 0.4 | 0.0 | 0.8 |
| Anthropometrics | | | | | | | | |
| Height (m) ² | 1.7 (0.1) | 1.7 (0.1) | 1.7 (0.1) | 1.7 (0.1)* | 1.7 (0.1)* | 1.7 (0.1)* | 1.7 (0.1)* | 1.7 (0.1)* |
| Weight (kg) ² | 74.8 (16.6) | 73.4 (15.6) | 75.2 (16.9) | 73.2 (14.2) | 77.3 (15.0)* | 74.8 (17.9) | 72.1 (16.3) | 76.4 (17.8) |
| BMI (kgm ²) ² | 25.6 (5.0) | 25.5 (5.0) | 25.7 (4.9) | 24.4 (3.9)* | 25.4 (4.6) | 25.9 (5.2) | 25.1 (4.9) | 26.7 (5.8)* |
| BMI Classification (%) | | | | | | | | |
| Underweight | 2.2 | 2.0 | 2.3 | 2.6 | 2.0 | 1.9 | 3.6 | 1.5 |
| Normal Weight | 50.9 | 53.0 | 49.1 | 59.1*** | 52.6 | 49.0 | 51.2 | 42.7** |
| Overweight | 31.2 | 31.0 | 31.2 | 28.1 | 32.1 | 31.6 | 29.3 | 35.2 |
| Obese | 15.7 | 14.0 | 17.4 | 10.2** | 13.4 | 17.5 | 16.0 | 20.6* |
| Physical Activity (%) ³ | | | | | | | | |
| Occupational | | | | | | | | |
| Light | 72.9 | 69.5 | 69.6 | 79.0* | 62.5*** | 82.2*** | 66.8* | 70.6 |
| Moderate | 25.0 | 27.5 | 28.1 | 20.4 | 34.1*** | 16.8*** | 31.2* | 26.0 |
| Heavy | 2.1 | 3.0 | 2.3 | 0.5 | 3.4 | 1.1 | 2.1 | 3.4 |
| Non-Occupational | | | | | | | | |
| Sedentary | 35.0 | 25.5** | 21.7*** | 31.5 | 23.4*** | 40.7** | 48.6*** | 50.2*** |
| Moderately active | 52.9 | 55.0 | 67.6*** | 61.3** | 64.8*** | 46.6** | 42.1** | 35.3*** |
| Active | 12.2 | 19.5*** | 10.7 | 7.3** | 11.8 | 12.7 | 9.3 | 14.6 |
| Reason for interest (%) | | | | | | | | |
| Personalised nutrition | 75.4 | 83.3*** | 82.0** | 77.2 | 78.7 | 78.7* | 55.6*** | 60.6*** |
| Knowing what | 80.7 | 73.1*** | 76.8 | 74.6** | 81.0** | 87.7*** | 86.8** | 73.6** |

| | | | | | | | | |
|---------------------|------|--------|------|---------|---------|--------|------|---------|
| foods are best | | | | | | | | |
| Losing weight | 48.8 | 44.6 | 47.7 | 45.2 | 36.6*** | 51.2 | 53.2 | 63.3*** |
| Gaining weight | 1.9 | 1.9 | 1.0 | 2.1 | 1.2 | 2.3 | 2.4 | 1.8 |
| Concerns for health | 87.5 | 92.5** | 90.6 | 81.1*** | 76.5*** | 91.0** | 90.6 | 88.5 |

¹Multinomial regression analyses were used to test for significant differences across categorical variables. For multinomial comparisons across countries, the overall average was used as the reference group. ANOVA and Fisher-Hayter pairwise comparisons were used for continuous variables. Results were deemed significant at * P<0.05, ** P<0.01 and *** P<0.001.

²Values are means ± SDs

³Physical activity was estimated from the food frequency questionnaire in 2763 individuals



* Total number of participants reporting one or more exclusion criteria

Fig 1. Food4Me Proof of Principle Study flow-chart

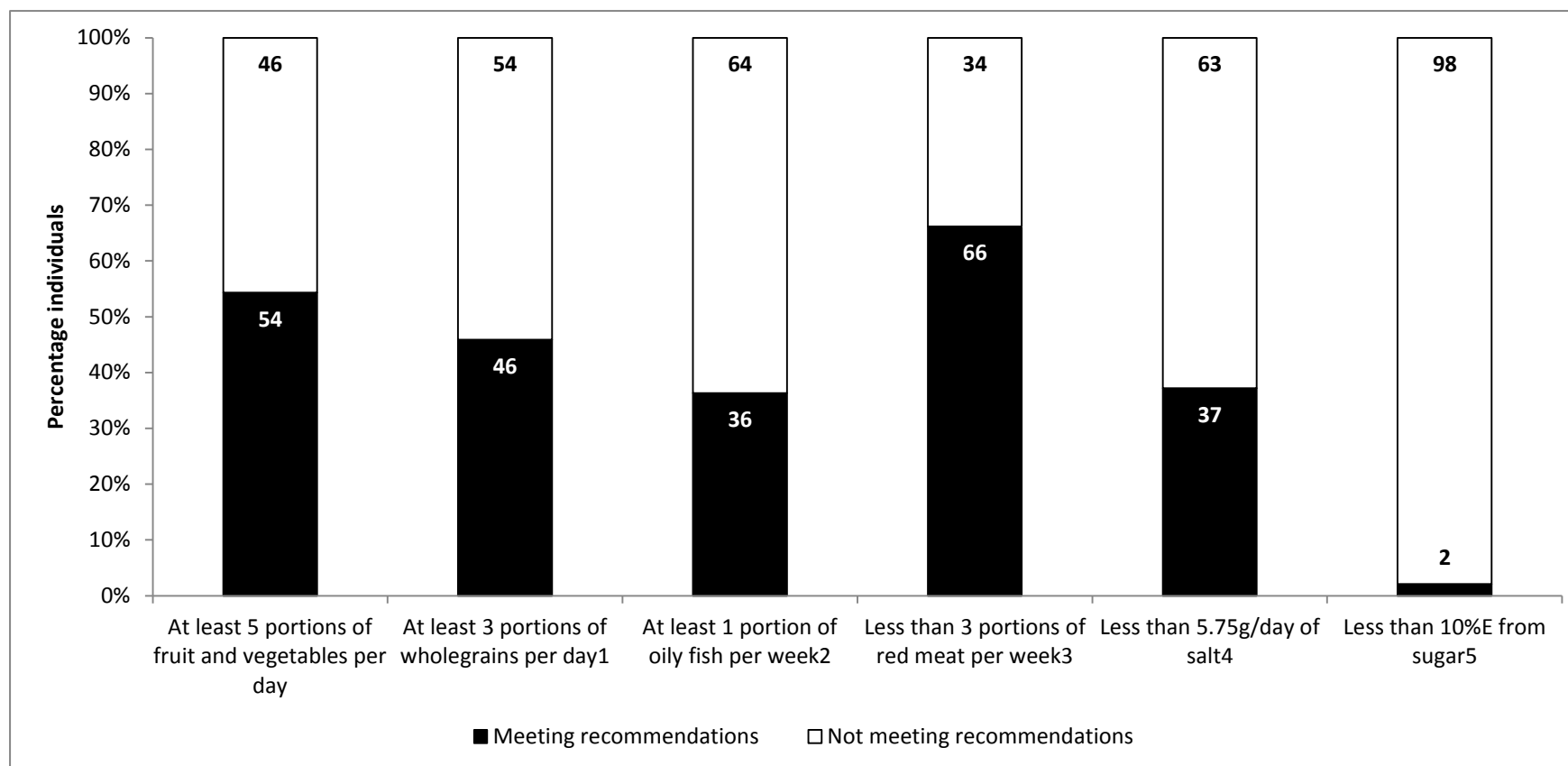


Fig 2. Percentage of participants meeting food-based dietary recommendations.

¹Equivalent to 48g/day; ²More than 150g/week of oily fish; ³Consume less than 450g/week of red or processed meat; ⁴Based on IoM recommendations[33]; ⁵Based on draft Scientific Advisory Committee for Nutrition (SACN) recommendations [27]

Online Resource 1 (Supplementary material)



1.1



1.2



1.3

Figure S1. Screen shots of the Food4Me 1.1 Website; 1.2 Facebook and 1.3 Twitter pages









Are you interested in receiving personalised dietary advice?

We need healthy volunteers aged 18 and older to take part in a study examining the concept of personalised nutrition.

What will the study involve?

- Individuals will be given healthy eating advice **tailored** specifically to their personal health status, lifestyle and genetic make-up.
- The latest **cutting edge technology** will be used to measure dietary intake, biomarkers of health and genes related to nutrition.
- This innovative study will be conducted through the internet modelling a **personalised nutrition service**.

If you are interested in the study, please go to the volunteer section of our website at:
www.food4me.org



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www.food4me.org

Are you interested in receiving free personalised dietary advice?

If you are aged 18 and above, and you want to improve your diet and health, sign up and make a change!

What will the study involve?

- ✓ Individuals will be given healthy eating advice **tailored** specifically to their personal health status, lifestyle and genetic make-up.
- ✓ The latest **cutting edge technology** will be used to measure your dietary intake, biomarkers of health and genes related to nutrition.
- ✓ This innovative study will be conducted through the **internet** and you will receive **free personalised dietary advice** during 6 months.

If you are interested in taking part, please go to the volunteer section of our website at: www.food4me.org

Any queries contact: food4me@reading.ac.uk




Figure S2. Examples of UK poster advertisements used during recruitment

Methods: Screening Questionnaires

Unlike in other countries, Dutch individuals had the opportunity to register and then choose whether or not to complete the first screening questionnaire. IP addresses and cookies were not used to identify individuals, as unique usernames prevented participants from entering duplicate entries from the same user and allowed individuals using the same computer, e.g. family members, to register for the study. No monetary incentives were offered for completing the screening questionnaires. Participants were informed that, if they were randomised into the intervention study, they would receive healthy eating and lifestyle advice, as well as non-diagnostic information relating to their health status during, or on completion of, the study. No randomization of adaptive questioning was employed. Automated completeness checks prevented participants from submitting incomplete questionnaire responses. Completion rates were estimated as the ratio of users who finished the survey to users who provided their consent to participate. Once questionnaires were submitted, participants could not change their responses and these responses were later extracted from the server into databases for statistical analysis. Under-reporting of dietary intakes via the FFQ was identified from a comparison between expected energy expenditure, based on a multiple (1.1) of predicted basal metabolic rate and reported energy intakes [34]. Participants who under-reported in their FFQ were asked to repeat the FFQ up to two times before being excluded. Participants had 7 days to complete the screening questionnaires. A reminder was sent at day 5; if they did not complete the screening FFQ by day 7, they were excluded from the study. Individuals who were deemed unsuitable for the study received an email notification that they did not match the inclusion criteria and so were excluded from further elements of the study.

Table S1 Health-related characteristics by sex and age group; data obtained from the second screening questionnaire¹

| | Sex | | P | Age | | P |
|---------------------|------|--------|--------|------|------|--------|
| | Male | Female | | <45 | ≥45 | |
| Total (n) | 1432 | 2379 | - | 2395 | 1416 | - |
| Medication use | | | | | | |
| Prescribed | 25.0 | 38.0 | <0.001 | 26.3 | 44.8 | <0.001 |
| Non-prescribed | 8.7 | 13.1 | <0.001 | 9.6 | 14.5 | <0.001 |
| Diseases | | | | | | |
| Cancer | 1.8 | 2.6 | 0.085 | 1.1 | 4.3 | <0.001 |
| High blood pressure | 12.6 | 7.3 | <0.001 | 3.6 | 18.9 | <0.001 |
| Heart disease | 3.4 | 0.9 | <0.001 | 0.7 | 3.8 | <0.001 |
| Liver disease | 2.4 | 1.2 | 0.007 | 1.4 | 2.1 | 0.083 |
| Kidney disease | 1.1 | 0.9 | 0.708 | 0.7 | 1.4 | 0.033 |
| Arthritis | 2.1 | 3.2 | 0.046 | 0.8 | 6.1 | <0.001 |
| Osteoporosis | 0.6 | 1.6 | 0.004 | 0.3 | 2.8 | <0.001 |
| Ulcers | 2.3 | 1.6 | 0.114 | 1.2 | 3.0 | <0.001 |
| Fibromyalgia | 0.1 | 1.1 | 0.001 | 0.3 | 1.5 | <0.001 |
| Diabetes | 1.2 | 0.8 | 0.230 | 0.4 | 1.8 | <0.001 |
| Lung disease | 3.0 | 2.7 | 0.519 | 2.7 | 2.9 | 0.742 |
| Allergies | 20.9 | 19.3 | 0.235 | 20.7 | 18.6 | 0.118 |
| Epilepsy | 0.5 | 0.4 | 0.759 | 0.6 | 0.2 | 0.095 |
| Thyroid disease | 1.5 | 11.3 | <0.001 | 5.4 | 11.3 | <0.001 |
| Diagnosed anaemia | 1.5 | 9.8 | <0.001 | 6.4 | 7.2 | 0.357 |
| Blood disorders | 0.8 | 2.0 | 0.005 | 1.8 | 1.3 | 0.248 |
| Alcoholism | 0.4 | 0.0 | 0.008 | 0.1 | 0.3 | 0.060 |
| Drug addiction | 0.4 | 0.1 | 0.071 | 0.2 | 0.3 | 0.651 |
| Depression | 5.2 | 8.4 | <0.001 | 6.0 | 9.3 | <0.001 |
| Smoker | 13.5 | 12.6 | 0.417 | 15.0 | 9.46 | <0.001 |
| Ex-smoker | 37.0 | 33.9 | 0.083 | 26.1 | 49.5 | <0.001 |

¹Chi squared tests and ANOVA were used to test for significant differences across categorical and continuous variables respectively.

²Values are means ± SDs

Table S2 Health-related characteristics of individuals by country; data obtained from the second screening questionnaire¹

| | All | Country | | | | | | |
|-------------------------------|------|---------|--------|--------|---------|---------|---------|---------|
| | | UK | IRE | GER | NED | ESP | POL | GRE |
| Total (n) | 3811 | 413 | 405 | 535 | 511 | 1206 | 340 | 401 |
| Medication | | | | | | | | |
| Prescribed | 33.2 | 30.8 | 32.6 | 38.5* | 47.4*** | 28.4** | 29.4 | 28.7 |
| Non-prescribed | 11.5 | 8.5 | 10.9 | 16.3** | 9.6 | 10.7 | 17.9*** | 8.0* |
| Clinically diagnosed diseases | | | | | | | | |
| Cancer | 2.3 | 3.2 | 3.0 | 2.6 | 4.5** | 1.2* | 0.9 | 1.8 |
| High blood pressure | 9.3 | 5.1** | 6.7 | 9.9 | 15.3*** | 9.0 | 12.9* | 5.5 |
| Heart disease | 1.9 | 0.2* | 1.2 | 2.2 | 3.9** | 1.7 | 3.2 | 0.5 |
| Liver disease | 1.7 | 0.5 | 0.7 | 0.4* | 0.6 | 3.2** | 3.2* | 0.8 |
| Kidney disease | 1.0 | 0.2 | 0.5 | 0.9 | 0.6 | 1.5 | 1.8 | 0.5 |
| Arthritis | 2.8 | 3.6 | 4.9* | 3.2 | 3.1 | 2.0 | 0.6* | 3.0 |
| Osteoporosis | 1.2 | 0.0 | 1.2 | 0.8 | 2.9** | 0.8 | 1.5 | 1.5 |
| Ulcers | 1.9 | 0.7* | 3.0 | 0.4 | 1.0 | 2.1 | 4.4** | 2.5 |
| Fibromyalgia | 0.8 | 0.7 | 0.5 | 0.4 | 1.8* | 0.8 | 0.3 | 0.5 |
| Diabetes | 0.9 | 0.5 | 0.5 | 0.6 | 1.8 | 0.8 | 0.9 | 1.8 |
| Lung disease | 2.8 | 1.0* | 0.7* | 1.7 | 5.5** | 3.1 | 2.9 | 3.7 |
| Allergies | 19.9 | 15.3* | 13.3** | 20.6 | 18.8 | 26.9*** | 14.1* | 15.7* |
| Epilepsy | 0.5 | 0.5 | 0.5 | 0.8 | 0.4 | 0.6 | 0.0 | 0.0 |
| Thyroid disease | 7.6 | 4.8* | 3.7** | 11.6** | 4.3** | 5.7* | 9.1 | 11.6** |
| Diagnosed anaemia | 6.7 | 6.1 | 3.2** | 0.6*** | 9.8* | 8.3 | 8.8 | 8.7 |
| Blood disorders | 1.6 | 1.0 | 1.0 | 0.4* | 0.6 | 2.3 | 2.9 | 2.2 |
| Alcoholism | 0.2 | 0.2 | 0.0 | 0.4 | 0.6 | 0.0 | 0.0 | 0.3 |
| Drug addiction | 0.2 | 0.5 | 0.0 | 0.0 | 0.4 | 0.0 | 0.0 | 0.5 |
| Depression | 7.2 | 12.6*** | 9.9 | 3.6** | 8.0 | 7.6 | 2.7** | 5.2 |
| Smoker | 12.9 | 5.6*** | 8.9* | 9.0* | 6.9*** | 16.5** | 8.8* | 30.2*** |
| Ex-smoker | 35.1 | 20.6*** | 30.3 | 37.4 | 48.5*** | 39.4* | 19.2*** | 31.8 |

¹Multinomial regression analyses were used to test for significant differences across categorical variables. For multinomial comparisons across countries, the overall average was used as the reference group. ANOVA and Fisher-Hayter pairwise comparisons were used for continuous variables. Results were deemed significant at * P<0.05, ** P<0.01 and *** P<0.001.

²Values are means ± SDs

Table S3. Mean intakes of key foods and food groups¹

| Dietary target | Fruit and vegetables | Wholegrain products | Oily fish | Red meat | Salt | Sugars |
|-----------------|----------------------|---------------------|----------------|----------------|-------------|---------------|
| | g/day (SD) | g/day (SD) | g/week (SD) | g/week (SD) | g/day (SD) | % energy (SD) |
| All | 651.4 (488.6) | 173.0 (208.5) | 171.0 (236.3) | 573.0 (516.9) | 7.56 (4.9) | 21.4 (6.6) |
| Sex | | | | | | |
| Male | 645.2 (410.8) | 188.6 (237.7) | 200.0 (237.2) | 713.0 (597.3) | 8.56 (4.79) | 20.4 (6.3) |
| Female | 655.0 (528.7) | 163.9 (188.8) | 154.2 (234.2) | 491.4 (443.8) | 6.97 (4.83) | 21.4 (6.6) |
| <i>P</i> | 0.611 | 0.003 | <0.001 | <0.001 | <0.001 | <0.001 |
| Age category | | | | | | |
| <45 years | 633.4 (519.6) | 156.7 (198.9) | 169.8 (222.8) | 594.5 (548.4) | 7.64 (4.50) | 21.5 (6.5) |
| ≥45 years | 681.3 (430.5) | 200.2 (220.9) | 173.1 (257.5) | 537.2 (457.5) | 7.43 (5.45) | 21.2 (6.6) |
| <i>P</i> | 0.013 | <0.001 | 0.719 | 0.005 | 0.272 | 0.238 |
| Country | | | | | | |
| UK | 687.0 (425.3) | 176.2 (192.5) | 168.2 (191.6) | 466.7 (392.2)* | 7.3 (4.1) | 22.9 (6.4)* |
| Ireland | 696.7 (881.9) | 222.7 (152.8)* | 163.0 (224.7) | 592.4 (492.0) | 7.7 (3.70) | 21.3 (6.3) |
| Germany | 675.8 (398.4) | 182.2 (161.6) | 104.9 (142.6)* | 445.1 (599.3)* | 6.9 (4.1) | 21.6 (6.3) |
| The Netherlands | 647.5 (351.3) | 319.2 (290.2)* | 152.0 (213.8) | 482.3 (445.8)* | 8.3 (4.4)* | 20.6 (6.1) |
| Spain | 641.8 (419.9) | 73.6 (110.6)* | 260.3 (249.8)* | 746.1 (537.3)* | 7.9 (6.3) | 21.3 (6.9) |
| Poland | 595.8 (436.8) | 214.9 (268.7)* | 132.8 (176.9) | 536.1 (538.7) | 8.2 (4.8) | 21.8 (7.0) |

¹Multinomial regression analyses were used to test for significant differences across categorical variables. For multinomial comparisons across countries, the overall average was used as the reference group. ANOVA and Fisher-Hayter pairwise comparisons were used for continuous variables. Results across countries were deemed significant at * $P < 0.05$

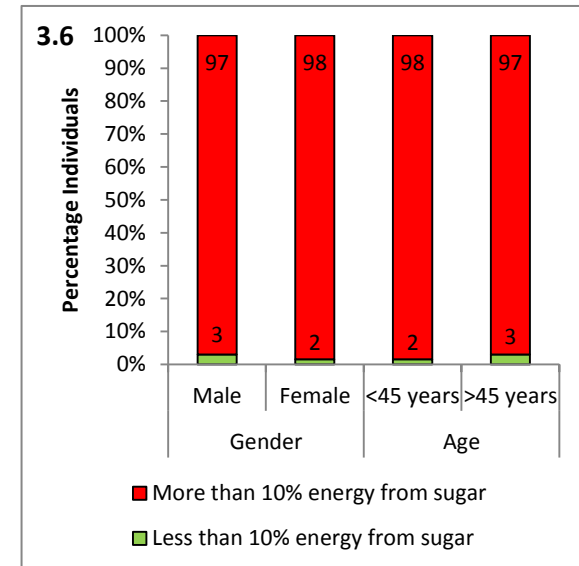
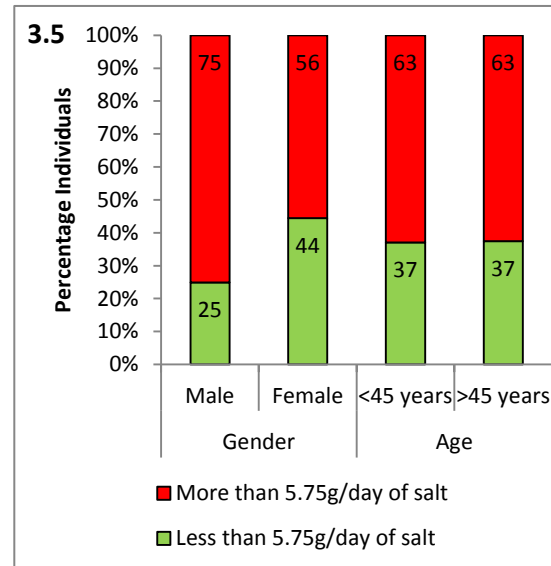
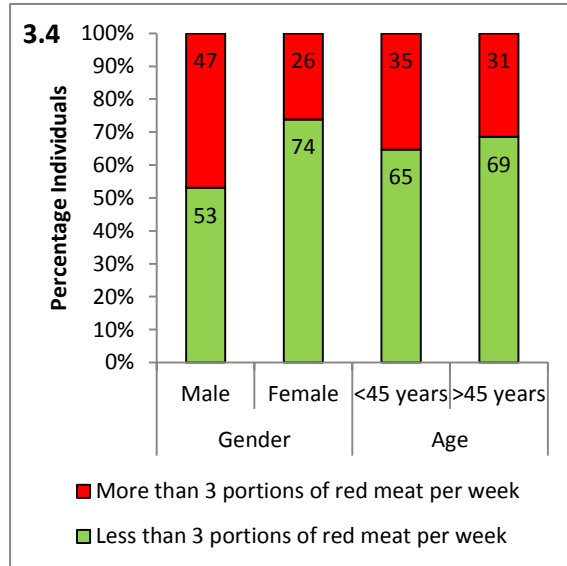
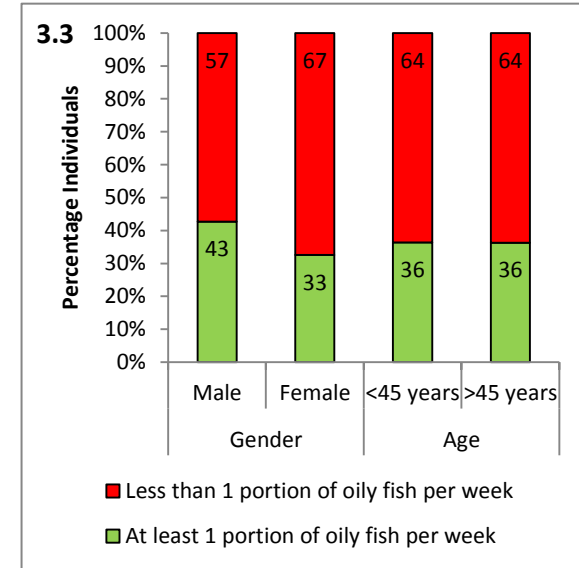
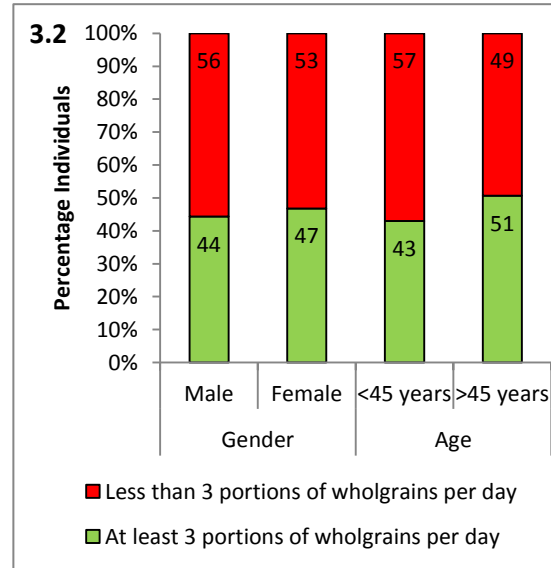
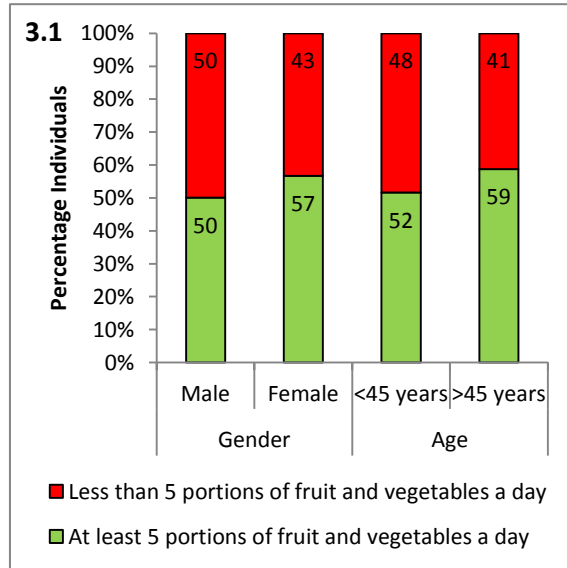


Figure S3. Percentage of individuals meeting the dietary recommendations for 3.1 At least 5 portions of fruit and vegetables a day (400g/day); 3.2 At least 3 portions of whole grain per day (48g/day); 3.3 At least 1 or more servings of oily fish per week (150g/week); 3.4 Less than 3 portions of red or processed meat per week (450g/week); 3.5 Less than 5.75g salt per day; 3.6 Less than 10% energy from sugars by age and sex.

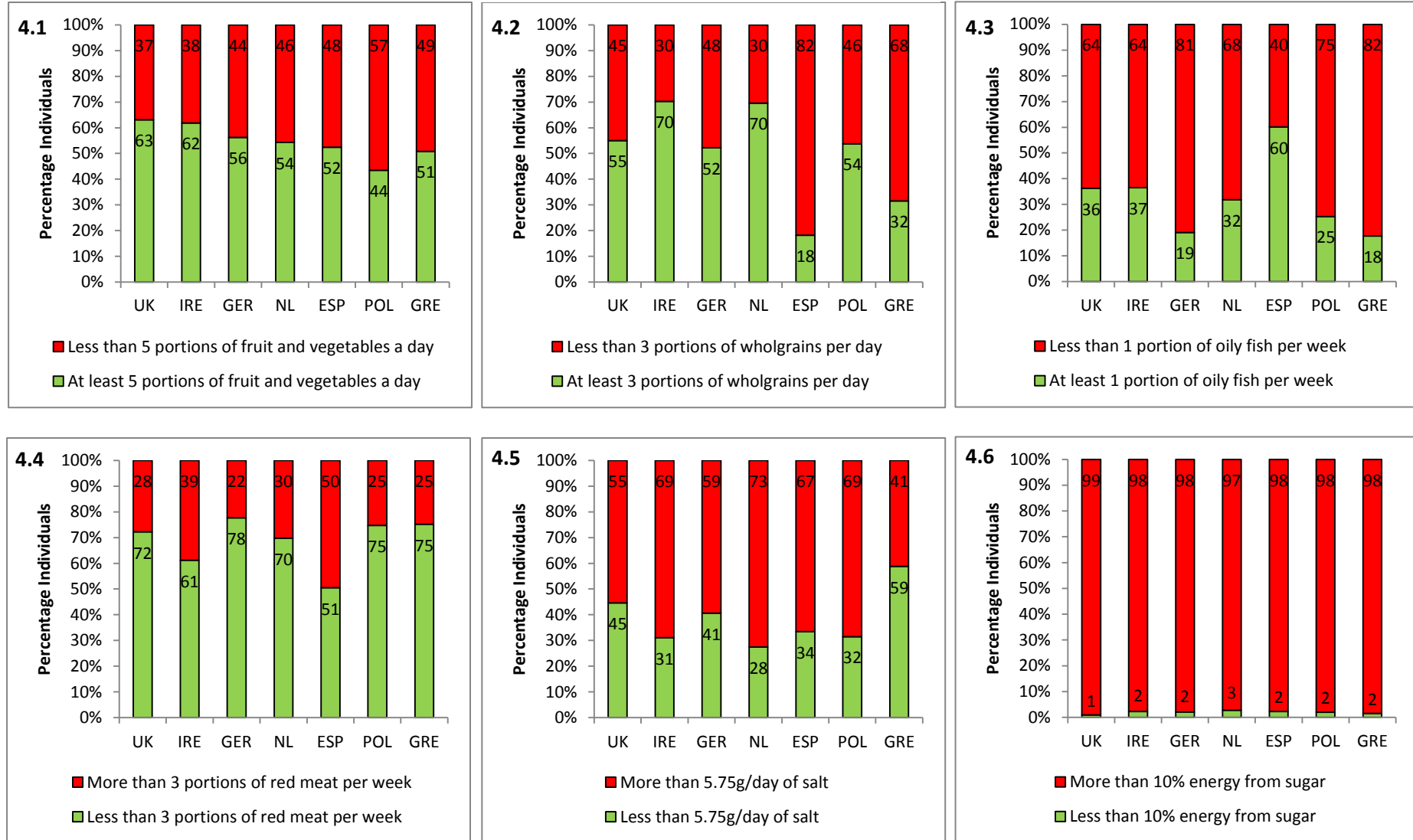


Figure S4. Percentage of individuals meeting the dietary recommendations for 4.1 At least 5 portions of fruit and vegetables a day (400g/day); 4.2 At least 3 portions of whole grain per day (48g/day); 4.3 At least 1 or more servings of oily fish per week (150g/week); 4.4 Less than 3 portions of red or processed meat per week (450g/week); 4.5 Less than 5.75g salt per day; 4.6 Less than 10% energy from sugars by country

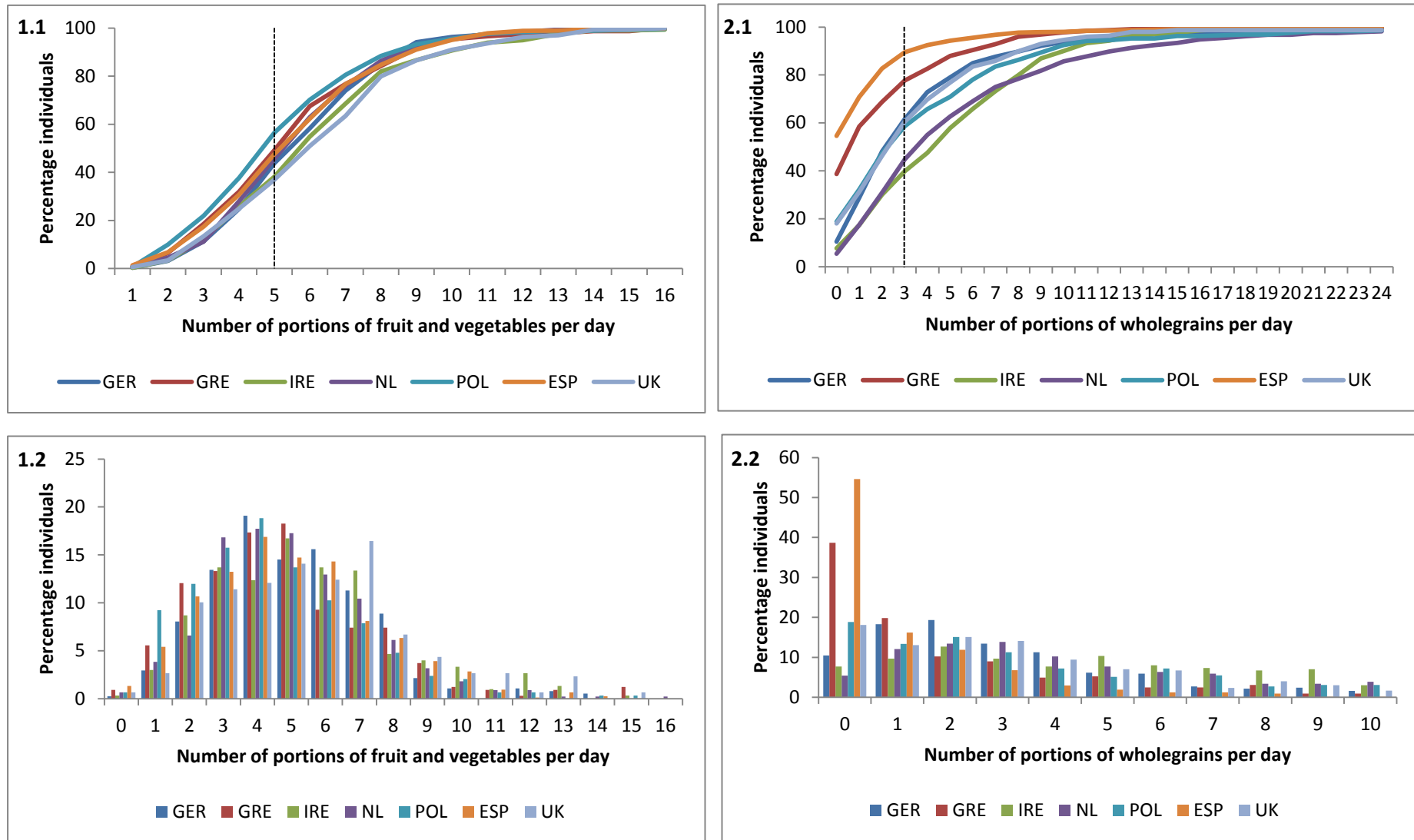


Figure S5. Cumulative (.1) and relative percentage (.2) of portion consumption of 1 fruit and vegetables and 2 wholegrain by country. The recommended number of portions is indicated by the dotted line.

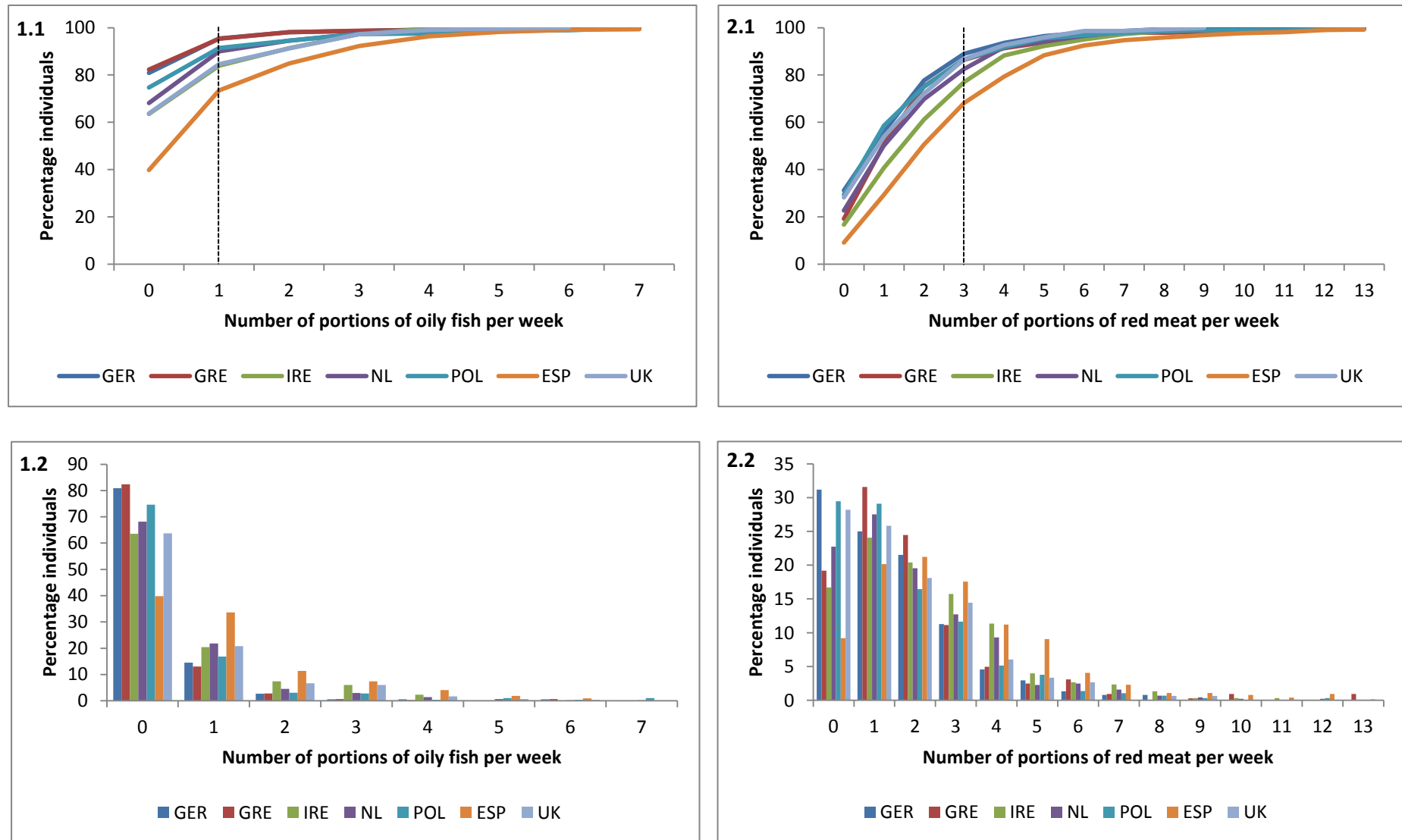


Figure S6. Cumulative (.1) and relative percentage (.2) of portion consumption of 1 fruit and vegetables and 2 wholegrain by country. The recommended number of portions is indicated by the dotted line.