HIGHER VEGETABLE PROTEIN CONSUMPTION, ASSESSED BY AN ISOENERGETIC MACRONUTRIENT EXCHANGE MODEL, IS ASSOCIATED WITH A LOWER PRESENCE OF OVERWEIGHT AND OBESITY IN THE WEB-BASED FOOD4ME EUROPEAN STUDY

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SHORT TITLE: Vegetable protein intake and risk of obesity

Abstract

The objective was to evaluate differences in macronutrient intake and to investigate the possible association between consumption of vegetable protein and the risk of
overweight/obesity, within the Food4Me randomised, online intervention. Differences in macronutrient consumption among the participating countries grouped by EU Regions (Western Europe, British Isles, Eastern Europe and Southern Europe) were assessed. Relation of protein intake, within isoenergetic exchange patterns, from vegetable or animal sources with risk of overweight/obesity was assessed through the multivariate nutrient density model and a multivariate-adjusted logistic regression.

A total of 2413 subjects who completed the Food4Me screening were included, with self-reported data on age, weight, height, physical activity and dietary intake.

As success rates on reducing overweight/obesity are very low, form a public health perspective, the elaboration of policies for increasing intakes of vegetable protein and reducing animal protein and sugars, may be a method of combating overweight/obesity at a population level.

**Trial registration:** The Food4Me trial was registered as a RCT (NCT01530139) at clinicaltrials.gov ([http://clinicaltrials.gov/show/NCT01530139](http://clinicaltrials.gov/show/NCT01530139)).

**Keywords**

Food4Me Study; Macronutrient intake; Overweight and Obesity; Protein quality; Vegetable protein.

**Introduction**

The current worldwide increase in overweight and obesity prevalence represents a serious threat to health and quality of life in most societies (Ng et al., 2014). The growing concern for being healthy is taking a lead role in the understanding of social culture and population welfare (Kumanyika et al., 2008). This issue is supported by research evidence, in which according to the recent report of GBD Risk Factors, dietary factors are one of the leading risk factors of non-communicable diseases, and importantly, are high preventable (Phillips et al., 2014, Cecchini et al., 2010, G. B. D. Risk Factors Collaborators 2016).

Lifestyle choices, mainly dietary and physical activity patterns, are key factors for preventing the onset of obesity features, type 2 diabetes, cardiovascular diseases, accompanying comorbidities and cancer (Mathers 2015, Raguso et al., 2006, Milte and McNaughton 2015, Grosso et al., 2017). Recent studies on the relationship between
protein consumption and prevention/management of obesity, as well as interactions between macronutrient intake and age, sex and physical activity, have revealed controversial or inconsistent results (Riddle et al., 2016, Levine et al., 2014, Hernandez-Alonso et al., 2016, Larsen et al., 2010, Navas-Carretero et al., 2016).

In this context, the development of new epidemiological concepts and tools may contribute to envisage public policies and to help health professionals modify risk factors of disease onset to implement adequate therapeutic approaches during all life stages (Srikanthan and Karlamangla 2014, Stenholm, Rantanen, et al., 2007, Stenholm, Sainio, et al., 2007, Woods et al., 2016). Precision nutrition data, based on genetics, clinical history, likes/dislikes, lifestyles, perinatal nutrition, social/cultural constraints, etc., tries to harmonize accurate prediction equations and algorithms with sensitive procedures to give more individualised dietary advice for health management (Forster et al., 2016, Bauer et al., 2014). Furthermore, the increasing use of internet, as well as the reduced timetables of working families to attend to the health professional practice, creates a necessity to develop procedures that help professionals to acquire and provide valid information from users, in order to prescribe personalised and valuable nutritional advice (San-Cristobal et al., 2013, Gibney and Walsh 2013, Fallaize et al., 2015).

Moreover, in research and primary care there is a growing concern about the relative lack of success of public health policies in developed as well as developing countries, and the necessity to combine personalised with public health nutrition advices (Hafekost et al., 2013, Kumanyika et al., 2008), where the role of energy-yielding macronutrients on the obesity epidemic needs to be ascertained, although the intake of micronutrients and other food derived compounds will receive attention in the future, when more precise information about micronutrients, food processing effects, etc., is available.

To address such issues, the current analysis used data from the Food4Me study (www.food4me.org), which is an European, randomised controlled trial, designed to investigate the effect of different types of personalised nutrition advice using a web-based intervention platform (Celis-Morales, Livingstone, Marsaux, et al., 2015).

In this context, the authors hypothesized that an increased consumption of protein from vegetable origin instead of protein from animal origin, using models of isocaloric nutrient exchange, would be associated with a reduction in overweight/obesity.

Therefore, the aim of the present study was to evaluate the differences in macronutrient
intake among participating centres, with populations presenting different features, in the Food4Me study, and to assess the role of the substitution of animal and vegetable protein sources by other macronutrients, on the prevalence of overweight or obesity.

**Subjects and methods**

**Study design**

The current investigation is an ancillary cross-sectional analysis from the Food4Me randomised controlled trial (Food4Me 2011), including data from 2413 participants. Food4Me was a Pan-European project designed for evaluating the opportunities and challenges of personalised nutrition (Food4Me 2011). The Food4Me study included four arms, conducted in 7 European Countries. The intervention was designed to emulate a real-life web-based personalised nutrition service. The extended protocol of the present intervention has been described elsewhere (Celis-Morales, Livingstone, Marsaux, et al., 2015).

**Ethical approval and participant recruitment**

Ethical approval for the study was obtained from all the local research ethics committees at each participating centre. All participants interested in the study were required to sign online consent forms at two stages in the screening process: before sending the first data and before completing the screening food frequency questionnaire (FFQ).

Recruitment for the study was conducted between August 2012 and August 2013, in 7 European centres, as an internet-based personalised nutrition intervention. The recruitment centres for the Food4Me study 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); and Technical University of Munich (Germany). To facilitate recruitment, each centre used one or more of the following strategies: Local and National advertising of the study, via newspapers, radio, the internet, posters, flyers or social media; press releases through the internet (online news media), radio, newspaper; and word of mouth (Livingstone et al., 2016). Participants had to voluntarily register their details on the Food4Me website, participant section (Food4Me 2011), which was designed for the purposes of the study.
Inclusion and exclusion criteria

The Food4Me inclusion criteria were set as wide as possible to include the most representative populations excluding known diseases or prescribed diets. Inclusion criteria were: age > 18 years; to have internet access at home or easily accessible; to be healthy (self-perceived) and without any known food allergy/intolerance.

The exclusion criteria were: pregnancy or planning to be pregnant the next 6 months; following a prescribed diet, including for weight loss, in the last 3 months; to have very limited or no access to the internet; suffering from diabetes, coeliac disease, Crohn’s disease, or any other metabolic conditions implying an alteration of nutritional requirements such as known non-controlled thyroid disorders or food allergies and intolerances.

Additionally, for the specific analysis in the current study, all those participants who reported energy intakes lower than 1.1-fold of the calculated basal metabolic rate (1.1*BMR), or higher than 5000 kcal/day as well as those who provided unrealistic data on height or weight were excluded.

Data collection

Once the interested individuals had registered on the Food4me website, and signed the informed consent forms, they were then asked to fill in online-based questionnaires. Participants were requested to provide age and sex, ease of internet access, pregnancy, food intolerances and/or allergies, in order to automatically proceed to the inclusion/exclusion of the participants. In addition, self-reported socio-demographic information, health-related issues, anthropometrics data and dietary intakes were collected.

Dietary intake evaluation

An online food frequency questionnaire (FFQ) was completed, where participants provided details of the frequency and portion size of 157 commonly-used food items they had consumed within the previous month, as described elsewhere (Forster et al., 2014).
This questionnaire provided the list of food items, categorised in food groups, and for each item, the participants had to mark the frequency they had consumed it, expressed as times per month/week/day. In relation to the estimated portion size, for each item, appropriate photographs were shown, in order to help the participant to fill in the most realistic weight of the portion, according to these pictures.

Intakes of foods and nutrients were then computed in real time, using a common food composition database, with a small number of local food-item differences (McCance et al., 2002). Both the online FFQ and the food database were developed and validated specifically for the Food4Me trial (Forster et al., 2014, Fallaize et al., 2014, Marshall et al., 2016). In the current analyses, the focus was specifically set on macronutrient and energy intakes of the participants, and the percentual exchange of one macronutrient by another.

**Anthropometric and physical activity assessment**

Body weight and height were self-measured and self-reported via internet following the online instructions available at the website. These self-reported data evidenced good agreement with standard methods, as it was demonstrated in a face-to-face validation of a sample of the Food4Me participants (Celis-Morales, Livingstone, Woolhead, et al., 2015). Physical activity was assessed with the self-reported occupational and non-occupational activities using the Baecke questionnaire (Marsaux et al., 2016). Participants were asked to categorise their occupational activity as light (e.g. administrative and managerial), moderate (e.g. sales worker) or heavy (e.g. equipment operator) and their non-occupational activity as sedentary (no activity or little walking/cycling/exercise), moderately active (intense exercise 20-45 minutes, at least twice per week) or very active (intense exercise during at least one hour daily) as published (Marsaux et al., 2016).

**Statistical analyses**

Results from descriptive analyses are presented as means and Standard Deviation (SD) for continuous variables or as percentages for categorical variables. Data were analysed using Stata (version 12; Statacorp LP, College Station, TX, USA).

To facilitate the analytical process, participating centres were grouped according to the United Nations Composition of geographical regions, geographical sub-regions, and
selected economic and other groupings (Nations). Thus, Ireland and the United Kingdom were analysed together as “British Isles”, Germany and The Netherlands were grouped as “Western Europe”, Poland was representative of “Eastern Europe”, and finally Spain and Greece were grouped as “Southern Europe”.

To assess the influence of specific variables and to minimize biases, subjects were dichotomised. Thus, participants were categorised by the median age in those below 40 years old and those who were 40 years or older. To study the influence of BMI and sex, dichotomised categorical variables were used (under or above 25 kg/m², and men/women, respectively).

To detect differences between regions, as well as between categorized variables, one factor ANOVA and multivariate linear regression analyses were performed. Results were considered statistically significant for p values <0.05.

The multivariate nutrient density model developed by Hu et al (Hu et al,. 1999) was performed to evaluate the influence of protein intake and the isoenergetic substitution of protein sources on the risk of being overweight/obese. In this study, presence or absence of overweight/obesity was considered the event outcome and dependent variable. A multivariate-adjusted logistic regression was performed to calculate the Prevalence Risk Ratio (PRR) of being overweight, using as independent variables the macronutrient proportions (% of total energy intake), excluding protein for which the isoenergetic exchange was analysed, and adjusting for total energy intake, geographical region, age, sex and physical activity level (Moslehi et al,. 2015). This approach allowed assessment of the effect (risk) of the exchange of 1% (total energy intake) of protein by 1% (total energy intake) of other macronutrients on being overweight or obese.

Sensitivity analysis for over and under estimation of food intake was performed, using the cut-offs proposed by Goldberg and Black (Black 2000) based on the ratio of reported energy intake/estimated energy requirements, to prevent biases in the risk estimation model.

**Results**

A total of 5562 subjects across Europe were interested in receiving personalized nutrition advice and registered on the Food4Me website (Figure 1), while 2402 did not complete the whole screening process. Subsequently, these 3160 individuals signed the
two informed consents, filled in the two screening questionnaires and the food frequency questionnaire. Finally, 2413 participants were considered eligible as they provided valid data and fulfilled inclusion criteria.

**Participant characteristics**

Women represented the 64.69% of the total sample, with the biggest difference in Eastern Europe, where 75% of registered participants were women (Table 1). Therefore, data analyses were adjusted for sex. Anthropometric measures showed slight, but significant differences between regions (p<0.001). Notably, height was significantly greater in Western Europe compared with the other regions by 4cm on average. Differences in BMI were also statistically significant, with participants in Western and Eastern Europe presenting the lowest BMI, whereas overweight/obesity was more prevalent in the British Isles and Southern Europe.

In relation to food intake, no differences in energy consumption (kcal/day) were observed, with mean energy intake ranging from 2558 to 2608 kcal/day across regions. However, macronutrient distribution was significantly different (p<0.001) across European regions (Table 1). Southern European participants consumed the highest fat content in their diet (p<0.001), mainly attributable to olive oil, as MUFA intake was higher (15.2±3.8 %E, p<0.001) and SFA intake was lower (13.5±3.1 %E) in southern European participants compared with other EU Regions. Protein intake was significantly higher in Southern Europe (18.6±4.1 %E) compared with Western Europe (16.0±2.9 %E), Eastern Europe (16.8±3.3 %E) and the British Isles (16.3±3.1 %E). Southern Europeans consumed the largest proportion of animal protein (12.2%E) and the lowest percentage of vegetable protein (4.7%), compared with other regions. Western Europe and British Isles participants consumed more dietary fibre (33 g/day), while Eastern Europeans showed the highest intake of carbohydrates (49.2±7.1 %E) and simple sugars (22.0±6.6 %E) compared to the other regions.

When participants were grouped according to age (Table 2), the youngest participants were lighter and had lower BMI (70.6 kg and 24.1 kg/m² vs 76.9 kg and 26.4 kg/m²; p<0.001 in both measures), as well as higher physical activity level (1.53 vs 1.50 arbitrary units, p<0.001). Dietary intake was similar between age groups, and there were only very highly significant differences (p<0.001) for intake of PUFA and dietary fibre, which was higher in the group > 40 years-old (Table 2). Also, a significant difference
(p=0.02) in protein intake was observed, as younger participants consumed more than older subjects.

As expected, when men were compared with women, significant differences (p<0.001) were found in all the analysed outcomes, concerning anthropometry, physical activity level and dietary intake (table 2), except for protein and carbohydrates intake.

Finally, regression analysis of BMI as dependent variable and protein intake (%E) as the independent variable, adjusted for age, sex, country of origin, total energy intake and physical activity level, showed a weak but significant positive association (β= 0.12; p < 0.001). Comparing participants with BMI below 25 kg/m² (n=1322) with those presenting overweight or obesity (BMI ≥ 25 kg/m²; n=1091), this second group were significantly older and practised significantly less physical activity (table 2). In relation to nutrient intake, those presenting overweight reported higher energy intakes (p<0.001). In addition, the overweight group showed a slight but significantly higher proportion of total fat (p=0.046) and lower carbohydrates (p<0.001) in their diets. Also, those subjects with overweight reported significantly higher intakes of total protein (p<0.001).

**Contribution of food groups to reported protein intakes**

When the sources of protein were evaluated separately (Figure 2a), animal products were the major contributors to dietary protein intake in all regions, although differences between regions were highly significant (p<0.001) for the main protein sources: meat, fish and eggs (33.5% Western Europe, 37.4% Eastern Europe; 39.5% British Isles; and 46.7% Southern Europe); dairy products (19.4% Western Europe; 19.9% Eastern Europe; 17.1% British Isles; 16.6% Southern Europe); bread, grains, pasta and rice (27.8% Western Europe; 25.7% Eastern Europe; 22.8% British Isles; 18.8% Southern Europe) or vegetables (7.6% Western Europe; 5.9% Eastern Europe; 9.1% British Isles; 7.8% Southern Europe).

Moreover, protein sources in overweight/obese subjects, differed significantly from those reported by participants with BMI < 25 kg/m² (Figure 2b). Although meat, fish and eggs were the major contributors for protein in both groups, subjects with lower BMI consumed less animal products than overweight/obese subjects (37.9% vs 42.9%, p<0.001, respectively), while the percentages of cereals, grains, pasta and rice (24.0%),...
and fruits and vegetables (8.3%) were significantly higher among normal weight subjects than in overweight participants (22% of protein from cereals and 7.1% from fruits and vegetables, p<0.001).

**Influence of isoenergetic protein exchange on the risk of overweight/obesity**

No relevant changes in relative risks were found within the sensitivity analyses performed according to available cut-offs.

When the isocaloric nutrient exchange analysis was performed, according to the method described in the statistics section, the focus was set on evaluating the effect of increasing vegetable protein consumption in isolation from other macronutrients. Therefore, the Prevalence Risk Ratio of increasing 1% of total energy from vegetable protein, and the reduction in 1% of total energy of other nutrients is given (Figure 3). The risk of overweight/obesity was reduced if vegetable protein replaced animal protein (p=0.012), also observed in relation to sugar consumption (p=0.006). The calculated Prevalence Risk Ratio of replacing 1%E of sugar by 1%E of vegetable protein was of 0.940 (95%CI: 0.900-0.982), i.e. a reduction of about 6% in the risk of being overweight/obese. With regards to total fat exchange, although not significant, the substitution of total fat by vegetable protein showed a marginal trend to clearly protect against obesity (p=0.079).

The comparison of subjects categorised by tertiles for animal and vegetable protein consumption showed significant differences in BMI (Figure 4), with the highest animal protein consumption (above 11.4% of total energy consumption), associated to the highest BMI, and those participants consuming less vegetable protein had BMI > 25 kg/m².

**Discussion**

A major finding was that a higher vegetable protein intake is associated with reduced risk of developing overweight or obesity, whereas a positive association was found for animal protein consumption and the risk of overweight/obesity. Moreover, the current results revealed that self-reported data, collected via the Internet may be useful for acquiring nutritional and socio-demographic information in large cohorts. Although it cannot be completely confirmed whether this is an accurate reflection of the real food intake, this knowledge will enable the future elaboration of a more accurate and directed
personalised advice. It must be drawn to one’s attention that the FFQ used within the present study had already been validated (Fallaize et al., 2014, Forster et al., 2014, Marshall et al., 2016), with respect to the accuracy in estimating changes in dietary habits, being this feasible and simple tool to estimate nutrient intake for screening, which may be considered more a strength than a limitation (Forster et al., 2016).

In agreement with previous studies on dietary habits, Food4Me online participants from Western Europe, Eastern Europe and the British Isles, reported a larger consumption of saturated fats, whereas Greece and Spain presented the largest intakes of MUFA, possibly because their main fat staple source is traditionally olive oil (Osler and Schroll 1997, Lasheras et al., 2000, Trichopoulou et al., 2003). Furthermore, differences in meat and fish consumption were reflected in protein intake (larger in southern countries), and in agreement with other studies, reduced intake of grain and potatoes was associated with lower carbohydrates intake in Southern Europe and countries (Naska et al., 2006, Trichopoulou et al., 2007). However, it should be noted that dietary patterns are changing in Mediterranean and other countries towards more westernized patterns (San-Cristobal et al., 2015, da Silva et al., 2009, Vardavas et al., 2010).

In relation to differences found between men and women in nutrients intake, it is interesting to highlight that women in the Food4Me study seemed to present “a priori” a less favourable macronutrient profile, according to their reported total fat, saturated fat and sugars consumption, with a lower intake of dietary fibre. These differences seemed inconsistent given that women had a lower BMI and a significantly higher percentage of men were overweight or obese. In this context, the misreporting of overweight/obese subjects may have played a role (Jessri et al., 2016). When normal weight subjects were compared to overweight/obese participants, differences in sugar consumption were observed, being higher in the normal weight group, which may be the result of an inverse causality phenomena, indicating that the expected cause-effect response has not been observed due to a bias in reporting, as previously noted in other studies (Santiago et al., 2015, Santiago et al., 2013). Also, it must be pointed out that a previous analysis of dietary patterns in the Spanish Food4Me participants identified four dietary patterns, which were consistently associated with subjects’ weight-status (San-Cristobal et al., 2015). In the Spanish cohort, the “compensatory” pattern, characterised by an overconsumption of both beneficial and detrimental food items, was related to the
highest BMI, and a similar association might occur in this larger sample (San-Cristobal et al., 2015).

The association observed between protein consumption and BMI has also been reported in several life-stages in previous studies (Alkerwi et al., 2015, Hernandez-Alonso et al., 2016, Lin et al., 2015). The PREDIMED trial (Hernandez-Alonso et al., 2016) as well as the ORISCAV-LUX study (Alkerwi et al., 2015) showed an association between higher risk of obesity and death with higher total and animal protein consumption, but apparently did not report data on protein intake from vegetable sources. With a similar approach, the HELENA study reported an association between total and animal protein consumption and higher risk of obesity in European adolescents (Lin et al., 2015).

Interestingly, the HELENA study evidenced a protective effect of vegetable protein consumption in the development of obesity among young Europeans, which is in agreement with our data.

In other studies, the dietary intake of protein is positively associated with percent body fat in middle-aged and older adults (Vinknes et al., 2011), and that cysteine intake may be the causal factor (Elshorbagy et al., 2012). Thus, it would be of interest to examine the amino acid pattern of the protein sources of our European populations to achieve new insights. Nevertheless, and taking into account the origin of protein, vegetable protein is accompanied by many other micronutrients and compounds which may play a role in metabolism, and these effects must not be disregarded, given the possibility of interaction of phytochemical activity with energy metabolism [50].

In another trial carried out with patients presenting features of the metabolic syndrome, within the RESMENA study (Zulet et al., 2011, de la Iglesia et al., 2014), it was observed that protein quality may have an important impact in overweight/obesity, but also in related diseases (Lopez-Legarrea et al., 2014). In this context, consuming vegetable protein sources under energy restriction was specifically associated to a reduction of inflammatory markers, which allowed to hypothesize that obesity could also be tackled through this anti-inflammatory process. The positive effect of diets differing in macronutrient composition on weight loss has also been previously shown by this research group (Abete et al., 2009). In a study with hypoenergetic diets, additional benefits in consuming high-legumes or high-protein (30%E), were observed (7% and 8% weight loss, respectively, compared to 5% weight loss with a control diet).
and mitochondrial oxidation, which led the authors to conclude that an increase in
energy expenditure led to a higher basal metabolic rate in the volunteers (Abete et al.,
2009). These researchers also observed how inflammatory and lipid markers, and blood
pressure improved after the nutritional intervention enriched in protein (Hermsdorff et
al., 2011).

Focusing on weight maintenance, some nutritional interventions revealed better or
similar responses to weight control with diets containing higher protein proportions
(Larsen et al., 2010, Navas-Carretero et al.. 2016, Keogh et al., 2007, Brinkworth et al.,
2004, Clifton et al., 2014). In this context, nutritional interventions such as the
DIOGENES study (Larsen et al., 2010) have shown that diets with higher protein
content (30%E) and lower glycemic index may have a marginal effect on maintaining
the weight loss at 6 months (Larsen et al., 2010) and at 12 months (Aller et al., 2014).
Indeed, a more thorough analysis of these results led to the conclusion that gender may
also need to be considered as another factor to integrate in the complex process leading
to precision nutrition, in order to prescribe the best possible dietary patterns for each
subject (Navas-Carretero et al., 2016).

Other studies have reported similar positive results, when analysing weight loss and
maintenance on higher protein diets compared with control diets as well as on
cardiovascular risk markers (Keogh et al., 2007, Brinkworth et al., 2004). In any case,
it must be considered that in most of these trials and nutritional interventions, the
effectiveness of good adherence to the prescribed diet is essential to achieve successful
and sustainable weight loss and maintenance results (Clifton et al., 2014).

Different sources of protein have been investigated through intervention studies and
epidemiological cohorts showing distinct health responses depending on the animal and
vegetable protein intake, where animal protein sources are associated with increased
risk of developing obesity-related diseases (Lin et al., 2015). However, white meats or
fish products have not been often related to these outcomes (Battaglia Richi et al.,
2015), or as in RESMENA study, fish-protein has been correlated, as well as vegetable
protein, with positive effects on inflammation (Lopez-Lagarrea et al., 2014). The results
of the present analysis, suggest that the risk of overweight or obesity was lower when
higher amounts of protein from vegetable origin are consumed, whereas animal protein
(in general) has been associated with an increased risk for overweight or obesity.
Interestingly, substitution of 1% E from sugars by vegetable protein demonstrated a lower prevalence of obesity, in agreement with studies reporting that refined carbohydrates may be implicated in the obesity epidemics (Stanhope 2016). The same trend, although with a marginal statistical evidence, was found with the exchange of 1%E from fat by vegetable protein confirming the benefit of vegetable protein increase in the diet (Lopez-Lagarrea et al., 2014, Feskens et al., 2014).

Current data point to the differential effect and potential interactions of isolated nutrients. Moreover, it shows the importance of the nutrient sources and provides an opportunity for further investigation of dietary patterns. This strategy would take into account the combination of nutrients in a food matrix, behavioural influences, and interaction between different genes (Bauer et al., 2014, Fallaize et al., 2013, Kelly et al., 2016, San-Cristobal et al., 2015), leading personalised nutrition to the next step of precision nutrition, by considering lifestyle, social environment, and clinical features among others (Ferguson et al., 2016).

Data collection may be considered a weak point in the present study, given that all measurements were self-reported. However, as mentioned previously, the FFQ used to obtain the analysed data have been validated to ensure the accuracy of measurements (Fallaize et al., 2014, Forster et al., 2014, Marshall et al., 2016). In addition, checking of anthropometrical and genetic markers as proxy for identity was carried out at each intervention centre in a random subsample of the volunteers enrolled in the intervention study (Celis-Morales, Livingstone, Woolhead, et al., 2015). These results showed a strong validity and agreement between the self-reported data and data collected by trained researchers (Celis-Morales, Livingstone, Woolhead, et al., 2015). It is also worth mentioning as a strength of the study, the nutrient substitution model used in the analyses, which has been widely used since it was developed (Hu et al., 1999) and allows to study the association of the substitution of isolated nutrients, as well as some interactions, while energy intake is kept constant, as previously reported (Moslehi et al., 2015, Skilton et al., 2008, Vergnaud et al., 2013).

Nevertheless, some limitations that the current analysis might present must also be mentioned. In this sense, the consideration of “crude” isolated macronutrients on overweight or obesity might disregard some synergistic effects of micronutrients contained in foods that are source of vegetable or animal protein, such as...
phytochemicals, vitamins or fibre, and minerals, respectively. It must also be noted that the use of BMI as a marker of adiposity, may underestimate cases of high adipose tissue within a normal weight. However, and acknowledging the limitations, the measure of BMI is still widely used as a proxy screening tool in population studies [10, 47, 48].

The need of developing valid, feasible, effective and economic personalised medicine strategies will also have an impact on precision nutrition, with individual and public health perspectives. In this context, the Food4Me study hypothesized that web-based contact with subjects interested in improving their nutritional status is feasible, and internet-based personalised nutritional advice (Celis-Morales, Livingstone, Marsaux, et al., 2015) may be a future tool for preventing and managing non-communicable diseases, although the impact of these type of interventions in subjects suffering from specific diseases needs to be assessed. Taking into account differences in macronutrient intakes among countries, a “One size fits all” strategy may be inappropriate, while more specific messages, such as increasing vegetable protein consumption, may be easier to deliver. Indeed, personalising nutritional advice based on the phenotype of individuals, as well as their previous dietary habits may advance our understanding of precision nutrition, because dietary habits differ substantially in European regions (Livingstone et al., 2016). Although from a public health nutrition point of view, general recommendations are advisable, the need for combining general messages with nutrient-specific targets depending on the region is becoming urgent to reduce the epidemic of obesity and accompanying diseases, such as diabetes, hypertension and dislipemia (Pavlovic et al., 2007, Jankovic et al., 2015, Kirwan et al., 2016), and messages stressing the role of protein and the possible effects depending on the protein quality and sources may be beneficial in this public health actions.

Conclusion

In conclusion, the present results shed light on the differential role of protein quality in the occurrence of overweight/obesity, stressing the importance of increasing vegetable protein sources in our diet, in substitution of animal protein and simple sugars. Differences found in macronutrient intakes depending on region of origin, sex, age and physical activity also point to the importance of personalised nutrition in targeting successful messages for a healthier lifestyles.

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Author responsibilities were as follows: SNC and RSC drafted the paper and performed the statistical analysis for the manuscript. SNC and RSC are joint first authors. JAM was the responsible of Spanish centre of intervention. MG, JCM, JAM, CCM, MCW, ERG, LB, WHMS, HD, CAD, JAL, YM and IT, contributed to the research design. SNC, RSC, KML, CFM, CO’D, HF, CW, ALM, RF, GM, CPL, MJ, and AS conducted the intervention. All authors contributed to a critical review of the manuscript during the writing process. All authors approved the final version to be published.

Ethical Standards Disclosure
All procedures performed in the study were in accordance with the ethical standards of the corresponding research committees of each of the seven participating centres, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all individual participants included in the study.

The Research Ethics Committees evaluating the study protocol were those with the appropriate authority in each study site: University College Dublin, Ireland; University of Maastricht, Netherlands; Universidad de Navarra, Spain; Harokopio University, Greece; The University of Reading, United Kingdom; National Food and Nutrition Institute, Poland; Technische Universitaet Muenchen, German.

Being the study coordinator Ireland, the relevant Health Authority in Food4Me study was the Research Ethics Committee of Ireland.

The Food4Me trial was registered as a RCT (NCT01530139) at clinicaltrials.gov (http://clinicaltrials.gov/show/NCT01530139).

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### Table 1. Demographic anthropometric and dietary characteristics of subjects in the Food4Me study by European Regions at screening.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Western Europe</th>
<th>British Isles</th>
<th>Southern Europe</th>
<th>Eastern Europe</th>
<th>p¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>n (n females)</td>
<td>2413 (1561)</td>
<td>742 (462)</td>
<td>518 (341)</td>
<td>900 (568)</td>
<td>253 (190)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>40.2 ± 13.0</td>
<td>46.0 ± 14.1</td>
<td>37.8 ± 13.1</td>
<td>38.1 ± 10.3</td>
<td>35.7 ± 12.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.71 ± 0.09</td>
<td>1.74 ± 0.09</td>
<td>1.70 ± 0.09</td>
<td>1.69 ± 0.09</td>
<td>1.69 ± 0.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.6 ± 15.5</td>
<td>74.8 ± 14.5</td>
<td>72.9 ± 15.7</td>
<td>74.0 ± 16.1</td>
<td>70.4 ± 15.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.2 ± 4.7</td>
<td>24.7 ± 4.0</td>
<td>25.2 ± 4.7</td>
<td>25.9 ± 5.0</td>
<td>24.6 ± 4.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Physical activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>factor (AU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight (&lt;18.5 kg/m²)</td>
<td>2.3%</td>
<td>2.4%</td>
<td>2.1%</td>
<td>1.8%</td>
<td>4.3%</td>
<td></td>
</tr>
<tr>
<td>Normal weight (18.5 – 24.99 kg/m²)</td>
<td>52.5%</td>
<td>56.6%</td>
<td>55.0%</td>
<td>46.9%</td>
<td>54.9%</td>
<td>&lt;0.001²</td>
</tr>
<tr>
<td>Overweight (25 – 29.9 kg/m²)</td>
<td>31.1%</td>
<td>30.5%</td>
<td>28.6%</td>
<td>34.2%</td>
<td>26.9%</td>
<td>&lt;0.001²</td>
</tr>
<tr>
<td>Obesity (&gt;30 kg/m²)</td>
<td>14.1%</td>
<td>10.5%</td>
<td>14.3%</td>
<td>17.1%</td>
<td>13.8%</td>
<td></td>
</tr>
<tr>
<td>Energy (kcal/d)</td>
<td>2602 ± 797</td>
<td>2609 ± 744</td>
<td>2632 ± 823</td>
<td>2592 ± 823</td>
<td>2558 ± 802</td>
<td>0.641</td>
</tr>
<tr>
<td>Total fat (% E)</td>
<td>35.7 ± 6.4</td>
<td>35.4 ± 6.5</td>
<td>35.6 ± 6.2</td>
<td>36.4 ± 6.6</td>
<td>34.5 ± 5.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SFA (% E)</td>
<td>14.0 ± 3.4</td>
<td>14.2 ± 3.6</td>
<td>14.2 ± 3.4</td>
<td>13.5 ± 3.1</td>
<td>14.5 ± 3.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MUFA (% E)</td>
<td>13.8 ± 3.4</td>
<td>13.0 ± 2.9</td>
<td>13.2 ± 3.0</td>
<td>15.2 ± 3.8</td>
<td>11.9 ± 2.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PUFA (% E)</td>
<td>5.6 ± 1.4</td>
<td>5.9 ± 1.4</td>
<td>5.8 ± 1.5</td>
<td>5.2 ± 1.3</td>
<td>5.8 ± 1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Protein (% E)</td>
<td>17.1 ± 3.6</td>
<td>16.0 ± 2.9</td>
<td>16.3 ± 3.1</td>
<td>18.6 ± 4.1</td>
<td>16.8 ± 3.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Animal Protein (% E)</td>
<td>10.3 ± 4.3</td>
<td>8.7 ± 3.6</td>
<td>9.5 ± 3.6</td>
<td>12.2 ± 4.7</td>
<td>9.9 ± 3.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vegetable Protein (% E)</td>
<td>5.1 ± 1.7</td>
<td>5.5 ± 1.7</td>
<td>5.0 ± 1.5</td>
<td>4.7 ± 1.7</td>
<td>5.1 ± 1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total carbohydrates (% E)</td>
<td>46.4 ± 8.2</td>
<td>46.9 ± 8.0</td>
<td>47.1 ± 8.1</td>
<td>44.7 ± 8.5</td>
<td>49.2 ± 7.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Simple sugars (% E)</td>
<td>21.3 ± 6.4</td>
<td>20.8 ± 5.9</td>
<td>22.0 ± 6.2</td>
<td>21.1 ± 6.7</td>
<td>22.0 ± 6.6</td>
<td>0.002</td>
</tr>
<tr>
<td>Dietary fibre (g)</td>
<td>30.8 ± 13.3</td>
<td>33.4 ± 14.2</td>
<td>33.1 ± 13.6</td>
<td>27.3 ± 11.8</td>
<td>30.8 ± 12.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are expressed as mean and SD (Standard Deviation) or percentages. Western = Netherlands and Germany; British Isles = United Kingdom and Ireland; BMI: Body Mass Index; AU = Arbitrary Units; SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; %E = % of total energy intake. ¹Differences between European Regions analysed by one way ANOVA. ²Chi-square p-value for distribution. Different superscript letters mean significant differences among regions (p<0.05) in Tukey post-hoc analysis.
Table 2. Differences among Food4Me screenees according to age, sex and BMI.

<table>
<thead>
<tr>
<th></th>
<th>&lt;40 years</th>
<th>≥40 years</th>
<th>p¹</th>
<th>Male</th>
<th>Female</th>
<th>p¹</th>
<th>BMI&lt;25 kg/m²</th>
<th>BMI&gt;25kg/m²</th>
<th>p¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (n females)</td>
<td>1236 (826)</td>
<td>1177 (735)</td>
<td></td>
<td>852</td>
<td>1561</td>
<td></td>
<td>1322 (958)</td>
<td>1091 (603)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>29.4 ± 5.7</td>
<td>51.5 ± 8.0</td>
<td>&lt;0.001</td>
<td>42.0</td>
<td>39.2</td>
<td>&lt;0.001</td>
<td>37.4 ± 13.0</td>
<td>43.5 ± 12.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.71 ± 0.09</td>
<td>1.70 ± 0.09</td>
<td>0.602</td>
<td>1.79</td>
<td>1.66</td>
<td>&lt;0.001</td>
<td>1.70 ± 0.09</td>
<td>1.71 ± 0.09</td>
<td>0.118</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.6 ± 14.9</td>
<td>76.8 ± 15.6</td>
<td>&lt;0.001</td>
<td>83.8</td>
<td>68.1</td>
<td>&lt;0.001</td>
<td>64.0 ± 9.2</td>
<td>85.3 ± 13.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.1 ± 4.3</td>
<td>26.4 ± 4.7</td>
<td>&lt;0.001</td>
<td>26.3</td>
<td>24.7</td>
<td>&lt;0.001</td>
<td>22.0 ± 1.89</td>
<td>29.2 ± 3.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI status*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Under-weight</td>
<td>3.2% ± 1.4%</td>
<td></td>
<td></td>
<td>0.5%</td>
<td>3.3%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Weight</td>
<td>62.8% ± 41.6%</td>
<td>&lt;0.001²</td>
<td></td>
<td>42.3%</td>
<td>58.0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>25.2% ± 37.2%</td>
<td></td>
<td></td>
<td>41.5%</td>
<td>25.4%</td>
<td>&lt;0.001²</td>
<td></td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Obesity</td>
<td>8.7% ± 19.8%</td>
<td></td>
<td></td>
<td>15.7%</td>
<td>13.3%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical activity factor (AU)*</td>
<td>1.53 ± 0.11</td>
<td>1.50 ± 0.09</td>
<td>&lt;0.001</td>
<td>1.54</td>
<td>1.50</td>
<td>&lt;0.001</td>
<td>1.53 ± 0.10</td>
<td>1.50 ± 0.10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Energy (kcal/d)</td>
<td>2599 ± 808</td>
<td>2606 ± 785</td>
<td>0.617</td>
<td>2888</td>
<td>2446</td>
<td>&lt;0.001</td>
<td>2481 ± 774</td>
<td>2750 ± 800</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total fat (% E)</td>
<td>35.6 ± 6.1</td>
<td>35.7 ± 6.7</td>
<td>0.702</td>
<td>34.8</td>
<td>36.2</td>
<td>&lt;0.001</td>
<td>35.4 ± 6.3</td>
<td>36.1 ± 6.5</td>
<td>0.046</td>
</tr>
<tr>
<td>SFA (% E)</td>
<td>13.9 ± 3.2</td>
<td>14.0 ± 3.5</td>
<td>0.8019</td>
<td>13.5</td>
<td>14.2</td>
<td>&lt;0.001</td>
<td>13.8 ± 3.3</td>
<td>14.1 ± 3.4</td>
<td>0.0168</td>
</tr>
<tr>
<td>MUFA (% E)</td>
<td>13.8 ± 3.3</td>
<td>13.7 ± 3.5</td>
<td>0.4697</td>
<td>13.4</td>
<td>14.0</td>
<td>&lt;0.001</td>
<td>13.5 ± 3.4</td>
<td>14.0 ± 3.4</td>
<td>0.001</td>
</tr>
<tr>
<td>PUFA (% E)</td>
<td>5.5 ± 1.3</td>
<td>5.8 ± 1.5</td>
<td>&lt;0.001</td>
<td>5.5</td>
<td>5.7</td>
<td>0.001</td>
<td>5.6 ± 1.4</td>
<td>5.6 ± 1.4</td>
<td>0.5703</td>
</tr>
<tr>
<td>Protein (% E)</td>
<td>17.3 ± 3.7</td>
<td>16.9 ± 3.5</td>
<td>0.0205</td>
<td>17.2</td>
<td>17.1</td>
<td>0.572</td>
<td>16.9 ± 3.5</td>
<td>17.4 ± 3.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Animal Protein (%E)</td>
<td>10.5 ± 4.3</td>
<td>10.1 ± 4.3</td>
<td>0.010</td>
<td>10.4</td>
<td>10.3</td>
<td>0.553</td>
<td>9.9 ± 4.2</td>
<td>10.8 ± 4.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vegetable Protein (%E)</td>
<td>5.0 ± 1.6</td>
<td>5.1 ± 1.7</td>
<td>0.616</td>
<td>5.1</td>
<td>5.0</td>
<td>0.098</td>
<td>5.2 ± 1.7</td>
<td>4.9 ± 1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total carbohydrates (% E)</td>
<td>46.7 ± 7.9</td>
<td>46.1 ± 8.6</td>
<td>0.0531</td>
<td>46.1</td>
<td>46.5</td>
<td>0.359</td>
<td>47.1 ± 8.1</td>
<td>45.5 ± 8.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Simple sugars (% E)</td>
<td>21.5 ± 6.1</td>
<td>21.1 ± 6.6</td>
<td>0.1457</td>
<td>20.2</td>
<td>21.9</td>
<td>&lt;0.001</td>
<td>21.8 ± 6.2</td>
<td>20.7 ± 6.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dietary fibre (g/day)</td>
<td>29.7 ± 13.1</td>
<td>32.0 ± 13.5</td>
<td>&lt;0.001</td>
<td>32.5</td>
<td>29.9</td>
<td>&lt;0.001</td>
<td>31.1 ± 13.8</td>
<td>30.5 ± 12.8</td>
<td>0.300</td>
</tr>
</tbody>
</table>

Values are expressed as mean and SD (Standard Deviation).
BMI = Body Mass Index; AU = Arbitrary Units; SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; %E = % of total energy intake; NA=Not applicable. ¹Differences between groups analysed by one way ANOVA. ²Chi-square p-value for distribution.
* BMI Categories: Underweight (< 18.5 kg/m²); Normal weight (18.5 – 24.99 kg/m²); Overweight (25 – 29.9 kg/m²); Obesity (> 30 kg/m²).
FIGURE TITLES AND FOOTNOTES

Figure 1. Flow-chart for the participants in the online Food4Me screening included in the present study.

Figure 2. Contribution of different Food Groups to protein intake in each EU Region (a), and divided by BMI (<25 kg/m² vs >25 kg/m²) (b).

[Footnote] Protein intake differed between regions and BMI in meat, fish and eggs (p<0.001); cereal, grain, pasta and rice (p<0.001), as well as in dairy products and fruits and vegetables (p<0.001 in both comparisons).

Figure 3. Prevalence Risk Ratio (PRR) of overweight or obesity in Food4Me screenees.

[Footnote] Calculation of PRR was performed according to the isoenergetic substitution (1 %E) of macronutrients by vegetable protein, following the nutrient-density model (n=2413).

Figure 4. Differences in BMI by tertiles of animal protein and vegetable protein intake, in the Food4Me Screenees

[Footnote] Different superscript letters represent significant mean differences between tertiles (p<0.05) in Tukey post-hoc analysis.