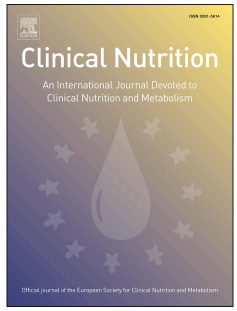
Accepted Manuscript



High dietary protein intake is associated with an increased body weight and total death risk

Pablo Hernández-Alonso, Jordi Salas-Salvadó, Miguel Ruiz-Canela, Dolores Corella, Ramón Estruch, Montserrat Fitó, Fernando Arós, Enrique Gómez-Gracia, Miquel Fiol, José Lapetra, Josep Basora, Lluis Serra-Majem, Miguel Ángel Muñoz, Pilar Buil-Cosiales, Carmen Saiz, Mònica Bulló

PII: S0261-5614(15)00091-6

DOI: 10.1016/j.clnu.2015.03.016

Reference: YCLNU 2544

To appear in: *Clinical Nutrition*

Received Date: 9 January 2015

Revised Date: 20 March 2015

Accepted Date: 28 March 2015

Please cite this article as: Hernández-Alonso P, Salas-Salvadó J, Ruiz-Canela M, Corella D, Estruch R, Fitó M, Arós F, Gómez-Gracia E, Fiol M, Lapetra J, Basora J, Serra-Majem L, Muñoz MÁ, Buil-Cosiales P, Saiz C, Bulló M, High dietary protein intake is associated with an increased body weight and total death risk, *Clinical Nutrition* (2015), doi: 10.1016/j.clnu.2015.03.016.

High dietary protein intake is associated with an increased body weight and total death risk.

Pablo Hernández-Alonso [a], Jordi Salas-Salvadó [a, b, *], Miguel Ruiz-Canela [b, c], Dolores Corella [b, d], Ramón Estruch [b, e], Montserrat Fitó [b, f], Fernando Arós [b, g], Enrique Gómez-Gracia [b, h], Miquel Fiol [i], José Lapetra [j], Josep Basora [a, b], Lluis Serra-Majem [k], Miguel Ángel Muñoz [l], Pilar Buil-Cosiales [b,c], Carmen Saiz [b,d] and Mònica Bulló [a, b, *]

Author affiliations

[a]Human Nutrition Unit from the Department of Biochemistry and Biotechnology, Hospital Universitari de Sant Joan de Reus, Faculty of Medicine and Health Sciences, IISPV (Institut d'Investigació Sanitària Pere Virgili), Universitat Rovira i Virgili, Reus, Spain.

[b]CIBERobn (Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición), Institute of Health Carlos III, Madrid, Spain.

[c]Department of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain.

[d]Department of Preventive Medicine, University of Valencia, València, Spain.

[e]Department of Internal Medicine, Institut d'Investigacions Biomediques August Pi Sunyer (IDIBAPS), Hospital Clínic, University of Barcelona, Barcelona, Spain.

[f]Cardiovascular Risk and Nutrition Research Group (Regicor Study Group) Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Barcelona, Spain.

[g]Department of Cardiology, University Hospital Txagorritxu, Vitoria, Spain.

[h]Department of Preventive Medicine, University of Malaga, Malaga, Spain.

[i]Institute of Health Sciences, University of Balearic Islands and Hospital Son Espases, Palma de Mallorca, Spain.

[j]Department of Family Medicine, Primary Care Division of Sevilla, San Pablo Health Center, Sevilla, Spain.

[k]Department of Clinical Sciences, University of Las Palmas de Gran Canaria, Las Palmas, Spain.

[l]Primary Health Care Division and Research, Instituto de Investigaciones Biomédicas August Pi i Sunyer-Jordi Gol, Barcelona, Spain

Address correspondence and reprint requests to:

Mònica Bulló and Jordi Salas-Salvadó. Human Nutrition Unit, Faculty of Medicine and Health Sciences, Universitat Rovira i Virgili. C/ Sant Llorenç 21, 43201 Reus (SPAIN). Telephone number: +34 977759312 / Fax number: +34 977759322

e-mail address: monica.bullo@urv.cat and jordi.salas@urv.cat

Manuscript information:

Running title: Protein intake, body weight and death

Word Count of the Abstract: 247

Word Count of the Introduction section: 452

Word Count of the Discussion section: 1328

Word Count (full manuscript: introduction to discussion): 3534

Number of figures: 2

Number of tables: 4

Number of supplementary files: two tables

Abbreviations: BMI, body mass index; BW, body weight; CI, confidence interval; CVD, cardiovascular disease; E%, percentage of energy; EPIC, European Prospective Investigation into Cancer and Nutrition; FFQ, food frequency questionnaire; g prot/kg BW/d, g protein /kg body weight/day; HDL, high-density lipoprotein; HPD, high-protein diet; HPFS, Health Professionals' Follow-up Study; HR, hazard ratio; LC, low carbohydrate; LCHP, LC-high protein; LDL, low-density lipoprotein; LPD, low-protein diet; NHS, Nurses' Health Study; q-trend, quadratic-trend; RCT, randomised controlled trial; T2D, type 2 diabetes; WC, waist circumference.

ABSTRACT

Background & Aims: High dietary protein diets are widely used to manage overweight and obesity. However, there is a lack of consensus about their long-term efficacy and safety. Therefore, the aim of this study was to assess the effect of long-term high-protein consumption on body weight changes and death outcomes in subjects at high cardiovascular risk.

Methods: A secondary analysis of the PREDIMED trial was conducted. Dietary protein was assessed using a food-frequency questionnaire during the follow-up. Cox proportional hazard models were used to estimate the multivariate-adjusted hazard ratio (HR) and 95% confidence intervals (95%CI) for protein intake in relation to the risk of body weight and waist circumference changes, cardiovascular disease, cardiovascular death, cancer death and total death.

Results: Higher total protein intake, expressed as percentage of energy, was significantly associated with a greater risk of weight gain when protein replaced carbohydrates (HR: 1.90; 95%CI: 1.05, 3.46) but not when replaced fat (HR: 1.69; 95%CI: 0.94, 3.03). However, no association was found between protein intake and waist circumference. Contrary, higher total protein intake was associated with a greater risk of all-cause death in both carbohydrate and fat substitution models (HR: 1.59; 95%CI: 1.08, 2.35; and HR: 1.66; 95%CI: 1.13, 2.43, respectively). Animal protein was associated with an increased risk of fatal and non-fatal outcomes when protein substituted carbohydrates or fat.

Conclusions: Higher dietary protein intake is associated with long-term increased risk of body weight gain and overall death in a Mediterranean population at high cardiovascular risk.

Keywords: Protein; cardiovascular; body weight; death; risk

INTRODUCTION

The alarming rise in overweight and obesity in developing countries has generated a plethora of dietary strategies for managing body weight. Moderate- or high-protein diets have gained in popularity and have been widely promoted for losing weight, preserving lean body mass, and maintaining weight loss [1]. Advocates of these diets often recommend protein intakes at or above 1.2g protein/kg body weight/day (g prot/kg BW/d) or >25E% (percentage of energy) consumed. These amounts are substantially higher than usual recommendations for healthy adults which are set at 0.8g prot/kg BW/d [2–4] or recent recommendations for healthy older subjects set at 1.0-1.2g protein/kg BW/day [5].

Several randomised controlled trials (RCTs) have investigated the short-term effects of high-protein (HPD) versus low-protein (LPD) diets and reported that HPD have advantages in terms of adiposity and blood lipid profile [6–8]. Similarly, in a pooled analysis of 15 RCTs lasting between 28 days and 12 months, HPD showed favourable effects on obesity and cardiovascular risk markers [9]. In addition, a meta-analysis of weight-loss studies conducted in adults consuming either a HPD (>15E%) or a LPD (<15E%) with a follow-up of at least 12 months, demonstrated a greater body weight (BW) loss and an improvement of triglyceride and insulin levels in HPD. However, no differences were observed in concentrations of HDL and LDL-cholesterol or fasting glucose [10]. Despite the generalised use of HPD, there is no consensus about their long-term efficacy and safety. A meta-analysis of RCTs with a minimum 12-month follow-up demonstrated that high-protein diets (up to 25E%) had neither beneficial nor detrimental effects on weight, body composition and fat distribution, or cardiovascular risk [11]. Data from large-scale, long-term cohort studies have shown a positive association between protein intake and weight gain [12–14] and suggest that physiological mechanisms supporting the beneficial effect of high protein intake in weight control could depend on body mass index and waist circumference [14]. Additionally, in two recent systematic reviews conducted in healthy adults [15] and older adults [16], including prospective cohort, case-control and long-term intervention studies, the association between protein consumption and different clinical outcomes ranged from probable or suggestive to inconclusive. Safer intakes were between 15-20E% of total protein, and inconclusively harmful were above 20-23E%. Risk of all-cause death and type 2 diabetes (T2D) seemed to increase with long-term total protein intake of 20-23E%.

Since there is a lack of consensus about the long-term associations between the amount and type of dietary protein, weight control and death, the aim of the present study was to analyse, in the same population, both the long-term body weight changes and the incidence of several fatal clinical outcomes resulting from total, animal and vegetable protein consumption in a high cardiovascular risk cohort.

METHODS

Study population

This prospective cohort analysis was based on the PREDIMED (*PREvención con DIeta MEDiterránea*) cohort, which is a large, parallel group, multicenter, controlled, randomized clinical trial conducted in 7,447 older adults at high cardiovascular risk. The aim was to assess the effects of Mediterranean diet on the primary prevention of diseases with cardiovascular origin. The detailed study protocol was already published [17]. Eligible participants were men (55-80 years) and women (60-80 years), without cardiovascular disease (CVD) at enrolment, and who had either T2D or three or more of the following criteria: smoking, hypertension, high LDL-C, hypertriglyceridemia, HDL-C level ≤40 mg/dL, overweight or obesity (BMI ≥25 kg/m^2) or family history of CVD. Exclusion criteria included severe chronic illness; abuse of drug or alcohol and history of allergy or intolerance to either olive oil or nuts. All participants signed the informed consent according to a protocol approved by the institutional review boards.

Dietary assessment

Dietary intake was measured at baseline and at each annual visit by using a 137-item food-frequency questionnaire [18]. Detailed information about the development, reproducibility and validity of the questionnaire in the PREDIMED cohort has been previously reported [19]. Spanish food-composition tables were used to derive energy and nutrient intake [20]. Animal protein was mainly derived from meat, poultry, fish and dairy products, whereas vegetable protein was from legumes, fruits and nuts.

Anthropometric and biochemical measurements

Body weight, height and waist circumference were measured at baseline and at each annual visit by trained personnel. We used the Minnesota leisure-time physical activity questionnaire to determine the physical activity of each participants' leisure-time [21].

Ascertainment of changes in body weight and waist circumference

To evaluate changes in BW we defined 'Successful weight change' when participants lost or gained ≥10% of BW [22]. Other participants were included in the 'maintaining weigh' category. Changes in waist circumference were defined according to the metabolic syndrome criteria (i.e. ≥88 cm for women and ≥102 cm for men) [23] and classified into three categories (incidence, reversion and maintenance).

Ascertainment of death and CVD event

Cardiovascular events (i.e. myocardial infarction, stroke or death from cardiovascular causes), and death by cardiovascular, cancer and all-cause were the primary outcomes for the long-term safety evaluation of protein intake. Different methodologies were employed in order to determine outcomes related to CVD and death: repeated contact with participants, contact with family physicians, and annual review of medical records and consultation of the National Death Index. Information regarding the health and medication status of the participants was collected from yearly programmed visits and medical records.

Statistical analyses

Baseline characteristics of participants were presented as mean±SD or percentages. For total protein consumption, we performed a dual analysis as follows: (a) evaluated as E% of protein and then categorized into quintiles; (b) evaluated as g prot/kg BW/d, establishing three categories (<1; 1-1.5; >1.5 g prot/kg BW/d) with the middle category as the reference, according to the latest recommendations from the ESPEN [5]. Sources of protein intake (i.e. animal and vegetable) and ratio between them, were evaluated as E%, and then categorized into quintiles.

We excluded from the analysis those subjects with incomplete dietary data and those who had extremes of total energy intake (>4000 or <800 kcal/day in men and >3500 or <500 kcal/day in women). Total energy intake was used to adjust all nutrients [24]. Total time of follow-up time was computed as the difference between the date of the cardiovascular event, death, or end of follow-up and the date of randomization. WC_{BMI} was calculated as the gender-specific linear regression of WC on BMI.

In order to explore the correlation between body weight or abdominal obesity and protein consumption, we computed the cumulative average of the BMI, BW and WC_{BMI} throughout the study's follow-up period. The cumulative average of BMI, BW or WC_{BMI} was used as the dependent variable, and protein consumption (both continuous and quintile-defined) was used as independent variable.

Cox proportional hazards regression models were fitted to estimate hazard ratios (HR) and the corresponding 95%CIs for BW and WC_{BMI} changes, cardiovascular event and death, cancer death, and all-cause death. BW and WC_{BMI} changes were calculated as the difference between the cumulative average of non-baseline visits and the baseline visit, to account for body oscillations throughout the follow-up. We used the cumulative average approach (with data from baseline to the last FFQ before onset of disease) to assign an individual's protein intake because it minimizes measurement error by using all previous dietary assessments during follow-up [25].

To assess the type of relationship between protein intake and outcome, we entered protein intake as both a linear and a quadratic term in the model. *P* for quadratic trend (*P* q-trend) was calculated using the median value of each quintile in a polynomial analysis of the Cox regression models. Because the assessment of the U-shaped relationship between protein intake and the different outcomes was significant, the third quintile (Q3) of protein consumption was established as the reference. Interaction tests for sex and intervention group were not statistically significant for either BW and WC_{BMI} changes, or fatal and non-fatal outcomes. Macronutrient energy substitution models were used, where energy from protein replaced fat or carbohydrate [26]. Therefore, the estimated regression coefficient has to be interpreted as the estimated effect of protein (according to quintiles) replacing E% of the omitted macronutrient while the energy of the other macronutrients is assumed to be constant. Model 1 was adjusted for intervention group, node, sex, age, BMI, smoking status (former or current smoker), leisure-time physical activity (metabolic equivalent task-min/d) and cumulative average alcohol intake (continuous, with an added quadratic term). Model 2 and model 3 were also adjusted for the prevalence of diabetes, hypertension, hypercholesterolemia, family history of coronary heart disease, use of aspirin, antihypertensive medication, oral antidiabetic medication, insulin medication, hypocholesterolemic medication, and nutritional variables: percentage of total energy intake, energy from fats (in model 2), energy from carbohydrates (in model 3), energy-adjusted omega-3 fatty acids and fiber, and glycemic index.

Additional Cox regression models were used to assess the risk of increases BW or WC_{BMI}, and the risk of CV event, total death, cardiovascular death and cancer death in terms of cumulative average E% of protein intake. On the basis of previous publications, we established three categories: normal ([15-20]E%), low (<15E%) and high (>20E%) [15,16,27].

All statistical tests were two-tailed, and the significance level was set at $P < 0.05$. The Bonferroni post-hoc test was used to correct for multiple comparisons. Statistical analysis was performed using SPSS.20 for Windows (SPSS Inc, Chicago, IL).

RESULTS

Of the 7,447 subjects in the PREDIMED cohort, 7,216 were included in this secondary analysis. Subjects with extremes of total energy intake (n=153) and with incomplete dietary data at baseline were excluded (n=78). The baseline characteristics of the study population according to the quintile of cumulative average E% from total protein are shown in Table 1. Participants in the highest quintile of dietary protein had a higher prevalence of diabetes and a family history of CV disease, but a lower prevalence of hypertension. During a median follow-up of 4.8 years, the following events were detected: 186 of weight loss, 149 of weight gain (10% cut-off), 486 of WC_{BMI} incidence and 378 of WC_{BMI} reversion. A total of 323 deaths (81 cardiovascular, 130 cancer and 112 other causes) and 277 cardiovascular events occurred.

Protein intake, changes in body weight and waist circumference

After possible confounders had been adjusted for, in both continuous and quintile-defined variables, and carbohydrate and fat substitution models, higher BMI and BW were observed to have positive significant relationships with total E% protein intake, protein from animal sources and animal-to-vegetable protein ratio but not vegetable protein intake (Table S1). In the case of $WC_{BMI,}$ this positive association was also observed for vegetable protein, but not for the animal-to-vegetable protein ratio.

In Cox regression analysis, subjects in the highest quintile of total dietary protein intake showed a significant 90% greater risk of increasing BW (higher or equal to 10%) than subjects in the reference quintile (third quintile; Figure 1) when protein replaced carbohydrates. No significant association between total dietary protein intake and changes in WC_{BMI} was observed (Figure 2). No significant associations were observed between the source of protein and BW changes, although subjects in the lowest quintile of vegetable protein showed an unexpected lower risk of weight loss (Figure 1) and both WC_{BMI} incidence and reversion (Figure 2) in carbohydrate and fat substitution models. Similarly, no significant associations between total dietary protein intake and changes in either body weight or WC_{BMI} were observed when total protein intake was evaluated as g/kg BW/d (Table 3). In a sensitivity analysis, subjects were divided into the following groups: normal (15-20E%), low (<15E%) and high (>20E%) protein consumption (Table S2). Risk of body weight gain was significantly higher in the high-protein intake group than in the normal group, when protein replaced carbohydrates, and a borderline significance when replaced fat (HR: 2.03; 95% CI: 1.07, 3.86: *P* q-trend = 0.03; HR: 1.87; 95% CI: 0.99, 3.53; *P* q-trend = 0.03, respectively), even after adjusting for potential confounders. We failed to find any association with risk of WC_{BMI} incidence or reversion in the sensitivity analysis (Table S2).

Protein intake and fatal and non-fatal outcomes

Table 2 shows the HRs for cardiovascular events and other cause-specific death for total protein intake. Participants in the highest quintile of dietary protein intake had a 59% and 66% greater risk than those in the middle quintile of all-cause death in the carbohydrate or fat substitution models respectively, even after adjusting for potential confounders. However, total dietary protein intake showed no significant association with either cardiovascular events, or cardiovascular or cancer death (Table 2). A positive association was also observed between the intake of >1.5 g protein/kg BW/d and the risk of cardiovascular and all-cause death compared with 1.0-1.5 g/kg BW/d category (Table 3).

In both carbohydrate and fat substitution models, subjects in the highest quintile of animal protein showed a significant risk of cardiovascular event, and cardiovascular, cancer and all-cause death (Table 4). Accordingly, a higher animal-to-vegetable protein ratio was associated with a higher risk of cancer death and all-cause death, whereas a higher risk of cancer death was also observed in the lowest quintile of vegetable protein intake when protein replaced carbohydrates or fats (Table 4). In addition, when our population was divided into normal, low and high protein consumption, the high protein intake was observed to have a significant relationship with cancer death and all-cause death (Table S2) compared with the middle category.

DISCUSSION

The main findings of the present study indicate that long-term high protein intake seems to be associated with an increased risk of weight gain, and overall death in middle-aged subjects and older adults at high cardiovascular risk, compared with moderate consumption. Moreover, higher animal protein consumption was associated with an increased risk of cardiovascular event and cardiovascular, cancer and total death, compared with moderate consumption.

Dietary protein intake and body weight

The beneficial effects ascribed to dietary protein –higher thermogenesis, increased satiety and decreased subsequent meal energy intake [12,28]– have led to it being used as an effective dietary strategy for losing weight and fighting overweight and obesity. It has also been claimed that high dietary protein intake is useful for improving blood pressure and lipid profile [29,30], decreasing insulin levels [31] and controlling T2D [32]. However, whereas there is general consensus about its beneficial short-term effects, the long-term effects on BW and metabolic risk markers are more controversial, ranging from non-effective to harmful [15]. According to a recent meta-analysis of 15 RCT of HPD (≥25E% as protein) with follow-up periods between 12-24 months, they have neither beneficial nor detrimental effects on BW, waist circumference or body composition compared to LPD (≤20E% as protein) [11]. In contrast, one of the largest prospective studies conducted so far –with a total of 89,432 subjects from the EPIC cohort and a mean follow-up of 6.5 years– failed to find any association between high intake of energy as protein and weight loss. However, it did report a positive relationship between consumption of animal protein and weight gain [12]. Similarly, a higher intake of total protein or animal protein was associated with a greater risk of overweight and obesity in men after 7-years of follow-up [33]. A recent sophisticated analysis, in which characteristics of an RCT were mimicked in the observational data from a cohort study, showed that subjects with a high protein intake had a significantly lower weight gain when matched on dietary variables in combination with body characteristics. However, when matched only on dietary variables, protein intake was not observed to have any effect on annual weight changes. The authors suggested that physiological mechanisms that potentially explain the relationship between protein intake and weight control could be related to the amount of body fat [14]. In our study, even after adjusting for BMI and other potential confounders, we found significant increases in body weight in those subjects with a higher consumption of total protein (expressed as E%, but not as g prot/BW/d) and a non-significant increased risk of weight gain in those with a higher consumption of animal protein, suggesting that sources of protein may have a different long-term effect on BW. However, we failed to find any significant association between dietary protein intake and WC_{BMI}, as a degree of abdominal obesity.

Dietary protein intake and fatal and non-fatal outcomes

Concern is increasing not only about the role that protein plays in body weight, but also about the extent to which HPD affect risk of death. In a recent systematic review, the analysis of seven large-scale prospective cohort studies in healthy adults inconclusively suggested a possible relationship between cardiovascular disease or risk of all-cause death and total or animal protein intake, while an inverse association was suggested between cardiovascular death and vegetable protein intake [15]. In agreement with most studies, we found a higher risk of all-cause death in those subjects in the highest quintile or category of total protein intake (i.e. E% or g prot/BW/d), and animal protein consumption. However, we failed to find that vegetable protein was related to lower risk. The association between total dietary protein intake and all-cause death was even stronger when subjects with a protein intake of >20E% were compared with subjects with a normo-protein intake (15-20E%).

Additionally, we observed a non-significant trend to a higher risk of cardiovascular death related to the highest consumption of total protein. However, in agreement with the hypothesis that the source of dietary protein will have different effects on the cause of death, the higher consumption of animal protein was associated with a significantly increased risk of cardiovascular disease and cardiovascular and cancer death but not the consumption of vegetable protein. Similarly, the animal-to-vegetable protein ratio was positively associated with cancer death and all-cause death, suggesting that the amount of protein derived from animal sources accounts for a considerable proportion of the association between overall protein intake and all-cause death. Our results are in agreement with those derived from a pooled analysis of 85,168 subjects from the NHS and 44,548 subjects from the HPFS [34] which show that protein from animal sources was related to higher cancer death.

Several mechanisms may explain the increased risk of all-cause death associated with a higher intake of protein. An increase in protein consumption may lead to increased glomerular pressure and renal disease [35], and, in turn, to a higher risk of cardiovascular death [36]. Also, animal and vegetable protein has different effects on the glucose and lipid metabolism [15]. In a recent meta-analysis of 15 long-term RCTs, however, no significant differences were reported in insulin circulating levels after a low-fat diet that was either low or high in protein [11]. In addition, the different effect of animal or vegetable protein on death risk could be due to differences in the amino acid composition as is the case with insulin resistance status [31,37,38]. Essential sulphur-containing amino acids, which are mainly present in animal-containing foods, have also been associated with increased BMI in middle-aged men [39]. Moreover, some experimental studies conducted in animals have suggested that the dietary macronutrient composition could modulate the hypothalamic orexin/hypocretin system, thus promoting food consumption [40]. Likewise, the decrease in food intake associated with a protein-enriched diet could be counterbalanced by the hypothalamic melanocortin system to protect the body against weight variation [41].

Strengths and limitations

Our study has some potential limitations that should be mentioned. Because it was conducted in middle-aged subjects and older adults with a high risk of CVD, our findings cannot be extrapolated to the general population. Moreover, PREDIMED is a clinical trial and the sub-analysis has been conducted as an observational cohort, so although the statistical analyses have been adjusted for each intervention group, a potential residual effect of dietary intervention on the final results cannot be discounted. Also, although other major potential confounders have been adjusted in the current approach, we cannot completely rule out the possibility of residual confounding from measured and unmeasured factors. The lack of specific measurements of body composition and protein metabolism, such as DEXA and urinary nitrogen, could limit our findings. Finally, it should also be taken into account that the E% evaluated by quintiles of protein in the PREDIMED cohort fluctuates from 13.9 to 19.5, a narrower range than has been found by other studies based on hyperproteic diets.

In contrast, our study is the first to have evaluated the efficacy and safety of protein consumption at the same time. It has a large sample of subjects, followed up subjects for a medium-long period, and accurately ascertained CV events and death. We also used the cumulative average method, which corrects for fluctuating values of protein consumption, and changes in body weight or fat distribution during the follow-up. Finally, we used macronutrient energy substitution models to analyse the association between dietary protein intake and several outcomes when this intake replaced carbohydrates or fats.

Conclusions

Taken together, the results of our study do not support the generalised use of high protein diets as a tool for better weight control in the long term and indicate that in middle-aged subjects or older adults these diets can have potentially adverse health consequences related to cardiovascular disease and cancer. There is a huge need for further molecular and clinical studies to elucidate the mechanisms by which the quantity and source of protein can differentially affect body composition and fatal and non-fatal outcomes, before recommend a high protein intake for a long-term.

ACKNOWLEDGMENTS

The authors thank the participants for their enthusiastic collaboration, the PREDIMED personnel for excellent assistance and the personnel of all affiliated primary care centers.

STATEMENT OF AUTHORSHIP

The authors' responsibilities are as follows: MB and JS-S had full access to all the data in the study and take full responsibility for the integrity and accuracy of the data analysis. Study concept and design: MB and JS-S. Acquisition of data: MB, MR-C, DC, RE, MF, FA, EG-G, MF, JL, JB, LS-M, MAM, PB-C, CS and JS-S. Analysis and interpretation of data: PH-A, MB, JS-S. Drafting the manuscript: PH-A, MB, JS-S. Critical revision of the manuscript for important intellectual content: PH-A, MB, MR-C, DC, RE, MF, FA, EG-G, MF, JL, JB, LS-M, MAM, PB-C, CS and JS-S. Statistical analysis: PH-A, MB, JS-S.

Funding: MB, MR-C, DC, RE, MF, FA, EG-G, MF, JL, JB, LS-M, MAM, PB-C, CS, JS-S. All authors had final approval of the submitted manuscript and published versions.

CONFLICT OF INTEREST STATEMENT

JS-S serves on the board of the International Nut and Dried Fruit Council, and receives grant support through his institution from the International Nut and Dried Fruit Council. LS-M serves on the boards of the Mediterranean Diet Foundation. No other potential conflict of interest relevant to this article was reported.

FUNDING SOURCES

We acknowledge CIBERobn and RTIC RD 06/0045 and FIS PI12/0153 which are initiatives of ISCIII, Spain, and are grateful for the grants from the Centro Nacional de Investigaciones Cardiovasculares CNIC 06/2007, Fondo de Investigación Sanitaria (PI07/0473;PI10/02658;PI12/0153) Ministerio de Ciencia e Innovación (AGL-2009-13906-C02, AGL2010-22319-C03), Ministerio de Sanidad-Plan Nacional de drogas (2010/087) and Fundación Mapfre 2010, the Government of the Basque Country (IT386-10), the University of the Basque Country (UFI 11/32) and the Catalan government (joint contract with ISCIII (Miguel Servet 06/00100)). PH-A is the recipient of a predoctoral fellowship from the Generalitat de Catalunya's Department of Universities [FI-DGR 2014]. The funding sources played no role in the experimental design, the collection, analysis or interpretation of data, the writing of the report, or the decision to submit the paper for publication.

REFERENCES

1. Clarke R. Long-term weight loss and prevention of cardiovascular disease. Circulation. 2011.124:2801–2.

2. World Health Organization. Protein and amino acid requirements in human nutrition: Report of a joint WHO/FAO/UNU expert consultation. World Health Organization; 2007.

3. Institute of Medicine. Dietary Reference Intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids (macronutrients). Washington , DC: The National Academies Press;

4. European Food Safety Authority (EFSA). Outcome of a public consultation on the draft scientific opinion on the EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA) on dietary reference values for protein. Parma, Italy; 2012.

5. Deutz NEP, Bauer JM, Barazzoni R, Biolo G, Boirie Y, Bosy-Westphal A, et al. Protein intake and exercise for optimal muscle function with aging: Recommendations from the ESPEN Expert Group. Clin Nutr. 2014.33:929–36.

6. Noakes M, Keogh JB, Foster PR, Clifton PM. Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women. Am J Clin Nutr. 2005.81:1298–306.

7. Skov AR, Toubro S, Rønn B, Holm L, Astrup A. Randomized trial on protein vs carbohydrate in ad libitum fat reduced diet for the treatment of obesity. Int J Obes Relat Metab Disord. 1999.23:528–36.

8. Layman DK, Boileau RA, Erickson DJ, Painter JE, Shiue H, Sather C, et al. A reduced ratio of dietary carbohydrate to protein improves body composition and blood lipid profiles during weight loss in adult women. J Nutr. 2003.133:411–7.

9. Santesso N, Akl EA, Bianchi M, Mente A, Mustafa R, Heels-Ansdell D, et al. Effects of higher- versus lower-protein diets on health outcomes: a systematic review and meta-analysis. Eur J Clin Nutr. Macmillan Publishers Limited; 2012.66:780–8.

10. Clifton PM, Condo D, Keogh JB. Long term weight maintenance after advice to consume low carbohydrate, higher protein diets - A systematic review and meta analysis. Nutr Metab Cardiovasc Dis. 2014.24:224–35.

11. Schwingshackl L, Hoffmann G. Long-term effects of low-fat diets either low or high in protein on cardiovascular and metabolic risk factors: a systematic review and meta-analysis. Nutr J. 2013.12:48.

12. Halkjær J, Olsen A, Overvad K, Jakobsen MU, Boeing H, Buijsse B, et al. Intake of total, animal and plant protein and subsequent changes in weight or waist circumference in European men and women: the Diogenes project. Int J Obes. 2011.35:1104–13.

13. Vergnaud A-C, Norat T, Mouw T, Romaguera D, May AM, Bueno-de-Mesquita HB, et al. Macronutrient composition of the diet and prospective weight change in participants of the EPIC-PANACEA study. PLoS One. 2013.8:e57300.

14. Ankarfeldt MZ, Angquist L, Jakobsen MU, Overvad K, Tjønneland A, Halkjaer J, et al. Interactions of dietary protein and adiposity measures in relation to

subsequent changes in body weight and waist circumference. Obesity. 2014.22:2097–103.

15. Pedersen AN, Kondrup J, Børsheim E. Health effects of protein intake in healthy adults: a systematic literature review. Food Nutr Res. 2013.57.

16. Pedersen AN, Cederholm T. Health effects of protein intake in healthy elderly populations: a systematic literature review. Food Nutr Res. 2014.58.

17. Martínez-González MÁ, Corella D, Salas-Salvadó J, Ros E, Covas MI, Fiol M, et al. Cohort profile: design and methods of the PREDIMED study. Int J Epidemiol. 2012.41:377–85.

18. Martin-Moreno JM, Boyle P, Gorgojo L, Maisonneuve P, Fernandez-Rodriguez JC, Salvini S, et al. Development and validation of a food frequency questionnaire in Spain. Int J Epidemiol. 1993.22:512–9.

19. Fernández-Ballart JD, Piñol JL, Zazpe I, Corella D, Carrasco P, Toledo E, et al. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. Br J Nutr. 2010.103:1808–16.

20. Moreiras O, Carbajal A, Cabrera L, Cuadrado C. Tablas de composición de los alimentos. (Food Composition Tables). 9th editio. Madrid: Pirámide; 2005. 456 p.

21. Schröder H, Fitó M, Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, et al. A short screener is valid for assessing Mediterranean diet adherence among older Spanish men and women. J Nutr. 2011.141:1140–5.

22. NHLBI Obesity Education Initiative Expert Panel on the Identification, Evaluation and T of O in A (US). Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults: The Evidence Report. Bethesda (MD): National Heart, Lung, and Blood Institute. 1998. Available from: http://www.ncbi.nlm.nih.gov/books/NBK2003/;

23. Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International . Circulation. 2009.120:1640–5.

24. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr. 1997.65:1220S – 1228S; discussion 1229S – 1231S.

25. Hu FB, Stampfer MJ, Rimm E, Ascherio A, Rosner BA, Spiegelman D, et al. Dietary fat and coronary heart disease: a comparison of approaches for adjusting for total energy intake and modeling repeated dietary measurements. Am J Epidemiol. 1999.149:531–40.

26. Willett WC. Nutritional epidemiology. 3rd ed. New York: Oxford University Press; 2013.

27. Wolfe RR, Miller SL, Miller KB. Optimal protein intake in the elderly. Clin Nutr. 2008.27:675–84.

28. Halton TL, Hu FB. The effects of high protein diets on thermogenesis, satiety and weight loss: a critical review. J Am Coll Nutr. 2004.23:373–85.

29. Zhan S, Ho SC. Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. Am J Clin Nutr. 2005.81:397–408.

30. Teunissen-Beekman KFM, van Baak MA. The role of dietary protein in blood pressure regulation. Curr Opin Lipidol. 2013.24:65–70.

31. Krajcovicova-Kudlackova M, Babinska K, Valachovicova M. Health benefits and risks of plant proteins. Bratisl Lek Listy. 2005.106:231–4.

32. Dong J-Y, Zhang Z-L, Wang P-Y, Qin L-Q. Effects of high-protein diets on body weight, glycaemic control, blood lipids and blood pressure in type 2 diabetes: meta-analysis of randomised controlled trials. Br J Nutr. 2013.110:781–9.

33. Bujnowski D, Xun P, Daviglus ML, Van Horn L, He K, Stamler J. Longitudinal association between animal and vegetable protein intake and obesity among men in the United States: the Chicago Western Electric Study. J Am Diet Assoc. 2011.111:1150–5.e1.

34. Fung TT, van Dam RM, Hankinson SE, Stampfer M, Willett WC, Hu FB. Low-carbohydrate diets and all-cause and cause-specific mortality: two cohort studies. Ann Intern Med. 2010.153:289–98.

35. Friedman AN. High-protein diets: potential effects on the kidney in renal health and disease. Am J Kidney Dis. 2004.44:950–62.

36. Tonelli M, Wiebe N, Culleton B, House A, Rabbat C, Fok M, et al. Chronic kidney disease and mortality risk: a systematic review. J Am Soc Nephrol. 2006.17:2034–47.

37. Tremblay F, Lavigne C, Jacques H, Marette A. Role of dietary proteins and amino acids in the pathogenesis of insulin resistance. Annu Rev Nutr. 2007.27:293–310.

38. Janghorbani M, Hu FB, Willett WC, Li TY, Manson JE, Logroscino G, et al. Prospective study of type 1 and type 2 diabetes and risk of stroke subtypes: the Nurses' Health Study. Diabetes Care. 2007.30:1730–5.

39. Virtanen JK, Voutilainen S, Rissanen TH, Happonen P, Mursu J, Laukkanen JA, et al. High dietary methionine intake increases the risk of acute coronary events in middle-aged men. Nutr Metab Cardiovasc Dis. 2006.16:113–20.

40. Karnani MM, Apergis-Schoute J, Adamantidis A, Jensen LT, de Lecea L, Fugger L, et al. Activation of central orexin/hypocretin neurons by dietary amino acids. Neuron. 2011.72:616–29.

41. Hagan MM, Rushing PA, Schwartz MW, Yagaloff KA, Burn P, Woods SC, et al. Role of the CNS melanocortin system in the response to overfeeding. J Neurosci. 1999.19:2362–7.

TABLES

Table 1. Baseline characteristics of study participants by quintiles (Q) of total protein intake

	Cumulative average percentage of energy from total protein intake					
	Q1 (n = 1443)	**Q2** (n = 1443)	**Q3** (n = 1444)	**Q4** (n = 1443)	**Q5** (n = 1443)	***P* value**
Female sex, % (n)	37.8 (546)	50.2 (724)	58.7 (848)	67.4 (972)	73.1 (1055)	<0.001
Age, years	67 ± 6	67 ± 6	67 ± 6	67 ± 6	67 ± 6	0.01
BMI, kg/m^2	29.7 ± 3.7	29.7 ± 3.7	30.0 ± 3.6	30.1 ± 4	30.3 ± 4.2	<0.001
Weight, kg	78.7 ± 11.6	76.8 ± 12.0	76.7 ± 11.7	76.1 ± 12.3	75.5 ± 12.0	<0.001
Waist circumference, cm	101 ± 10	100 ± 11	101 ± 10	100 ± 11	100 ± 11	0.004
Leisure-time physical activity, MET-min/day	245 ± 269	229 ± 222	231 ± 237	225 ± 239	225 ± 226	<0.001
Smoking status, % (n)						<0.001
Never	47.1 (679)	55.6 (803)	62.6 (904)	69.9 (1008)	72.4 (1045)	
Current	22.7 (327)	15.3 (221)	13.3 (192)	9.6 (139)	8.7 (125)	
Former	30.3 (437)	29.0 (419)	24.1 (348)	20.5 (296)	18.9 (273)	
Educational level, % (n)						<0.001
Primary education	73.7 (1058)	74.9 (1068)	76.2 (1072)	79.9 (1127)	81.6 (1146)	
Secondary education	17.4 (250)	17.3 (246)	15.9 (224)	14.6 (206)	12.1 (170)	
Academic/graduate	8.9 (128)	7.8 (111)	7.9 (111)	5.5 (78)	6.3 (88)	
Prevalence of diabetes, % (n)	36.9 (533)	46.4 (669)	48.1 (695)	51.9 (749)	59.8 (863)	<0.001
Prevalence of hypertension, % (n)	83.6 (1206)	82.8 (1195)	84.6 (1222)	82.7 (1193)	80.0 (1154)	0.02
Prevalence of hypercholesterolemia, % (n)	71.7 (1034)	72.3 (1044)	73.3 (1058)	72.5 (1046)	71.4 (1030)	0.81
Family history of CVD, % (n)	19.6 (283)	20.2 (291)	22.3 (322)	24.4 (352)	25.1 (362)	<0.001
Medication use, % (n)						
Aspirin	22.9 (331)	23.8 (344)	23.0 (332)	20.7 (299)	21.3 (307)	0.52
Oral antidiabetic drugs	23.8 (344)	30.4 (439)	32.0 (462)	34.6 (499)	40.1 (579)	<0.001
Insulin	3.7 (54)	5.6 (81)	7.0 (101)	7.2 (104)	10.9 (157)	<0.001
Antihypertensive drugs	74.3 (1072)	71.5 (1032)	73.9 (1067)	71.2 (1027)	72.8 (1050)	0.44
Statins	45.3 (653)	47.4 (684)	47.7 (689)	50.5 (728)	50.2 (725)	0.07
Total energy intake, Kcal/d	2453.2 ± 572.5	2346.4 ± 526.0	2256.1 ± 520.4	2153.2 ± 498.5	1972.0 ± 469.5	<0.001
Protein, g/day	83.0 ± 20.0	89.7 ± 20.3	92.7 ± 20.7	94.6 ± 21.6	96.6 ± 21.6	<0.001
Total protein, % of energy	13.6 ± 1.6	15.4 ± 1.6	16.6 ± 1.7	17.7 ± 1.8	19.8 ± 2.4	<0.001
Animal protein, % of energy	8.3 ± 1.8	10.0 ± 1.9	11.1 ± 2.0	12.2 ± 2.1	14.2 ± 2.7	<0.001
Vegetable protein, % of energy	5.3 ± 1.1	5.4 ± 1.0	5.4 ± 1.0	5.5 ± 1.1	5.6 ± 1.1	<0.001
Animal-to-vegetable protein ratio	1.7 ± 0.6	2.0 ± 0.7	2.2 ± 0.8	2.4 ± 0.8	2.7 ± 1.1	<0.001
Carbohydrates, % of energy	42.7 ± 7.6	42.0 ± 7.3	41.7 ± 7.1	41.5 ± 6.8	41.0 ± 6.8	<0.001
Fats, % of energy	39.2 ± 7.1	40.0 ± 7.0	39.6 ± 6.8	39.2 ± 6.6	38.1 ± 6.4	<0.001
Saturated fat	9.7 ± 2.1	10.1 ± 2.3	10.1 ± 2.2	10.1 ± 2.2	9.9 ± 2.3	<0.001
Monounsaturated fat	19.9 ± 4.8	20.0 ± 4.8	19.7 ± 4.6	19.4 ± 4.4	18.6 ± 4.1	<0.001

Polyunsaturated fat	6.4 ± 2.2	6.4 ± 2.1	6.3 ± 2.0	6.2 ± 2.0	5.8 ± 1.9	<0.001
Alcohol, % of energy	4.5 ± 5.4	2.7 ± 3.8	2.1 ± 3.2	1.6 ± 2.9	1.1 ± 2.1	<0.001
Fiber, energy adjusted g/d	23.3 ± 7.8	24.6 ± 7.5	25.1 ± 7.3	26.0 ± 7.4	27.2 ± 7.0	<0.001
Omega-3 fatty acids, energy-adjusted, g/d	1.9 ± 0.7	2.1 ± 0.8	2.2 ± 0.8	2.3 ± 0.8	2.4 ± 0.8	<0.001
Glycemic index	54.9 ± 5.8	54.4 ± 5.7	53.8 ± 5.7	53.1 ± 5.6	51.8 ± 5.9	<0.001

Data are expressed as mean ± SD or % (n). *P* values for comparisons across quintiles of cumulative average energy from total protein (Pearson χ^2 test for categorical variables or one-factor analysis of variance for continuous variables) as appropriate. BMI, body mass index; MET, metabolic equivalent task; CVD, cardiovascular disease.

Table 2. Hazard ratios of cardiovascular events or death for different causes according to quintiles (Q) of total protein intake

	Quintiles of cumulative average percentage of energy from total protein intake					
	Q1 (n = 1443)	Q2 (n = 1443)	Q3 (n = 1444)	Q4 (n = 1443)	Q5 (n = 1443)	*P* q-trend
Median (% of energy)	13.87	15.40	16.47	17.63	19.45	
Cardiovascular event, % (n)	4.9 (70)	3.4 (49)	3.9 (56)	3.0 (44)	4.0 (58)	
Person-years, n	6244	6325	6348	6190	5934	
Crude Model	1.27 (0.89, 1.80)	0.88 (0.60, 1.29)	1.00 (Referent)	0.80 (0.54, 1.19)	1.12 (0.77, 1.61)	0.03
Model 1	1.08 (0.74, 1.57)	0.82 (0.56, 1.22)	1.00 (Referent)	0.90 (0.60, 1.34)	1.31 (0.90, 1.91)	0.05
Model 2	0.89 (0.60, 1.33)	0.75 (0.51, 1.12)	1.00 (Referent)	0.91 (0.60, 1.37)	1.33 (0.88, 2.01)	0.16
Model 3	0.89 (0.59, 1.32)	0.75 (0.51, 1.11)	1.00 (Referent)	0.92 (0.61, 1.38)	1.38 (0.92, 2.07)	0.14
Cardiovascular death, % (n)	1.7 (24)	1.3 (19)	0.9 (13)	0.7 (10)	1.0 (15)	
Person-years, n	6333	6336	6173	6108	6129	
Crude Model	1.89 (0.96, 3.71)	1.46 (0.72, 2.97)	1.00 (Referent)	0.78 (0.34, 1.79)	1.25 (0.59, 2.62)	0.10
Model 1	1.25 (0.60, 2.60)	1.20 (0.59, 2.45)	1.00 (Referent)	0.98 (0.43, 2.24)	1.81 (0.85, 3.88)	0.13
Model 2	1.02 (0.47, 2.25)	1.08 (0.52, 2.27)	1.00 (Referent)	1.03 (0.44, 2.42)	2.09 (0.92, 4.78)	0.17
Model 3	1.03 (0.46, 2.30)	1.09 (0.52, 2.31)	1.00 (Referent)	1.03 (0.44, 2.42)	2.10 (0.93, 4.75)	0.16
Cancer death, % (n)	2.6 (37)	1.5 (22)	1.9 (27)	1.2 (17)	1.9 (27)	
Person-years, n	6333	6336	6173	6108	6129	
Crude Model	1.39 (0.85, 2.28)	0.82 (0.47, 1.44)	1.00 (Referent)	0.65 (0.36, 1.20)	1.10 (0.64, 1.87)	0.04
Model 1	1.09 (0.63, 1.87)	0.74 (0.42, 1.31)	1.00 (Referent)	0.76 (0.41, 1.41)	1.40 (0.80, 2.43)	0.05
Model 2	0.91 (0.51, 1.60)	0.69 (0.39, 1.22)	1.00 (Referent)	0.78 (0.42, 1.45)	1.48 (0.83, 2.67)	0.19
Model 3	0.80 (0.45, 1.43)	0.66 (0.37, 1.18)	1.00 (Referent)	0.77 (0.42, 1.44)	1.44 (0.80, 2.59)	0.21
All-cause death, % (n)	6.6 (95)	4.0 (57)	3.9 (57)	3.1 (45)	4.8 (69)	
Person-years, n	6333	6336	6173	6108	6129	
Crude Model	1.70 (1.23, 2.36)	1.00 (0.70, 1.45)	1.00 (Referent)	0.81 (0.55, 1.20)	1.31 (0.92, 1.87)	<0.001
Model 1	1.40 (0.98, 2.00)	0.91 (0.63, 1.32)	1.00 (Referent)	0.91 (0.62, 1.35)	1.61 (1.12, 2.32)	<0.001
Model 2	1.22 (0.84, 1.77)	0.88 (0.60, 1.28)	1.00 (Referent)	0.93 (0.63, 1.39)	1.59 (1.08, 2.35)	<0.001
Model 3	1.17 (0.80, 1.70)	0.86 (0.59, 1.25)	1.00 (Referent)	0.95 (0.64, 1.42)	1.66 (1.13, 2.43)	<0.001

Cox proportional hazards models were used to assess the risk of death by quintiles of cumulative average total protein intake (%E). Data is presented as HRs and 95% CIs. Multivariable models were adjusted for intervention group, node, sex, age in years, body mass index (kg/m^2), smoking status (former or current smoker), leisure time physical activity (metabolic equivalent task-min/d), cumulative average alcohol intake (continuous, adding a quadratic term), prevalence of diabetes, hypertension, hypercholesterolemia, family history of coronary heart disease, use of aspirin, antihypertensive medication, oral antidiabetic medication, insulin medication and hypocholesterolemic medication; and nutritional variables as follows: quintiles of cumulative average percentage of total energy intake, energy from fats (in model 2), energy from carbohydrates (in model 3), energy-adjusted omega-3 fatty acids and fiber, and glycemic index. Extremes of total energy intake were excluded. *P* for q-trend stands for the evaluation of the quadratic model. (a) stands for significant ($P < 0.05$) in linear trend.

Table 3. Hazard ratios of body weight and WC_{BMI} changes, and cardiovascular event or mortality for different causes according to intake of g protein/kg BW per day into three categories.

	Categories of cumulative average protein intake (g protein/kg BW/day)			
	< 1.0 (n = 1737)	[1.0, 1.5] (n = 4461)	> 1.5 (n = 1018)	*P* q-trend
Median (g protein/kg BW/day)	0.89	1.21	1.64	
Anthropometry outcomes				
Body weight gain (10% cut-off), % (n)	2.2 (37)	2.1 (93)	1.9 (19)	
Crude Model	1.24 (0.84, 1.81)	1.00 (Referent)	0.89 (0.55, 1.47)	0.77
Model 1	1.13 (0.76, 1.66)	1.00 (Referent)	0.93 (0.56, 1.54)	0.90
Model 2	1.06 (0.64, 1.74)	1.00 (Referent)	0.98 (0.53, 1.84)	0.91
Body weight loss (10% cut-off), % (n)	2.6 (45)	2.4 (105)	3.6 (36)	
Crude Model	1.31 (0.92, 1.86)	1.00 (Referent)	1.47 (1.01, 2.14)	0.03
Model 1	1.19 (0.83, 1.69)	1.00 (Referent)	1.43 (0.97, 2.11)	0.08
Model 2	1.13 (0.72, 1.77)	1.00 (Referent)	1.25 (0.74, 2.09)	0.28
WC_{BMI} incidence, % (n)	6.5 (106)	7.3 (311)	7.2 (69)	
Crude Model	1.09 (0.87, 1.36)	1.00 (Referent)	0.97 (0.74, 1.25)	0.79
Model 1	0.90 (0.72, 1.13)	1.00 (Referent)	1.26 (0.96, 1.64)	0.50
Model 2	1.05 (0.79, 1.38)	1.00 (Referent)	1.04 (0.74, 1.47)	0.66
WC_{BMI} reversion, % (n)	5.8 (94)	5.5 (229)	5.8 (55)	
Crude Model	1.34 (1.05, 1.70)	1.00 (Referent)	1.02 (0.76, 1.37)	0.15
Model 1	1.13 (0.88, 1.45)	1.00 (Referent)	1.12 (0.83, 1.52)	0.28
Model 2	1.04 (0.76, 1.42)	1.00 (Referent)	1.20 (0.81, 1.76)	0.34
Fatal and non-fatal outcomes				
Cardiovascular event, % (n)	3.8 (66)	3.7 (163)	4.7 (48)	
Crude Model	1.15 (0.86, 1.53)	1.00 (Referent)	1.30 (0.94, 1.80)	0.10
Model 1	0.94 (0.69, 1.28)	1.00 (Referent)	1.53 (1.08, 2.18)	0.14
Model 2	0.92 (0.64, 1.32)	1.00 (Referent)	1.42 (0.93, 2.16)	0.32
Cardiovascular mortality, % (n)	1.0 (17)	1.0 (43)	2.1 (21)	
Crude Model	1.14 (0.65, 1.99)	1.00 (Referent)	2.15 (1.28, 3.62)	0.04
Model 1	0.82 (0.45, 1.48)	1.00 (Referent)	3.14 (1.75, 5.62)	0.03
Model 2	0.92 (0.46, 1.84)	1.00 (Referent)	2.77 (1.35, 5.68)	0.06
Cancer mortality, % (n)	2.2 (39)	1.8 (80)	1.1 (11)	
Crude Model	1.41 (0.96, 2.07)	1.00 (Referent)	0.61 (0.32, 1.14)	0.70
Model 1	1.33 (0.88, 2.02)	1.00 (Referent)	0.69 (0.35, 1.33)	0.82
Model 2	0.93 (0.56, 1.53)	1.00 (Referent)	0.95 (0.48, 2.02)	0.81
All-cause mortality, % (n)	5.5 (95)	3.9 (174)	5.3 (54)	
Crude Model	1.58 (1.23, 2.03)	1.00 (Referent)	1.36 (1.00, 1.85)	<0.001
Model 1	1.42 (1.09, 1.86)	1.00 (Referent)	1.48 (1.06, 2.06)	<0.001
Model 2	1.28 (0.93, 1.76)	1.00 (Referent)	1.54 (1.04, 2.29)	<0.001

BMI, (body mass index), WC_{BMI} (waist circumference corrected by BMI). Cox proportional hazards models were used to assess the risk of weight change (10% from baseline), WC_{BMI} change (incidence and reversion), cardiovascular event, and death by different causes, by categories of cumulative average grams of protein/kg body weight (BW) per day. Data is presented as HRs and 95% CIs. Multivariable models were adjusted for intervention group, node, sex, age in years, smoking status (former or current smoker), leisure time physical activity (metabolic equivalent task-min/d), cumulative average alcohol intake (continuous, adding a quadratic term) (model 1). Model 2 was also adjusted for: prevalence of diabetes, hypertension, hypercholesterolemia, family history of coronary heart disease, use of aspirin, antihypertensive medication, oral antidiabetic medication, insulin medication and hypocholesterolemic medication; and nutritional variables as follows: quintiles of cumulative average percentage of total energy intake, fats (in g protein/kg body weight/ day), carbohydrates (in g protein/kg body weight/day), energy-adjusted omega-3 fatty acids and fiber, and glycemic index. Extremes of total energy intake were excluded. P for q-trend stands for the evaluation of the quadratic model...

"Weight gain" and "Weight loss" were defined when participants gained or loss ≥10% of body weight, respectively. "WC_{BMI} incidence" and "WC_{BMI} reversion" were defined according to the metabolic syndrome criteria (i.e. ≥88 cm for women and ≥102 cm for men). Comparisons of both weight and WC_{BMI} groups were performed against the "maintenance group" (i.e. no weight gain/loss, or no WC_{BMI} incidence/reversion, as appropriate).

Table 4. Hazard ratios of cardiovascular events or death for different causes according to quintiles (Q) of different sources of protein intake

		Quintiles of cumulative average percentage of energy from protein intake from different sources					
		Q1	Q2	Q3	Q4	Q5	*P* q-trend
Animal protein, median E%		8.25	9.84	10.95	12.11	13.90	
Cardiovascular event, % (n)		4.7 (68)	3.6 (52)	3.1 (45)	3.7 (53)	4.1 (59)	
	Crude Model	1.63 (1.12, 2.37)	1.18 (0.79, 1.77)	1.00 (Referent)	1.20 (0.81, 1.79)	1.44 (0.97, 2.12)	0.01
	Model 2	1.13 (0.74, 1.71)	1.00 (0.67, 1.51)	1.00 (Referent)	1.45 (0.97, 2.19)	1.88 (1.23, 2.88)	0.02
	Model 3	1.14 (0.74, 1.73)	1.01 (0.67, 1.52)	1.00 (Referent)	1.46 (0.97, 2.19)	1.88 (1.23, 2.86)	0.03
Cardiovascular death, % (n)		1.6 (23)	1.1 (16)	0.9 (13)	0.8 (11)	1.2 (18)	
	Crude Model	1.92 (0.97, 3.79)	1.26 (0.61, 2.63)	1.00 (Referent)	0.85 (0.38, 1.91)	1.53 (0.75, 3.13)	0.03
	Model 2	1.14 (0.53, 2.43)	0.90 (0.42, 1.92)	1.00 (Referent)	1.24 (0.54, 2.86)	3.06 (1.39, 6.74)	0.01
	Model 3	1.17 (0.54, 2.52)	0.91 (0.43, 1.96)	1.00 (Referent)	1.23 (0.54, 2.83)	2.98 (1.36, 6.51)	0.02
Cancer death, % (n)		2.6 (38)	1.2 (17)	1.6 (23)	1.7 (24)	1.9 (28)	
	Crude Model	1.78 (1.06, 2.99)	0.75 (0.40, 1.41)	1.00 (Referent)	1.06 (0.60, 1.88)	1.34 (0.77, 2.33)	0.01 (a)
	Model 2	1.15 (0.64, 2.05)	0.61 (0.32, 1.16)	1.00 (Referent)	1.17 (0.65, 2.10)	1.85 (1.03, 3.34)	0.02
	Model 3	1.03 (0.57, 1.85)	0.58 (0.31, 1.11)	1.00 (Referent)	1.23 (0.68, 2.21)	1.81 (1.00, 3.31)	0.04
All-cause death, % (n)		6.3 (91)	3.8 (55)	3.9 (56)	3.4 (49)	5.0 (72)	
	Crude Model	1.76 (1.26, 2.45)	1.01 (0.69, 1.46)	1.00 (Referent)	0.88 (0.60, 1.30)	1.42 (1.00, 2.01)	<0.001
	Model 2	1.27 (0.87, 1.84)	0.88 (0.60, 1.29)	1.00 (Referent)	1.10 (0.74, 1.63)	1.86 (1.27, 2.73)	<0.001
	Model 3	1.24 (0.86, 1.81)	0.88 (0.60, 1.29)	1.00 (Referent)	1.12 (0.76, 1.65)	1.92 (1.31, 2.82)	<0.001
Vegetable protein, median E%		4.48	5.06	5.49	5.94	6.59	
Cardiovascular event, % (n)		4.6 (66)	3.6 (52)	3.4 (49)	3.7 (54)	3.9 (56)	
	Crude Model	1.29 (0.89, 1.87)	1.04 (0.70, 1.54)	1.00 (Referent)	1.16 (0.79, 1.71)	1.40 (0.96, 2.06)	0.05
	Model 2	0.91 (0.60, 1.40)	0.94 (0.62, 1.41)	1.00 (Referent)	1.23 (0.82, 1.83)	1.36 (0.86, 2.15)	0.61
	Model 3	0.84 (0.55, 1.29)	0.91 (0.60, 1.36)	1.00 (Referent)	1.24 (0.83, 1.85)	1.38 (0.88, 2.17)	0.74

Cardiovascular death, % (n)		1.4 (20)	1.0 (15)	1.5 (21)	1.0 (14)	0.8 (11)	
	Crude Model	0.89 (0.48, 1.64)	0.71 (0.36, 1.37)	1.00 (Referent)	0.71 (0.36, 1.39)	0.66 (0.32, 1.37)	0.69
	Model 2	0.52 (0.25, 1.07)	0.56 (0.28, 1.14)	1.00 (Referent)	0.78 (0.38, 1.59)	0.73 (0.30, 1.75)	0.14
	Model 3	0.45 (0.22, 0.95)	0.57 (0.28, 1.15)	1.00 (Referent)	0.80 (0.39, 1.63)	0.78 (0.32, 1.88)	0.22
Cancer death, % (n)		2.9 (42)	1.4 (20)	1.4 (20)	1.6 (23)	1.7 (25)	
	Crude Model	2.04 (1.19, 3.48)	0.98 (0.52, 1.81)	1.00 (Referent)	1.22 (0.67, 2.22)	1.60 (0.89, 2.89)	0.003
	Model 2	1.82 (1.01, 3.30)	0.96 (0.50, 1.82)	1.00 (Referent)	1.31 (0.71, 2.42)	1.37 (0.69, 2.72)	0.05
	Model 3	1.84 (1.01, 3.35)	0.96 (0.51, 1.82)	1.00 (Referent)	1.30 (0.70, 2.40)	1.39 (0.70, 2.75)	0.04
All-cause death, % (n)		6.1 (88)	4.0 (57)	4.2 (60)	3.7 (54)	4.4 (64)	
	Crude Model	1.38 (0.99, 1.92)	0.93 (0.65, 1.34)	1.00 (Referent)	0.96 (0.66, 1.38)	1.36 (0.96, 1.94)	0.003
	Model 2	1.04 (0.72, 1.52)	0.86 (0.59, 1.25)	1.00 (Referent)	1.01 (0.70, 1.48)	1.28 (0.84, 1.94)	0.16
	Model 3	1.02 (0.70, 1.49)	0.85 (0.58, 1.24)	1.00 (Referent)	1.01 (0.70, 1.48)	1.32 (0.88, 2.00)	0.14
Animal-to-vegetable protein ratio, median E%		1.40	1.75	2.03	2.31	2.85	
Cardiovascular event, % (n)		4.4 (63)	3.5 (50)	4.2 (60)	2.7 (39)	4.5 (65)	
	Crude Model	1.18 (0.83, 1.68)	0.85 (0.58, 1.23)	1.00 (Referent)	0.63 (0.42, 0.95)	1.13 (0.79, 1.60)	0.02
	Model 2	0.94 (0.64, 1.38)	0.81 (0.55, 1.18)	1.00 (Referent)	0.67 (0.44, 1.01)	1.24 (0.84, 1.81)	0.07
	Model 3	0.90 (0.61, 1.34)	0.81 (0.55, 1.20)	1.00 (Referent)	0.67 (0.44, 1.01)	1.21 (0.81, 1.79)	0.12
Cardiovascular death, % (n)		1.2 (17)	1.4 (20)	1.0 (14)	0.8 (11)	1.3 (19)	
	Crude Model	1.38 (0.68, 2.80)	1.45 (0.73, 2.88)	1.00 (Referent)	0.76 (0.34, 1.67)	1.41 (0.71, 2.82)	0.18
	Model 2	1.08 (0.50, 2.32)	1.36 (0.67, 2.76)	1.00 (Referent)	0.79 (0.35, 1.78)	1.73 (0.82, 3.64)	0.22
	Model 3	1.19 (0.54, 2.64)	1.48 (0.71, 3.08)	1.00 (Referent)	0.84 (0.36, 1.94)	2.09 (0.95, 4.59)	0.11
Cancer death, % (n)		2.2 (32)	1.7 (24)	1.2 (17)	1.6 (23)	2.4 (34)	
	Crude Model	2.16 (1.20, 3.89)	1.45 (0.78, 2.70)	1.00 (Referent)	1.32 (0.70, 2.47)	2.10 (1.17, 3.76)	0.01
	Model 2	1.43 (0.77, 2.68)	1.34 (0.71, 2.51)	1.00 (Referent)	1.44 (0.76, 2.73)	2.67 (1.43, 4.97)	0.01
	Model 3	1.34 (0.71, 2.53)	1.32 (0.70, 2.48)	1.00 (Referent)	1.54 (0.81, 2.93)	2.82 (1.49, 5.33)	0.01
All-cause death, % (n)		5.1 (74)	4.6 (67)	3.7 (54)	3.7 (53)	5.2 (75)	

Crude Model	1.57 (1.10, 2.22)	1.27 (0.89, 1.82)	1.00 (Referent)	0.95 (0.65, 1.39)	1.45 (1.02, 2.06)	0.002
Model 2	1.22 (0.84, 1.78)	1.23 (0.86, 1.77)	1.00 (Referent)	1.01 (0.69, 1.48)	1.67 (1.15, 2.44)	0.01
Model 3	1.20 (0.82, 1.76)	1.23 (0.86, 1.78)	1.00 (Referent)	1.04 (0.71, 1.53)	1.69 (1.15, 2.49)	0.02

E% (percentage of energy). Cox proportional hazards models were used to assess the risk of death by quintiles of cumulative average protein intake (E%) from animal, vegetable and animal-to-vegetable protein ratio, as convenient. Data is presented as HRs and 95% CIs. Multivariable models were adjusted for intervention group, node, sex, age in years, body mass index (kg/m^2), smoking status (former or current smoker), leisure time physical activity (metabolic equivalent task-min/d), cumulative average alcohol intake (continuous, adding a quadratic term), prevalence of diabetes, hypertension, hypercholesterolemia, family history of coronary heart disease, use of aspirin, antihypertensive medication, oral antidiabetic medication, insulin medication and hypocholesterolemic medication; and nutritional variables as follows: quintiles of cumulative average percentage of total energy intake, energy from fats (in model 2), energy from carbohydrates (in model 3), energy-adjusted omega-3 fatty acids and fiber, glycemic index and mutual adjustment for animal protein and vegetable protein (in quintiles). Extremes of total energy intake were excluded. *P* for q-trend stands for the evaluation of the quadratic model. (a) stands for significant ($P < 0.05$) in linear trend.

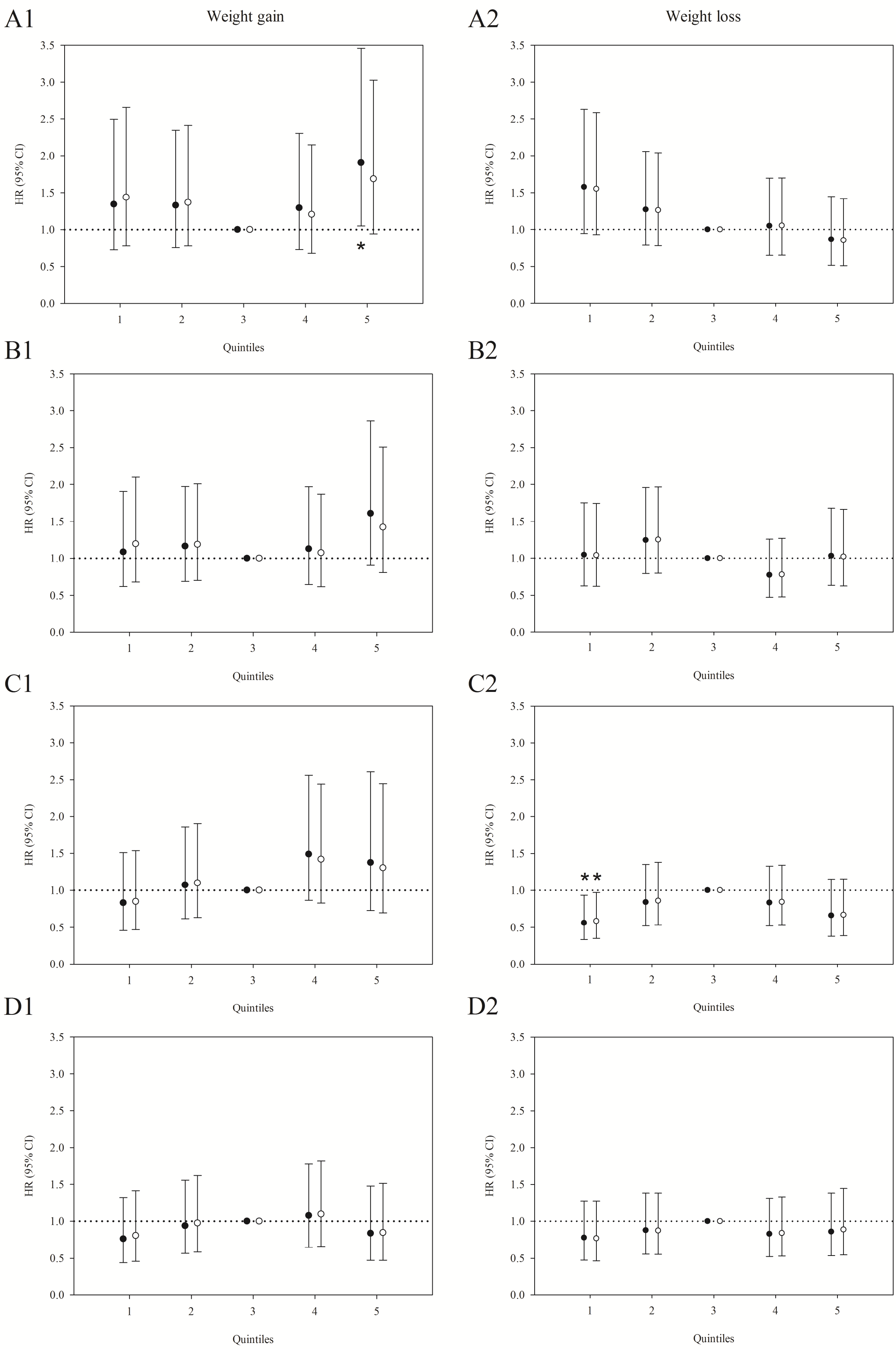
FIGURE LEGENDS

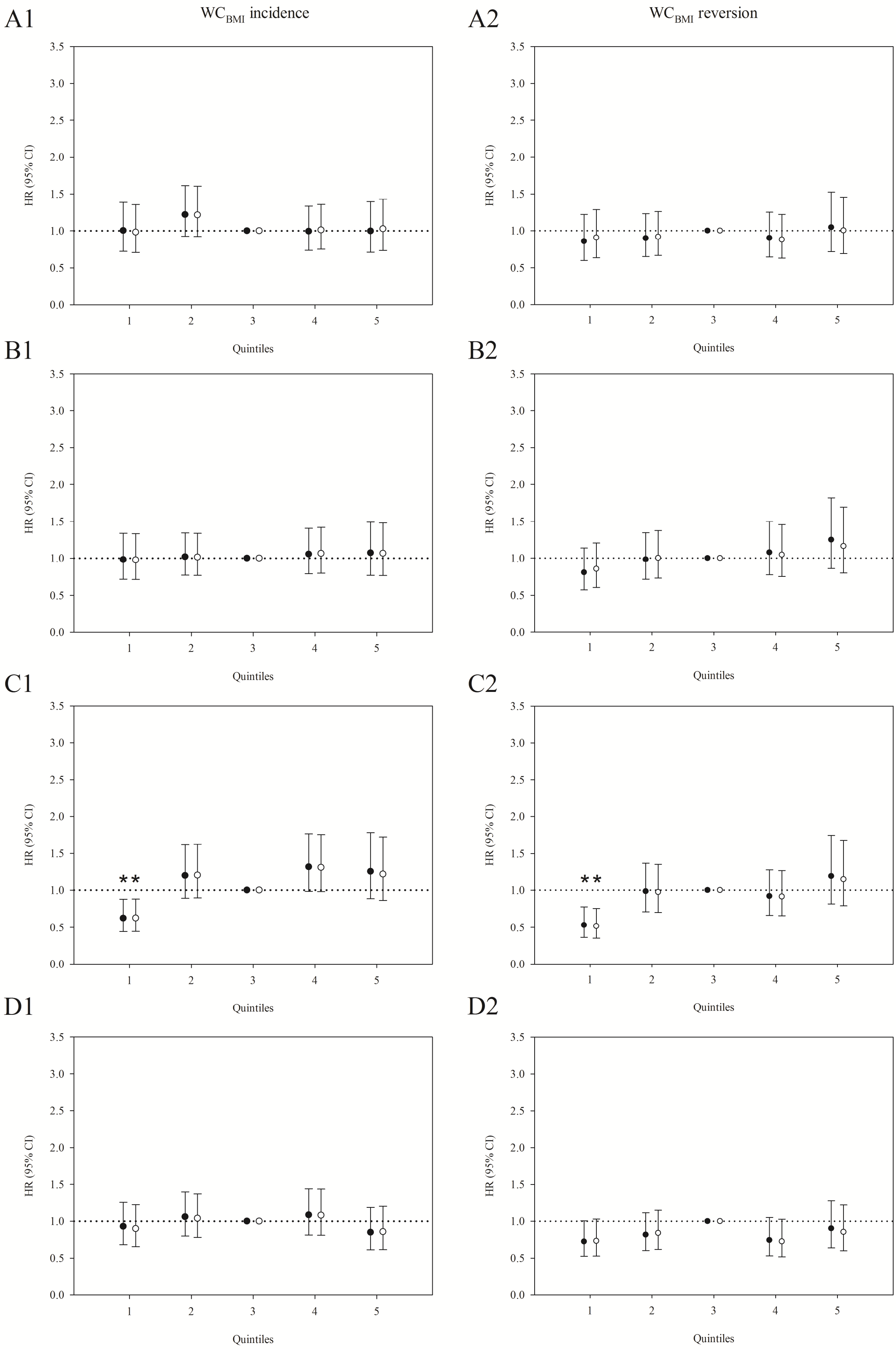
Figure 1. Associations between protein intake (in quintiles) and weight change (10% from baseline)**.**

Cox proportional hazards models were used to assess the risk of weight gain (left columns) or weight loss (right columns) by quintiles of cumulative average protein intake (in E%). Black circles (model 3) and white circles (model 4). Total protein (A1 and A2), animal protein (B1 and B2), vegetable protein (C1 and C2) and animal-to-vegetable protein ratio (D1 and D2) were evaluated. Results are from fully adjusted models: intervention group, node, sex, age, baseline body mass index (kg/m2), smoking status (former or current smoker), leisure time physical activity (metabolic equivalent task-min/d), cumulative average alcohol intake (continuous, with an added quadratic term), prevalence of diabetes, hypertension, hypercholesterolemia, family history of coronary heart disease, use of aspirin, antihypertensive medication, oral antidiabetic medication, insulin medication and hypocholesterolemic medication. The following nutritional variables were also used: quintiles of cumulative average percentage of total energy intake, energy from fats (in model 2), energy from carbohydrates (in model 3), energy-adjusted omega-3 fatty acids and fiber, and glycemic index. Extremes of total energy intake were excluded. * stands for $P < 0.05$. A “weight loss” event was defined as losing 10% or more of baseline weight; and a “weight gain” event was defined as gaining 10% or more of baseline weight. Comparisons were performed against the maintenance group (body weight gain lower than 10%, and body weight loss lower than 10%).

Figure 2. Associations between protein intake (in quintiles) and WC_{BMI} change (incidence and reversion)**.**

Cox proportional hazards models were used to assess the risk of WC_{BMI} incidence (left columns) or WC_{BMI} reversion (right columns) by quintiles of cumulative average protein intake (in E%). Black circles (model 3) and white circles (model 4). Total protein (A1 and A2), animal protein (B1 and B2), vegetable protein (C1 and C2) and animal-to-vegetable protein ratio (D1 and D2) were evaluated. Results are from fully adjusted models: intervention group, node, sex, age, baseline body mass index (kg/m2), smoking status (former or current smoker), leisure time physical activity (metabolic equivalent task-min/d), cumulative average alcohol intake (continuous, with an added quadratic term), prevalence of diabetes, hypertension, hypercholesterolemia, family history of coronary heart disease, use of aspirin, antihypertensive medication, oral antidiabetic medication, insulin medication and hypocholesterolemic medication. The following nutritional variables were also used: quintiles of cumulative average percentage of total energy intake, energy from fats (in model 2), energy from carbohydrates (in model 3), energy-adjusted omega-3 fatty acids and fiber, and glycemic index. Extremes of total energy intake were excluded. * stands for $P < 0.05$. Changes in waist circumference were defined according to the metabolic syndrome criteria (i.e. ≥88 cm for women and ≥102 cm for men) and classified into three categories (incidence, reversion and maintenance). Comparisons were performed against the maintenance group. WC_{BMI} (waist circumference corrected by BMI).


A1
Weight gain
A2
Weight loss
B1
B2
C1
C2
D1
D2
HR (95% CI)
Quintiles
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
*
* *



A1
WC_{BMI} incidence
A2
WC_{BMI} reversion
B1
B2
C1
C2
D1
D2
HR (95% CI)
Quintiles
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
**

Highlights

- Higher protein intake is related to a high risk of weight gain and death at long-term.
- Higher animal protein intake is related to fatal and non-fatal cardiovascular outcomes.
- No association was found between protein intake and abdominal obesity.

Supplementary information

for

High dietary protein intake is associated with an increased body weight and total death risk.

by

Pablo Hernández-Alonso [a], Jordi Salas-Salvadó [a, b, *], Miguel Ruiz-Canela [b, c], Dolores Corella [b, d], Ramón Estruch [b, e], Montserrat Fitó [b, f], Fernando Arós [b, g], Enrique Gómez-Gracia [b, h], Miquel Fiol [i], José Lapetra [j], Josep Basora [a, b], Lluis Serra-Majem [k], Miguel Ángel Muñoz [l], Pilar Buil-Cosiales [b,c], Carmen Saiz [b,d] and Mònica Bulló [a, b, *]

Author affiliations

[a]Human Nutrition Unit from the Department of Biochemistry and Biotechnology, Hospital Universitari de Sant Joan de Reus, Faculty of Medicine and Health Sciences, IISPV (Institut d'Investigació Sanitària Pere Virgili), Universitat Rovira i Virgili, Reus, Spain. [b]CIBERobn (Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición), Institute of Health Carlos III, Madrid, Spain. [c]Department of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain. [d]Department of Preventive Medicine, University of Valencia, València, Spain. [e]Department of Internal Medicine, Institut d'Investigacions Biomediques August Pi Sunyer (IDIBAPS), Hospital Clínic, University of Barcelona, Barcelona, Spain. [f]Cardiovascular Risk and Nutrition Research Group (Regicor Study Group) Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Barcelona, Spain. [g]Department of Cardiology, University Hospital Txagorritxu, Vitoria, Spain. [h]Department of Preventive Medicine, University of Malaga, Malaga, Spain. [i]Institute of Health Sciences, University of Balearic Islands and Hospital Son Espases, Palma de Mallorca, Spain. [j]Department of Family Medicine, Primary Care Division of Sevilla, San Pablo Health Center, Sevilla, Spain. [k]Department of Clinical Sciences, University of Las Palmas de Gran Canaria, Las Palmas, Spain. [l]Primary Health Care Division and Research, Instituto de Investigaciones Biomédicas August Pi i Sunyer-Jordi Gol, Barcelona, Spain

*Corresponding authors:

Mònica Bulló and Jordi Salas-Salvadó. Human Nutrition Unit, Faculty of Medicine and Health Sciences, Universitat Rovira i Virgili. C/ Sant Llorenç 21, 43201 Reus (SPAIN). Telephone number: +34 977759312 / Fax number: +34 977759322

e-mail address: monica.bullo@urv.cat and jordi.salas@urv.cat

Table S1. Linear regression analysis of BMI, body weight or waist circumference and protein consumption

			Body mass index (kg/m^2)*		Body weight (kg)†		Waist circumference (cm)¶	
	Variable	**Model**	**B (95% CI)**	***P-***	**B (95% CI)**	***P-***	**B (95% CI)**	***P***
Total protein								
	Continuous	Crude	0.09 (0.05, 0.13)	<0.001	-0.43 (-0.55, -	<0.001	-0.17 (-0.24, -	<0.001
		Model 2	0.08 (0.03, 0.13)	0.002	0.26 (0.13, 0.40)	<0.001	0.15 (0.08, 0.23)	<0.001
		Model 3	0.04 (0.00, 0.09)	0.061	0.14 (0.01, 0.27)	0.033	0.18 (0.11, 0.25)	<0.001
	Quintiles	Crude	0.15 (0.08, 0.21)	<0.001	-0.72 (-0.91, -	<0.001	-0.31 (-0.41, -	<0.001
		Model 2	0.13 (0.06, 0.21)	0.001	0.37 (0.16, 0.58)	0.001	0.21 (0.09, 0.33)	<0.001
		Model 3	0.08 (0.01, 0.16)	0.025	0.20 (-0.01, 0.40)	0.061	0.25 (0.13, 0.36)	<0.001
Animal protein								
	Continuous	Crude	0.12 (0.08, 0.16)	<0.001	-0.28 (-0.40, -	<0.001	-0.18 (-0.24, -	<0.001
		Model 2	0.08 (0.04, 0.13)	<0.001	0.28 (0.15, 0.40)	<0.001	0.10 (0.03, 0.17)	<0.001
		Model 3	0.05 (0.01, 0.10)	0.017	0.18 (0.06, 0.31)	0.005	0.13 (0.06, 0.20)	<0.001
	Quintiles	Crude	0.20 (0.14, 0.26)	<0.001	-0.50 (-0.69, -	<0.001	-0.33 (-0.44, -	<0.001
		Model 2	0.13 (0.06, 0.20)	<0.001	0.42 (0.21, 0.62)	<0.001	0.14 (0.02, 0.25)	<0.001
		Model 3	0.09 (0.02, 0.16)	0.013	0.27 (0.07, 0.47)	0.009	0.18 (0.06, 0.29)	<0.001
Vegetable protein								
	Continuous	Crude	-0.15 (-0.24, -	0.002	-0.65 (-0.93, -	<0.001	0.05 (-0.10, 0.21)	0.52
		Model 2	-0.04 (-0.16,	0.441	-0.20 (-0.52,	0.23	0.23 (0.05, 0.41)	0.01
		Model 3	-0.02 (-0.14,	0.701	-0.14 (-0.46,	0.408	0.29 (0.11, 0.47)	<0.001
	Quintiles	Crude	-0.14 (-0.21, -	<0.001	-0.52 (-0.72, -	<0.001	0.06 (-0.05, 0.16)	0.30
		Model 2	-0.08 (-0.17,	0.060	-0.23 (-0.47,	0.07	0.22 (0.09, 0.36)	<0.001
		Model 3	-0.06 (-0.15,	0.166	-0.17 (-0.41,	0.180	0.29 (0.15, 0.43)	<0.001
Animal-to-vegetable protein								
	Continuous	Crude	0.49 (0.35, 0.63)	<0.001	0.15 (-0.29, 0.59)	0.50	-0.48 (-0.71, -	<0.001
		Model 2	0.30 (0.14, 0.46)	<0.001	1.05 (0.60, 1.50)	<0.001	0.07 (-0.18, 0.32)	0.59
		Model 3	0.24 (0.07, 0.41)	0.006	0.85 (0.37, 1.32)	0.001	0.08 (-0.19, 0.35)	0.55
	Quintiles	Crude	0.22 (0.16, 0.29)	<0.001	-0.06 (-0.26,	0.54	-0.25 (-0.35, -	<0.001
		Model 2	0.13 (0.06, 0.20)	<0.001	0.41 (0.21, 0.61)	<0.001	0.04 (-0.07, 0.16)	0.43
		Model 3	0.10 (0.03, 0.18)	0.007	0.31 (0.10, 0.53)	0.004	0.05 (-0.07, 0.17)	0.38

Protein (in percentage of energy, expressed as both continuous variables and quintiles), BMI (body mass index), body weight and WC_{BMI} (waist circumference) were computed as the cumulative average of all visits during follow-up. Results are from both the crude and adjusted models: intervention group, baseline anthropometry (BMI*, body weight† or waist circumference¶), node, sex, age in years, smoking status (former or current smoker), leisure time physical activity (metabolic equivalent task-min/d), cumulative average alcohol intake (continuous, adding a quadratic term), prevalence of diabetes, hypertension, hypercholesterolemia, family history of coronary heart disease, use of aspirin, antihypertensive medication, oral antidiabetic medication, insulin medication and hypocholesterolemic medication; and nutritional variables as follows: quintiles of cumulative average percentage of total energy intake, energy from fats (in model 2), energy from carbohydrates (in model 3), energy-adjusted omega-3 fatty acids and fiber, and glycemic index. Extremes of total energy intake were excluded.

Table S2. Hazard ratios of body weight and WC_{BMI} changes, and cardiovascular event or mortality for different causes according to E% protein intake into three categories

	Low-protein <15 E% (n = 1744)	**Normo-protein** [15, 20] E% (n = 4978)	**High-protein** >20 E% (n = 494)	*P* q-trend
Median (% of energy)	14.06	16.95	20.89	
Anthropometry outcomes				
Body weight gain (10% cut-off), % (n)	2.4 (41)	1.9 (94)	2.9 (14)	
Crude Model	1.27 (0.88, 1.84)	1.00 (Referent)	2.00 (1.14, 3.51)	0.01
Model 2	1.16 (0.74, 1.79)	1.00 (Referent)	2.03 (1.07, 3.86)	0.03
Model 3	1.24 (0.80, 1.93)	1.00 (Referent)	1.87 (0.99, 3.53)	0.03
Body weight loss (10% cut-off), % (n)	2.3 (40)	2.7 (132)	2.9 (14)	
Crude Model	0.90 (0.63, 1.28)	1.00 (Referent)	1.47 (0.84, 2.54)	0.44
Model 2	1.07 (0.71, 1.62)	1.00 (Referent)	1.08 (0.59, 1.98)	0.69
Model 3	1.07 (0.71, 1.62)	1.00 (Referent)	1.05 (0.57, 1.91)	0.77
WC_{BMI} incidence, % (n)	7.4 (121)	7.1 (336)	6.1 (29)	
Crude Model	1.15 (0.93, 1.41)	1.00 (Referent)	1.23 (0.84, 1.80)	0.14
Model 2	0.99 (0.77, 1.27)	1.00 (Referent)	1.44 (0.94, 2.19)	0.15
Model 3	0.97 (0.76, 1.25)	1.00 (Referent)	1.46 (0.96, 2.21)	0.15
WC_{BMI} reversion, % (n)	6.3 (102)	5.6 (259)	3.7 (17)	
Crude Model	1.25 (0.99, 1.57)	1.00 (Referent)	0.93 (0.57, 1.52)	0.60
Model 2	0.86 (0.65, 1.13)	1.00 (Referent)	1.21 (0.71, 2.04)	0.91
Model 3	0.89 (0.67, 1.18)	1.00 (Referent)	1.16 (0.68, 1.95)	0.90
Fatal and non-fatal outcomes				
Cardiovascular event, % (n)	4.6 (81)	3.5 (173)	4.7 (23)	
Crude Model	1.33 (1.02, 1.74)	1.00 (Referent)	1.58 (1.03, 2.45)	0.01
Model 2	1.00 (0.73, 1.38)	1.00 (Referent)	1.54 (0.95, 2.49)	0.08
Model 3	0.99 (0.72, 1.35)	1.00 (Referent)	1.61 (1.00, 2.58)	0.12
Cardiovascular mortality, % (n)	1.6 (28)	1.0 (48)	1.0 (5)	
Crude Model	1.67 (1.05, 2.66)	1.00 (Referent)	1.27 (0.51, 3.20)	0.18
Model 2	0.93 (0.52, 1.66)	1.00 (Referent)	1.61 (0.58, 4.49)	0.48
Model 3	0.91 (0.51, 1.62)	1.00 (Referent)	1.69 (0.61, 4.71)	0.48
Cancer mortality, % (n)	2.5 (43)	1.5 (74)	2.6 (13)	
Crude Model	1.64 (1.13, 2.39)	1.00 (Referent)	2.14 (1.18, 3.85)	<0.001
Model 2	1.08 (0.68, 1.72)	1.00 (Referent)	2.29 (1.18, 4.46)	0.01
Model 3	0.99 (0.63, 1.57)	1.00 (Referent)	2.57 (1.34, 4.95)	0.03
All-cause mortality, % (n)	6.2 (108)	3.6 (180)	7.1 (35)	
Crude Model	1.71 (1.35, 2.17)	1.00 (Referent)	2.38 (1.66, 3.42)	<0.001
Model 2	1.23 (0.92, 1.64)	1.00 (Referent)	2.38 (1.58, 3.59)	<0.001
Model 3	1.18 (0.89, 1.58)	1.00 (Referent)	2.53 (1.68, 3.79)	<0.001

E% (percentage of energy), BMI, (body mass index), WC_{BMI} (waist circumference corrected by BMI). Cox proportional hazards models were used to assess the risk of weight change (10% from baseline), WC_{BMI} change (incidence and reversion), cardiovascular event, and death by different causes, by group-defined total protein intake (low (<15 E%), normal ([15,20] E%) and high (>20 E%) protein consumption). Data is presented as HRs and 95% CIs. Multivariable models were adjusted for intervention group, node, sex, age in years, body mass index (kg/m^2), smoking status (former or current smoker), leisure time physical activity (metabolic equivalent task-min/d), cumulative average alcohol intake (continuous, adding a quadratic term), prevalence of diabetes, hypertension, hypercholesterolemia, family history of coronary heart disease, use of aspirin, antihypertensive medication, oral antidiabetic medication, insulin medication and hypocholesterolemic medication; and nutritional variables as follows: quintiles of cumulative average percentage of total energy intake, energy from fats (only in model 2), energy from carbohydrates (only in model 3), energy-adjusted omega-3 fatty acids and fiber, and glycemic index. Extremes of total energy intake were excluded. *P* for q-trend stands for the evaluation of the quadratic model.
"Weight gain" and "Weight loss" were defined when participants gained or loss ≥10% of body weight, respectively. "WC_{BMI} incidence" and "WC_{BMI} reversion" were defined according to the metabolic syndrome criteria (i.e. ≥88 cm for women and ≥102 cm for men). Comparisons of both weight and WC_{BMI} groups were performed against the "maintenance group" (i.e. no weight gain/loss, or no WC_{BMI} incidence/reversion, as appropriate).