Public Health Nutrition: 24(8), 2215–2224 doi:10.1017/S1368980020000427

The association of animal and plant protein with successful ageing: a combined analysis of MEDIS and ATTICA epidemiological studies

Alexandra Foscolou[1], Elena Critselis[1], Stefanos Tyrovolas[1,2], Christina Chrysohoou[3], Nenad Naumovski[4,5], Labros S Sidossis[1,6], Loukianos Rallidis[7], Antonia-Leda Matalas[1] and Demosthenes Panagiotakos[1,5,6,*]

[1]Department of Nutrition and Dietetics, School of Health Science and Education, Harokopio University, 17671 Kallithea, Attica, Greece: [2]Parc Sanitari Sant Joan de Déu, Fundació Sant Joan de Déu, CIBERSAM, Universitat de Barcelona, Barcelona, Spain: [3]First Cardiology Clinic, School of Medicine, University of Athens, Athens, Greece: [4]Faculty of Health, University of Canberra, Bruce, Canberra, Australia: [5]Collaborative Research in Bioactives and Biomarkers (CRIBB) Group, University of Canberra, Bruce, Canberra, Australia: [6]Department of Kinesiology and Health, School of Arts and Sciences, Rutgers University, New Brunswick, NJ, USA: [7]Second Cardiology Clinic, School of Medicine, University of Athens, Athens, Greece

Submitted 26 September 2019: Final revision received 24 December 2019: Accepted 29 January 2020: First published online 21 May 2020

Abstract

Objective: The aim of the present study was to investigate the differences between the consumption of plant-based *v.* animal-based protein-rich diets on successful ageing, as well as to identify the optimal combination of dietary protein intake for facilitating successful ageing in people aged >50 years.

Design: A combined analysis was conducted in older adults of the ATTICA and MEDIS population-based cross-sectional studies. Anthropometrical, clinical and sociodemographic characteristics, lifestyle parameters, dietary habits and level of protein intake were derived through standard procedures. Successful ageing was evaluated using the validated Successful Aging Index (SAI) composed of ten health-related social, lifestyle and clinical characteristics.

Setting: Athens area and twenty Greek islands.

Participants: A total of 3349 Greek women and men over 50 years old.

Results: Participants with high consumption of plant proteins were more likely to be male, physically active, with higher daily energy intake, higher adherence to the Mediterranean diet and higher level of SAI ($P < 0{\cdot}001$). Participants with 'Low animal & High plant' and 'High animal & High plant' protein consumption had a 6 and 7 % higher SAI score, respectively, compared with the other participants ($P < 0{\cdot}001$). In contrast, 'Low animal & Low plant' and 'High animal & Low plant' protein intake was negatively associated with SAI as compared to the combination of all other consumption categories ($P < 0{\cdot}02$).

Conclusions: The consumption of a plant-based protein-rich diet seems to be a beneficial nutritional choice that should be promoted and encouraged to older people since it may benefit both individual's health and prolong successful ageing.

Keywords
Plant protein
Animal protein
Successful ageing
Older adults
Mediterranean

The consumption of high-protein diets (i.e., diets including >15 % of total daily energy consumption being derived from all sources of proteins) is associated with numerous health benefits including favourable weight control and diminished age-related muscle loss and functional decline[1,2]. Particularly among older adults, preliminary evidence reveals that the consumption of such diets may also facilitate successful ageing[3], namely the process of developing and maintaining the functional ability enabling the well-being in older age[4]. It is well established that the process of ageing is very complex, affecting a wide range of metabolic and immunological functions (among others)[5]. These changes are one of the primary factors associated with the predisposition to the development of several chronic problems such as CVD, variety of cancers and neurodegenerative diseases[6]. However,

*Corresponding author: Email dbpanag@hua.gr

there is evidence supporting that protein intake may affect cardiometabolic health and this fact may depend on the protein source[7]. Additionally, some pathophysiological mechanisms that can be affected by diet include cell signalling through insulin sensitivity factors, a nutrient sensing complex related to growth, altered gene expression and the effects of microbial metabolites produced by the gut microbiota[8–10]. However, it is proposed that vegetarian[11] and plant-based, as opposed to animal-based, protein-rich diets may differentially benefit and promote human health[12]. Importantly, the food matrix where proteins are imbedded and carried as a part of the diet itself (such as polyphenolic and bioactive composition) plays an important role of providing potentially beneficial health effects.

The authors of a recent study, after evaluating the associations between animal and plant protein intake and all-cause and cause-specific mortality, found that higher protein intake from plant sources was inversely associated with all-cause mortality and cardiovascular-related mortality. Accordingly, substitution of animal with plant proteins was associated with a significant diminution of all-cause mortality as well as cardiovascular and cancer-related mortality[13]. Similar findings have been documented from other age-related disorders, including sarcopenia[14,15]. Furthermore, the effects of consuming plant-based, as opposed to animal-based, protein-rich diets are most prominent among older individuals[16]. However, evidence is currently lacking regarding such differential impacts upon successful ageing in the elderly.

Hence, the aim of the present study was to investigate the differential association between the consumption of plant-based, as opposed to animal-based, protein-rich diets upon successful ageing in people aged >50 years in Greece.

Methodology

Two cross-sectional, population-based, large-scale epidemiological studies (ATTICA[17] and the MEDiterranean Islands Study (MEDIS))[18] conducted in Greece were combined for the purposes of the present analysis. Beyond the increase of the studied sample, that is, by the combination of the ATTICA and MEDIS studies, and consequently the statistical power of the results, the pooled data set merged diverge reference populations, since these two studies represent urban and rural/insular individuals, respectively. Thus, this combination provides a great opportunity to achieve better representativeness of the entire population by including a variety of environmental and cultural particularities. To combine these studies, a harmonisation procedure was applied following established methodologies (see below).

Sample

The study sample consisted of participants of the ATTICA and MEDIS studies, aged >50 years old, residing in urban and insular Greek areas. As previously described[17], the ATTICA study was a population-based observational study implemented in the greater metropolitan Athens area, a purely urban area, during 2001–2002. At baseline, all participants were free of CVD and cancer, as assessed through a detailed clinical evaluation by the study's physicians. From the original sample (*n* 2583) over 18 years old, of the ATTICA sample, a sub-group of *n* 1128 individuals aged >50 years old were analysed for the purposes of the present work. In addition, as previously described[18], based on the MEDIS study, 3000 older people from Mani (Greek peninsula region) and twenty-six Mediterranean islands of five countries were enrolled during 2005–2017 (MEDIS study). Individuals who resided in assisted-living centres, had a clinical history of CVD or cancer, or had left the island for a considerable period of time during their life (i.e., >5 years) were excluded. Of the 3138 MEDIS study participants aged >50 years living in the insular Mediterranean region (Cyprus, Spain, Italy, Turkey and Greece), a sub-group of *n* 2221 individuals from Greek islands, living in rural or semi-urban areas, were analysed in this work. For both aforementioned studies, a group of trained health scientists (including cardiologists, general practitioners, physicians, dietitians, public health nutritionists and nurses) collected all information using standard, validated questionnaires and clinical procedures.

Measurements

Harmonisation procedures

The combination of the two data sets demanded a careful harmonisation of the variables that they were not common in the studies (i.e., smoking and dietary habits and physical activity status). The harmonised data set included study-specific variables that were transformed in the least common basis, following standard procedures and having the same definition and format across the studies, as suggested by Maelstrom Research guidelines for rigorous retrospective data harmonisation[19]. Specifically, variables of both FFQ were first transformed to harmonised standard food variables and then protein sources and intake were calculated. Thus, the degree of correspondence of the food sources between the FFQ was in complete agreement.

Sociodemographic data

The sociodemographic characteristics assessed within the context of the present investigation were age (years), gender (male/female) and smoking status. In the combined data set, current smokers were defined as those who smoked at least one cigarette or any type of tobacco per day at the time of the interview. Former smokers were defined as those who previously smoked but had quit

https://doi.org/10.1017/S1368980020000427 Published online by Cambridge University Press

within the previous year. Current and former smokers were combined as ever smokers. The remaining participants were defined as nonsmokers.

Physical activity levels
Physical activity was transformed into metabolic equivalent (MET) minutes per week, using the shortened, translated and validated Greek version of the self-reported International Physical Activity Questionnaire[20], for the ATTICA study and similar questions for the MEDIS study. Individuals who reported at least 3 MET-minutes per week were classified as physically active. All others were defined as physically inactive.

Anthropometric and clinical characteristics
Weight and height were measured using standard procedures to attain volunteer's BMI (kg/m^2). Overweight was defined as BMI between 25·0 and 29·9 kg/m^2, while obesity was defined as BMI > 29·9 kg/m^2. In both studies, type 2 diabetes mellitus was determined by measuring fasting plasma glucose and in accordance with the American Diabetes Association diagnostic criteria (fasting blood glucose >7 mmol/l (i.e., >126 mg/dl) or use of antidiabetic medication). Participants who had blood pressure levels >140/90 mmHg or who were administered antihypertensive medications were classified as hypertensive. Fasting blood lipids levels (including HDL-cholesterol, LDL-cholesterol and TAG) were also recorded. Hypercholesterolaemia was defined as total serum cholesterol levels >5·7 mmol/l (i.e., >200 mg/dl) or the use of lipid-lowering agents according to the National Cholesterol Education Program Adult Treatment Panel III guidelines[21]. The CV for the blood measurements was less than 5 %. A cumulative proxy of total cardiometabolic risk, indicating the overall burden of known cardiometabolic risk factors (i.e., obesity and history of hypertension, type 2 diabetes mellitus and hypercholesterolaemia) was constructed (score range 0–4), wherein participants having none of the aforementioned risk factors were assigned a score of 0, having one factor a score of 1, etc.

Dietary habits assessment
Among ATTICA study participants, the evaluation of dietary habits was based on a semi-quantitative FFQ, originally developed for the European Prospective Investigation into Cancer and Nutrition study[22]. The Greek version of the European Prospective Investigation into Cancer and Nutrition questionnaire was provided by the Unit of Nutrition of Athens Medical School, after being translated according to standard literature guidelines[23]. Participants were requested to report the average intake (per week or per day) of several food items that they have been consuming (during the last 12 months). Similar to the ATTICA study, dietary habits in the MEDIS study were assessed through a similar semi-quantitative, validated and reproducible FFQ[24]. Energy and macronutrient intake were then calculated based on the participants' harmonised responses on the FFQ. The frequency of consumption of various food types (i.e., meat and meat products, fish, milk and other dairy products, fruits, vegetables, greens and salads, legumes, cereals, pasta and olive oil) on a daily, weekly or monthly basis was assessed. In both studies, energy and macronutrient intake were calculated based on the participants' responses on the FFQ. Using the harmonised food variables and based on food composition tables (USDA) as well as using previously described categorisation of protein-containing foods[25], protein intake – from both studies – was grouped into animal- and plant-based sources in the harmonised data set. Animal protein intake was measured based on the assessment of the frequency of meat, poultry, fish and dairy consumption, whereas plant protein intake was assessed based on the frequency of cereal, potato, vegetable and legume consumption. Specifically, information regarding frequency of intake based on 'daily', 'weekly' (i.e., 1–2, 3–5 times per week), 'monthly' basis (i.e., 2–3 times per month), 'rare', or 'never' was collected. It was not possible to harmonise data regarding nut and seed intake. In order to separately investigate the association between each protein category and amount and successful ageing, participants who 'never' or 'rarely' consumed meat, poultry, fish and/ or dairy were classified as those consuming 'low animal protein intake', whereas those who never or rarely consumed cereals, potatoes, vegetables and legumes were classified as having 'Low plant protein intake'. Accordingly, those who consumed 2–3 times per month or 1–2 times per week the animal origin foods or plant origin foods were classified as 'Moderate animal protein intake' or 'Moderate plant protein intake', respectively, whereas participants who consumed the animal origin foods or plant origin foods 3–5 times per week or daily were classified as consuming 'High animal protein intake' or 'High plant protein intake', respectively. In order to investigate the comprehensive association between different types of protein-rich diets, participants were classified into the following mutually exclusive categories based on the frequency of weekly consumption of animal- and plant-based proteins: (a) 'Low animal & Low plant protein diet': protein intake from both animal and plant sources was <1–2 times/week, (b) 'Low animal & High plant protein': animal protein intake was <1–2 times/week and simultaneously plant protein intake ≥1–2 times/week, (c) 'High animal & Low plant protein': animal protein intake was ≥1–2 times/week and simultaneously protein from plant sources <1–2 times/week and (d) 'High animal & High plant protein': both animal and plant protein intakes were ≥1–2 times per week.

Furthermore, to evaluate the level of adherence to the Mediterranean diet, a previously developed and validated MedDietScore with theoretical range 0–55 was used, with higher values indicating greater adherence[26].

Successful Aging Index
A Successful Aging Index (SAI), ranging from 0 to 10, which has been previously developed and validated[18], using ten

https://doi.org/10.1017/S1368980020000427 Published online by Cambridge University Press

attributes that reflect and have been found associated with the ageing process, was applied in both studies for assessing successful ageing. The index encompasses health-related social, lifestyle and clinical factors, including education, financial status, physical activity, BMI, depression, participation in social activities with friends and family, number of yearly excursions, total number of clinical CVD risk factors (i.e., history of hypertension, diabetes, hypercholesterolaemia and obesity) and level of adherence to the Mediterranean diet[18].

Statistical analysis

Continuous variables are presented as mean ± SD, and categorical variables as frequencies. Associations between continuous variables and group of participants (i.e., low/moderate/high plant protein consumers or low/moderate/high animal protein consumers) are evaluated with ANOVA. To correct for the inflation of Type-I error in multiple comparisons, Bonferroni's correction was applied. Associations between categorical variables and group of participants were evaluated using the χ^2 test. Linear regression analysis was used to evaluate the association between plant or animal protein intake (low animal-based and low plant-based protein diets, low animal-based and high plant-based protein diets, high animal-based and low plant-based protein diets or high animal-based and high plant-based protein diets) (independent variables) and participants' characteristics (i.e., age, gender and smoking habits) and the SAI (dependent outcome), after adjusting for total energy intake. Similar analyses were conducted based on the type of protein intake. Study was included in the models as a latent variable, and relevant interactions were tested with protein intake. Results are presented as unstandardised beta coefficients ± SE and *P*-value. STATA software version 13 was used for all calculations.

Results

A comparison of the sociodemographic, lifestyle and clinical characteristics of participants based on the frequency of plant protein intake is presented in Table 1. Participants with high consumption of plant proteins were more frequently male, physically active, with higher mean total daily energy intake, higher adherence to the Mediterranean diet and higher level of SAI, as compared to participants with moderate or low intake of plant protein (*all* $P < 0{\cdot}0001$). However, participants with high intake of plant protein were more likely to be smokers ($P < 0{\cdot}001$). In contrast, as shown in Table 2, participants with high consumption of animal protein were less likely to be physically active ($P = 0{\cdot}002$), to have higher total daily energy intake ($P < 0{\cdot}001$) and higher adherence to the Mediterranean diet ($P < 0{\cdot}001$), as compared to participants with moderate or low intake of animal proteins. The mean amount of plant protein intake was 48 ± 6·6 g/d, whereas the mean amount of animal protein intake was 42 ± 6·6 g/d for ATTICA and MEDIS studies' participants.

As residual confounding may exist in observational studies, Table 3 presents results from linear regression models that evaluated the association of animal protein intake and plant protein intake on successful ageing (outcome) among ATTICA and MEDIS studies' participants. Following adjustments for age, sex, smoking habits and total energy intake as kcal/d, 'Animal protein intake' was not significantly associated with SAI levels ($P = 0{\cdot}15$) (*Model 1*), whereas 'Plant protein intake' was positively associated with SAI ($P < 0{\cdot}001$) (*Model 2*). Actually, those participants with 'Moderate or High' weekly plant protein intake had a 5 % higher score compared to those participants with 'Low' weekly plant protein intake ($P < 0{\cdot}001$) (Table 3). To avoid collinearity since MedDietScore was a part of SAI, all analyses were performed excluding MedDietScore from SAI.

A significant correlation was observed between protein intake and sex (Spearman rho = 0·171, $P < 0{\cdot}001$), as well as a significant interaction between sex and animal or plant protein intake ($P_{\text{for interaction}}$ 0·005 and 0·06, respectively); thus, to further evaluate the research hypothesis, multiple linear regression analysis was applied. Stratification by sex revealed that animal protein intake was again not significantly associated with SAI in both females and males (*all* $P > 0{\cdot}05$), whereas female participants with 'Moderate or High' plant protein intake had 4·6 ± 0·7 % higher SAI score ($P < 0{\cdot}001$) compared to female participants with 'Low' plant protein intake; the effect of the higher plant protein intake among men was more prominent (i.e., 5·3 ± 0·6 % higher SAI score, $P < 0{\cdot}001$) (Table 3).

Finally, investigating the association between protein category (i.e., plant origin protein and animal origin protein) and successful ageing (Table 4), it was revealed that following adjustment for age, sex, smoking habits and total daily energy intake as kcal/d, 'Low animal & Low plant' (*Model 1*) *and* 'High animal & Low plant' (*Model 2*) protein intake were negatively associated with successful ageing (b ± SE: –0·725 ± 0·064, $P < 0{\cdot}001$ and –0·183 ± 0·080, $P = 0{\cdot}02$, respectively). In contrast, 'Low animal & High plant' (*Model 4*) and 'High animal & High plant' (*Model 3*) protein consumption was positively associated with successful ageing (b ± SE: 0·674 ± 0·079, $P < 0{\cdot}001$ and 0·605 ± 0·082, $P < 0{\cdot}001$, respectively). In other words, those participants who consumed a diet high in plant and animal protein had a 6 % higher SAI score compared with all the other participants, whereas those participants consuming a diet with low animal protein but high plant protein had a 6·7 % higher SAI score compared with all the other participants (*all* $P < 0{\cdot}001$). When analyses were stratified by sex, the results showed that female participants who had 'Low animal & High plant' protein intake and 'High animal & High plant' protein intake had 7·1 % (±1·2 %) and 7·9 % (±1·2 %), respectively, higher SAI score compared to the

https://doi.org/10.1017/S1368980020000427 Published online by Cambridge University Press

https://doi.org/10.1017/S1368980020000427 Published online by Cambridge University Press

Table 1 Sociodemographic, lifestyle and clinical characteristics of the participants based on the weekly frequency of plant protein intake*

	Plant protein											
	All participants		Low intake		Moderate intake		High intake					
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*P*	P^1	P^2	P^3
n	3349		439		1488		555					
Age (years)	69·2	10·2	69·9	10·8	72·6	8·8	72·2	8·4	<0·001	<0·001	1·000	<0·001
Male												
n	1751		211		786		351		<0·001	1·000	0·002	0·114
%	52		48		53		63					
Ever smokers (yes)												
n	1358		174		534		234		0·003	1·000	<0·001	<0·001
%	43		41		38		47					
Physically active (active)												
n	1372		160		570		294		<0·001	1·000	<0·001	<0·001
%	41		37		39		54					
BMI (kg/m²)	28	4	28	5	28	4	28	4	0·233	0·638	0·451	1·000
Protein intake (g/d)	49	22	45	23	46	21	60	23	<0·001	1·000	<0·001	<0·001
Energy intake (kcal/d)	1766	738	1567	556	1638	580	1927	659	<0·001	0·193	<0·001	<0·001
MedDietScore (0–55)	29	7	29	6	31	5·5	34	5·3	<0·001	<0·001	<0·001	<0·001
Hypertension (yes)												
n	1881		266		912		340		0·161	1·000	0·237	0·328
%	86		88		87		84					
Diabetes (yes)												
n	696		90		340		132		0·450	0·884	1·000	0·668
%	21		21		23		24					
Hypercholesterolaemia (yes)												
n	1747		245		742		281		0·093	0·092	1·000	0·315
%	53		56		50		51					
CVD risk factors (0–4)	1·6	1·1	1·7	1·1	1·7	1·1	1·7	1·1	0·628	1·000	1·000	1·000
SAI (0–10)	3·1	1·2	2·2	1·2	2·7	1·3	3·4	1·3	<0·001	<0·001	<0·001	<0·001

P, *P*-values derived from ANOVA for continuous variables or the χ^2chi-square test for the categorical variables; P^1, between 'Low intake' and 'Moderate intake' group; P^2, between 'Moderate intake' and 'High intake' group; P^3 between 'Low intake' and 'High intake' group; after correcting for the inflation of Type-I error with the Bonferroni's rule; SAI, Successful Aging Index.

*To convert kcal to kJ multiply by 4·184.

Table 2 Sociodemographic, lifestyle and clinical characteristics of the participants based on the weekly frequency of animal protein intake*

	Animal protein											
	All participants		Low intake		Moderate intake		High intake					
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	P^0	P^1	P^2	P^3
n	3349		451		1467		632					
Age (years)	69·2	10·2	69·8	11·6	72·6	8·2	70·9	9·8	<0·001	<0·001	<0·001	0·139
Male												
n	1751		234		781		365		0·094	1·000	0·171	0·168
%	52		52		53		58					
Ever smokers (yes)												
n	1358		193		542		246		0·059	0·052	1·000	0·416
%	43		46		40		41					
Physically active (active)												
n	1372		218		591		242		0·002	0·008	0·992	0·002
%	41		49		41		39					
BMI (kg/m^2)	28	4	28	5	28	4	28	4	0·447	0·614	1·000	1·000
Protein intake (g/d)	49	22	47	25	45	18	66	27	<0·001	0·492	<0·001	<0·001
Energy intake (kcal/d)	1766	738	1555	716	1617	528	2070	720	<0·001	0·590	<0·001	<0·001
MedDietScore (0–55)	29	7	29	6·7	31	5·3	32	6·1	<0·001	<0·001	0·079	<0·001
Hypertension (yes)												
n	1881		242		926		379		0·002	0·002	0·202	0·290
%	86		81		89		85					
Diabetes (yes)												
n	696		104		348		120		0·054	1·000	0·051	0·328
%	21		23		24		19					
Hypercholesterolaemia (yes)	1747	53	257	52	728	50	331	53	0·062	0·324	0·761	0·449
CVD risk factors (0–4)	1·6	1·1	1·6	1·1	1·7	1·1	1·6	1·1	0·455	1·000	0·643	1·000
SAI (0–10)	3·1	1·2	3·1	1·2	2·6	1·3	3·1	1·3	<0·001	<0·001	<0·001	1·000

P, *P*-values derived from ANOVA for continuous variables or the chi-square test for the categorical variables; P^1, between 'Low intake' and 'Moderate intake' group; P^2, between 'Moderate intake' and 'High intake' group; P^3 between 'Low intake' and 'High intake' group; after correcting for the inflation of Type-I error with the Bonferroni's rule; SAI, Successful Aging Index.
*To convert kcal to kJ multiply by 4·184.

Table 3 Results from linear regression models that evaluated the association between the weekly frequency of plant protein intake (independent variable), as well as animal protein intake (independent variable), and successful ageing (dependent outcome) among ATTICA and MEDIS study participants*

	b	SE	*P*
All participants			
Model 1: Weekly frequency of plant protein intake (Moderate/High *v.* Low)	0·506	0·045	<0·001
Model 2: Weekly frequency of animal protein intake (Moderate/High *v.* Low)	0·086	0·060	0·15
Females			
Model 1: Weekly frequency of plant protein intake (Moderate/High *v.* Low)	0·460	0·065	<0·001
Model 2: Weekly frequency of animal protein intake (Moderate/High *v.* Low)	0·039	0·089	0·66
Males			
Model 1: Weekly frequency of plant protein intake (Moderate/High *v.* Low)	0·532	0·062	<0·001
Model 2: Weekly frequency of animal protein intake (Moderate/High *v.* Low)	0·133	0·081	0·10

b, unstandardised B-coefficient.
*'All participants' models were adjusted for age, sex, study, smoking habits and total daily energy intake (kcal/d). 'Females' and 'Males' models were adjusted for age, study, smoking habits and total daily energy intake (kcal/d). To convert kcal to kJ multiply by 4·184.

Table 4 Results from linear regression models that evaluated the association of several combinations of protein intake (i.e., low animal-based and low plant-based protein diet, low animal-based and high plant-based protein diet, high animal-based and low plant-based protein diet or high animal-based and high plant-based protein diet *v.* all other scenarios) on successful ageing (outcome), among ATTICA and MEDIS study participants

	b	SE	*P*
All participants			
Model 1: Low animal & Low plant protein intake *v.* all other	–0·725	0·064	<0·001
Model 2: High animal & Low plant protein intake *v.* all other	–0·183	0·080	0·02
Model 3: High animal & High plant protein intake *v.* all other	0·605	0·082	<0·001
Model 4: Low animal & High plant protein intake *v.* all other	0·674	0·079	<0·001
Females			
Model 1: Low animal & Low plant protein intake *v.* all other	–0·660	0·088	<0·001
Model 2: High animal & Low plant protein intake *v.* all other	–0·212	0·112	0·059
Model 3: High animal & High plant protein intake *v.* all other	0·789	0·122	<0·001
Model 4: Low animal & High plant protein intake *v.* all other	0·709	0·122	<0·001
Males			
Model 1: Low animal & Low plant protein intake *v.* all other	–0·782	0·092	<0·001
Model 2: High animal & Low plant protein intake *v.* all other	–0·142	0·113	0·21
Model 3: High animal & High plant protein intake *v.* all other	0·472	0·110	<0·001
Model 4: Low animal & High plant protein intake *v.* all other	0·638	0·103	<0·001

b, unstandardised B-coefficient.
*'All participants' models were adjusted for age (per 1 year), sex (m/f), study, smoking habits (y/n) and total energy intake (kcal/d). 'Females'/'Males' models were adjusted for age (per 1 year), study, smoking habits (y/n) and total energy intake (kcal/d). To convert kcal to kJ multiply by 4·184.

other female participants ($P < 0·001$). On the other hand, the effect of 'Low animal & High plant' and 'High plant & Low plant' protein intake was less prominent among male participants (6·4 ± 1·0 and 4·7 ± 1·1 %, respectively) on SAI score (*all* $P < 0·001$). No significant interactions were observed between 'Study' and 'Animal/plant protein' intake on the outcome (i.e., SAI) (all $P > 0·30$), and therefore, no stratified analysis was performed by study.

Discussion

The present combined analysis of two population-based epidemiological studies (namely, ATTICA and MEDIS) reveals that, following the adjustment for potential confounding effects, the consumption of a plant-based protein-rich diet was associated with more favourable successful ageing in adults aged >50 years old. In contrast, no significant association was detected between the consumption of animal-based protein-rich diets and successful ageing among both females and males. Moreover, when examining the differential effects of various combinations of plant- and animal-based protein intakes, it was revealed that the frequent consumption of plant-based protein diets beneficially impacts successful ageing, regardless of the frequency of animal-based proteins consumed. This effect was more prominent among female participants. These findings support previous reports suggesting that high plant protein consumption has beneficial impacts on health and particularly successful ageing[3].

The consumption of high animal and low plant protein diets, both individually and /or concomitantly, is associated with the onset and progression of several chronic diseases, most prominently including CVD[27]. Concomitant consumption of low plant-based and high animal-based protein diets has been associated with inflammation and stress response, as well as decreased expression of genes associated with neuropeptide signalling and synaptic

https://doi.org/10.1017/S1368980020000427 Published online by Cambridge University Press

transmission[28]. Therefore, protein intake is associated not only with the onset and progression of chronic diseases but also potentially with individuals' overall cognitive and functional decline, notable parameters of successful ageing. Yet, it has to be stated that animal protein intake is also associated with benefits for successful ageing. More specifically, an analysis of the Framingham Offspring cohort found that individuals aged over 60 years old benefited from animal protein intake, by maintaining muscle strength and supporting the prevention of mobility impairment[29]. This effect can be attributed to the higher levels of leucine in typically animal sources, which has a key role in stimulating translation initiation and muscle protein anabolism[15]. Therefore, consumption of adequate levels of animal-based proteins is essential for maintenance of muscle strength and, ultimately, prevention of functional decline within the context of successful ageing.

Several potential pathways are proposed to be implicated in the association between the consumption of different protein sources and ageing[30]. One potential pathway is involving the increase in plasma thyroxine levels after the soya protein intake[31]. Nevertheless, the hypocholesterolaemic effect of dietary soya intake in particular is well documented and from the compositional perspective, soya beans (and many other legumes and some nuts) contain additional well-established cholesterol lowering compounds such as isoflvones, lecithins, saponins as well as fibre that may provide beneficial cardiovascular benefits on their own[32].

Another pathway that can potentially explain this finding is that the dietary methionine is very high in animal food products. While most legumes and some non-animal food products are protein dense, these are also relatively low in this amino acid. Methionine is a sulphur-containing amino acid that is naturally present in the diet, and it is a pre-cursor to S-adenosylmethionine which, after the removal of methyl group, can form homocysteine. Consequently, homocysteine has been identified as an independent risk factor for the development of CVD and it is implicated in some of the other diseases as well such as Alzheimer's disease. In support of this notion, the findings of the Kuopio Ischemic Heart Disease Risk Factor Study has identified that long-term, moderately high dietary methionine may increase the risk for developing the acute coronary events in middle-aged Finish men free of prior CHD[33]. Additionally, a previous study by Preis *et al.*[34] has identified that there were no associations between the dietary protein intake and risk of total IHD. However, the same study has suggested that higher intake of proteins from animal food sources may be associated with the increase in risk for developing the IHD in otherwise healthy males (40–75 years of age).

Based on the above observational findings, public health policymakers should consider focusing on promoting the consumption of high-protein diets, primarily derived from plant sources, since these diets may promote successful ageing. In support to this, based on the most recent National Health and Nutrition Examination Surveys, individuals' diets are on average composed of 46 % of the total protein intake derived from animals, 16 % from dairy products and 30 % from plant sources[35]. In addition, a significant proportion of the general population consumes relatively low levels of proteins daily, irrespectively whether these are of plant- or animal-based origin. Taken into account that 7·7 % of adolescent females and 7·2–8·6 % of older adult women, and 16·0 % of men aged 51–70 years old have relatively low daily protein consumption, these particular population groups are presumed to be at particular enhanced risk for manifesting health outcomes associated with low protein intake[36]. Finally, it has been proposed that plant-based diets combined with small meat, fish and diary consumption could offer significant improvements in the overall health status[37], while dietary patterns consisting of high fruit consumption and limited meat and fried foods may improve the likelihood of successful ageing[38]. Therefore, further public health actions are necessary to promote the enhanced consumption of plant-based protein-rich diets, particularly among individuals at highest risk for developing CVD[39], in the context of a Mediterranean dietary pattern and without excessive energy intake[40].

To the best of our knowledge, this is one of the first studies examining the type of protein diet consumed in relation to successful ageing in older adults. As to this effect, two different population-based studies were applied; it is anticipated that the effects of a population bias have been minimised and the external validity of findings is consequently augmented. However, the findings of the present study should be considered under the following limitations. First, dietary habits were measured on a single occasion based on a FFQ leading to a higher degree of uncertainty or measurement error. Nutrient intake was calculated through food composition tables using the information retrieved through FFQ; thus, over/underestimation of true intake levels may exist and a misclassification bias may have been introduced. Nuts intake and their role in protein intake were not taken into account. Finally, the observational nature of the cross-sectional design does not allow for causal associations to be conclusively drawn.

Conclusions

The consumption of high-protein diets has been associated not only with health benefits but also with health risks. However, the effect of high plant or animal protein consumption has little been investigated, especially regarding the successful ageing process. Based on the findings of the present work, the consumption of a plant-based protein-rich diet seems to be a beneficial nutritional choice that should be promoted and encouraged to older people,

https://doi.org/10.1017/S1368980020000427 Published online by Cambridge University Press

particularly in the Mediterranean region, since it may benefit both individual's health and prolong successful ageing.

Acknowledgements

Acknowledgements: The authors are particularly grateful to the men and women from all areas who participated in the ATTICA and MEDIS studies, as well as to both studies investigators. *Financial support:* The ATTICA study is supported by research grants from the Hellenic Cardiology Society (HCS2002) and the Hellenic Atherosclerosis Society (HAS2003). The MEDIS study was funded by research grants from the Hellenic Heart Foundation, the Graduate Program of the Department of Nutrition and Dietetics, Harokopio University and the Rutgers University, NJ, USA (GA #5884). Stefanos Tyrovolas was supported by the Foundation for Education and European Culture (IPEP), the Sara Borrell postdoctoral program (reference no. CD15/00019 from the Instituto de Salud Carlos III (ISCIII – Spain) and the Fondos Europeo de Desarrollo Regional (FEDER). Demosthenes Panagiotakos, Stefano Tyrovolas, Elena Critselis and Alexandra Foscolou have been funded for ATHLOS project to study trajectories of healthy ageing (European Union's Horizon 2020 research and innovation program, grant agreement No 635316). *Conflict of interest:* None. *Authorship:* Conceptualisation: D.P. and A.F.; Methodology: D.P.; Formal analysis: A.F. and D.P.; Writing-original draft preparation: A.F.; Writing – review and editing: E.C., S.T., C.C., N.N., L.S.S., L.R. and A.L.M.; Supervision: D.P. *Ethics of human subject participation:* The ATTICA study was approved by the Bioethics Committee of Athens Medical School and was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association. The MEDIS study was approved by the Institutional Ethics Board of Harokopio University (16/19-12-2006) and followed the ethical recommendations of the World Medical Association (52nd WMA General Assembly, Edinburgh, Scotland, October 2000). In both studies, participants were informed of the study aims and procedures and provided written informed consent for study participation prior to enrollment.

References

1. Astrup A, Raben A & Geiker N (2015) The role of higher protein diets in weight control and obesity-related comorbidities. *Int J Obes* **39**, 721–726.
2. Bradlee ML, Mustafa J, Singer MR *et al.* (2017) High-protein foods and physical activity protect against age-related muscle loss and functional decline. *J Gerontol A Biol Sci Med Sci* **73**, 88–94.
3. Foscolou A, Magriplis E, Tyrovolas S *et al.* (2019) The association of protein and carbohydrate intake with successful aging: a combined analysis of two epidemiological studies. *Eur J Nutr* **58**, 807–817.
4. World Health Organization (2015) Ageing and life course. What is Healthy Ageing? https://www.who.int/ageing/healthy-ageing/en/ (accessed February 2019).
5. Calder PC, Carding SR, Christopher G *et al.* (2018) A holistic approach to healthy ageing: how can people live longer, healthier lives? *J Hum Nutr Diet* **31**, 439–450.
6. Negpal R, Mainali R, Ahmadi S *et al.* (2018) Gut microbiome and aging: physiological and mechanistic insights. *Nutr Healthy Aging* **4**, 267–285.
7. Hruby A & Jacques PF (2018) Dietary protein and changes in markers of cardiometabolic health across 20 years of follow-up in middle-aged Americans. *Public Health Nutr* **21**, 2998–3010.
8. Logan J & Bourassa MW (2018) The rationale for a role for diet and nutrition in the prevention and treatment of cancer. *Eur J Cancer Prev* **27**, 406–410.
9. Garcia-Mantrana I, Selma-Royo M, Alcantara C *et al.* (2018) Shift on gut microbiota associated to Mediterranean diet adherence and specific dietary intakes on general adult population. *Front Microbiol* **9**, 890.
10. Bouchard-Mercier A, Paradis AM, Rudkowska I *et al.* (2013) Association between dietary patterns and gene expression profiles of healthy men and women: a cross-sectional study. *Nutr J* **12**, 24.
11. Godos J, Bella F, Sciacca S *et al.* (2017) Vegetarianism and breast, colorectal and prostate cancer risk: an overview and meta-analysis of cohort studies. *J Hum Nutr Diet* **30**, 349–359.
12. Pedersen AN, Kondrup J & Børsheim E (2013) Health effects of protein intake in healthy adults: a systematic literature review. *Food Nutr Res* **57**, doi: 10.3402/fnr.v57i0.21245.
13. Budhathoki S, Sawada N, Iwasaki M *et al.* (2019) Association of animal and plant protein intake with all-cause and cause-specific mortality in a Japanese cohort. *JAMA Intern Med* **179**, 1509–1518.
14. Baumgartner RN (2000) Body composition in healthy aging. *Ann N Y Acad Sci* **904**, 437–448.
15. Paddon-Jones D, Campbell WW, Jacques PF *et al.* (2015) Protein and healthy aging. *Am J Clin Nutr* **101**, 1339S–1345S.
16. Lonnie M, Hooker E, Brunstrom JM *et al.* (2018) Protein for life: review of optimal protein intake, sustainable dietary sources and the effect on appetite in ageing adults. *Nutrients* **10**, pii: E360.
17. Panagiotakos DB, Georgousopoulou EN, Pitsavos C *et al.* (2015) Ten-year (2002–2012) cardiovascular disease incidence and all-cause mortality, in urban Greek population: the ATTICA Study. *Int J Cardiol* **180**, 178–184.
18. Tyrovolas S, Haro JM & Mariolis A *et al.* (2014) Successful aging, dietary habits and health status of elderly individuals: a k-dimensional approach within the multi-national MEDIS study. *Exp Gerontol* **60**, 57–63.
19. Fortier I, Raina P, Van den Heuvel ER *et al.* (2017) Maelstrom Research guidelines for rigorous retrospective data harmonization. *Int J Epidemiol* **46**, 103–105.
20. Papathanasiou G, Georgoudis G, Papandreou M *et al.* (2009) Reliability measures of the short International Physical Activity Questionnaire (IPAQ) in Greek young adults. *Hellenic J Cardiol* **50**, 283–294.
21. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (2001) Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* **285**, 2486–2497.
22. Katsouyanni K, Rimm EB, Gnardellis C *et al.* (1997) Reproducibility and relative validity of an extensive semi-quantitative frequency questionnaire using dietary records

and biochemical markers among Greek schoolteachers. *Int J Epidemiol* **26**, Suppl. 1, S118–S227.
23. Maneesriwongul W & Dixon JK (2004) Instrument translation process: a methods review. *J Adv Nurs* **48**, 175–186.
24. Tyrovolas S, Pounis G, Bountziouka V *et al.* (2010) Repeatability and validation of a short, semi-quantitative food frequency questionnaire designed for older adults living in Mediterranean areas: the MEDIS-FFQ. *J Nutr Elder* **29**, 311–324.
25. Song M, Fung TT, Hu FB *et al.* (2016) Association of animal and plant protein intake with all-cause and cause-specific mortality. *JAMA Intern Med* **176**, 1453–1463.
26. Panagiotakos DB, Pitsavos C & Stefanadis C (2006) Dietary patterns: a Mediterranean diet score and its relation to clinical and biological markers of cardiovascular disease risk. *Nutr Metab Cardiovasc Dis* **16**, 559–568.
27. Richter CK, Skulas-Rav AC, Champagne CM *et al.* (2015) Plant protein and animal proteins: do they differentially affect cardiovascular disease risk? *Adv Nutr* **6**, 712–728.
28. Swanson KS, Vester BM, Apanavicius C *et al.* (2009) Implications of age and diet on canine cerebral cortex transcription. *Neurobiol Aging* **30**, 1314–1326.
29. McLean RR, Mangano KM, Hannan MT *et al.* (2015) Dietary protein intake is protective against loss of grip strength among older adults in the Framingham Offspring Cohort. *J Gerontol A Biol Sci Med Sci* **71**, 356–361.
30. Forsythe WA 3rd (1995) Soy protein, thyroid regulation and cholesterol metabolism. *J Nutr* **125**, Suppl. 3, 619S–623S.
31. McCarty MF, Barroso-Aranda J & Contreras F (2009) The low-methionine content of vegan diets may make methionine restriction feasible as a life extension strategy. *Med Hypotheses* **72**, 125–128.
32. Ramdath DD, Padhi EM, Sarfaraz S *et al.* (2017) Beyond the cholesterol-lowering effect of soy protein: a review of the effects of dietary soy and its constituents on risk factors for cardiovascular disease. *Nutrients* **9**, pii: E324.
33. Virtanen JK, Voutilainen S, Rissanen TH *et al.* (2006) High dietary methionine intake increases the risk of acute coronary events in middle-aged men. *Nutr Metab Cardiovasc Dis* **16**, 113–120.
34. Preis SR, Stampfer MJ, Spiegelman D *et al.* (2010) Dietary protein risk of ischemic heart disease in middle-aged men. *Am J Clin Nutr* **92**, 1265–1272.
35. Fulgoni VL 3rd (2008) Current protein intake in America: analysis of the National Health and Nutrition Examination Survey, 2003–2004. *Am J Clin Nutr* **87**, 1554S–1557S.
36. Forman D & Bulwer BE (2006) Cardiovascular disease: optimal approaches to risk factor modification of diet and lifestyle. *Curr Trear Options Cardiovasc Med* **8**, 47–57.
37. McEvoy CT, Temple N & Woodside JV (2012) Vegetarian diets, low-meat diets and health: a review. *Public Health Nutr* **15**, 2287–2294.
38. Hodge AM, O'Dea K, English DR *et al.*, 2014) Dietary patterns as predictors of successful ageing. *J Nutr Health Aging* **18**, 221–227.
39. Aiking H (2011) Future protein supply. *Trends Food Sci Technol* **22**, 112–120.
40. Mathers JC (2015) Impact of nutrition on the ageing process. *Br J Nutr* **113**, Suppl., S18–S22.

Public Health Nutrition