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Plant protein substitution for animal protein and its association with cardiovascular risk factors and inflammatory biomarkers in elderly men: A substitution analysis

Hanieh Abbasi[a], Noushin Fahimfar[b,c,1], Milad Nazarzadeh[d,e] and Leila Azadbakht[a,f,*]

[a]*Department of Community Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran*

[b]*Osteoporosis Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran*

[c]*Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

[d]*Deep Medicine, Oxford Martin School, University of Oxford, Oxford, UK*

[e]*Nuffield Department of Women's and Reproductive Health, Medical Science Division, University of Oxford, Oxford, UK*

[f]*Diabetes Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran*

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Abstract.

BACKGROUND: Substituting different types of protein intake may be associated with cardiovascular risk factors and inflammatory biomarkers. However, there are few studies conducted on elders and the findings are contradictory.

OBJECTIVE: We decided to examine the association of substituting plant protein for animal protein with cardiovascular risk factors and inflammatory biomarkers among elderly men.

METHOD: The current cross-sectional study included 357 elderly men chosen from health centres in southern Tehran, Iran. They provide written consent to be included in the study. We used a validated and reliable food frequency questionnaire (FFQ) to assess dietary intake. All biochemical factors like lipid profile, fasting blood sugar (FBS), high sensitivity C-reactive protein (hs-CRP), interleukin 6 (IL6), tumor necrosis factor-α (TNF-α) were measured. Waist circumference (WC) and blood pressure (BP) were also assessed. The substitution analysis by STATA was used to examine the aforementioned association.

RESULTS: Substituting animal protein with plant protein had significant beneficial association with WC (OR: –4.28; 95% CI: –8.51, –0.62; Ptrend = 0.047) and LDL/HDL (OR: –0.26; 95% CI: –0.48, –0.05; Ptrend = 0.018).

CONCLUSION: In elderly men, substituting animal protein with plant protein had favorable association with some of cardiovascular risk factors including WC and LDL/HDL but there was no significant association for inflammatory biomarkers.

Keywords: Elders, protein intake, substitution, cardiovascular risk factors, inflammation

[1]Considered as co-correspondent

*Corresponding author: Leila Azadbakht, Ph.D., Department of Community Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, P. O. Box: 1416643931, Tehran, Iran. Tel.: + 98/218/895 556; Fax: + 98/218/8984 861; E-mail: azadbakhtleila@gmail.com.

1. Introduction

In 2021, Iran's elderly population comprised more than 10% of the total population, and it is projected to grow, with the elderly population expected to exceed 19.4% by 2041 and increase to 26.1% by 2051, according to Iran's health ministry [1]. An aging population presents many challenges and raises concerns about the future pace of economic growth, healthcare operations, and financial health, as well as the health status of the elderly population [2]. Cardiovascular disease (CVD) is expected to be a significant health problem among the elderly, as reported by the American Heart Association [3]. Worldwide, the number of people with CVD increased from 271 million in 1990 to 523 million in 2019, with two-thirds of cardiovascular deaths occurring in the elderly population, according to the Global Burden of Cardiovascular Diseases and Risk Factors study [4]. In Iran, CVDs accounted for 46% of all deaths and 20–23% of the disease burden based on the previous global burden of diseases (GBD) reports in 2010 and 2015 [6–8]. Additionally, national statistics indicate that the prevalence of CVD in Iran's elderly population was 39.9% in 2015 [9]. Various factors may contribute to the rising prevalence of cardiovascular disease (CVD), such as socioeconomic and cultural changes, dietary patterns, lack of physical activity, metabolic changes, and physical risk factors [10]. According to previous research, nutrition is considered to be one of the most influential factors in the development of CVD [11–16]. Previous studies have examined the relationship between dietary macronutrients, such as protein, and CVDs, and have found significant associations [17–20]. Different types of protein may have varying effects on CVD risk factors, and substituting one type of protein for another may be beneficial in reducing certain risk factors [21–31]. The present study aims to investigate the effects of protein substitution on CVD risk factors. A higher intake of protein, particularly from plant sources, has been associated with lower blood pressure levels and a reduced risk of CVD [21]. Additionally, plant proteins such as soy protein have been shown to be effective in reducing plasma cholesterol levels [22]. However, some studies have found reverse associations or no significant relationship [26–29]. Furthermore, the substitution of different types of protein intake may also affect CVD risk factors [30–32]. Substituting red meat with legumes and nuts, for example, has been associated with favorable effects on blood glucose and diabetes risk [30]. Moreover, substituting dairy products and meat with plant protein may have protective role against unhealthy aging [31].

Thus, findings on the association between the consumption of different protein sources and CVD risk factors are still controversial. Furthermore, there are few studies on the elderly population. The aforementioned reasons justify our aim to evaluate the association of substituting plant protein for animal protein with cardiovascular risk factors and inflammatory biomarkers in an elderly population. This may provide beneficial information about the potential association of each type of protein on cardiovascular risk factors for future studies.

2. Materials and Methods

2.1. Study population

In the current cross-sectional study, 365 elder men were included from health centres in southern Tehran, Iran (March to August 2017). Ethical approval was given by TUMS as Tehran University of Medical Sciences (TUMS) supervises health centers in southern Tehran (grant number: 48040). Men referred to medical centres for primary care were contacted by staff to be included in this study. All participants were required to provide written consent for inclusion in the study. Inclusion criteria were: a) Iranian male participants; b) Over 60 years old; c) no self-report of past determination of malignant diseases and d) no change in their usual diet as a result of predominant maladies or as a dietitian proposal.

Hypertension was used as the main dependent variable for calculating the study sample size as the highest sample size was obtained using this variable [33]. A total sample size of 340 elder men was estimated to be recruited. Clustered random sampling was utilized to decide the number of members to be chosen from each health center. After data collection ($n = 365$), participants with very low (<800 kcal/day) or high-calorie intake (>4200 kcal/day) were excluded and finally 357 elder men remained for statistical analysis.

2.2. Dietary assessment

Through face-to-face interviews conducted by a trained nutritionist, a valid 168-item semi-quantitative food frequency questionnaire (FFQ)

was filled out for all participants. Dietary intakes were changed from serving sizes and household measurements to grams. A modified version of the NUTRITIONIST IV software (version 7.0; N-Squared Computing, Salem, OR, USA) designed for Iranian foods, was utilized to compute nutrient intake [34]. Many studies have reported the validity and reliability of FFQ to be used in Iranian [35, 36] and non-Iranian elderly [37]. We estimated the protein content of each food item based on USDA nutrition facts, and then categorized them into animal protein and plant protein.

2.3. Biochemical assessment

A venous blood sample taken after 12 hours of fasting was used for biochemical evaluation. Commercial enzymatic reagents (Pars Azmoon, Tehran, Iran) were used to evaluate the concentration of fasting serum glucose (FBS) [glucose oxidase] and triglyceride (TG) [glycerol phosphate oxidase]. An ultrasensitive latex-enhanced immunoturbidimetric assay (Randox Laboratory Ltd., Belfast, UK) was used to evaluate the plasma concentration of highly sensitive C reactive protein (hs-CRP). Other inflammatory biomarkers were evaluated using the enzyme-linked immunosorbent assay (ELISA) method (Boster Biological Technology for IL-6 and TNF-a, China).

2.4. Anthropometric assessment

Anthropometric measures like body weight, height, and waist circumference were assessed by a trained assistant. A portable digital weight scale (SECA 813; Seca, Hamburg, Germany) was used to measure the body weight (measurement accuracy of 100 grams) of lightly dressed participants. To measure participants' height, they were asked to stand against a wall in a normal, motionless position, and a tape measure with an accuracy of 0.5 cm was used. The waist was measured using a tape measure with an accuracy of 0.5 cm and the midpoint between the top of the waist and the bottom of the ribs was chosen. BMI was calculated by dividing the participant's weight (kg) by their height (m2).

2.5. Assessment of other covariates

A validated and reliable questionnaire was used to determine socioeconomic status (SES) [38]. Work, education, car and home ownership, modern appliances, number of rooms and family members, and travel in the past year were considered. For other covariates, a questionnaire considering age, family, and smoking status was used. Participants were asked about the history of their chronic diseases such as diabetes, hyperlipidemia, hypertension, myocardial infarction, stroke, angina and thyroid disease. They were also questioned about their drugs such as diabetes drugs, heart disease drugs, lipid-lowering and thyroid drugs.

Participants were asked to sit still for about 10 minutes, their bladder were emptied and they did not smoke. The blood pressure (BP) was measured twice at 1-minute intervals. In addition, they did not consume caffeinated beverages and did not exercise within 1 hour before the measurement. Mean time of activity was presented as (MET-h/week).

2.6. Statistical analysis

Kolmogorov-Smirnov tests and histogram curves were evaluated to determine the normal distribution of the covariates. Participants were classified based on their dietary intake of animal protein (AP) and plant protein (PP). General characteristics were described for animal and plant protein quartiles. One-way analysis of variance (ANOVA) was used to test the distribution of elderly men with categorical variables and Chi-square test was used to test the distribution of elderly men with continuous variables within the quartiles of animal and plant protein intakes. Furthermore, one-way analysis of covariance (ANCOVA) with energy intake adjustment was used to compare the distribution of nutrients and food group intakes across quartiles of animal and plant protein intake.

The substitution based on replacing 5% of animal protein with 5% of the plant protein and investigating the effects on conventional cardiovascular risk factors and inflammatory factors, were estimated considering the energy partition and nutrient density paradigms while holding the total intake of that nutrient constant [39].

In brief, in the partition model, the model expression is $f(Y) = \beta 1\mathrm{A} + \beta 2\mathrm{B} + \beta 3\mathrm{C}$ and parameters for the substitution effect (substituting A for C) would be β1–β3 [39]. Confounding factors including age, body mass index, marital status, physical activity, socio-economic status, smoking status, diseases and drugs were selected based on previous association studies. SPSS software version 26.0 and STATA

version 14.0 were used to analyze the data and P values < 0.05 were considered statistically significant. The reporting of this work is compliant with STROBE guidelines.

3. Results

Participants' general characteristics across quartiles of animal protein and plant protein and in total population are shown in Table 1. The study included 357 elderly men in total with mean age of 64.97 ± 6.51 and mean BMI of 25.36 ± 3.19. Participants in the highest quartile of animal protein had the highest BMI with a mean of $26.32\,kg/m^2$. In addition, they had lower SES ($p < 0.001$), lower percentage of smoking ($p = 0.006$) and lower percentage of using anti-diabetic drugs ($p = 0.006$), lipid lowering drugs ($p < 0.001$) and heart disease drugs ($p < 0.001$). Participants in the highest quartile of plant Protein had lower SES ($p < 0.001$) and lower percentage of smoking ($p < 0.001$), using of anti-diabetic drugs ($p < 0.001$) and heart disease drugs ($p = 0.007$). Of 357 elderly men, 17.1 % were smokers, 23.8% had chronic diseases and 21.8% used heart disease drugs.

Dietary intakes of the participants across quartiles of AP and PP are presented in Table 2. Participants in the highest quartile of AP, had higher intakes of phosphorus ($p < 0.001$), potassium ($p < 0.001$), calcium ($p < 0.001$), magnesium ($p < 0.001$), sodium ($p = 0.013$), zinc ($p < 0.001$) and dairy products ($p < 0.001$). They had lower intake of carbohydrates ($p < 0.001$).

Participants in the highest quartile of PP had higher intakes of fiber ($p < 0.001$), magnesium ($p = 0.005$), iron ($p < 0.001$), fruits and vegetables ($p < 0.001$). They had lower intakes of calcium ($p = 0.013$) and dairy products ($p < 0.001$).

The association between substituting 5% of plant protein for animal protein with cardiovascular risk factors and inflammatory biomarkers is indicated in Table 3. There were significant inverse association between 5% substitution of plant protein for animal protein with WC ($\beta = -4.28$, 95%CI: –8.51, –0.62, $P = 0.047$) and LDL/HDL ($\beta = -0.26$, 95%CI: –0.48, –0.05, $P = 0.018$). No significant association was found for other variables.

4. Discussion

This study demonstrated that dietary protein substitution may have significant effects on cardiovascular risk factors but not inflammatory factors. Specifically, a 5% increase in plant protein intake in substitution for animal protein resulted in a significant inverse association with LDL/HDL and WC.

Previous research has explored the relationship between different types of protein intake and inflammatory factors and cardiovascular risk factors, primarily in adult populations. However, the relevance of this evidence to older adults is limited due to differences in body fat distribution and amount between these groups [40].

In the current study, we observed a significant inverse association between a 5% substitution of plant protein for animal protein and WC. This finding is supported by a study conducted by Ki-Byeong Park et al on older Korean adults, which also found a significant inverse association between plant protein intake and WC [25]. Additionally, a study by Yi Lin et al in Belgium on both males and females found a similar association [41]. Two cohort studies by Xianwen Shang et al. [42] and Adela Hruby and Paul F [43] also reported a favorable association between plant protein intake and WC. Xianwen Shang et al, conducted a cohort study on people aged 27–80 and they found that a 5% substitution of plant protein with animal protein increases WC by about 0.9 cm [42] which is following our findings. Furthermore, in a study on the Iranian population aged 35–70 an inverse association between plant protein intake and WC was found [29]. However, a study by J Halkjær on European men and women did not find a significant association between plant protein intake and WC [27]. Further research is needed to confirm these findings and determine the potential mechanisms underlying the relationship between plant protein intake and WC. The inconsistent findings in the two studies may be attributed to the inclusion of women in the named study, while all participants in the current study are men. In contrast to most studies, our study focused solely on the protein content of food items, while other macronutrients and micronutrients available in different food items were not considered as favorable exposure. Therefore, the probable reason for our findings is related to the amino acid content of plant and animal proteins. The inverse association between increasing plant protein intake and WC may be due to the limitation of Leucine and Histidine, as the amounts of these two amino acids in plant protein are low [44]. They can stimulate insulin secretion. However, higher intake of non-essential amino acids, which are abundant in plant protein, may lead to the downregulation of insulin secretion and increased glucagon secretion,

Table 1
General characteristics of participants across the quartiles of AP and PP

	AP					PP					Total
Characteristics	T1	T2	T3	T4	P_{value}†	T1	T2	T3	T4	P_{value}†	
n	**90**	88	**89**	**90**		89	87	93	88		**357**
Age (year)	62.40 ± 5.20	66.69 ± 6.48	65.11 ± 8.15	65.70 ± 5.07	<0.001	65.45 ± 6.25	65.41 ± 8.96	66.27 ± 5.46	62.66 ± 3.93	0.001	64.97 ± 6.51
Weight (kg)	72.22 ± 10.44	70.37 ± 9.05	72.38 ± 10.68	73.72 ± 10.61	0.185	74.75 ± 8.70	72.94 ± 12.69	68.27 ± 9.70	72.96 ± 8.36	<0.001	72.18 ± 10.25
Physical activity (Met/h)	0.84 ± 0.09	0.86 ± 0.11	0.84 ± 0.11	0.84 ± 0.11	0.440	0.84 ± 0.11	0.83 ± 0.11	0.86 ± 0.11	0.84 ± 0.09	0.173	0.84 ± 0.11
BMI kg/m2)	24.74 ± 2.87	25.20 ± 3.06	25.16 ± 3.22	26.32 ± 3.42	0.007	25 ± 2.49	24.77 ± 3.27	25.50 ± 3.16	26.15 ± 3.62	0.021	25.36 ± 3.19
WC (cm)	95.56 ± 12.26	95.49 ± 7.14	96.68 ± 6.72	96.82 ± 6.93	0.607	98.92 ± 6.91	96.44 ± 8.45	93.50 ± 11.29	95.82 ± 5.64	<0.001	96.14 ± 8.58
SES, n(%)											
Low	35(38.9)	35(39.8)	27(30.3)	44(48.9)	<0.001	32(36.0)	42(48.3)	27(29.0)	40(45.5)	<0.001	141 (39.5)
Moderate	46(51.1)	44(50.0)	30(33.7)	34(37.8)		49(55.1)	38(43.7)	36(38.7)	31(35.2)		154 (43.1)
High	9(10.0)	9(10.2)	32(36.0)	12(13.3)		8(9.0)	7(8.0)	30(32.3)	17(19.3)		62 (17.4)
Smoking, n(%)											
No	67 (74.4)	79 (89.8)	69 (77.5)	81(90.0)	0.006	61(68.5)	75(86.2)	92(98.9)	68(77.3)	<0.001	296 (82.9)
Yes	23 (25.6)	9 (10.2)	20 (22.5)	9(10.0)		28(31.5)	12(13.8)	1(1.1)	20(22.7)		61 (17.1)
Disease, n (%)											
No	62 (68.9)	59 (67.0)	55 (61.8)	43(47.8)	0.016	49 (55.1)	54 (62.1)	66 (71.0)	50 (56.8)	0.117	219 (61.3)
Yes	28 (31.1)	29 (33.0)	34 (38.2)	47 (52.2)		40 (44.9)	33 (37.9)	27 (29.0)	38 (43.2)		138 (38.7)
Anti-diabetic drugs, n (%)											
No	60 (66.7)	72 (81.8)	62 (69.7)	78(21.8)	0.004	71 (79.8)	67 (77)	57 (61.3)	77 (87.5)	<0.001	272 (76.2)
Yes	30 (33.3)	16 (18.2)	27 (30.3)	12 (13.3)		18 (20.2)	20 (23)	36 (38.7)	11 (12.5)		85 (23.8)
Lipid lowering drugs, n (%)											
No	76 (84.4)	68 (77.3)	65 (73.0)	86(95.6)	<0.001	77 (86.5)	75 (86.2)	74 (79.6)	69 (78.4)	0.332	295 (82.6)
Yes	14 (15.6)	20 (22.7)	24 (27.0)	4 (4.4)		12 (13.5)	12 (13.8)	19 (20.4)	19 (21.6)		62 (17.4)
Thyroid drugs, n (%)											
No	85 (94.4)	88 (100.0)	86 (96.6)	88 (97.8)	0.154	85 (95.5)	84 (96.6)	93 (100)	85 (96.6)	0.276	347 (97.2)
Yes	5 (5.6)	0 (0.0)	3 (3.4)	2 (2.2)		4 (4.5)	3 (3.4)	0 (0)	3 (3.4)		10 (2.8)
Heart disease drugs, n (%)											
No	77 (85.6)	66 (75.0)	54 (60.7)	82 (91.1)	<0.001	73 (82.0)	70 (80.5)	61 (65.6)	75 (85.2)	0.007	279 (78.2)
Yes	13 (14.4)	22 (25.0)	35 (39.3)	8 (8.9)		16 (18.0)	17 (19.5)	32 (34.4)	13 (14.8)		78 (21.8)

P value less than 0.05 was considered significant. Values are based on average ± standard deviation or reported percentage. analysis of variance ANOVA for quantitative data and Chi-2 test for qualitative data have been used. AP: animal protein; PP: plant protein; BMI: body mass index; WC: waist circumference.

Table 2
Energy-adjusted dietary intakes across quartiles of AP, PP

Food groups	AP (g/day)					PP(g/day)				
Nutrients*	T1	T2	T3	T4	P_{value}†	T1	T2	T3	T4	P_{value}†
N	**90**	88	**89**	**90**		**89**	**87**	**93**	**88**	
Energy	1819.52 ± 55.48	1974.24 ± 56.11	2203.92 ± 56.11	2613.84 ± 55.48	<0.001	1593.16 ± 45.57	1916.25 ± 46.09	2398.60 ± 44.58	2702.37 ± 46.09	<0.001
Protein	61.30 ± 1.17	73.07 ± 1.14	83.90 ± 1.13	114.03 ± 1.22	<0.001	80.64 ± 2.54	86.85 ± 2.23	81.28 ± 2.16	83.93 ± 2.54	0.155
Carbohydrate	344.54 ± 4.53	338.23 ± 4.43	339.01 ± 4.37	318.86 ± 4.72	0.001	331.73 ± 5.27	328.89 ± 4.63	342.28 ± 4.50	337.16 ± 5.28	0.251
Fat	61.02 ± 1.79	64.79 ± 1.75	59.62 ± 1.73	58.37 ± 1.87	0.077	58.44 ± 2.06	62.75 ± 1.81	59.56 ± 1.76	63.13 ± 2.06	0.200
Fiber	10.81 ± 0.40	14.07 ± 0.39	12.00 ± 0.38	11.16 ± 0.41	<0.001	8.17 ± 0.40	9.56 ± 0.35	13.97 ± 0.34	15.85 ± 0.40	<0.001
Phosphorus	1.29 ± 0.04	1.55 ± 0.04	1.78 ± 0.04	2.55 ± 0.04	<0.001	1.90 ± 0.07	1.89 ± 0.06	1.75 ± 0.06	1.65 ± 0.07	0.063
Potassium	3.14 ± 0.08	4.04 ± 0.08	3.95 ± 0.08	5.11 ± 0.08	<0.001	3.82 ± 0.12	4.07 ± 0.11	4.35 ± 0.10	4.00 ± 0.12	0.007
Calcium	0.98 ± 0.05	1.17 ± 0.05	1.40 ± 0.05	2.44 ± 0.05	<0.001	1.68 ± 0.08	1.63 ± 0.07	1.42 ± 0.07	1.28 ± 0.08	0.013
Magnesium	254.27 ± 6.62	327.48 ± 6.47	299.44 ± 6.38	396.35 ± 6.89	<0.001	288.97 ± 9.45	316.30 ± 8.29	331.01 ± 8.05	341.43 ± 9.46	0.005
Sodium	7.11 ± 2.14	6.17 ± 2.09	8.71 ± 2.06	15.89 ± 2.22	0.013	8.82 ± 2.46	14.51 ± 2.16	8.08 ± 2.10	6.67 ± 2.46	0.071
Sodium/Potassium	2.91 ± 0.48	1.65 ± 0.47	2.36 ± 0.47	3.47 ± 0.50	0.054	2.88 ± 0.55	3.80 ± 0.49	1.98 ± 0.47	1.80 ± 0.56	0.041
Zinc	6.74 ± 0.28	8.30 ± 0.28	8.41 ± 0.27	13.31 ± 0.29	<0.001	9.61 ± 0.42	10.09 ± 0.37	8.48 ± 0.36	8.67 ± 0.42	0.023
Iron	11.34 ± 0.34	13.82 ± 0.33	12.49 ± 0.32	12.10 ± 0.35	<0.001	9.39 ± 0.33	11.64 ± 0.29	12.61 ± 0.28	16.13 ± 0.33	<0.001
Food Groups*										
Grains	330.25 ± 15.56	353.37 ± 15.19	327.64 ± 15.00	322.92 ± 16.19	0.515	322.53 ± 17.70	356.20 ± 15.52	308.43 ± 15.09	348.70 ± 17.73	0.079
Fruits	375.32 ± 19.27	516.34 ± 18.82	447.02 ± 18.57	485.30 ± 20.06	<0.001	295.23 ± 20.87	437.73 ± 18.30	512.73 ± 17.79	576.90 ± 20.90	<0.001
Vegetables	367.12 ± 18.45	426.79 ± 18.02	406.39 ± 17.78	385.46 ± 19.21	0.095	273.35 ± 19.51	336.78 ± 17.11	473.55 ± 16.63	498.66 ± 19.55	<0.001
Dairy Product	370.98 ± 25.14	456.43 ± 24.56	588.68 ± 24.23	1076.90 ± 26.17	<0.001	804.55 ± 39.69	728.26 ± 34.81	546.08 ± 33.83	419.87 ± 39.76	<0.001

AP: animal protein; PP: plant protein; *Mean ± SE; *P* value less than 0.05 was considered significant. †Calculated by analysis of variance (*ANOVA*) for energy intake and multivariate analysis of covariance (ANCOVA) for other dietary variables. All the variables, except energy, adjusted for energy intake.

Table 3

Substitution model for increasing 5% energy intake of plant protein at expense of 5% energy intake of animal protein

Characteristics	Crude	95% CI	P_{value}	Model 1	95% CI	P_{value}	Model 2	95% CI	P_{value}
WC (cm)	–3.68	–7.37, 0.02	0.051	–4.52	–8.35, –0.69	0.021	–4.28	–8.51, –0.62	**0.047**
SBP (mmHg)	0.82	0.12, 1.52	0.021	0.91	0.21, 1.62	0.011	0.33	–0.47, 1.14	0.414
DBP (mmHg)	0.15	–0.19, 0.49	0.399	–0.09	–0.43, 0.24	0.579	–0.21	–0.60, 0.18	0.290
FBS (mg/dl)	7.66	–1.29, 16.63	0.093	9.37	0.19, 18.55	0.046	5.94	–3.95, 15.83	0.238
TG (mg/dl)	–10.07	–26.30, 6.17	0.223	–11.68	–28.98, 5.62	0.185	–6.86	–24.98, 11.25	0.457
HDL (mg/dl)	0.15	–3.64, 3.93	0.938	1.31	–2.69, 5.31	0.521	3.25	–0.66, 7.17	0.103
LDL (mg/dl)	–6.59	–15.70, 2.52	0.156	–7.64	–17.03, 1.74	0.110	–4.49	–13.63, 4.64	0.334
TC (mg/dl)	–2.09	–13.15, 8.96	0.710	–3.58	–14.71, 7.56	0.528	3.69	–6.78, 14.17	0.488
LDL/HDL	–0.15	–0.36, 0.06	0.160	–0.26	–0.48, –0.04	0.018	–0.26	–0.48, –0.05	**0.018**
Hs-CRP (mg/dl)	0.09	–0.49, 0.69	0.750	–0.52	–1.08, 0.04	0.069	–0.28	–0.82, 0.26	0.310
IL-6 (pg/ml)	–0.08	–0.40, 0.23	0.604	–0.10	–0.44, 0.24	0.568	3.92	–17.47, 25.31	0.719
Fibrinogen (mg/dl)	6.89	–13.42, 27.20	0.505	0.21	–20.67, 21.10	0.984	–0.07	–0.46, 0.32	0.733
TNF-α (pg/ml)	0.01	–0.02, 0.04	0.641	0.01	–0.02, 0.05	0.520	–0.005	–0.04, 0.04	0.822
ALT (IU/L)	0.76	–5.59, 7.12	0.813	–1.50	–8.09, 5.09	0.655	3.19	–4.13, 10.51	0.392
AST (IU/L)	–1.91	–7.84, 4.01	0.526	–5.40	–11.52, 0.70	0.083	–2.32	–9.17, 4.53	0.506

P value less than 0.05 was considered significant. *Crude: Not adjusted for any variables. *Model 1: The model was adjusted for age, energy intake, marital status, socioeconomic status, physical activity, smoking and BMI. *Model 2: Model 1 + diseases and drugs, fiber, total fat and total carbohydrate. TG: triglyceride; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBS: fasting blood sugar; HDL-C: high density lipoprotein-cholesterol; WC: waist circumference; LDL-C, low-density lipoprotein-cholesterol; TC, total cholesterol; hs-CRP, high-sensitive C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; AP: animal protein; PP: plant protein; TP: total protein.

which stimulates gluconeogenesis, hepatic lipid oxidation, lipolysis, and decreased IGF-1 and cholesterol synthesis, ultimately leading to improved body composition [44].

The aforementioned mechanism may also be responsible for LDL reduction as a result of hepatic lipid oxidation and reduction of cholesterol synthesis. Similarly, we found that a 5% substitution of animal protein with plant protein is inversely associated with LDL/HDL. In support of our findings, Shuangli Meng et al conducted a cross-sectional study in China and found an inverse significant association between plant protein intake and LDL/HDL [43]. In a meta-analysis of 112 clinical trials conducted by Siying S. Li et al, findings showed that the substitution of animal protein with plant protein is associated with lower LDL and a 4% reduction in cardiovascular disease [45]. In line with previous findings, Hang Zhao et al conducted a meta-analysis of 32 studies and eventually found that plant protein consumption in comparison with animal protein is associated with higher HDL, lower LDL and LDL/HDL [46].

4.1. Limitation and strength

Due to our knowledge, this is the first observational study about the association of dietary protein substitution and cardiovascular risk factors and inflammatory biomarkers in Iranian elderly men. There are few studies conducted on elderly population. In addition, unlike most of the studies, we only estimated the amount of protein in each food item and focused on the amino acid content. However, it is not possible to draw causal conclusions due to cross-sectional design. Therefore, more comprehensive studies are needed about this subject, especially on older adults.

5. Conclusion

In the current study, the substitution of animal protein with plant protein was found to be associated with lower WC and LDL/HDL, which are important risk factors for cardiovascular disease. The finding shows that plant protein consumption may be more beneficial compared to animal protein.

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Author contributions

Hanieh Abbasi contributed to the conception and design, analysis, interpretation of data and drafting of the article. N. Fahimfar and M. Nazarzade contributed to the analysis. L.Azadbakht contributed to the conception and design and acquisition of data. All authors read and approved the final manuscript.

Ethics statement

Ethical approval was given by TUMS as health centres in southern Tehran are supervised by the Tehran University of Medical Sciences (TUMS) (grant number: 48040).

Conflict of interest

The authors declare no conflicts of interest.

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