ORIGINAL RESEARCH

Nutritional Epidemiology and Public Health

CURRENT DEVELOPMENTS IN NUTRITION

Dietary Protein and Changes in Biomarkers of Inflammation and Oxidative Stress in the Framingham Heart Study Offspring Cohort

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ABSTRACT

Background: Chronic inflammation is thought to be a major characteristic of aging, which may increase need for substrates, specifically protein, to support anti-inflammatory processes.

Objectives: The aim of this study was to assess associations between dietary protein and changes in biomarkers of inflammation and oxidative stress over the long term in a community-dwelling population.

Methods: In 2061 participants of the Framingham Heart Study Offspring cohort who attended exams 7 (1998–2001; mean ± SD age 60.0 ± 8.8 y, 56% female) and 8 (2005–2008), total, animal, and plant protein intakes were assessed by food-frequency questionnaire at each exam, energy adjusted, and averaged. We defined an inflammation and oxidative stress score as the sum of rank-normalized values of 9 circulating biomarkers (C-reactive protein, osteoprotegerin, P-selectin, tumor necrosis factor receptor II, soluble intercellular adhesion molecule-1, interleukin 6, monocyte chemoattractant protein 1, and lipoprotein phospholipase A2 mass and activity), and urinary isoprostanes, along with 2 subscores. Adjusted least-square means of changes in the scores and log individual biomarkers in quartile categories of intake were estimated with the use of linear regression models, across mean ± SD 6.6 ± 0.7 y of follow-up.

Results: Protein intake was inversely associated with changes in the inflammation and oxidative stress score (mean ± SE in Q1 compared with Q4: 0.77 ± 0.17 compared with 0.31 ± 0.19; *P*-trend = 0.02), indicating overall inflammation/oxidative stress increased less in those with the highest intake than in those with the lowest. Favorable associations were observed for plant protein (Q1 compared with Q4: 0.89 ± 0.25 compared with 0.14 ± 0.25; *P*-trend = 0.001), but only trended toward significance for animal protein (Q1 compared with Q4: 0.70 ± 0.26 compared with 0.31 ± 0.26; *P*-trend = 0.05). Total protein and plant protein intakes were also inversely associated with changes in monocyte chemoattractant protein 1 (total: Q1 compared with Q4: 0.19 ± 0.01 compared with 0.15 ± 0.01 log-pg/mL; *P*-trend = 0.03; plant: Q1 compared with Q4: 0.21 ± 0.01 compared with 0.16 ± 0.01 log-pg/mL; *P*-trend = 0.003).

Conclusions: Dietary protein, particularly from plant sources, may be associated with beneficial changes in the inflammatory burden in aging populations. *Curr Dev Nutr* 2019;3:nzz019.

Keywords: protein intake, inflammation, oxidative stress, monocyte chemoattractant protein-1, C-reactive protein, aging, epidemiology, cohort

Manuscript received October 10, 2018. Initial review completed February 4, 2019. Revision accepted March 25, 2019. Published online March 28, 2019.

This research was supported by the USDA—Agricultural Research Service (ARS), agreement no. 58-1950-4-003, and by in part by the North American Branch of the International Life Sciences Institute (ILSI NA). ILSI NA is a public, nonprofit foundation that provides a forum to advance understanding of scientific issues related to the nutritional quality and safety of the food supply by sponsoring research programs, educational seminars and workshops, and publications. ILSI NA receives support primarily from its industry membership. The Framingham Heart Study is supported by contract no. HHSN268201500001I from the NIH National Heart, Lung and Blood Institute with additional support from other sources. The views expressed in this article are of those of the authors and do not necessarily represent the views of the funding organization. The sponsors had no role in the conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication.

Author disclosures: AH and PFJ, no conflicts of interest.

Online Supporting Material, Supplemental Figure 1 and Supplemental Tables 1–6 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/cdn/.

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Abbreviations used: CRP, C-reactive protein; ICAM-1, soluble intracellular adhesion molecule 1; IS, inflammation and oxidative stress score; LPL-A2, lipoprotein phospholipase A2; MCP-1, monocyte chemoattractant protein 1; NSAID, nonsteroidal anti-inflammatory drug; OPG, osteoprotegerin; PAI, physical activity index; RDA, Recommended Dietary Allowance; TNFRII, tumor necrosis factor receptor II.

Introduction

Chronic, low-grade, systemic inflammation is thought to be a major characteristic of aging [so-called "inflammaging" (1)], contributing to age-associated frailty, morbidity, and mortality (2–6). Instances of higher overall inflammatory status, such as those of older individuals or in chronically inflamed disease states, may lead to an increased need for substrates (i.e., protein) to support anti-inflammatory processes. Meeting protein needs in aging populations may therefore be important not just for maintenance of lean mass (7–9), strength (7, 8, 10), and physical function (9, 11, 12), but also for counteracting inflammation, oxidation, and their downstream catabolic effects

(13). Several studies have observed that proinflammatory cytokines are inversely associated with muscle strength and physical performance (14–18), both of which have been positively associated with at least adequate protein intake (9, 12).

However, higher protein intake is also known to upregulate the IGF/Akt/mTOR cascade, which acts as a key driver of the aging process (19). Higher protein intake has been associated with higher concentrations of certain circulating inflammatory biomarkers, such as C-reactive protein (CRP) (20, 21), although the dietary source of the protein may be relevant (22–26). A considerable proportion of dietary protein, notably in most Western populations, comes from animal sources (i.e., dairy, poultry, meat), and some of this protein intake has been shown to be associated with proinflammatory and pro-oxidative states (22, 27–32). Thus, protein intake may in fact have an overall null effect on inflammation and oxidative stress in aging populations, both providing substrates for anti-inflammatory and antioxidative processes [e.g., cysteine for glutathione synthesis (19)] and supporting anabolism (13), but also inducing a proinflammatory state, potentially depending on its source. One long-term study has attempted to link protein intake with age-related frailty and inflammation: conducted in a community-dwelling elderly population, the authors observed that in those experiencing high levels of inflammation, low protein intake exacerbated age-related loss in muscle strength over 3 y of follow-up, although relations between protein intake and changes in biomarkers themselves were not reported (18).

Although short-term trials (ranging from postprandial to 2 y) of protein intake on health, including inflammatory biomarkers as primary or secondary outcomes, are common, there are relatively few studies of relations between long-term habitual protein intake and circulating inflammatory biomarkers in generally healthy populations. To our knowledge, no study has examined habitual protein intake in relation to long-term (>5 y) changes in biomarkers of inflammation and oxidative stress. Prior studies have all been cross-sectional, and frequently focused on just 1 or 2 biomarkers or on a specific protein food source, rather than total protein intake (21, 25, 28, 31, 33). Therefore, in the present study, we sought to assess associations between habitual protein intake and changes in circulating biomarkers of inflammation and oxidative stress, including an overall inflammation and oxidative stress score and 2 subscores, over ~7 y of follow-up in community-dwelling participants of the Framingham Heart Study Offspring cohort.

Methods

Participants

The National Heart, Lung, and Blood Institute's Framingham Heart Study Offspring cohort is a community-based, longitudinal study of cardiovascular disease that began in 1971 (34). In the seventh examination cycle (1998–2001; mean age 61.5 y) of the Offspring cohort, 3539 participants of the original 5124 underwent a standard medical examination, consisting of laboratory and anthropometric assessments, as well as dietary intake assessment. Of the 3539 participants who attended exam 7, we excluded from the present analyses those who were missing information on relevant inflammation and oxidative stress biomarkers (n = 397), who were nonfasting (n = 98), missing valid dietary data (n = 270), missing covariates (n = 8), and finally, missing follow-up measures at exam 8 (2005–2008; n = 3021; mean age 66.8 y) needed for calculating change (n = 705). Thus, 2061 participants were included in the primary longitudinal analysis (**Online Supporting Material, Supplemental Figure 1**).

The original data-collection protocols were approved by the Institutional Review Board at Boston University Medical Center, and written informed consent was obtained from all participants. The present study protocol was reviewed by the Tufts University Health Sciences Institutional Review Board.

Protein and dietary intake

The Harvard semiquantitative, 126-item FFQ was used to assess dietary intake at both examinations (35). The FFQs included lists of foods for which participants were asked to report the frequency of consumption of standard serving sizes of each item over the previous year. The range of possible responses was never/<1 time/mo to ≥6 times/d. Invalid FFQs were those which estimated daily caloric intake as <600 kcal/d, or ≥4000 kcal/d for women or ≥4200 kcal/d for men, or those which had ≥12 blank items (36). Total protein intake was calculated as the sum of protein intake from contributions to protein from individual line items. The validity and reliability of the FFQs have been previously described (35, 37–39). The relative validity of the FFQ for protein intake shows reasonable correlation with estimates from dietary records and urinary nitrogen (35, 37–39).

All foods and nutrients, including protein intake, were energy adjusted with the use of the residual method (36, 40). We averaged the intake values from exams 7 and 8, then created quartile categories of the averaged intake. Other dietary factors derived from the FFQ included estimated intake of energy, alcohol, carbohydrates, fatty acids, and the dietary glycemic index (41, 42).

Inflammation and oxidative stress biomarkers

As shown in **Supplemental Table 1**, 10 biomarkers of inflammation and oxidative stress which were assessed at the 7th examination were subsequently repeated at the 8th examination. Fasting blood samples were collected and stored at −80°C. Serum C-reactive protein (CRP) was measured with a high-sensitivity assay (Dade Behring BN100 nephelometer; Dade Behring Diagnostic). The following biomarkers were measured in duplicate with the use of commercially available ELISA kits: plasma osteoprotegerin (OPG; BioMedica GmbH, distributed by ALPCO Diagnostics); plasma P-selectin, plasma tumor necrosis factor receptor II (TNFRII), serum-soluble intercellular adhesion molecule 1 (ICAM-1), serum IL-6, and serum monocyte chemoattractant protein 1 (MCP-1; R&D Systems, Inc.); and plasma lipoprotein phospholipase A2 (LPL-A2) mass and activity (GlaxoSmithKline, distributed by diaDexus) (43). Urinary isoprostanes were quantified on morning urine specimens through the use of ELISA (Cayman Chemical) indexed to urinary creatinine (Abbot Spectrum CCX) (44).

Intra-assay CVs were 3.2% for CRP, 3.7% for OPG, 3.0% for P-selectin, 2.2% for TNFRII, 3.7% for ICAM-1, 3.1% for IL-6, 3.8% for MCP-1, 7.0% for LPL-A2 mass, 6.0% for LPL-A2 activity, 2–4% for urinary creatinine, and 9.1% for urinary isoprostanes (43, 44).

Values of individual biomarkers were log transformed prior to analysis, except for in the creation of the inflammation and oxidative stress score and subscores, described below.

Inflammation and oxidative stress score

To create the inflammation and oxidative stress score (IS) at each exam, individual biomarker values were first rank normalized, standardized as *z* scores, and then summed to compute the score, adapted from a score previously described in the Framingham Offspring cohort (45). The overall IS was calculated as the sum of the standardized rank values of 9 biomarkers available at both exams 7 and 8 (i.e., CRP, ICAM-1, LPL-A2 mass, LPL-A2 activity, IL-6, MCP-1, OPG, P-selectin, and TNFRII). Given the number of missing observations for urinary isoprostanes at exam 7 (Supplemental Table 1), these were excluded from the primary score. We also generated 2 subscores for secondary analyses: a cytokine subscore (sum of IL-6, TNFRII, and OPG) and an oxidation subscore (sum of LPL-A2 mass, LPL-A2 activity, and urinary isoprostanes). Higher values of the IS and the subscores indicate higher inflammation or oxidative stress.

Clinical and lifestyle covariates.

Nondietary covariates were assessed by in-person interview and examination at both examinations 7 and 8, and included baseline age (years), sex (male, female), current smoking status (yes, no), physical activity (metabolic-equivalent task h/d), nonsteroidal anti-inflammatory drug (NSAID) use (any, none), BMI, history of cardiovascular disease, and treatment for hypertension, diabetes, or dyslipidemia (all yes/no).

Statistical analysis

We assessed Pearson correlations between log-transformed biomarkers at each exam. Dietary protein was averaged across examinations 7 and 8 to account for long-term intake, and divided into quartile categories. Our primary outcome was the change in the overall IS. Secondary outcomes included changes in the cytokine and oxidation subscores, and changes in the logged values of the individual biomarkers. Change in each outcome was calculated as the value at examination 8 minus the value at examination 7. Our primary analysis estimated associations between total dietary protein intake, and animal and plant protein intake, and the change in the overall IS. Secondary analyses included the following: *1*) assessing protein intake against changes in the cytokine and oxidation subscores; *2*) assessing protein intake against changes in logged values of the individual biomarkers; and *3*) assessing associations between animal protein and plant protein intake against changes in the subscores, and the individual biomarkers. In addition, results indicated that a single biomarker (MCP-1) was strongly associated with protein intake and could be largely responsible for associations of protein with changes in the overall IS, thus sensitivity analyses included generating an IS without MCP-1 to assess whether associations were present even without MCP-1 as a score component. The FFQs inquired about the prior year's consumption, recorded on or in the weeks leading up to the date of the examination at which blood was drawn, thus theoretically allowing for a prospective analysis with respect to changes in biomarkers. However, because these data can be viewed as being collected concurrently with biomarker data, the present analyses may not be understood to be strictly prospective. To address this possibility, and to confirm the associations observed in the primary analysis, we conducted sensitivity analyses based on dietary data averaged from examinations 6 (1995–1998) and 7, and assessed these associations with changes in biomarkers between examinations 7 and 8, in an unambiguously prospective approach. (Note that the relevant biomarkers were not assessed at examination 6.)

In regression models, we estimated least-square means of the change in the outcome in each quartile category of protein intake adjusted for age, sex, energy intake, smoking status, and the baseline (examination 7) value of the outcome (model 1); physical activity, NSAID use, BMI, history of cardiovascular disease, treatment for hypertension, diabetes, or dyslipidemia, and alcohol intake (model 2); and dietary factors: the glycemic index and the PUFA:SFA ratio (model 3). Models of animal or plant protein were mutually adjusted for the other protein source. Further adjusting for educational attainment or change in weight did not substantively alter results. *P*-trend across quartile categories of protein intake was assessed by assigning the median value in each quartile category and treating it as a continuous variable in regression models. We statistically assessed potential effect modification of total protein intake on outcomes by age ($<$60.0 compared with $\geq$60.0 y), sex, BMI, and NSAID use through the use of first-order interaction terms. We also conducted analyses excluding current smokers. Finally, we created "substitution" models of associations with changes in the overall IS for total protein (i.e., modeling total protein intake holding energy and either fat or carbohydrate intake constant, adjusted as for all other covariates in model 2, above), and for plant protein for animal protein, and vice versa (i.e., holding energy, fat, and carbohydrate intake constant, otherwise adjusted as for model 2, above). Statistical significance was set at a nominal α level of 0.05. All analyses were conducted in SAS version 9.4.

Results

At examination 7, the mean ± SD age of participants was 60.0 ± 8.8 y, 56% were female, mean BMI was 27.9 ± 5.2 kg/m^2, and mean protein intake was 78.9 ± 26.1 g/d (17% of energy). Participants who reported consuming higher dietary protein tended to be younger, female, have higher BMI and waist circumference, and were more likely to be treated for diabetes, and less likely to be smokers (**Table 1**). They reported lower carbohydrate and alcohol intake, and higher fiber and fat intake, specifically unsaturated fat intake. Trends of logged age- and sex-adjusted biomarker concentrations indicated that ICAM-1, P-selectin, TNFRII, and creatinine-corrected urinary isoprostanes, as well as the overall IS, were lower in those reporting higher protein intake at examination 7. The strongest Pearson correlations between biomarkers at examination 7 were between LPL-A2 activity and LPL-A2 mass ($r = 0.52$, $P < 0.001$), and between IL-6 and CRP ($r = 0.46$, $P < 0.001$). These remained the strongest correlations at examination 8 ($r = 0.68$ and 0.50, respectively, both $P < 0.001$). The overall IS at examination 7 was correlated with the IS at examination 8 ($r = 0.64$, $P < 0.001$). Over a mean ± SD 6.6 ± 0.7 y of follow-up, mean absolute, unadjusted amounts of LPL-A2 mass and activity, IL-6, OPG, CRP, and isoprostanes decreased, whereas P-selectin, ICAM-1, MCP-1, and TNFRII increased.

Protein intake was inversely associated with the change in IS (mean ± SE in Q1 compared with Q4: 0.77 ± 0.17 compared with 0.31 ± 0.19; *P*-trend = 0.02) after adjusting for age, sex, smoking status, and the baseline score, indicating overall inflammation/oxidative stress increased less in those with the highest protein intake than it did in those with the lowest protein intake (**Table 2**). This association remained

TABLE 1 Adjusted means of baseline characteristics per quartile category of average protein intake in 2061 participants of the Framingham Heart Study Offspring cohort at examination 7[1]

	Quartile category of averaged protein intake (g/d)				
Median in quartile category	**Q1: 67.4**	**Q2: 77.3**	**Q3: 85.0**	**Q4: 95.9**	***P*-trend**
Characteristic					
Age, y	61.0 ± 0.4	60.0 ± 0.4	59.8 ± 0.4	59.2 ± 0.4	0.001
Sex, % female	43 ± 2	53 ± 2	61 ± 2	65 ± 2	<0.001
BMI, kg/m^2	27.0 ± 0.2	27.6 ± 0.2	28.4 ± 0.2	28.4 ± 0.2	<0.001
NSAID use, %	40 ± 2	41 ± 2	41 ± 2	44 ± 2	0.20
Current smoker, %	16 ± 1	11 ± 1	10 ± 1	5 ± 1	<0.001
PAI, MET-h/wk	38.2 ± 0.3	37.7 ± 0.3	37.3 ± 0.3	37.6 ± 0.3	0.09
Clinical characteristics					
SBP, mm Hg	125.7 ± 0.7	125.6 ± 0.7	125.1 ± 0.7	126.3 ± 0.7	0.68
DBP, mm Hg	74.0 ± 0.4	73.9 ± 0.4	74.2 ± 0.4	74.6 ± 0.4	0.22
Hypertension treatment, %	26 ± 2	32 ± 2	32 ± 2	30 ± 2	0.16
Diabetes treatment, %	1 ± 1	4 ± 1	5 ± 1	9 ± 1	<0.001
Triglycerides, mg/dL	135.0 ± 3.6	135.5 ± 3.5	129.0 ± 3.5	129.8 ± 3.6	0.19
Cholesterol, mg/dL	202.8 ± 1.6	202.5 ± 1.5	201.1 ± 1.5	200.0 ± 1.6	0.17
HDL cholesterol, mg/dL	54.8 ± 0.7	53.9 ± 0.7	54.9 ± 0.7	54.6 ± 0.7	0.99
LDL cholesterol, mg/dL	121.0 ± 1.5	121.5 ± 1.4	120.4 ± 1.4	119.5 ± 1.5	0.40
Dyslipidemia treatment, %	17 ± 2	21 ± 2	18 ± 2	20 ± 2	0.40
History of CVD, %	11 ± 1	12 ± 1	9 ± 1	8 ± 1	0.10
Dietary characteristics					
Protein, g/d	64.1 ± 0.4	75.4 ± 0.4	82.1 ± 0.4	94.9 ± 0.4	<0.001
Protein, % energy	14.1 ± 0.1	16.6 ± 0.1	18.2 ± 0.1	20.8 ± 0.1	<0.001
Protein, g · kg BW^{-1} · d^{-1}	0.9 ± 0.0	1.0 ± 0.0	1.1 ± 0.0	1.2 ± 0.0	<0.001
Meets protein RDA, %	60 ± 2	83 ± 2	86 ± 2	94 ± 2	<0.001
Animal protein, g/d	42.0 ± 0.5	51.8 ± 0.5	58.3 ± 0.5	70.7 ± 0.5	<0.001
Animal protein, % energy	9.3 ± 0.1	11.4 ± 0.1	13.0 ± 0.1	15.5 ± 0.1	<0.001
Animal protein, g · kg BW^{-1} · d^{-1}	0.6 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	<0.001
Plant protein, g/d	22.1 ± 0.3	23.6 ± 0.3	23.9 ± 0.3	24.2 ± 0.3	<0.001
Plant protein, % energy	4.9 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.3 ± 0.1	<0.001
Plant protein, g · kg BW^{-1} · d^{-1}	0.30 ± 0.005	0.32 ± 0.005	0.32 ± 0.005	0.32 ± 0.005	0.11
Carbohydrates, g/d	240.3 ± 1.8	235.6 ± 1.8	228.5 ± 1.8	215.1 ± 1.8	<0.001
Dietary fiber, g/d	16.6 ± 0.3	18.7 ± 0.3	19.0 ± 0.3	19.6 ± 0.3	<0.001
Glycemic index	54.4 ± 0.2	54.4 ± 0.2	54.0 ± 0.2	52.6 ± 0.2	<0.001
Fat, g/d	62.2 ± 0.6	63.1 ± 0.6	63.9 ± 0.6	64.9 ± 0.6	0.002
PUFA, g/d	11.6 ± 0.2	11.9 ± 0.2	12.0 ± 0.2	12.5 ± 0.2	<0.001
SFA, g/d	22.3 ± 0.3	22.1 ± 0.3	22.3 ± 0.3	22.5 ± 0.3	0.50
PUFA:SFA ratio	0.51 ± 0.13	0.89 ± 0.12	0.59 ± 0.12	0.58 ± 0.13	0.94
MUFA, g/d	22.9 ± 0.3	23.2 ± 0.3	23.6 ± 0.3	23.7 ± 0.3	0.02
Alcohol, g/d	14.8 ± 0.6	9.8 ± 0.6	8.7 ± 0.6	7.3 ± 0.6	<0.001
Biomarker concentrations[2]					
CRP, mg/L	0.72 ± 0.05	0.79 ± 0.05	0.72 ± 0.05	0.74 ± 0.05	0.98
ICAM-1, ng/mL	5.52 ± 0.01	5.50 ± 0.01	5.48 ± 0.01	5.46 ± 0.01	<0.001
IL-6, pg/mL	1.03 ± 0.03	0.97 ± 0.03	0.99 ± 0.03	0.96 ± 0.03	0.13
Corrected isoprostanes, ng/mmol[3]	4.96 ± 0.03	4.87 ± 0.03	4.83 ± 0.03	4.75 ± 0.03	<0.001
MCP-1, pg/mL	5.73 ± 0.01	5.74 ± 0.01	5.70 ± 0.01	5.72 ± 0.01	0.17
OPG, pmol/L	1.65 ± 0.01	1.66 ± 0.01	1.66 ± 0.01	1.64 ± 0.01	0.68
LPL-A2 activity, nmol · mL^{-1} · min^{-1}	4.94 ± 0.01	4.92 ± 0.01	4.92 ± 0.01	4.91 ± 0.01	0.11
LPL-A2 mass, ng/mL	5.67 ± 0.01	5.63 ± 0.01	5.64 ± 0.01	5.64 ± 0.01	0.18
P-selectin, ng/mL	3.59 ± 0.02	3.54 ± 0.02	3.55 ± 0.02	3.53 ± 0.02	0.01
TNFRII, pg/mL	7.61 ± 0.01	7.59 ± 0.01	7.58 ± 0.01	7.57 ± 0.01	0.01
Inflammation score[4]	0.50 ± 0.19	0.07 ± 0.19	−0.16 ± 0.19	−0.41 ± 0.19	<0.001

[1]Characteristics are presented as means ± SEs unless otherwise indicated. BW, body weight; CRP, C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; ICAM-1, soluble intracellular adhesion molecule 1; LPL-A2, lipoprotein phospholipase A2; MCP-1, monocyte chemoattractant protein 1; MET, metabolic-equivalent task; NSAID, non-steroidal anti-inflammatory drug; OPG, osteoprotegerin; PAI, physical activity index; RDA, Recommended Dietary Allowance; SBP, systolic blood pressure; TNFRII, tumor necrosis factor receptor II.

[2]Individual biomarker concentrations were log-transformed prior to analysis. Values presented are log values.

[3]$n = 1690$ for urinary creatinine and urinary creatinine-corrected urinary isoprostanes.

[4]The inflammation and oxidative stress score is the sum of rank-normalized values of CRP, OPG, ICAM-1, IL-6, MCP-1, LPL-A2 mass and activity, P-selectin, and TNFRII.

TABLE 2 Adjusted least-square means of change in inflammation and oxidative stress score per averaged dietary protein in quartile categories of intake in 2061 participants of the Framingham Heart Study Offspring cohort[1]

Dietary intake	Model[3]	Quartile category of protein intake[2]				*P*-trend
		Q1	Q2	Q3	Q4	
Protein, g/d	1	0.766 ± 0.172	0.575 ± 0.176	0.369 ± 0.179	0.310 ± 0.185	0.02
	2	0.839 ± 0.258	0.598 ± 0.252	0.362 ± 0.254	0.284 ± 0.252	0.007
	3	0.813 ± 0.261	0.583 ± 0.253	0.362 ± 0.254	0.313 ± 0.255	0.02
Animal protein, g/d	1	0.624 ± 0.176	0.655 ± 0.175	0.410 ± 0.176	0.402 ± 0.184	0.19
	2	0.651 ± 0.258	0.644 ± 0.251	0.389 ± 0.253	0.360 ± 0.254	0.11
	3	0.702 ± 0.261	0.642 ± 0.251	0.362 ± 0.253	0.313 ± 0.258	0.05
Plant protein, g/d	1	0.784 ± 0.170	0.473 ± 0.178	0.524 ± 0.180	0.227 ± 0.183	0.01
	2	0.822 ± 0.252	0.479 ± 0.257	0.513 ± 0.253	0.196 ± 0.253	0.006
	3	0.891 ± 0.254	0.508 ± 0.257	0.519 ± 0.252	0.141 ± 0.254	0.001

[1]Values are least-square adjusted means ± SEs of the outcome, the change in the inflammation and oxidative stress score, modeled as the difference in the score between examination 8 and examination 7. The score is the sum of rank-normalized values of CRP, OPG, ICAM-1, IL-6, MCP-1, LPL-A2 mass and activity, P-selectin, and TNFRII. A higher value of the outcome indicates a larger change (increase) in inflammation/oxidative stress, whereas a lower value indicates a smaller change (increase if positive, decrease if negative), and thus less inflammation/oxidative stress. CRP, C-reactive protein; CVD, cardiovascular disease; ICAM-1, soluble intracellular adhesion molecule 1; LPL-A2, lipoprotein phospholipase A2; MCP-1, monocyte chemoattractant protein 1; NSAID, nonsteroidal anti-inflammatory drug; OPG, osteoprotegerin; TNFRII, tumor necrosis factor receptor II.

[2]Median values in quartile categories of intake were as follows: for total protein, 67.4, 77.3, 85.0, and 95.9 g/d; for animal protein, 42.6, 52.5, 60.3, and 71.6 g/d; and for plant protein, 19.8, 22.9, 25.5, and 29.9 g/d.

[3]Models were adjusted as follows: *1*) age, sex, energy intake, smoking status, and the baseline (examination 7) value of the score; *2*) baseline physical activity, NSAID use, BMI, cardiovascular disease history, treatment for hypertension, diabetes, or dyslipidemia, and alcohol intake; *3*) glycemic index, and the PUFA:SFA ratio. For animal and plant protein, model 3 was also adjusted for the other protein source (e.g., animal protein adjusted for plant protein as well as glycemic index and the PUFA:SFA ratio).

significant in the fully adjusted model. When protein was separated by source (i.e., plant or animal), the beneficial association was observed for plant protein only (Q1 compared with Q4: 0.89 ± 0.25 compared with 0.14 ± 0.25; *P*-trend = 0.001), and only trended toward significance for animal protein (Q1 compared with Q4: 0.70 ± 0.26 compared with 0.31 ± 0.26; *P*-trend = 0.05) in fully adjusted models including mutual adjustment for the other protein source.

Associations between protein intake and changes in logged values of individual biomarkers indicated that total protein intake was only statistically significantly inversely associated with MCP-1 (mean ± SE in Q1 compared with Q4: 0.19 ± 0.01 compared with 0.15 ± 0.01 logged pg/mL; *P*-trend = 0.03) (**Supplemental Table 2**), and this inverse association was seen for plant protein (Q1 compared with Q4: 0.21 ± 0.01 compared with 0.16 compared with 0.01 logged pg/mL; *P*-trend = 0.003) (**Supplemental Table 3**), but not animal protein (**Supplemental Table 4**). There were no statistically significant associations of animal protein with individual biomarkers in fully adjusted models (Supplemental Table 4). In sensitivity analyses removing MCP-1 from the overall IS, the association between protein intake and the modified IS was attenuated but was borderline significant in the fully adjusted model (Q1 compared with Q4: 0.82 ± 0.24 compared with 0.42 ± 0.24; *P*-trend = 0.049), suggesting associations with MCP-1 were predominantly responsible for the observed association with the overall IS. In sensitivity analyses assessing habitual dietary intake as the average of examinations 6 and 7 (as opposed to examinations 7 and 8), results for total, animal, and plant protein on the overall IS as well as MCP-1 were largely unchanged relative to the initial dietary approach (**Supplemental Table 5**).

With respect to the oxidation and cytokine subscores, total protein intake was only inversely associated with the cytokine subscore, not the oxidation subscore (**Supplemental Table 6**). Although neither animal nor plant protein showed statistically significant associations with the cytokine subscore, inverse trends in both protein sources were evident.

Results were somewhat attenuated but remained statistically significant after excluding current smokers ($n = 217$, 10.5% of participants). There were no significant interactions on outcomes between protein intake and sex, age, BMI, or NSAID use (all *P*-interaction >0.05).

Substitution models suggested that whether total protein replaced carbohydrates or fat, changes in IS were favorable: (mean ± SE β per 10 g/d protein instead of carbohydrate: −0.17 ± 0.06; $P = 0.005$; mean ± SE β per 10 g/d protein instead of fat: −0.14 ± 0.07; $P = 0.03$]. In addition, plant protein substituted for animal protein showed a favorable association (mean ± SE β per 10 g/d plant protein instead of animal protein: −0.41 ± 0.15; $P = 0.005$), whereas animal protein in place of plant protein showed no association (mean ± SE β per 10 g/d animal protein instead of plant protein 0.11 ± 0.09; $P = 0.20$). Models that used protein sources as percentages of energy yielded similar results.

Discussion

In the present study, we observed that higher protein intake was associated with favorable changes in overall inflammation/oxidative stress levels over ∼7 y in a community-based population, when inflammation/oxidative stress was defined as a score consisting of 9 biomarkers of inflammation and oxidative stress. We observed that the concentrations of one biomarker in particular, MCP-1, appeared to be largely responsible for the overall relation of our inflammation and oxidative stress score, and that the inverse associations with protein intake and changes in this biomarker were stronger than for the other individual biomarkers. In addition, we observed that when protein was considered by its dietary source, plant protein, but not animal protein, showed favorable associations with changes in both overall inflammation/oxidative stress as assessed by the overall score, as well as MCP-1, in particular. Substitution models confirmed that higher

protein intake—whether replacing fats or carbohydrates—and plant protein—replacing animal protein—were favorably associated with changes in overall inflammation/oxidative stress as assessed by the overall score. To our knowledge, ours is the first study to assess habitual protein intake in relation to changes in inflammation and oxidative stress over this long a time period.

Of the individual biomarkers, we were surprised to have *only* observed associations with MCP-1, given prior reports of relations between dietary protein and biomarkers such as IL-6 and CRP (13, 20, 22, 27, 32). However, there is sufficient variation in the both experimental and observational results to suggest that the relations are equivocal and may perhaps depend more on the distribution of the remaining macronutrients or on the underlying dietary pattern in generally healthy populations, or on a combination of these, and prior experiments provide only mixed evidence. For example, in the DiOGenes study, a low-protein diet lowered circulating CRP in adults trying to maintain weight loss over 26 wk more than a high-protein diet (20). An 8-wk trial of an energy-restricted high (30% energy) compared with a low (15% energy) protein diet indicated that high compared with low protein, and meat protein in particular, but not plant or fish protein, increased a score that included CRP, IL-6, TNF-α, and PAI-1 concentrations (27). Another 12-wk trial of protein intake in the context of 10% energy restriction, this one in overweight, older participants, indicated that a high-protein diet (1.7 g · kg body weight^{-1} · d^{-1}) compared to a normal-protein diet (0.9 g · kg body weight^{-1} · d^{-1}) induced differential expression in 530 genes in white adipose tissue, notably a downregulation in expression of genes linked to immune cell infiltration, adaptive immune response and inflammasome in the normal-protein group, but not the high-protein group (46). However, a 6-wk ad libitum high animal compared with high plant protein (30% energy) diet in overweight or obese individuals with nonalcoholic fatty liver disease and type 2 diabetes showed no differences in IL-6 or MCP-1 between diets, a decrease in IL-18 only in the high animal protein group, and a decrease in TNF-α only in the plant protein group (24). These varying results suggest that protein quantity and source play roles in inflammation, but that the underlying dietary and health contexts may also be important.

In the present study, MCP-1 was only somewhat correlated with the overall inflammation and oxidative stress score ($r = 0.42$ and 0.40 at examinations 7 and 8, respectively) and poorly correlated with other biomarkers (range $r = 0.03$ for LPL-A2 mass to 0.22 for IL-6). MCP-1, also known as chemokine CCL2, is a well-characterized chemokine linked with myocardial infarction (47), atherosclerosis (48), as well as type 2 diabetes (49). The family of MCPs recruits monocytes to sites of trauma, infection, and ischemia, and MCP-1 itself is a strong chemotactic factor for dendritic cells, memory T cells, and basophils, as well as monocytes (50, 51). In macrophage-rich atherosclerotic plaques, it is induced by oxidized LDL cholesterol and produced in endothelial and smooth-muscle cells, and thus is a proposed link between oxidized lipoproteins and early monocyte recruitment to the vessel wall. In addition, MCP-1 is highly expressed in adipose tissue by adipocytes and macrophages, not only signaling further recruitment of macrophages, but also upregulating an inflammatory cascade and downstream impairment of insulin sensitivity (50–52). It was also found to be stimulated by glucose-dependent insulinotropic peptide, a nutrient-induced intestinal hormone, in response to a calorie load. Infusions of glucose-dependent insulinotropic peptide significantly increased MCP-1 mRNA in adipose tissue, as well as circulating plasma concentrations, independently of circulating insulin or glucose. Those findings suggest links between gut hormones and adipose tissue inflammation via MCP-1 (52).

However, of the few experiments examining protein and MCP-1, short-term dietary studies have suggested that total protein intake may not be as relevant to MCP-1 concentrations as is the type of protein. In a 1-mo-long trial with participants who were overweight or obese , dairy-supplemented, but not soy-supplemented, eucaloric diets suppressed MCP-1 by 10% (26). A 6-wk crossover study of dairy in participants with metabolic syndrome indicated that women, but not men, had lower MCP-1 following 12 wk of low-fat dairy consumption compared with carbohydrate control (53). However, neither whey nor casein (60 g/d) over 12 wk affected MCP-1 in abdominally obese adults (54). And, as mentioned, there was no difference in MCP-1 concentrations between eucaloric high animal compared with plant protein diets over 6 wk in participants with fatty liver disease and type 2 diabetes (24). Similar to summary relations between protein intake and other inflammation or oxidative stress biomarkers, the relation between protein and MCP-1 seems to show the same inconsistencies.

As alluded to above, food sources have been shown to have differential effects on different biomarkers of inflammation. According to one systematic review, dairy intake is broadly equivocal with respect to biomarkers such as CRP, IL-6, and TNF-α (55). Dietary patterns rich in plant protein sources (i.e., nuts, legumes, whole grains, vegetables) have been associated with lower levels of inflammation (22, 32, 56, 57), substituting whole for refined grains appears to modulate select cytokines (e.g., TNF-α) (58, 59), whereas fruits and vegetables affect still others (e.g., IL-6) (57, 59). On the other hand, soy, an ostensibly good source of plant protein, seems to have little to no effect on biomarkers of inflammation and oxidation (60–62). Nevertheless, higher habitual intake of polyphenols, notably flavonoids, and flavonoid-containing foods are relatively consistently associated with lower levels of inflammation (57, 63–65, 45, 66). Plant protein sources may contain high concentrations of polyphenols and a host of other potentially inflammation-reducing constituents, which may be contributing to the lower levels of overall inflammation and oxidative stress we observed with higher plant protein intake.

It should be noted that plant protein intake in this study was relatively low, on average just 5.3% of energy, compared with animal protein (12.4% energy). Investigations in populations with higher percentages of protein from plants are merited. Total protein amounts remain nevertheless relevant in aging populations even in the absence of consistently discernible effects on inflammation specifically, owing to potentially favorable associations of higher protein intake with cardiometabolic health (67) and physical function (8, 10, 11). It has been demonstrated that too little protein (e.g., half the RDA compared with the RDA) can have deleterious effects on immune function, lean mass, and muscle function in older women (9). On the other hand, protein in excess of the RDA may not confer benefits; a recent study in older men failed to show a benefit to twice the RDA (compared with the RDA) on preservation of lean mass or function (12). However, benefits of protein in aging may depend on underlying inflammation/oxidative stress status. A longitudinal study in an elderly Italian population found no significant relation between protein intake and muscle strength in

the total population, but did see a relation among those with high concentrations of CRP, IL-6, or TNF-α in whom lower protein intake was associated with greater decline in muscle strength over 3 y of follow-up (18). Authors did not report the relations between protein intake and circulating biomarkers of inflammation.

Strengths and Limitations

We benefited from a large, well-characterized cohort followed for an average of 7 y with repeated measures of exposures and outcomes from which changes in circulating and urinary biomarkers of inflammation and oxidative stress could be derived. Although averages of multiple biomarker measures may have provided a more accurate picture of long-term inflammation status, our goal was to prospectively assess whether usual protein intake in a sample of aging adults might affect the well-established age-related changes in inflammatory response. Our findings may be biased by the availability of a relatively limited set of repeatedly measured biomarkers of inflammation and oxidation. Our overall score depended on biomarkers previously assessed by the investigators of the Framingham Heart Study, and does not reflect all possible biomarkers, nor possibly even the most important biomarkers of inflammation or oxidative stress. In addition, it is possible that these biomarkers may not be the most biologically relevant to assess in relation to protein intake. That said, these biomarkers are among the most commonly measured in observational and experimental studies; they are broadly associated with chronic diseases and their risk factors, as well as aging; and diet has been shown to be associated with their concentrations.

Population-based observational cohorts generally use FFQs to assess diet in large samples, but they have limitations, including recall, social desirability, and self-report biases. FFQs provide good estimates of relative intake, but only approximate absolute intake. Thus they give us the ability to distinguish between high and low consumers of a given nutrient, but may not translate directly to clinical applications. In grouping by animal and plant protein food sources, we invariably incorporate other components of those foods, such as saturated fat, fiber, and polyphenols, which were not individually adjusted for in regressions. However, we adjusted for overall diet quality (as glycemic index and fatty acid ratio) in these analyses, which may account for many differences in dietary quality potentially associated with different protein food sources. In addition, residual confounding by unmeasured dietary or other lifestyle factors may also influence our results. We did not adjust our nominal α level of significance for the number of outcomes (10 biomarkers, 2 subscores, 1 overall score), and instead combined the biomarkers in a single overall score of inflammation and oxidative stress. If we were to apply a correction (e.g., Bonferroni) for multiple testing, we would likely reach a more conservative conclusion. Finally, participants of the Framingham Offspring cohort are predominantly Caucasian Americans, which may limit the generalizability of our findings.

Conclusions

The findings of our study, which includes one of the most comprehensive assessments of inflammation and oxidative stress status and protein intake across a very long follow-up period, lend support to the existing literature suggesting that higher protein intake, particularly from plant-based foods, is associated with lower risk of "inflammaging."

Acknowledgments

The authors' responsibilities were as follows—PFJ: designed the research; AH: analyzed the data and wrote the manuscript; and both authors contributed to interpreting the data, and edited, reviewed, approved, and are responsible for the final content of the manuscript.

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