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ORIGINAL RESEARCH

Proteins as Mediators of the Association Between Diet Quality and Incident Cardiovascular Disease and All-Cause Mortality: The Framingham Heart Study

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BACKGROUND: Biological mechanisms underlying the association of a healthy diet with chronic diseases remain unclear. Targeted proteomics may facilitate the understanding of mechanisms linking diet to chronic diseases.

METHODS AND RESULTS: We examined 6360 participants (mean age 50 years; 54% women) in the Framingham Heart Study. The associations between diet and 71 cardiovascular disease (CVD)-related proteins were examined using 3 diet quality scores: the Alternate Healthy Eating Index, the modified Mediterranean-style Diet Score, and the modified Dietary Approaches to Stop Hypertension diet score. A mediation analysis was conducted to examine which proteins mediated the associations of diet with incident CVD and all-cause mortality. Thirty of the 71 proteins were associated with at least 1 diet quality score ($P<0.0007$) after adjustment for multiple covariates in all study participants and confirmed by an internal validation analysis. Gene ontology analysis identified inflammation-related pathways such as regulation of cell killing and neuroinflammatory response (Bonferroni corrected $P<0.05$). During a median follow-up of 13 years, we documented 512 deaths and 488 incident CVD events. Higher diet quality scores were associated with lower risk of CVD ($P\leq0.03$) and mortality ($P\leq0.004$). After adjusting for multiple potential confounders, 4 proteins (B2M [beta-2-microglobulin], GDF15 [growth differentiation factor 15], sICAM1 [soluble intercellular adhesion molecule 1], and UCMGP [uncarboxylated matrix Gla-protein]) mediated the association between at least 1 diet quality score and all-cause mortality (median proportion of mediation ranged from 8.6% to 25.9%). We also observed that GDF15 mediated the association of the Alternate Healthy Eating Index with CVD (median proportion of mediation: 8.6%).

CONCLUSIONS: Diet quality is associated with new-onset CVD and mortality and with circulating CVD-related proteins. Several proteins appear to mediate the association of diet with these outcomes.

Key Words: cardiovascular disease ■ diet quality ■ mediator ■ mortality ■ proteomics

A healthy diet is recommended as an important lifestyle factor to reduce the risk of developing cardiovascular disease (CVD) and other chronic diseases.[1] Substantial epidemiological evidence has shown that a healthy diet, as assessed by diet quality score, is associated with reduced risk of a broad range of clinical outcomes including CVD. For example, higher diet quality scores, estimated using the Alternate Healthy Eating Index (AHEI), the Mediterranean-style Diet Score (MDS), or the Dietary Approaches to Stop Hypertension (DASH) diet score, were associated with lower incident coronary heart disease[2–5] and lower all-cause mortality.[6] Although the association between these diet quality scores and clinical outcomes has

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CLINICAL PERSPECTIVE

What Is New?

- A healthy diet is associated with circulating cardiovascular disease-related protein biomarkers, largely representing regulators of inflammatory pathways in a group of middle-aged and older participants in the Framingham Heart Study.
- Four proteins—B2M (beta-2-microglobulin), GDF15 (growth differentiation factor 15), sICAM1 (soluble intercellular adhesion molecule 1), and UCMGP (uncarboxylated matrix Gla-protein) may mediate the association of diet with health outcomes.

What Are the Clinical Implications?

- Our findings provide novel evidence to better understand the mechanisms linking healthy diet with new-onset cardiovascular disease and all-cause mortality.

Nonstandard Abbreviations and Acronyms

ADM	adrenomedullin
AHEI	Alternate Health Eating Index
B2M	beta-2-microglobulin
DASH	Dietary Approaches to Stop Hypertension
FFQ	Food Frequency Questionnaire
GDF15	growth differentiation factor 15
GO	gene ontology
GRN	granulin
HPX	hemopexin
LDLR	low-density lipoprotein receptor
MDS	Mediterranean-style Diet Score
MMP	matrix metallopeptidase
MPO	myeloperoxidase
PAI1	plasminogen activator inhibitor 1
pQTL	protein quantitative trait loci
sICAM1	soluble intercellular adhesion molecule 1
UCMGP	uncarboxylated matrix Gla-protein

been well established, the underlying mechanisms remain elusive.

A proteomics approach may play a critical role in the elucidation of underlying mechanisms of the well-observed diet-disease associations.[7] A previous FHS (Framingham Heart Study) investigation examined the association of 71 CVD-related proteins with CVD and all-cause mortality.[8] Diet may play a pivotal role in regulating these proteins; however, current research examining the association of habitual diet quality with protein biomarkers is limited. To address this knowledge gap, we used data derived from the FHS, a large community-based observational study, to test our hypothesis that the association between diet quality and new-onset CVD and all-cause mortality is mediated, at least partly, by CVD-related proteins. We assessed the cross-sectional association between diet quality and 71 CVD-related proteins. Gene ontology (GO) is a widely used bioinformatic tool to systematically examine protein function and test the potential protein-protein interaction.[9] We, therefore, conducted GO functional enrichment analysis to better understand the potential biological mechanisms of diet-associated proteins. We further tested the potential mediation effects of these proteins on the prospective association of diet quality with incident CVD and mortality.

METHODS

Anonymized data and materials used for this analysis have been made publicly available through the database of Genotypes and Phenotypes repository and can be accessed at the following accession number: phs000007.v29.p10.

Study Participants

The present study included FHS participants who attended the seventh examination of the Offspring cohort (1998–2001; n=3530) or the first examination of the Third Generation cohort (2002–2005; n=4095).[10,11] Among these participants (n=7350), 71 CVD-related protein biomarkers were measured by the SABRe CVD (Systems Approach to Biomarker Research in Cardiovascular Disease) Initiative that was established by the National Heart, Lung, and Blood Institute.[8] After exclusion of participants without Food frequency questionnaire (FFQ) data (n=750) and missing covariates (n=240) at baseline, we analyzed data collected from up to 6360 participants. Participant selection is shown in Figure 1. All FHS protocols and procedures were approved by the Institutional Review Board for Human Research at Boston University Medical Center and all participants provided written informed consent. The current analyses were approved by the Tufts University Institutional Review Board.

Diet Quality Scores

The FHS used a previously validated 126-item FFQ to assess habitual dietary intake for the year preceding each examination.[12] The FFQ used in the present study was administered at the seventh examination of the Offspring cohort and the first examination of the Third Generation cohort. Dietary data were excluded if the

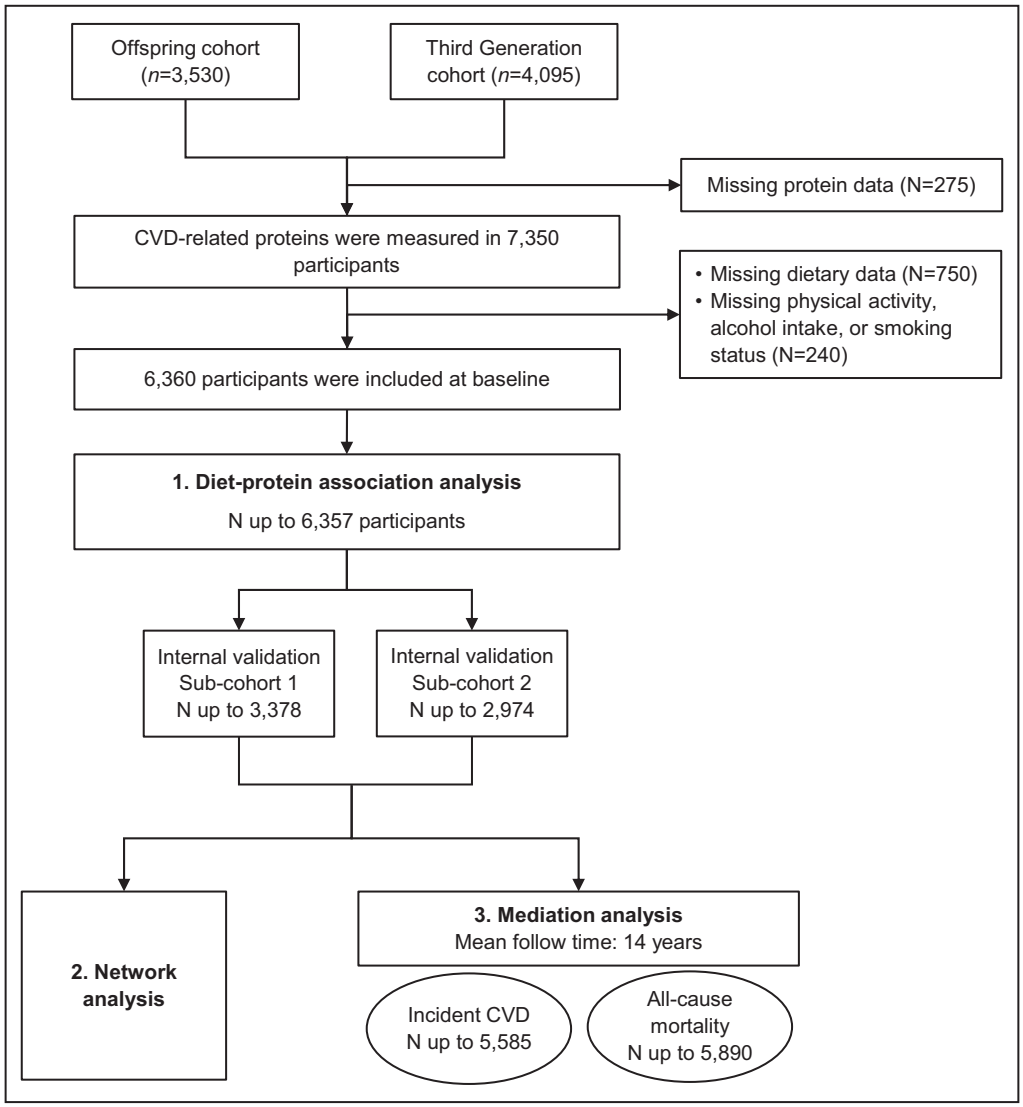

Figure 1. The flow diagram of participant selection and study overview.
The number of participants in each model was varied according to the presence of each protein data. CVD indicates cardiovascular disease.

reported energy intake was <2.5 MJ/day (600 kcal/day) for both men and women, ≥16.7 MJ/day (4000 kcal/day) for women, ≥17.5 MJ/day (4200 kcal/day) for men, or if more than ≥13 food items were left blank.[13] The FFQ was used to calculate 3 commonly used diet quality scores: the AHEI,[2] a modified DASH score,[5,14] and a modified MDS.[3,15] Although the 3 scores use different scoring strategies, they have largely similar components (Table S1), and higher scores represent a healthier diet.

The AHEI is composed of 11 components including vegetables, fruits, nuts and legumes, whole grains, red and processed meat, sugar-sweetened beverages and fruit juice, eicosapentaenoic and docosahexaenoic acids, other polyunsaturated fatty acids (without eicosapentaenoic acid and docosahexaenoic acid), trans fatty acids, sodium, and alcohol.[5] Each component score ranges from 0 (unhealthy) to 10 (healthy), with a higher score assigned to moderate alcohol drinking and higher intakes of vegetables, fruits, whole grains, nuts and legumes, eicosapentaenoic and docosahexaenoic acids, and polyunsaturated fatty acids. Lower scores are assigned to higher intakes of sugar-sweetened beverages and fruit juice, red and processed meat, trans fatty acids, and sodium.[2] The component scores are summed to generate the overall score ranging from possible value of 0 to 110, where a higher score reflects better concordance with the current Dietary Guidelines for Americans.[2,16]

The modified DASH score is calculated on the basis of energy-adjusted intakes of 8 dietary components including vegetables, fruits, nuts and legumes, whole grains, low-fat dairy, red and processed meat, sugar-sweetened beverages, and sodium.[5,14] For

each component, with the exception of for red and processed meat, sugar-sweetened beverages, and sodium, we assigned a value of 1 (lowest quintile of intake) to 5 (highest quintile of intake). The order of the scores was reversed for red and processed meat, sugar-sweetened beverages, and sodium, whereby the highest quintile was assigned a value of 1 and the lowest quintile was assigned a value of 5. The scores of all 8 components were then summed to create an overall DASH score ranging from 8 to 40, with higher scores indicating better adherence to the DASH dietary pattern.

The modified MDS was calculated as a measure of adherence to the Mediterranean diet.[15,17] The MDS is composed of 9 components including vegetables, fruits, nuts, legumes, whole grains, fish, red and processed meat, ratio of monounsaturated to saturated fatty acids, and alcohol.[3] Instead of using median intake as a threshold to dichotomize each component as described in a previous study,[15] we categorized consumption of each component into sex- and cohort-specific quartiles.[17] A score of 0 to 3 was assigned to each component based on quartile rank, with the exception of red and processed meat and alcohol. For red and processed meat, the order of the scores was reversed (ie, the highest quartile was assigned a score of 0). For alcohol, we assigned a value of 1 to intake ≥10 and ≤25 g/day for men or ≥5 and ≤15 g/day for women and a value of 0 for all other intakes. All component scores were summed to generate an overall MDS, ranging from 0 (lowest diet quality) to 25 (highest diet quality).

Target Proteins

The selection of target proteins has been previously described.[8] Briefly, the SABRe CVD Initiative measured 85 CVD-related proteins. These proteins were quantified using a modified Sandwich ELISA method, multiplexed on a Luminex xMAP platform (Sigma-Aldrich, St. Louis, MO), using frozen fasting plasma samples collected at the same time that the FFQ was administered.[18,19] Because >80% samples had values below the lower detection limits, 14 proteins were excluded from the present analysis.[8] A 7-point calibration curve (in triplicate) was used to calibrate protein quantification. Quality control (QC) used both the "High" and "Low" spike control (QC1 and QC2, respectively). The method provided acceptable reproducibility of assay performance (Table S2), with mean intra-assay coefficient of variation of 7.8 (range: 2.3–28.9; interquartile range: 6.6) for QC1 and 6.8 (range: 1.2–15; interquartile range: 5.2) for QC2 and inter-assay coefficient of variation of 8.9 (range: 2.5–21.9; interquartile range: 8.2) for QC1 and 7.9 (range: 2.3–24.5; interquartile range: 5) for QC2. Complete list and selection criteria of the 71 CVD-related proteins analyzed in the present study are presented in Table S2.

Covariates and Clinical Outcome Ascertainment

Data on smoking status (never, past, or current), alcohol intake (servings/week), body mass index (BMI; kg/m^2), and blood pressure (mm Hg) were obtained using questionnaires and physical examinations following standard protocols.[11] Physical activity score was calculated based on the intensity and time spent for each type of activity assessed by the physical activity questionnaire.[20] Participants are under continuous surveillance for CVD events and mortality. A panel of 3 physicians was formed to review all pertinent information including medical and hospital records, death certificates, communication with personal physicians, and next-of-kin interviews. Primary outcomes were all-cause mortality and incident CVD including non-fatal CVD (coronary heart disease, myocardial infarction, angina, coronary insufficiency, cerebrovascular accident, atherothrombotic infarction of the brain, transient ischemic attack, cerebrovascular disease, and intermittent claudication) and CVD death (fatal coronary heart disease and death due to stroke, peripheral arterial disease, heart failure, or other cardiovascular causes).[21]

Statistical Analysis

Our 3 main analyses are as follows (Figure 1): (1) examine the cross-sectional association between diet quality and target proteins, (2) visualize the interrelation between diet-related proteins and biological pathways (ie, the functional network), and (3) perform a mediation analysis to test if diet-associated proteins mediate the longitudinal association between diet quality and incident CVD and all-cause mortality.

Diet-Protein Association

We primarily analyzed the 3 diet quality scores (AHEI, DASH, and MDS) on a continuous scale. To facilitate comparisons of the associations between the 3 diet quality scores and proteins, we standardized each score by dividing its SD. Log-transformed CVD-related proteins (n=71) were regressed on age, sex, and cohort index to obtain residuals and the residuals were then inverse normal transformed to a mean of 0 and SD of 1 for subsequent statistical analysis. Linear mixed effect models (implemented using the R *nlme* package)[22] were used to account for family structure in our study sample with adjustment for sex, age, energy intake, smoking status, physical activity score, alcohol intake, and BMI.

We used a 2-step strategy to identify diet-associated proteins. In the first-step analysis, we examined

diet-protein associations in all study participants. We applied Bonferroni correction with adjustment for the number of proteins to account for multiple testing, that is, to determine if a protein was statistically significant, we required that this protein had 2-tailed $P<0.0007$ (0.05/71). In the second-step analysis, we conducted the internal validation tests to examine robustness of the significant proteins identified in the first-step analysis. Based on pedigrees, we randomly divided our study participants into 2 independent subcohorts with the allocation ratio of 1:1. We considered a protein significant in the first-step analysis to be a diet-associated protein if that protein was associated with diet quality score at $P<0.05$ and had regression coefficients in the same direction in both subcohorts. In addition, we tested heterogeneity between the 2 subcohorts by calculating the Cochran's Q statistic using the R *meta* package[23] and required that diet-associated proteins should not have the heterogeneity $P<0.05$/the number of significant proteins in the first-step analysis.

Construction of Functional Network

Diet-related proteins were analyzed to identify closely related biological processes based on GO terms and interrelations of functional groups in biological network by using the Cytoscape plug-in ClueGO,[24] which enables the visualization of clustered networks and pathways. We used default selection criteria for relevant pathways, that is, a minimum of 3 proteins from the selected diet-related proteins, which accounted for at least 4% from the total number of proteins in the GO terms. The statistical test was based on the 2-sided hypergeometric option with a Bonferroni step-down correction.[25] The ClueGO network is created with Cohen's Kappa coefficient ≥0.5 and reflects the relationships between the terms based on the similarity of their associated proteins.[24]

Mediation Analysis

In a previous FHS analysis, 46 of the 71 proteins were associated with CVD or all-cause mortality.[8] We therefore conducted a mediation analysis using a modified approach proposed by Huang and Yang[26] to investigate whether a significant association between diet quality and clinical outcomes is mediated by these proteins. A linear mixed effect model was used to estimate the association between diet quality and CVD-related proteins, and a mixed effect Cox proportional hazard model (implemented using R *coxme* package)[27] was adopted to estimate the natural direct and indirect effects of diet quality on clinical outcomes. Family structure was accounted for by using a random intercept. We used the R code provided by the Huang and Yang[26] to calculate the 95% CIs and *P* values for the nature direct effect, indirect effect, and total effect based on a resampling method taking random draws (repeated for 1E+6 times) from multivariate normal distribution of estimates for model parameters. We considered natural indirect effect (ie, mediation) statistically significant if $P<0.05$/the number of the proteins significantly associated with diet quality. The proportion of mediation by a target protein was calculated as the ratio of indirect effect to the sum of both direct and indirect effect.

In mediation analysis for incident CVD, participants with history of CVD at baseline were excluded. We adjusted 2 sets of covariates. Covariates in model 1 included sex, age, energy intake, smoking status, physical activity score, alcohol intake, BMI, systolic blood pressure, use of antihypertension medications, high-density lipoprotein cholesterol, total cholesterol, and type 2 diabetes. In model 2, we additionally adjusted for education (with and without college education), smoking pack-years, estimated glomerular filtration rate (mL/min per 1.73^2), family history of CVD, aspirin use, and, for women only, menopausal status, oral contraceptive use, and hormone replacement therapy. Similarly, 2 sets of covariates were adjusted for in the mediation analysis for all-cause mortality. In model 1, we adjusted for the covariates included in the model 1 mediation analysis for incident CVD and history of CVD and cancer. In model 2, we included the same additional covariates adjusted for in the model 2 mediation analysis for incident CVD. We conducted sensitivity analysis with exclusion of the incident cases or death events occurring in the first 2 or 5 years after baseline. To test robustness of the proteins significant in the mediation analysis, we further adjusted for other diet-associated proteins that were correlated with the significant proteins. In this multi-marker analysis, we selected proteins with absolute pairwise Spearman correlation coefficients ≥0.3 and added these proteins as covariates.

We identified 71 independent *cis*-pQTL variants (protein quantitative trait loci; linkage disequilibrium R^2 <0.1 and minor allele frequency >0.01) for 18 of the diet-associated proteins based on our previous study.[28] These *cis*-pQTLs reside within 500kb from the transcription start site of the protein coding genes, suggesting that they may directly affect expression of the protein coding genes.[29,30] Therefore, analysis using *cis*-pQTLs provides evidence to support the causal roles of diet-associated proteins to clinical outcomes. Information on the 71 *cis*-pQTL variants and the 18 diet-associated proteins are presented in Table S3. We used mixed effect Cox models to examine the association of the 71 *cis*-pQTL variants with incident CVD and all-cause mortality in our study participants (n=7060). Covariates included in models were sex, age, and the first 2 genetic principal components. All statistical analyses were conducted using R statistical analysis

software (version 3.5.0; R Foundation for Statistical Computing, available: http://www.R-project.org).[31]

RESULTS

Participants' characteristics (n=6360; mean age 50 years; 54% women) at baseline are presented in Table 1. Women tended to have higher AHEI and DASH scores. Because MDS was constructed using sex-specific quartiles, MDS scores were similar in both men and women. Higher diet quality scores tended to be associated with a lower proportion of current smokers, more college education, less alcohol intake, and lower BMI. The 3 diet quality scores were correlated, with Pearson r ranging from 0.67 to 0.75 ($P<0.0001$ for all; Figure S1). We found no significant difference in participants' characteristics and distribution of CVD-related proteins in the 2 internal validation subcohorts after Bonferroni correction (Table S4).

Cross-Sectional Association of Diet Quality and CVD-Related Proteins

After adjustment for sex, age, energy intake, smoking status, physical activity score, alcohol intake, and BMI, we found that 34 of the 71 proteins were significantly associated with at least 1 diet quality score at $P<0.0007$ (Bonferroni corrected $P<0.05/71$; Table S5). Of these proteins, 31 were associated with AHEI, 25 with DASH, and 14 with MDS. As expected, the observed diet-protein association patterns were similar across the 3 diet quality scores, with pairwise Pearson correlations for t-statistics of 0.92, 0.96, and 0.94, respectively ($P<0.0001$ for all; Figure S2). Correlation coefficients between the 34 proteins ranged from −0.34 to 0.68 with a mean correlation coefficient of 0.12 (Figure S3). Similar associations between diet quality scores and proteins were obtained after excluding the patients with prevalent cases of CVD and type 2 diabetes (n=673; Figure S4).

The internal validation analysis confirmed that 30 of the 34 proteins met our criteria (Figure 2 and Table S6), that is, associated with at least 1 diet quality score with same direction and $P<0.05$ in both subcohorts. Twenty-eight proteins were associated with AHEI, 21 proteins were associated with DASH, and 12 proteins were associated with MDS. No significant heterogeneity was detected for the 30 proteins based on the Cochran's Q statistic $P<0.001$ threshold (0.05/34; Table S6).

Functional Network for Diet-Related Proteins

GO functional enrichment analysis for the 30 diet-associated proteins showed significant enrichment for 5 biological processes (Table S7). This analysis highlighted 3 functional groups (Figure 3). The most significant GO biological processes in these 3 functional groups were regulation of neuroinflammatory response ($P=5.6\times10^{-9}$ for GO:0150076), endothelial cell apoptotic process ($P=3.5\times10^{-6}$ for GO:2000351), and interleukin-8 production ($P=8.7\times10^{-6}$ for GO:0032677). Seven proteins (out of 30) were highly enriched in these networks including GRN (granulin; *GRN*), sICAM1 (soluble intercellular adhesion molecule-1; *sICAM1*), LDLR (low-density lipoprotein receptor; *LDLR*), MMP8 (matrix metallopeptidase 8; *MMP8*), MMP9 (*MMP9*), IGF1 (insulin-like growth factor 1; *IGF1*), and PAI1 (plasminogen activator inhibitor 1; *SERPINE1*).

Longitudinal Association of Diet Quality and Incident CVD and Mortality

After exclusion of participants with prevalent CVD at baseline, 5585 participants were included in the longitudinal analysis for incident CVD. We observed 413 nonfatal and 75 fatal CVD events, during a median follow-up time of 12 years. The analysis for all-cause mortality included 5890 participants and 512 death events that occurred during a median follow-up time of 14 years.

Higher diet quality scores were significantly associated with lower risk of incident CVD (fatal and nonfatal) and all-cause mortality (Table S8). Hazard ratios (HRs; 95% CI; *P*-trend) for incident CVD were 0.84 (0.76–0.93; $P<0.0001$), 0.82 (0.73–0.91; $P=0.0002$), and 0.89 (0.80–0.98; $P=0.025$) per SD increase of AHEI (12 points), DASH (5 points), and MDS (4 points), respectively. The HRs (95% CI; *P*-trend) for all-cause mortality were 0.86 (0.78–0.95; $P=0.004$), 0.85 (0.77–0.94; $P\leq0.002$), and 0.86 (0.77–0.95; $P=0.002$) per SD increase of AHEI, DASH, and MDS, respectively. In sensitivity analyses, exclusion of events occurring within 2 or 5 years after baseline resulted in similar associations of diet quality scores with incident CVD and all-cause mortality (Table S8).

We examined the 3-way associations between diet quality scores, proteins, and CVD and all-cause mortality. Because of the inverse association between diet quality and incidence CVD and all-cause mortality, we expected that a protein inversely associated with diet quality scores was likely to be positively associated with the clinical outcomes or vice versa. Overall, the observed cross-sectional association between diet quality scores and proteins are consistent with the findings in longitudinal association[8] or Mendelian randomization analysis[28] conducted in previous FHS analyses (Table S9). For example, we observed that better diet quality was associated with lower levels of cystatin C. In the prior FHS studies, Mendelian randomization analysis showed that higher levels of cystatin C were associated with increased risk of coronary heart

Table 1. Baseline Characteristics of Participants According to Tertiles of Diet Quality Score (n=6360)

	AHEI				DASH				MDS			
	T1	T2	T3	*P*-trend*	T1	T2	T3	*P*-trend	T1	T2	T3	*P*-trend
Age, y	50±14	51±14	48±13	<0.0001	49±14	50±14	50±14	0.002	49±13	50±14	50±14	<0.0001
Women, n (%)	862 (41)	1156 (54)	1421 (67)	<0.0001	1503 (59)	736 (43)	682 (32)	<0.0001	1222 (54)	1133 (55)	1084 (53)	0.99
College educated, n (%)	751 (36)	918 (44)	1152 (55)	<0.0001	939 (37)	768 (45)	1114 (53)	<0.0001	780 (35)	923 (45)	1118 (56)	<0.0001
Smoking status, n (%)				0.195				0.003				<0.0001
Never	1104 (53)	1225 (58)	1271 (61)		1284 (51)	1003 (59)	1313 (63)		1124 (50)	1172 (58)	1304 (65)	
Past	636 (30)	630 (30)	664 (32)		768 (31)	525 (31)	637 (31)		742 (33)	626 (31)	562 (28)	
Current	358 (17)	242 (12)	163 (8)		451 (18)	176 (10)	136 (7)		384 (17)	235 (12)	144 (7)	
Smoking pack-years	66±156	31±102	17±78	<0.0001	65±154	28±98	14±69	<0.0001	60±147	34±113	17±78	<0.0001
Alcohol, servings/wk	7±11	5±7	5±5	<0.0001	7±10	5±7	4±6	<0.0001	6±10	5±7	5±6	0.0002
Physical activity score	38±8	37±7	37±7	0.02	38±8	37±7	38±7	0.64	37±8	38±7	38±7	0.03
Body mass index, kg/m^2	28±5	28±6	26±5	<0.0001	28±6	28±5	26±5	<0.0001	28±6	28±5	27±5	<0.0001
Systolic blood pressure, mm Hg	123±17	122±17	119±17	<0.0001	122±16	122±18	119±17	<0.0001	121±17	122±18	120±17	0.04
Estimated glomerular filtration rate, mL/min per 1.73^2	93±19	92±19	93±18	0.902	94±19	92±19	92±18	<0.0001	95±19	92±19	92±18	<0.0001
Hypertension meds, n (%)	447 (21)	423 (20)	354 (17)	0.0002	503 (20)	350 (21)	371 (18)	0.06	406 (18)	444 (22)	374 (19)	0.57
High-density lipoprotein, mmol/L	0.7±0.7	0.8±0.7	1.0±0.8	<0.0001	0.8±0.7	0.8±0.7	0.9±0.8	<0.0001	0.8±0.7	0.8±0.7	0.9±0.8	0.0001
Aspirin use, n (%)	450 (21)	446 (21)	399 (19)	0.053	511 (20)	359 (21)	425 (20)	0.981	412 (18)	399 (20)	484 (24)	<0.0001
Hormone replacement therapy, n (%)	161 (8)	176 (8)	230 (11)	0.0002	167 (7)	151 (9)	249 (12)	<0.0001	188 (8)	185 (9)	194 (10)	0.139
Postmenopausal status, n (%)	464 (22)	531 (25)	591 (28)	<0.0001	491 (20)	455 (27)	641 (31)	<0.0001	535 (24)	523 (26)	529 (26)	0.054
Oral contraceptive use, n (%)	154 (7)	271 (13)	369 (18)	<0.0001	219 (9)	241 (14)	334 (16)	<0.0001	287 (13)	258 (13)	249 (12)	0.721
Type 2 diabetes, n (%)	129 (6)	159 (8)	111 (5)	0.24	155 (6)	129 (8)	115 (6)	0.41	127 (16)	153 (8)	119 (6)	0.65
Cancer, n (%)	77 (4)	81 (4)	63 (3)	0.23	86 (3)	62 (4)	73 (3)	0.89	65 (3)	75 (4)	81 (4)	0.04
CVD, n (%)	137 (7)	131 (6)	102 (5)	0.02	148 (6)	112 (7)	110 (5)	0.40	118 (5)	132 (6)	120 (6)	0.29
Family history of CVD, n (%)	1564 (75)	1574 (75)	1506 (72)	0.039	1875 (75)	1247 (73)	1522 (73)	0.140	1696 (75)	1480 (73)	1468 (73)	0.080

Data were expressed as means±SDs or absolute numbers (percentage). AHEI, Alternate Healthy Eating Index; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; MDS, Mediterranean-style Diet Score; and T, tertile.

*Test of linear trend across tertile categories of diet quality scores was performed by entering the median value of each tertile category into the model as a continuous variable Unadjusted *P*-trends were analyzed by Cochran–Armitage trend tests for categorical variables and linear mixed effects models for continuous variables.

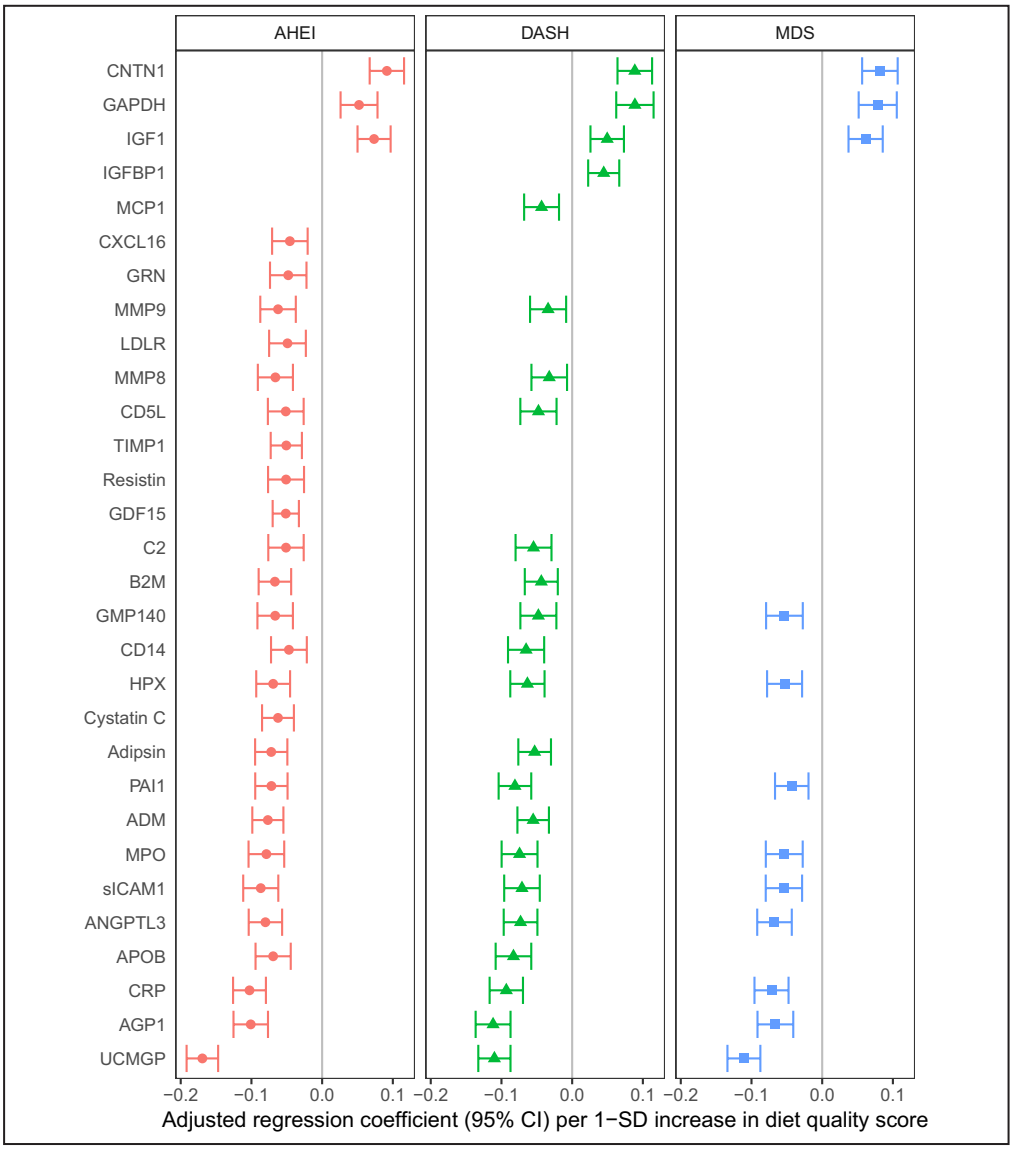

Figure 2. Adjusted regression coefficients and corresponding 95% CI for the associations between standardized diet quality scores and CVD-related proteins in all study participants. Linear mixed effects model was adjusted for sex, age, energy intake, smoking status, physical activity score, alcohol intake, and body mass index. Regression coefficients are depicted with ● for AHEI, ▲ for DASH, and ■ for MDS. The horizontal lines represent 95% CIs. The complete name of the abbreviated proteins can be found in Table S2. ADM indicates adrenomedullin; AGP1, arabinogalactan protein 1; AHEI, Alternate Healthy Eating Index; ANGPTL3, angiopoietin-like 3; APOB, apolipoprotein B; B2M, beta-2-microglobulin; CD14, cluster of differentiation 14; CNTN1, contactin 1; CRP, C-reactive protein; CVD, cardiovascular disease; CXCL16, chemokine ligand 16; DASH, Dietary Approaches to Stop Hypertension; GDF15, growth differentiation factor 15; GMP140, granule membrane protein 140; GRN, granulin; HPX, hemopexin; IGF1, insulin-like growth factor 1; IGFBP1, insulin-like growth factor binding protein 1; LDLR, low-density lipoprotein receptor; MCP1, monocyte chemoattractant protein 1; MDS, Mediterranean-style Diet Score; MMP, matrix metallopeptidase; MPO, myeloperoxidase; PAI1, plasminogen activator inhibitor 1; sICAM1, soluble intercellular adhesion molecule 1; TIMP1, tissue inhibitor of metalloproteinases 1; and UCMGP, uncarboxylated matrix Gla-protein.

disease,[28] and longitudinal analysis showed higher levels of cystatin C were associated with increased risk of incident CVD and all-cause mortality.[8]

In addition, we analyzed the association of 71 independent *cis*-pQTL variants for 18 diet-associated proteins with incident CVD and all-cause mortality. We found that participants who carried A allele of rs10908589, a *cis*-pQTL variant of CD5L, had increased CD5L levels (P=7.9×10^{-26})[28] and increased all-cause mortality (HR, 1.28; 95% CI, 1.13–1.44;

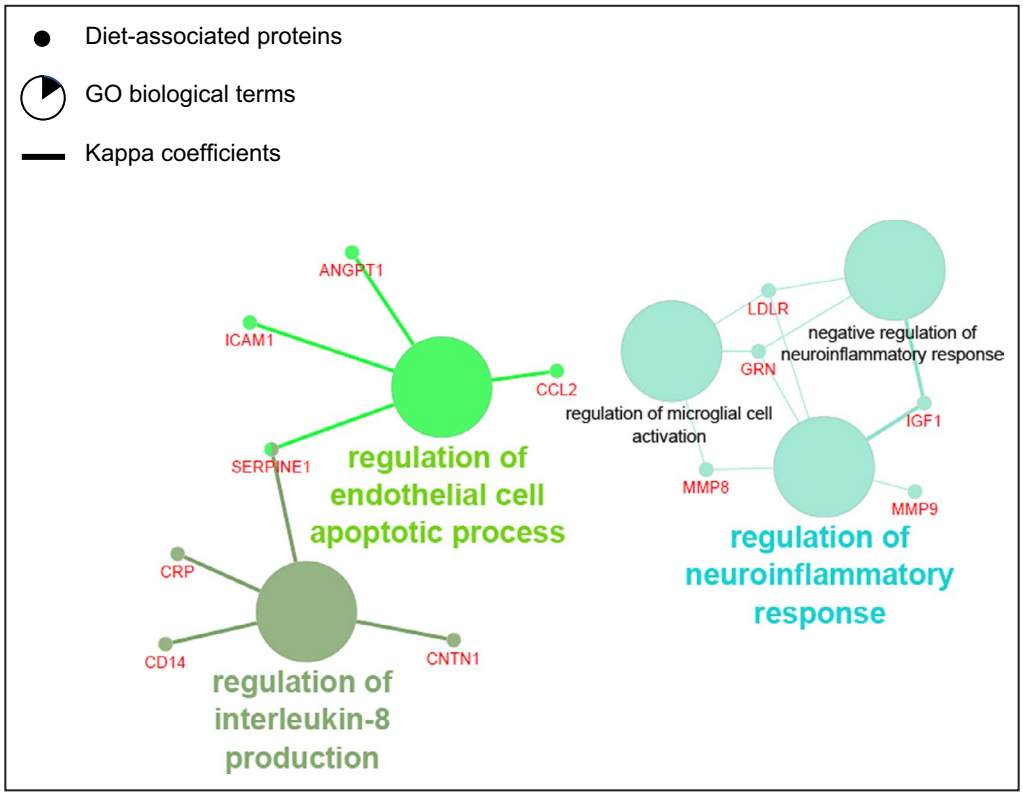

Figure 3. Functional network of diet-related proteins (*n*=30).
Three significant function groups including 5 enriched biological processes were identified, followed by a Bonferroni step-down correction for multiple testing. Nodes indicate enriched GO terms and the same color of nodes means that they are in the same pathway function group. The most significant term is highlighted by a large name label for each group. All terms are compared with each other, and pathway function groups are defined using the Cohen's Kappa coefficients, a measure taking into account how many genes are shared between 2 terms. Each dot represents diet-related target protein. Edges between nodes and dots represent interactions between protein and terms. The width of edges indicated the value of Cohen's Kappa coefficients. The network was generated by using ClueGO, a plug-in of Cytoscape. The complete name of the abbreviated proteins can be found in Table S2. ANGPT1 indicates angiopoietin 1; CCL2, C-C motif chemokine ligand 2; CD14, cluster of differentiation 14; CNTN1, contactin 1; CRP, C-reactive protein; GRN, granulin; ICAM1, intercellular adhesion molecule 1; IGF1, insulin-like growth factor 1; GO, gene ontology; LDLR, low-density lipoprotein receptor; MMP, matrix metallopeptidase; and SERPINE1, serpin family E member 1.

P=6.4×10^{-5}; Table S10). This observation was consistent with the expected 3-way associations, that is, higher diet quality scores were associated with lower CD5L levels and lower all-cause mortality. Two other independent *cis*-pQTLs associated with CD5L and 2 cis-pQTLs associated with GMP140 (granule membrane protein 140) and CD14 (cluster of differentiation 14) with nominal significance in the association analyses are also presented in Table S10.

Mediation Analysis of Target Proteins in Relation to Diet Quality and Clinical Outcomes

Among the 30 diet-associated proteins, 17 proteins were selected on the basis of significant associations with diet quality scores and with CVD outcomes and mortality. With adjustment for the mediation analysis model 1 covariates, 6 proteins (GDF15 [growth differentiation factor 15], UCMGP [uncarboxylated matrix Gla protein], sICAM1, ADM [adrenomedullin], CRP [C-reactive protein], and B2M [beta-2-microglobulin]) significantly mediated the association between at least 1 diet quality score and all-cause mortality at P for indirect effect <0.003 (0.05/17 proteins; Tables S11 through S13). Among them, UCMGP significantly mediated the association of all-cause mortality with AHEI (P=1.6×10^{-5}), DASH (P=1.2×10^{-4}), and MDS (P=9.0×10^{-5}). The median proportion of mediation (Table 2 and Table S14) by UCMGP was 20.7% (95% CI, 11.2–43.3%), 21.0% (95% CI, 9.4–82.9%), and 17.4% (95% CI, 8.8–47.4%) for AHEI, DASH, and MDS, respectively. Additional adjustment for education, smoking pack-years, estimated glomerular filtration rate, family history of CVD, aspirin

Table 2. Significant Mediation Effect of Diet-Associated Proteins on Longitudinal Associations of Diet With All-Cause Mortality and Incident CVD

Diet quality	Mediator	Hazard ratio (95% CI)		Model 1		Model 2	
				P value	Mediated proportion, %	*P* value	Mediated proportion, %
All-cause mortality							
AHEI	GDF15	0.957	(0.941–0.972)	1.2E-16	30.1	0.003	21.8
	UCMGP	0.966	(0.953–0.979)	1.6E-05	20.7	0.002	24.0
	Adrenomedullin	0.985	(0.977–0.992)	0.002	10.4	0.11	
	CRP	0.985	(0.976–0.993)	0.002	9.9	0.06	
	Beta-2-microglobulin	0.984	(0.975–0.992)	0.001	9.9	0.02	10.3
	Soluble intercellular adhesion molecule	0.987	(0.979–0.993)	0.002	9.3	0.03	8.6
Dietary Approaches to Stop Hypertension	UCMGP	0.979	(0.969–0.987)	1.2E-04	21.0	0.003	25.9
	CRP	0.986	(0.977–0.993)	0.002	15.2	0.06	
Mediterranean-style Diet Score	UCMGP	0.978	(0.968–0.987)	9.0E-05	17.4	0.003	19.1
Incident CVD							
AHEI	GDF15	0.982	(0.971–0.991)	0.002	11.3	0.02	8.6

Linear mixed effect and mixed effect Cox proportional hazard models were adopted to estimate the indirect (mediation) effect. Hazard ratios per 1 increase of SD of standardized diet quality score and *P* values were derived from mixed effect Cox proportional hazard models. Model 1 was adjusted for sex, age, energy intake, smoking status, physical activity score, alcohol intake, body mass index, systolic blood pressure, use of antihypertension medications, high-density lipoprotein and total cholesterol, type 2 diabetes, and history of CVD and cancer. Model 2 was additionally adjusted for estimated glomerular filtration rate, smoking pack-years, aspirin use, education, family history of CVD, use of hormone replacement therapy, postmenopausal status, and oral contraceptive use. The median proportion of mediation was calculated as the ratio of indirect effect to the sum of both direct and indirect effect. Complete mediation analysis results are in the Tables S11 through S17. AHEI indicates Alternate Healthy Eating Index; CRP, C-reactive protein; CVD, cardiovascular disease; growth differentiation factor 15; and UCMGP, uncarboxylated matrix gamma-carboxyglutamic acid protein.

use, and, for women only, menopausal status, oral contraceptive use, and hormone replacement therapy reduced the strength of the mediation effect (Table 2). Nonetheless, mediation *P* values for all-cause mortality remained significant or nominally significant for GDF15, UCMGP, B2M, and sICAM1.

Mediation analysis results for incident CVD are presented in Tables S15 through S17. GDF15 was significant in the mediation analysis for AHEI and incident CVD with *P* of 0.002 after adjusting for model 1 covariates (Table S15). The median proportion of mediation by GDF15 was 11.3% (95% CI, 4.9–31.6%). Similarly, additional adjustment for model 2 covariates reduced the strength of the mediation effect (Table 2), mediation *P*=0.02, and proportion of mediation by GDF15 was 8.6% (95% CI, 2.8–31.2%).

In sensitivity analyses, exclusion of events occurring within 2 or 5 years after baseline did not substantially change the strength of the mediation analysis results (Tables S18 and S19). After adjusting for correlated diet-associated proteins, mediation *P* values for the 4 proteins (B2M, sICAM1, GDF15, and UCMGP) remained similar in the mediation analysis for all-cause mortality with adjustment for model 2 covariates (Table S20). Also, mediation *P* value for GDF15 was similar tos that in the model 2 mediation analysis for incident CVD (Table S21).

DISCUSSION

We showed that diet quality, represented by 3 diet quality scores, was associated with 30 CVD-related protein biomarkers in a large group of middle-aged and older participants in the FHS. The majority of diet-associated proteins were involved in biological pathways related to inflammatory response. Our mediation analysis demonstrated that 6 proteins (ADM, B2M, GDF15, UCMGP, sICAM1, and CRP) may mediate the association between diet quality scores and all-cause mortality. In addition, GDF15 significantly mediated the longitudinal association between diet quality scores and new-onset CVD. Our study provides novel evidence that targeted proteomic analysis may be useful to highlight molecular pathways underlying the beneficial effects of healthy diet for disease prevention.

Our findings are consistent with the literature examining the beneficial effects of healthy diet on CVD and mortality.[1] Although the association between diet and protein biomarkers has not been well studied, we observed associations similar to those reported in previous studies.[32,33] The cross-sectional Toronto Nutrigenomics and Health study demonstrated that a Western-style dietary pattern was associated with 25 proteins involved in coagulation and lipid metabolism among 54 putative CVD biomarker proteins.[32] A Swedish study of 2

population-based cohorts analyzed 184 CVD-related circulating proteins and demonstrated that dietary patterns were associated with 21 proteins.[33] These dietary pattern-associated proteins are involved in multiple pathways such as inflammation and lipid metabolism. In the present study, we observed diet-protein associations consistent with those reported in 3 prior studies.[32–34] For example, the unhealthy dietary patterns characterized in these studies were associated with higher concentrations of PAI1 (plasminogen activator inhibitor 1), MPO (myeloperoxidase), APOB (apolipoprotein B), GDF15, and HPX (hemopexin). We found that higher diet quality scores, reflecting healthier diet, were associated with lower levels of these proteins.

The biological mechanisms underlying the relationship between healthy diet and CVD and other chronic diseases are not fully understood. It is postulated that adherence to healthy diet has the potential to reduce vascular damage by alleviating inflammatory responses.[35] A strong inverse association between overall diet quality and inflammation biomarkers has been demonstrated by few studies.[36,37] A study of the Women's Health Initiative cohort showed that higher MDS was favorably associated with a series of inflammation markers, including CRP and sICAM-1, which explained ≈30% of the observed MDS-CVD association.[37] Another study, also conducted in the Women's Health Initiative[34] showed that GDF15 may partly explain dietary effects on all-cause mortality. CRP and sICAM1 are well-known inflammation markers,[38] whereas ADM, B2M, and GDF15 have been linked with inflammatory response.[39–41] These data are consistent with our observations in the mediation analysis and, therefore, provide evidence to support inflammation as an important mechanism underlying the nexus between diet quality and human health.

The present study showed that UCMGP may be a strong mediator with respect to the association of diet quality with all-cause mortality. MGP is primarily secreted by vascular smooth muscle cells in the arterial wall.[42] The inactive form of MGP, UCMGP, undergoes posttranslational modifications depending upon availability of vitamin K.[43] Dark green leafy vegetables,[44] which are important constituents of healthy diet, are rich in vitamin K. Consistent with our observations, UCMGP is a risk factor for arterial calcification[45] and has been associated with an increased risk of mortality.[8] An observational study showed findings consistent with our results,[43] highlighting that proteins such as UCMGP may partly explain dietary effects on health outcomes.

The strengths of the present study include the use of comprehensive dietary, lifestyle, and clinical data; long-term follow-up of clinical outcomes; and well-quantified circulating targeted protein biomarkers in a large group of FHS participants. There are several limitations that warrant discussion. We examined the cross-sectional association between diet quality scores and target proteins, which limits our ability to infer causality between diet and protein biomarkers. Nevertheless, by examining the 3-way associations between diet quality score, proteins, and clinical outcomes, the cross-sectional analysis showed consistency with longitudinal analysis of diet-disease and protein-disease associations. In addition, the cross-sectional analysis was in line with associations generated from analyses using genetic variants. Our study population was predominately middle-aged and older White adults, which may limit the generalizability of the present findings to other populations. Dietary intake was assessed using semiquantitative FFQ, which may lead to misclassification. In addition, dietary constituents that were not selected for constructing the diet quality scores may play important roles in the regulation of protein biomarkers and affect the risk of CVD and mortality. Dietary quality may change over time; therefore, further analysis considering the change in diet quality is warranted. Proteins may mediate the relationship between diet quality and non-CVD mortality, which also need to be examined in future studies. Despite the fact that multiple potential confounders were adjusted for in the present analysis, the possibility of residual confounding could not be ruled out.

CONCLUSIONS

In conclusion, we demonstrated that diet quality, represented by 3 diet quality scores, was associated with 30 CVD-related, circulating protein biomarkers. We further showed that several proteins significantly mediated the long-term association of diet quality with incident CVD and all-cause mortality. Our findings provide novel evidence to better understand the mechanisms underlying the observed association of diet with CVD and all-cause mortality.

ARTICLE INFORMATION

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Disclosures

None.

Supplementary Material

Tables S1–S21
Figures S1–S4

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Supplemental Material

Table S1. Components of diet quality scores

	AHEI	DASH	MDS
Vegetable	√	√	√
Fruit	√	√	√
Sweetened beverage	√	√	
Nut	√	√	√
Legume			√
Whole grain	√	√	√
Fish			√
Red and processed meat	√	√	√
Low-fat dairy		√	
Monounsaturated/Saturated fatty acid ratio			√
Eicosapentaenoic & Docosahexaenoic acids	√		
Polyunsaturated fatty acids	√		
Trans-fat	√		
Alcohol	√		√
Sodium	√	√	

AHEI, Alternative Healthy Eating Index; DASH, Dietary Approaches to Stop Hypertension; MDS, Mediterranean-style diet score.

Table S2. Protein biomarker assay characteristics

Protein symbol	Full Protein Name	Protein-coding Gene Abbreviation	Selection criteria*	Intra-assay CV, %		Inter-assay CV, %	
				QC1	QC2	QC1	QC2
ADAM15	Disintegrin and metalloproteinase domain-containing protein 15	*ADAM15*	2	8.3	3.9	3.3	3
ADM	Adrenomedullin	*ADM*	1	5	6.3	10.9	6.3
sRAGE	Receptor for advanced glycation endproducts	*AGER*	1	14.5	15	5.6	4
A1M	Alpha-1-microglobulin	*AMBP*	2,3	3.9	3.9	7.9	7.3
Bikunin	AMBP-bikunin (BIKUNIN)	*AMBP*	2,3	2.9	7.1	6.3	9.8
ANGPTL3	Angiopoietin-like 3	*ANGPTL3*	1	17.6	12	4	4.8
APOA1	Apolipoprotein A-1	*APOA1*	1	28.9	11.8	7.1	7.3
APOB	Apolipoprotein B	*APOB*	1	3	6.6	13.4	13.4
B2M	Beta-2-microglobulin	*B2M*	2	3.7	4.2	17.7	14
BCHE	Butyrylcholine esterase	*BCHE*	2,3	8.6	8.4	14.9	8.9
Osteocalcin	Osteocalcin	*BGLAP*	1	8.8	10.7	5.1	6.3
C2	Complement C2	*C2*	4	3.2	3.9	16.1	11.9
MCP1	Monocyte chemotactic molecule 1	*CCL2*	1	11.1	13.7	3.3	6.1
CD14	Monocyte differentiation antigen CD14	*CD14*	1,2	3.5	3.6	15.4	14.5
CD163	Cluster of differentiation 163	*CD163*	2,3	8.5	4.8	3.4	4.3
CD40L	Soluble CD40 ligand	*CD40LG*	1	14.8	14.1	3.1	4.9
CD5L	CD5 antigen-like	*CD5L*	1	3	2.4	12.3	5.7
CDH13	Cadherin 13	*CDH13*	1	9.3	5.2	2.7	4.7
Adipsin	Adipsin	*CFD*	2	5.1	4.3	16.3	19
CLEC3B	Tetranectin	*CLEC3B*	2	8.5	5.8	5.4	2.8
CLU	Clusterin	*CLU*	1	11.2	9.1	16.5	12.6
CNTN1	Contactin 1	*CNTN1*	1	7.8	7.6	6.1	5.3
COL18A1	Collagen, type XVIII, alpha 1	*COL18A1*	1	17.1	12.9	3	5.9
Ceruloplasmin	Ceruloplasmin	*CP*	2	9.4	5.4	5	3.5
CRP	C-Reactive Protein	*CRP*	1	5.1	8.2	14.5	9.9
Cystatin C	Cystatin-C	*CST3*	1	3.2	3.1	9.8	5.1
SDF1	Stromal cell-derived factor 1	*CXCL12*	2	4.8	7	15.6	8.4
CXCL16	Chemokine (C-X-C motif) ligand 16	*CXCL16*	1,2	13.5	11.1	2.8	3.5
DPP4	Dipeptidyl dipeptidase	*DPP4*	3	7.8	3.4	2.5	2.3
EFEMP1	EGF containing fibulin-like extracellular matrix protein 1	*EFEMP1*	2	4.8	3.8	9.7	9.1
FGF23	Fibroblast growth factor 23	*FGF23*	1	13.4	14	5.5	5.7
FGG	Fibrinogen	*FGG*	2	3.8	2.9	9.7	7
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase	*GAPDH*	1	3.7	3.8	19	14.2
GDF15	Growth differentiation factor 15	*GDF15*	1,2	5.4	5	11.9	6.8
GP5	Glycoprotein V (platelet)	*GP5*	1	3.9	3.5	15.1	7.6
GRN	Granulin	*GRN*	1,2	4.2	1.2	6.4	4.4
HPX	Hemopexin	*HPX*	2	7.7	5.6	4.7	3.4
sICAM1	Intercellular adhesion molecule 1	*ICAM1*	1	4.5	4.5	11.2	9.3
IGF1	Insulin-like growth factor 1	*IGF1*	1	9.1	5.5	6.8	3.7
IGFBP1	Insulin-like growth factor-binding protein 1	*IGFBP1*	1,2	2.5	2.5	6.9	5.4
IGFBP2	Insulin-like growth factor binding protein 2	*IGFBP2*	2	2.8	6	8.7	10.2
IGFBP3	Insulin-like growth factor-binding protein 3	*IGFBP3*	1	3.9	4.4	15.2	18
sGP130	Interleukin-6 receptor beta	*IL6ST*	1	3	3	9.1	3.9
KLKB1	Plasma kallikrein	*KLKB1*	2	10.2	3.2	16.2	24.5
LDLR	LDL receptor	*LDLR*	2,3,4	8.9	4.7	3.4	3.9
Leptin	Leptin	*LEP*	1,2	15.8	7	9.6	3.2
Leptin-R	Leptin receptor	*LEPR*	1	10.7	9.1	5.5	6
LPA	Lipoprotein(a)	*LPA*	1,2	9.8	8.2	15.5	14
Myoglobin	Myoglobin	*MB*	1	6.6	8	6.6	6.2
MCAM	Melanoma cell adhesion molecule	*MCAM*	2	4.5	3.8	11.1	5.1
UCMGP	Uncarboxylated MGP	*MGP*	1,2	11.5	10.1	17.8	17.8
MMP8	Matrix metallopeptidase 8	*MMP8*	3,4	16.9	13.9	8.3	5.6
MMP9	Matrix metallopeptidase 9	*MMP9*	1,2	4.7	3.9	8.1	10
MPO	Myeloperoxidase	*MPO*	1,2	3.8	4.4	13.2	9.6
NCAM	Neural cell adhesion molecule	*NCAM1*	2	2.4	1.6	7.2	4.3
Notch1	Notch 1	*NOTCH1*	3	2.3	2.3	6.2	5.5
NTproBNP	N-terminal prohormone of brain natriuretic peptide	*NPPB*	1,2	13.3	10.3	4.4	7.2
NRCAM	Neuronal cell adhesion molecule	*NRCAM*	2	9	4.4	3.4	2.5
AGP1	Alpha-1 acid glycoprotein	*ORM1*	1,2	8.1	8.3	4.6	5.7
PMP2	Peripheral myelin protein 2	*PMP2*	2	5.8	6.6	3.1	4.7
PON1	Serum paraoxonase/arylesterase 1	*PON1*	2	4.2	3.9	7.4	7.6
PPBP	Pro-platelet basic protein	*PPBP*	3	3	3.8	6	6.4
REG1A	Lithostathine-1-alpha	*REG1A*	1	6.9	11.3	7.7	8.8
Resistin	Resistin	*RETN*	1	11.2	11	5	4.6
SAA1	Serum amyloid A1	*SAA1*	2	13.6	8.5	21.9	23.4
GMP140	Granule membrane protein 140	*SELP*	1,2	4.9	4.4	14.8	12.1
SERPINA10	Protein Z-dependent protease inhibitor	*SERPINA10*	2	3.4	4.7	7	6
PAI1	Plasminogen activator inhibitor 1	*SERPINE1*	1	10.8	12.3	3.6	4.2
TIMP1	Tissue inhibitor of metalloproteinases 1	*TIMP1*	1	4.3	5.4	13.2	8.5
TSC22D3	TSC22D3 domain family member 3	*TSC22D3*	3	9	12.2	9.3	15.9
VEGF	Vascular endothelial growth factor	*VEGFA*	1,2	14.7	14	4.3	6

CV: coefficient of variation

*Selection of biomarker based on:

1	Reported association with cardiovascular disease by literature review
2	Proteomics discovery via mass spectrometry in the Framingham Heart Study or other studies
3	Gene expression profiling analysis with cardiovascular disease and risk factors
4	Coding genes asscociated with cardiovascular disease identified by genome-wide association studies

Table S3. *Cis* -protein quantitative trait loci variants for diet-associated proteins (Yao et al. Nat Commun. 2018 Aug 15;9(1):3268.)

SNP	Protein	Chr	Position	Effect Allele	Other Allele	Effect allele frequency	β	SE	R-Squared	*P* value	Source
rs10796979	CD5L	1	157513404	C	A	0.854	0.16	0.026	0.005	1.1E-09	1000G
rs10908589	CD5L	1	157699867	A	T	0.795	0.241	0.023	0.016	7.9E-26	1000G
rs10908610	CD5L	1	157921375	A	T	0.528	0.117	0.019	0.006	5.4E-10	1000G
rs11056183	UCMGP	12	14926504	A	G	0.764	-0.174	0.029	0.005	1.4E-09	1000G
rs112211052	C2	6	31397654	A	G	0.928	0.419	0.043	0.014	4.2E-22	1000G
rs115313300	C2	6	31737416	G	A	0.975	0.35	0.058	0.005	1.7E-09	1000G
rs115978492	C2	6	31576863	C	A	0.935	0.321	0.044	0.008	2.3E-13	1000G
rs116298992	C2	6	32068495	C	T	0.978	0.709	0.066	0.017	8.3E-27	1000G
rs11880916	GDF15	19	18492578	G	A	0.755	0.345	0.026	0.025	1.7E-40	1000G
rs12426002	CNTN1	12	41041887	C	T	0.896	0.221	0.031	0.007	1.9E-12	1000G
rs12811939	CNTN1	12	41047721	A	G	0.636	-0.206	0.023	0.012	2.0E-19	1000G
rs13039514	Cystatin C	20	23610836	G	T	0.982	0.589	0.084	0.007	2.0E-12	1000G
rs149066340	C2	6	31486405	C	T	0.979	0.92	0.073	0.023	1.1E-36	1000G
rs17852402	sICAM1	19	10404519	G	A	0.861	-0.257	0.036	0.007	1.6E-12	1000G
rs1838343	CNTN1	12	41196230	C	T	0.58	0.207	0.021	0.015	6.5E-24	1000G
rs186738594	C2	6	31156072	C	T	0.954	0.363	0.047	0.009	1.3E-14	1000G
rs2211320	CRP	1	159693605	G	A	0.677	0.149	0.02	0.008	4.8E-14	1000G
rs2235302	GMP140	1	169580290	A	G	0.476	-0.159	0.019	0.013	1.6E-17	Exome
rs2273378	Cystatin C	20	23476389	A	G	0.899	0.193	0.03	0.006	1.8E-10	1000G
rs2297198	MMP9	20	44674283	C	T	0.799	-0.153	0.025	0.006	6.4E-10	1000G
rs2326100	CXCL16	17	4676452	G	A	0.552	-0.139	0.023	0.005	3.2E-09	1000G
rs2424595	Cystatin C	20	23644387	G	A	0.756	-0.126	0.021	0.005	2.0E-09	1000G
rs251351	CD14	5	140227218	T	C	0.63	-0.148	0.021	0.007	3.0E-12	1000G
rs2680703	MPO	17	56429673	A	G	0.596	-0.175	0.021	0.01	1.9E-17	1000G
rs2765501	CD5L	1	157804648	G	A	0.608	-0.285	0.018	0.034	4.8E-54	1000G
rs2787337	AGP1	9	117084228	T	C	0.381	-0.136	0.024	0.005	8.5E-09	1000G
rs28683560	UCMGP	12	15102681	G	C	0.793	0.131	0.023	0.005	1.9E-08	1000G
rs2923091	ADM	11	10358145	A	G	0.662	-0.204	0.025	0.01	2.3E-16	1000G
rs3138074	CD14	5	140015932	A	T	0.777	0.332	0.022	0.033	9.2E-53	1000G
rs34180877	CNTN1	12	41428205	T	G	0.955	0.252	0.045	0.004	2.8E-08	1000G
rs34523895	MPO	17	57034095	C	T	0.606	0.114	0.019	0.005	2.4E-09	1000G
rs35082135	MPO	17	56939694	A	G	0.621	-0.13	0.019	0.007	9.5E-12	1000G
rs35751322	sICAM1	19	10378677	A	T	0.824	0.153	0.026	0.005	5.3E-09	1000G
rs35911336	GDF15	19	18481825	A	G	0.833	-0.518	0.029	0.045	1.0E-71	1000G
rs35966900	MPO	17	56363693	T	C	0.824	-0.18	0.024	0.008	9.4E-14	1000G
rs3917771	GMP140	1	169571728	T	A	0.873	0.212	0.028	0.008	5.0E-14	1000G
rs3917827	GMP140	1	169564391	A	C	0.893	-0.181	0.03	0.005	1.4E-09	1000G
rs3917872	GMP140	1	169576667	T	G	0.983	0.85	0.091	0.013	6.0E-21	1000G
rs3918249	MMP9	20	44638136	C	T	0.348	0.173	0.019	0.012	2.9E-19	1000G
rs41495647	CNTN1	12	41016867	T	A	0.892	0.196	0.03	0.006	4.1E-11	1000G
rs4258871	Cystatin C	20	23746923	C	A	0.836	0.211	0.034	0.006	6.2E-10	1000G
rs4703	UCMGP	12	15095558	G	C	0.531	0.234	0.024	0.014	1.3E-22	1000G
rs4764127	UCMGP	12	14989012	T	A	0.919	0.314	0.043	0.008	1.5E-13	1000G
rs4768307	CNTN1	12	41155000	T	C	0.9	-0.294	0.032	0.012	1.1E-19	1000G
rs6049097	Cystatin C	20	23753552	T	C	0.608	-0.12	0.019	0.006	2.9E-10	1000G
rs6076118	Cystatin C	20	23689371	T	C	0.501	-0.129	0.018	0.007	2.4E-12	1000G
rs6136	GMP140	1	169563951	G	T	0.103	-0.609	0.031	0.07	3.8E-87	Exome
rs6666046	GMP140	1	169545422	T	A	0.709	0.151	0.02	0.008	9.3E-14	1000G
rs67682613	C2	6	31826705	G	A	0.858	-0.167	0.026	0.006	2.7E-10	1000G
rs7040440	AGP1	9	117091074	C	T	0.922	-0.348	0.046	0.008	3.3E-14	1000G
rs7122422	ADM	11	10067262	G	C	0.534	-0.125	0.018	0.007	1.0E-11	1000G
rs7135211	UCMGP	12	15052758	G	A	0.622	0.265	0.019	0.028	3.6E-44	1000G
rs72710043	CD5L	1	157816877	C	T	0.874	-0.2	0.034	0.005	5.8E-09	1000G
rs72798881	CD14	5	139856491	C	T	0.925	0.415	0.046	0.012	2.1E-19	1000G
rs732457	Resistin	19	7688838	A	G	0.747	-0.174	0.028	0.005	9.5E-10	1000G
rs73610708	Cystatin C	20	23539846	C	T	0.907	0.247	0.038	0.006	5.7E-11	1000G
rs74078563	CNTN1	12	41123685	T	G	0.874	0.162	0.028	0.005	4.0E-09	1000G
rs760694	GMP140	1	169568698	G	T	0.456	-0.236	0.018	0.024	5.0E-38	1000G
rs76782803	Cystatin C	20	23665192	G	A	0.886	0.416	0.032	0.023	1.8E-37	1000G
rs78061871	GDF15	19	18566637	G	A	0.856	-0.18	0.032	0.005	1.6E-08	1000G
rs78638091	CD14	5	139999730	T	C	0.962	0.349	0.052	0.007	1.6E-11	1000G
rs78958589	Cystatin C	20	23490713	C	T	0.976	0.415	0.065	0.006	1.4E-10	1000G
rs79141987	MPO	17	56398479	G	A	0.897	0.196	0.034	0.005	1.3E-08	1000G
rs7935957	HPX	11	6450200	A	T	0.788	-0.252	0.022	0.019	5.1E-30	1000G
rs79628425	CD5L	1	157715898	C	G	0.955	0.353	0.049	0.008	5.9E-13	1000G
rs8115833	Cystatin C	20	23639384	A	G	0.883	-0.265	0.031	0.011	5.0E-18	1000G
rs850733	GRN	17	42451305	G	A	0.609	0.221	0.026	0.011	1.5E-17	1000G
rs911119	Cystatin C	20	23612737	T	C	0.779	0.393	0.022	0.046	6.7E-73	1000G
rs9270936	C2	6	32572755	T	A	0.83	0.243	0.034	0.007	1.1E-12	1000G
rs9427315	CD5L	1	157771028	T	C	0.954	0.346	0.044	0.009	5.3E-15	1000G
rs9469019	C2	6	31473730	G	A	0.924	0.302	0.038	0.009	8.4E-16	1000G

Table S4. Characteristics of participants in two independent sub-cohorts

	Sub-cohort 1 (*n*=2,979)		Sub-cohort 2 (*n*=3,381)		*P* value
	Mean or *n*	SD or %	Mean or *n*	SD or %	
Age	50	14	49	14	0.803
Women (*n*, %)	1590	46	1849	54	0.306
Body mass index	28	6	27	5	0.414
Diet quality					
AHEI	54	12	53	12	0.475
DASH	24	5	24	5	0.237
MDS	12	4	12	4	0.971
Alcohol intake	5	7	6	8	0.059
Current smoker (*n*, %)	394	46	461	54	0.660
College educated (*n*, %)	1533	54	1316	46	0.361
Energy intake	1952	649	1972	663	0.153
Physical activity	38	7	38	7	0.946
CVD-related proteins					
A1M	-0.010	0.994	-0.016	0.992	0.898
ADAM15	0.011	1.001	0.010	0.988	0.557
Adipsin	-0.036	0.982	-0.012	1.007	0.216
ADM	-0.026	1.002	-0.012	1.003	0.195
AGP1	0.009	1.003	-0.039	0.995	0.127
ANGPTL3	0.020	1.001	-0.042	0.995	0.128
APOA1	-0.021	1.003	0.032	0.997	0.322
APOB	-0.007	1.007	-0.007	0.993	0.426
B2M	-0.012	0.990	-0.028	0.993	0.899
BCHE	0.037	1.001	-0.025	0.993	0.045
Bikunin	0.018	0.998	-0.007	0.984	0.386
NTproBNP	-0.020	0.988	0.007	0.984	0.531
C2	-0.006	1.003	-0.018	0.993	0.675
CD14	-0.024	0.985	-0.004	1.009	0.598
CD163	-0.011	1.002	0.004	0.995	0.430
CD40L	0.039	0.993	-0.017	0.985	0.130
NCAM	0.026	1.008	-0.025	0.982	0.297
CD5L	0.013	1.012	-0.039	0.982	0.059
CDH13	0.002	1.007	0.012	0.989	0.677
Ceruloplasmin	-0.021	1.026	0.022	0.984	0.131
CLU	-0.017	0.999	0.004	1.005	0.758
CNTN1	0.013	1.015	0.010	0.982	0.846
COL18A1	-0.023	0.993	-0.012	0.994	0.464
CRP	0.001	1.007	-0.038	0.992	0.801
CXCL16	0.023	0.993	-0.017	0.996	0.660
Cystatin C	-0.010	0.983	-0.029	0.995	0.843
DPP4	-0.030	0.974	0.024	1.016	0.193
EFEMP1	0.001	0.974	-0.029	1.002	0.899
FGG	0.031	1.002	-0.054	0.989	0.084
FGF23	0.015	1.003	-0.013	0.994	0.891
GAPDH	0.053	0.995	-0.011	1.007	0.373
GDF15	-0.004	0.965	-0.043	1.001	0.995
GMP140	0.031	0.996	-0.032	1.003	0.028
GP5	0.015	0.977	0.000	1.001	0.591
GRN	-0.024	1.006	0.012	0.994	0.189
HPX	0.008	1.006	-0.024	0.999	0.337
IGF1	0.002	1.009	0.026	0.999	0.805
IGFBP1	-0.056	1.007	0.052	0.986	0.002
IGFBP2	-0.038	0.993	0.004	1.002	0.278
IGFBP3	0.008	1.006	0.029	0.979	0.346
KLKB1	0.026	1.002	-0.019	1.003	0.507
LDLR	0.011	0.997	0.006	0.993	0.846
Leptin	0.010	0.997	0.000	0.990	0.508
Leptin-R	0.006	0.975	-0.002	0.976	0.749
LPA	0.002	1.000	-0.001	1.004	0.983
MCAM	0.015	0.981	-0.003	0.992	0.427
MCP1	-0.004	0.993	-0.016	1.004	0.634
MMP9	0.012	0.990	-0.027	0.999	0.372
MMP8	0.003	0.964	-0.010	0.995	0.375
MPO	-0.009	0.997	-0.020	0.993	0.427
Myoglobin	0.010	0.995	-0.027	0.993	0.086
Notch1	-0.017	1.010	0.001	0.983	0.301
NRCAM	0.014	0.994	0.015	0.992	0.744
Osteocalcin	-0.035	0.982	0.012	1.009	0.212
PAI1	0.018	1.011	-0.049	0.990	0.057
PMP2	0.017	1.002	0.007	0.993	0.998
PON1	-0.004	0.992	0.032	1.007	0.576
PPBP	0.015	1.000	0.009	0.990	0.656
SERPINA10	-0.009	0.990	0.011	0.990	0.028
REG1A	-0.019	0.992	-0.021	1.005	0.697
Resistin	0.024	0.986	-0.037	1.002	0.598
SAA1	0.020	1.003	-0.028	0.997	0.140
SDF1	0.010	1.001	0.008	0.987	0.472
sGP130	-0.002	1.000	-0.007	0.985	0.605
sICAM1	0.010	0.984	-0.036	1.002	0.508
sRAGE	-0.022	0.999	0.034	0.985	0.269
CLEC3B	-0.027	0.996	0.021	0.996	0.392
TIMP1	0.000	0.966	-0.024	1.009	0.629
TSC22D3	0.006	1.001	-0.010	0.996	0.516
UCMGP	0.001	0.984	-0.031	1.001	0.907
VEGF	0.020	0.975	-0.016	0.994	0.149

Data were expressed as means and SDs or frequency and percentage. A linear mixed effects model was used to test the difference between the discovery and validation samples for continuous variables, with family structure as random effect. For categorical variables, Chi-suqare test was used. AHEI, Alternate Healthy Eating Index; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; MDS, Mediterranean-style diet score.

Table S5. Cross-sectional association between diet quality scores and plasma concentration of CVD-related proteins in all study participants

Protein	AHEI			DASH			MDS		
	β	SE	*P* value	β	SE	*P* value	β	SE	*P* value
A1M	-0.033	0.012	0.007	-0.007	0.012	0.570	-0.011	0.013	0.406
ADAM15	-0.029	0.013	0.028	-0.003	0.013	0.850	-0.017	0.014	0.209
Adipsin	-0.072	0.012	4.4E-10	-0.053	0.012	5.6E-06	-0.037	0.012	0.002
ADM	-0.077	0.011	5.5E-12	-0.055	0.011	1.0E-06	-0.043	0.012	1.9E-04
AGP1	-0.101	0.012	3.2E-16	-0.112	0.012	3.9E-19	-0.066	0.013	2.3E-07
ANGPTL3	-0.080	0.012	1.9E-11	-0.073	0.012	1.4E-09	-0.067	0.012	4.9E-08
APOA1	0.006	0.012	0.602	-0.020	0.012	0.111	-0.013	0.013	0.301
APOB	-0.069	0.013	3.7E-08	-0.083	0.013	7.3E-11	-0.056	0.013	1.6E-05
B2M	-0.067	0.012	9.1E-09	-0.044	0.012	2.1E-04	-0.033	0.012	0.006
BCHE	-0.027	0.013	0.034	-0.032	0.013	0.013	-0.015	0.013	0.249
Bikunin	0.018	0.013	0.163	-0.004	0.013	0.733	0.010	0.013	0.459
NTproBNP	0.012	0.011	0.313	0.024	0.012	0.037	0.006	0.012	0.618
C2	-0.051	0.013	5.4E-05	-0.055	0.013	2.1E-05	-0.037	0.013	0.005
CD14	-0.047	0.013	2.4E-04	-0.065	0.013	4.7E-07	-0.041	0.013	0.002
CD163	-0.008	0.013	0.537	0.006	0.013	0.664	0.004	0.013	0.743
CD40L	-0.002	0.013	0.878	-0.005	0.013	0.708	0.002	0.013	0.862
NCAM	-0.007	0.012	0.556	0.021	0.012	0.072	0.022	0.012	0.065
CD5L	-0.051	0.013	5.8E-05	-0.048	0.013	2.3E-04	-0.033	0.013	0.013
CDH13	-0.031	0.013	0.020	-0.007	0.013	0.600	-0.017	0.014	0.204
Ceruloplasmin	-0.020	0.012	0.092	-0.019	0.012	0.108	-0.014	0.012	0.242
CLU	-0.003	0.013	0.813	-0.015	0.013	0.249	0.000	0.014	0.986
CNTN1	0.092	0.012	1.0E-13	0.089	0.012	8.9E-13	0.082	0.013	1.4E-10
COL18A1	-0.031	0.013	0.015	-0.007	0.013	0.565	0.001	0.013	0.966
CRP	-0.103	0.012	2.7E-18	-0.093	0.012	6.5E-15	-0.072	0.012	4.1E-09
CXCL16	-0.046	0.013	3.3E-04	-0.032	0.013	0.014	-0.034	0.013	0.009
Cystatin C	-0.062	0.011	4.2E-08	-0.038	0.012	0.001	-0.030	0.012	0.011
DPP4	0.011	0.013	0.405	0.017	0.013	0.192	0.014	0.014	0.317
EFEMP1	-0.020	0.011	0.072	-0.003	0.011	0.793	-0.008	0.011	0.465
FGG	-0.037	0.012	0.002	-0.030	0.012	0.013	-0.016	0.012	0.207
FGF23	-0.024	0.013	0.072	0.011	0.013	0.414	-0.007	0.014	0.627
GAPDH	0.052	0.013	7.7E-05	0.089	0.013	3.2E-11	0.079	0.014	8.4E-09
GDF15	-0.051	0.009	4.3E-08	-0.024	0.010	0.012	-0.019	0.010	0.056
GMP140	-0.066	0.013	1.7E-07	-0.048	0.013	2.0E-04	-0.053	0.013	4.7E-05
GP5	0.004	0.013	0.744	0.033	0.013	0.013	0.007	0.013	0.594
GRN	-0.048	0.013	2.4E-04	-0.032	0.013	0.015	-0.043	0.013	0.002
HPX	-0.069	0.012	1.1E-08	-0.063	0.012	2.4E-07	-0.053	0.013	2.2E-05
IGF1	0.073	0.012	5.7E-10	0.050	0.012	3.4E-05	0.061	0.012	5.2E-07
IGFBP1	0.002	0.011	0.865	0.045	0.011	5.9E-05	0.009	0.011	0.432
IGFBP2	0.011	0.012	0.329	0.020	0.012	0.087	0.002	0.012	0.889
IGFBP3	0.035	0.013	0.007	0.041	0.013	0.002	0.044	0.013	0.001
KLKB1	0.009	0.013	0.498	-0.017	0.013	0.188	0.001	0.013	0.950
LDLR	-0.049	0.013	1.9E-04	-0.022	0.013	0.097	-0.028	0.014	0.040
Leptin	-0.042	0.009	4.1E-06	-0.031	0.009	6.4E-04	-0.022	0.009	0.019
Leptin-R	0.012	0.013	0.339	0.023	0.013	0.077	0.017	0.013	0.208
LPA	0.012	0.013	0.356	0.019	0.013	0.151	0.027	0.013	0.049
MCAM	0.041	0.012	0.001	0.051	0.013	4.9E-05	0.041	0.013	0.001
MCP1	-0.051	0.012	3.2E-05	-0.043	0.012	5.2E-04	-0.033	0.013	0.009
MMP8	-0.066	0.013	1.4E-07	-0.032	0.013	0.011	-0.042	0.013	0.001
MMP9	-0.062	0.013	9.2E-07	-0.034	0.013	0.008	-0.041	0.013	0.002
MPO	-0.079	0.013	6.4E-10	-0.074	0.013	6.9E-09	-0.054	0.013	4.8E-05
Myoglobin	0.003	0.011	0.785	0.019	0.011	0.094	0.018	0.012	0.122
Notch1	0.010	0.013	0.451	0.021	0.013	0.121	0.011	0.014	0.415
NRCAM	-0.027	0.013	0.038	-0.010	0.013	0.448	-0.023	0.014	0.091
Osteocalcin	-0.004	0.013	0.758	-0.002	0.013	0.853	0.000	0.013	0.992
PAI1	-0.072	0.012	5.6E-10	-0.081	0.012	4.1E-12	-0.043	0.012	3.2E-04
PMP2	-0.025	0.013	0.060	0.002	0.013	0.859	-0.016	0.014	0.240
PON1	0.001	0.013	0.959	-0.008	0.013	0.519	-0.007	0.013	0.615
PPBP	-0.016	0.013	0.219	-0.008	0.013	0.541	0.000	0.014	0.984
SERPINA10	-0.036	0.013	0.006	0.003	0.013	0.821	-0.015	0.013	0.276
REG1A	0.000	0.013	0.979	-0.021	0.013	0.099	-0.014	0.013	0.281
Resistin	-0.051	0.013	7.2E-05	-0.028	0.013	0.028	-0.029	0.013	0.030
SAA1	-0.045	0.012	2.2E-04	-0.047	0.012	1.1E-04	-0.014	0.013	0.248
SDF1	0.028	0.013	0.035	0.045	0.013	6.4E-04	0.027	0.013	0.044
sGP130	-0.023	0.013	0.069	0.000	0.013	0.972	0.000	0.013	0.987
sICAM1	-0.087	0.013	4.8E-12	-0.071	0.013	2.5E-08	-0.054	0.013	3.1E-05
sRAGE	0.013	0.013	0.317	0.022	0.013	0.077	0.021	0.013	0.104
CLEC3B	-0.004	0.013	0.772	-0.015	0.013	0.268	-0.005	0.014	0.703
TIMP1	-0.051	0.011	5.5E-06	-0.034	0.011	0.003	-0.020	0.012	0.090
TSC22D3	0.006	0.013	0.627	0.002	0.013	0.861	0.008	0.014	0.541
UCMGP	-0.169	0.011	4.1E-50	-0.110	0.011	1.6E-21	-0.111	0.012	6.8E-21
VEGF	-0.025	0.013	0.058	-0.011	0.013	0.387	-0.015	0.013	0.274

Linear mixed effect models were utilized to account for family structure with adjustment for sex, age, energy intake, smoking status, physical activity, alcohol intake, and body mass index. The names of proteins highlighted in bold indicate significant proteins after bonferroni correction ($P < 0.0007$ [0.05/71]). AHEI, Alternate Healthy Eating Index; β; regression coefficient; DASH, Dietary Approaches to Stop Hypertension; MDS, Mediterranean-style diet score; SE, standard error.

Table S6. Cross-sectional association between diet quality scores and plasma concentration of CVD-related proteins in two independent sub-cohorts

Protein	AHEI								DASH								MDS							
	Sub-cohort 1			Sub-cohort 2			Q	Qp	Sub-cohort 1			Sub-cohort 2			Q	Qp	Sub-cohort 1			Sub-cohort 2			Q	Qp
	β	SE	*P* value	β	SE	*P* value			β	SE	*P* value	β	SE	*P* value			β	SE	*P* value	β	SE	*P* value		
Adipsin	-0.090	0.016	1.7E-08	-0.052	0.017	0.002	2.739	0.098	-0.063	0.016	9.4E-05	-0.040	0.017	0.017	0.955	0.328								
ADM	-0.075	0.015	1.1E-06	-0.077	0.016	1.7E-06	0.012	0.912	-0.059	0.016	1.8E-04	-0.051	0.016	0.002	0.127	0.721	-0.059	0.016	2.1E-04	-0.024	0.017	0.159	2.462	0.117
AGP1	-0.098	0.017	7.0E-09	-0.108	0.018	2.3E-09	0.150	0.698	-0.108	0.017	3.4E-10	-0.117	0.018	1.1E-10	0.137	0.711	-0.068	0.017	8.5E-05	-0.064	0.019	7.7E-04	3.7E-02	0.848
ANGPTL3	-0.085	0.016	2.0E-07	-0.075	0.018	2.2E-05	0.168	0.682	-0.062	0.017	1.7E-04	-0.085	0.018	1.6E-06	0.861	0.353	-0.061	0.017	2.5E-04	-0.074	0.018	5.7E-05	0.251	0.616
APOB	-0.087	0.017	3.3E-07	-0.048	0.019	0.010	2.369	0.124	-0.096	0.017	2.5E-08	-0.067	0.019	3.5E-04	1.285	0.257	-0.090	0.017	2.6E-07	-0.017	0.019	0.390	6.979	0.008
B2M	-0.063	0.016	8.5E-05	-0.071	0.017	2.8E-05	0.098	0.755	-0.035	0.016	0.033	-0.053	0.017	0.002	0.571	0.450								
C2	-0.060	0.017	0.001	-0.040	0.018	0.029	0.636	0.425	-0.055	0.018	0.002	-0.054	0.019	0.004	2.8E-04	0.987								
CD14	-0.055	0.018	0.002	-0.038	0.018	0.036	0.419	0.517	-0.073	0.018	6.7E-05	-0.057	0.018	0.002	0.369	0.543								
CD5L	-0.058	0.017	0.001	-0.046	0.019	0.017	0.244	0.622	-0.045	0.018	0.011	-0.054	0.019	0.005	0.118	0.732								
CNTN1	0.104	0.017	3.8E-10	0.077	0.018	2.7E-05	1.191	0.275	0.096	0.017	1.0E-08	0.080	0.018	1.3E-05	0.412	0.521	0.091	0.017	1.0E-07	0.071	0.019	2.1E-04	0.610	0.435
CRP	-0.111	0.016	5.8E-12	-0.093	0.017	9.6E-08	0.593	0.441	-0.097	0.016	2.8E-09	-0.088	0.017	5.3E-07	0.160	0.689	-0.080	0.016	1.4E-06	-0.062	0.018	6.2E-04	0.545	0.460
CXCL16	-0.051	0.017	0.003	-0.040	0.019	0.034	0.212	0.645																
Cystatin C	-0.060	0.016	1.3E-04	-0.063	0.017	1.5E-04	0.029	0.866																
GAPDH	0.045	0.018	0.013	0.060	0.019	0.002	0.302	0.583	0.093	0.018	5.5E-07	0.084	0.019	1.4E-05	0.108	0.743	0.084	0.019	7.2E-06	0.072	0.020	3.1E-04	0.191	0.662
GDF15	-0.069	0.013	1.6E-07	-0.031	0.013	2.0E-02	3.962	0.047																
GMP140	-0.074	0.017	2.5E-05	-0.058	0.019	2.0E-03	0.396	0.529	-0.039	0.018	0.028	-0.057	0.019	0.002	0.492	0.483	-0.057	0.018	0.002	-0.049	0.019	0.011	0.086	0.769
GRN	-0.035	0.018	0.048	-0.063	0.019	0.001	1.164	0.281								0.959								
HPX	-0.068	0.017	4.1E-05	-0.071	0.018	5.9E-05	0.022	0.882	-0.058	0.017	6.0E-04	-0.069	0.018	1.1E-04	0.223	0.637	-0.048	0.017	0.004	-0.057	0.018	0.002	0.125	0.724
IGF1	0.067	0.016	3.9E-05	0.080	0.017	3.8E-06	0.285	0.593	0.035	0.017	0.037	0.065	0.017	1.6E-04	1.664	0.197	0.053	0.017	0.002	0.072	0.018	5.8E-05	0.621	0.431
IGFBP1									0.057	0.015	1.9E-04	0.033	0.016	0.046	1.180	0.277								
LDLR	-0.039	0.018	0.029	-0.059	0.019	0.002	0.544	0.461																
Leptin	-0.059	0.012	1.7E-06	-0.020	0.013	0.133	5.432	0.020	-0.043	0.013	0.001	-0.017	0.013	0.214	2.820	0.093								
MCAM									0.036	0.017	0.039	0.069	0.018	1.7E-04	2.053	0.152								
MCP1	-0.033	0.017	0.055	-0.069	0.018	1.1E-04	2.289	0.130	-0.042	0.017	0.017	-0.044	0.018	0.015	0.010	0.921								
MMP8	-0.077	0.017	9.2E-06	-0.051	0.018	0.005	1.040	0.308																
MMP9	-0.067	0.017	1.3E-04	-0.056	0.018	0.003	0.184	0.668																
MPO	-0.085	0.017	1.2E-06	-0.070	0.019	1.9E-04	0.363	0.547	-0.066	0.018	1.9E-04	-0.082	0.019	1.2E-05	0.354	0.552	-0.048	0.018	0.007	-0.058	0.019	0.003	0.129	0.719
PAI1	-0.073	0.016	4.0E-06	-0.071	0.017	3.3E-05	0.010	0.919	-0.084	0.016	1.6E-07	-0.079	0.017	4.0E-06	0.053	0.819	-0.038	0.016	0.021	-0.050	0.018	0.005	2.8E-01	0.600
Resistin	-0.047	0.018	0.009	-0.057	0.019	0.002	0.156	0.693																
SAA1	-0.065	0.017	9.2E-05	-0.023	0.018	0.203	3.433	0.064	-0.059	0.017	4.5E-04	-0.035	0.018	0.049	1.151	0.283								
SDF1									0.057	0.018	0.002	0.033	0.019	0.091	0.890	0.345								
sICAM1	-0.094	0.017	7.3E-08	-0.077	0.018	2.3E-05	0.440	0.507	-0.057	0.018	0.001	-0.086	0.018	3.0E-06	1.296	0.255	-0.056	0.018	0.002	-0.051	0.019	0.007	0.049	0.826
TIMP1	-0.058	0.015	1.8E-04	-0.042	0.016	0.010	0.531	0.466																
UCMGP	-0.194	0.016	1.0E-34	-0.140	0.016	1.5E-17	5.627	0.018	-0.126	0.016	4.3E-15	-0.092	0.017	3.4E-08	2.272	0.132	-0.133	0.016	3.3E-16	-0.084	0.017	8.9E-07	4.211	0.040

Linear mixed effect models were utilized to account for family structure with adjustment for sex, age, energy intake, smoking status, physical activity, alcohol intake, and body mass index. Proteins were considered as significant when P <0.05 and regression coefficients have same direction in both sub-cohorts. Heterogeneity was tested between the two sub-cohorts by calculating the Cochran's Q statistic and P <0.001 (0.05/38) was considered to indicate statistical significance. β, regression coefficient; Q, Cochran's heterogeneity Q statistic; Qp, P-value for Q statistic; SE, standard error.

Table S7. Gene ontology (GO) terms of biological processes enriched to diet-associated proteins (*n*=30)

GOID	GOTerm	Term PValue	Term PValue Corrected with Bonferroni step down	Group PValue	Group PValue Corrected with Bonferroni step down	GOLevels	GOGroups	% Associated Genes	Number of Genes	Associated Genes Found
GO:0032677	regulation of interleukin-8 production	8.7E-06	8.7E-06	8.7E-06	8.7E-06	[4, 5, 6, 7]	Group1	4.35	4.00	[CD14, CNTN1, CRP, SERPINE1]
GO:2000351	regulation of endothelial cell apoptotic process	8.7E-07	3.5E-06	8.7E-07	1.7E-06	[7, 8]	Group2	7.69	4.00	[ANGPT1, CCL2, ICAM1, SERPINE1]
GO:0150077	regulation of neuroinflammatory response	1.1E-09	5.6E-09	1.1E-09	3.3E-09	[5, 6, 7]	Group3	14.71	5.00	[GRN, IGF1, LDLR, MMP8, MMP9]
GO:0150079	negative regulation of neuroinflammatory response	1.2E-06	3.6E-06	1.1E-09	3.3E-09	[5, 6, 7, 8]	Group3	20.00	3.00	[GRN, IGF1, LDLR]
GO:1903978	regulation of microglial cell activation	2.5E-06	5.1E-06	1.1E-09	3.3E-09	[5, 6, 7, 8]	Group3	15.79	3.00	[GRN, LDLR, MMP8]

Table S8. Sensitivity analysis with removal of events occurred in the first 2 years and 5 years after baseline in all study participants

Outcome	Exclusion of recent events	Diet	N	HR	LCI	UCI	*P* value
CVD (fatal + non-fatal)	All	AHEI	5585	0.840	0.760	0.930	1.2E-16
		DASH	5585	0.820	0.730	0.910	2.0E-04
		MDS	5585	0.890	0.800	0.983	0.025
	2 yrs	AHEI	5527	0.800	0.720	0.889	1.2E-16
		DASH	5527	0.825	0.746	0.913	1.2E-16
		MDS	5527	0.885	0.799	0.980	0.019
	5 yrs	AHEI	5396	0.758	0.673	0.855	1.2E-16
		DASH	5396	0.794	0.709	0.891	1.2E-16
		MDS	5396	0.843	0.751	0.946	0.004
All-cause mortality	All	AHEI	5890	0.860	0.780	0.950	0.004
		DASH	5890	0.850	0.770	0.940	0.002
		MDS	5890	0.860	0.770	0.950	0.002
	2 yrs	AHEI	5883	0.838	0.771	0.911	1.2E-16
		DASH	5883	0.885	0.817	0.959	0.003
		MDS	5883	0.869	0.801	0.943	0.001
	5 yrs	AHEI	5865	0.854	0.782	0.933	1.2E-16
		DASH	5865	0.892	0.819	0.971	0.008
		MDS	5865	0.868	0.795	0.946	0.001

Mixed effect Cox proportional hazards model for incident CVD was adjusted for sex, age, energy intake, smoking status, physical activity levels, alcohol intake, body mass index, systolic blood pressure, use of antihypertension medications, high density lipoprotein cholesterol, total cholesterol, and type 2 diabetes. For all-cause mortality, model was adjusted for the covariates included for incident CVD and history of CVD and cancer. AHEI, Alternate Healthy Eating Index; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; HR, Hazard Ratio; LCI, lower confidence interval; MDS, Mediterranean-style diet score; UCI, upper confidence interval.

Table S9. Direction of association between diet quality scores, proteins, and CVD and all-cause mortality

Protein	Present study	Ho et al (2018)		Yao et al (2018)
	Diet	CVD and CVD death	All-cause mortality	CHD
Adipsin	-	+	+	
ADM	-	+	+	
AGP1	-	+	+	
ANGPTL3	-			
APOB	-			
B2M	-	+	+	
C2	-			
CD14	-		+	
CD5L	-		+	
CNTN1	+		-	
CRP	-	+	+	
CXCL16	-			
Cystatin C	-	+	+	+
GAPDH	+			
GDF15	-	+	+	
GMP140	-			
GRN	-	+	+	
HPX	-			
IGF1	+		-	
IGFBP1	+	+	+	
LDLR	-	-		
MCP1	-		+	
MMP8	-		+	
MMP9	-	+	+	
MPO	-	+	+	+
PAI1	-			
Resistin	-	+	+	
sICAM1	-	+	+	
TIMP1	-	+	+	
UCMGP	-	+	+	

+ sign indicates a positive association and – sign indicates an inverse association. Directions (+, Positive; -, negative) were obtained from the previous Framingham Heart Study publications, Ho et al (J Am Heart Assoc. 2018 Jul 13;7(14):e008108.) and Yao et al (Nat Commun. 2018 Aug 15;9(1):3268.). CVD and CVD death included atherosclerotic CVD, heart failure, and CVD death. CVD, cardiovascular disease.

Table S10. Association of cis-pQTL and incident CVD and all-cause mortality in all study participants

SNP	Chr	Position	Effect allele	Other allele	Effect allele frequency	Protein	Association with protein			Association with trait				
							β	SE	*P* value	Trait	HR	LCI	UCI	*P* value
rs10908589	1	157699867	A	T	0.795	CD5L	0.241	0.023	7.9E-26	All-cause mortality	1.276	1.132	1.438	6.4E-05
rs2765501	1	157804648	G	A	0.608	CD5L	-0.285	0.018	4.8E-54	All-cause mortality	0.878	0.802	0.960	4.3E-03
rs10796979	1	157513404	C	A	0.854	CD5L	0.160	0.026	1.1E-09	All-cause mortality	1.146	1.006	1.304	4.0E-02
rs760694	1	169568698	G	T	0.456	GMP140	-0.236	0.018	5.0E-38	All-cause mortality	1.130	1.035	1.234	6.5E-03
rs3138074	5	140015932	A	T	0.777	CD14	0.332	0.022	9.2E-53	Incident CVD	1.214	1.050	1.403	8.9E-03

A mixed effect Cox proportional hazard model was used, accounting for family structure, with adjustment for sex, age, and the first two principal components estimated from single nucleotide polymorphisms. pQTL-outcome associations with *P* <0.05 were included. β (SE) estimates are per each additional effect allele. Positive β reflects higher circulating protein level. CVD, cardiovascular disease; HR, Hazard Ratio; LCI, lower confidence interval; pQTL, protein quantitative trait loci; UCI, upper confidence interval.

Table S11. Mediation analysis of CVD-related proteins in relation to AHEI and all-cause mortality in all study participants

Protein	Model	Direct effect				Indirect effect				Total effect			
		HR	LCI	UCI	*P* value	HR	LCI	UCI	*P* value	HR	LCI	UCI	*P* value
Adipsin	Model 1	0.862	0.793	0.936	4.4E-04	0.993	0.986	0.998	0.029	0.855	0.787	0.929	2.5E-04
	Model 2	0.882	0.782	0.994	4.0E-02	0.998	0.989	1.007	0.693	0.880	0.781	0.992	0.037
ADM	Model 1	0.878	0.808	0.954	0.002	0.985	0.977	0.992	0.002	0.865	0.796	0.939	0.001
	Model 2	0.895	0.793	1.008	0.069	0.992	0.983	1.001	0.111	0.888	0.788	1.001	0.051
AGP1	Model 1	0.868	0.799	0.943	0.001	0.990	0.983	0.997	0.015	0.860	0.792	0.934	3.7E-04
	Model 2	0.887	0.787	1.001	0.052	0.992	0.983	0.999	0.065	0.880	0.781	0.992	0.038
B2M	Model 1	0.864	0.795	0.939	0.001	0.984	0.975	0.992	0.001	0.850	0.782	0.924	1.5E-04
	Model 2	0.889	0.788	1.002	0.055	0.986	0.975	0.995	0.020	0.877	0.778	0.988	0.032
CD14	Model 1	0.864	0.796	0.938	0.001	0.992	0.986	0.997	0.017	0.857	0.790	0.931	2.8E-04
	Model 2	0.880	0.781	0.992	0.036	0.997	0.991	1.000	0.208	0.877	0.778	0.989	0.032
CNTN1	Model 1	0.868	0.798	0.943	0.001	0.991	0.984	0.997	0.017	0.860	0.791	0.934	3.8E-04
	Model 2	0.889	0.788	1.002	0.055	0.988	0.978	0.996	0.019	0.878	0.779	0.990	0.034
CRP	Model 1	0.874	0.804	0.950	0.001	0.985	0.976	0.993	0.002	0.861	0.793	0.935	4.3E-04
	Model 2	0.892	0.790	1.005	0.061	0.990	0.980	0.999	0.063	0.883	0.783	0.995	0.041
Cystatin C	Model 1	0.868	0.799	0.943	0.001	0.991	0.984	0.996	0.011	0.860	0.792	0.935	4.0E-04
	Model 2	0.886	0.786	0.999	0.048	0.996	0.990	1.002	0.256	0.883	0.783	0.995	0.042
GDF15	Model 1	0.902	0.830	0.981	0.016	0.957	0.941	0.972	1.2E-16	0.863	0.793	0.939	0.001
	Model 2	0.915	0.811	1.032	0.150	0.974	0.959	0.988	0.003	0.892	0.790	1.006	0.062
GRN	Model 1	0.867	0.798	0.942	0.001	0.993	0.987	0.998	0.025	0.861	0.793	0.935	4.2E-04
	Model 2	0.888	0.788	1.001	0.052	0.997	0.992	1.001	0.265	0.886	0.786	0.998	0.046
IGF1	Model 1	0.876	0.806	0.951	0.002	0.991	0.984	0.997	0.012	0.868	0.799	0.943	0.001
	Model 2	0.894	0.793	1.008	0.068	0.991	0.983	0.998	0.046	0.886	0.787	0.999	0.048
MMP8	Model 1	0.867	0.798	0.942	0.001	0.993	0.988	0.998	0.023	0.861	0.793	0.936	4.3E-04
	Model 2	0.885	0.785	0.998	0.047	0.998	0.992	1.002	0.352	0.883	0.784	0.996	0.043
MMP9	Model 1	0.874	0.805	0.949	0.001	0.991	0.984	0.996	0.012	0.866	0.797	0.941	0.001
	Model 2	0.897	0.796	1.011	0.076	0.993	0.985	0.999	0.068	0.891	0.790	1.004	0.059
MPO	Model 1	0.866	0.797	0.941	0.001	0.993	0.986	0.998	0.030	0.860	0.791	0.934	3.5E-04
	Model 2	0.889	0.789	1.002	0.054	0.993	0.984	1.000	0.093	0.882	0.783	0.994	0.040
sICAM1	Model 1	0.880	0.810	0.957	0.003	0.987	0.979	0.993	0.002	0.869	0.800	0.944	0.001
	Model 2	0.907	0.804	1.023	0.115	0.990	0.981	0.997	0.031	0.898	0.797	1.013	0.080
UCMGP	Model 1	0.877	0.807	0.953	0.002	0.966	0.953	0.979	1.6E-05	0.847	0.779	0.921	1.1E-04
	Model 2	0.901	0.798	1.017	0.092	0.967	0.947	0.986	0.002	0.871	0.772	0.983	0.025

Linear mixed and mixed effect Cox proportional hazard models was applied. Covariates in model 1 included sex, age, energy intake, smoking status, physical activity score, alcohol intake, body mass index, systolic blood pressure, use of antihypertension medications, high density lipoprotein cholesterol, total cholesterol, type 2 diabetes, and history of CVD and cancer. In model 2, we additionally adjusted for education, smoking pack-years, estimated glomerular filtration rate, family history of CVD, aspirin use, and menopausal status, oral contraceptive use and hormone replacement therapy (women only). AHEI, Alternate Healthy Eating Index; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; HR, Hazard Ratio; LCI, lower confidence interval; MDS, Mediterranean-style diet score; UCI, upper confidence interval.

Table S12. Mediation analysis of CVD-related proteins in relation to DASH and all-cause mortality in all study participants

Protein	Model	Direct effect				Indirect effect				Total effect			
		HR	LCI	UCI	*P* value	HR	LCI	UCI	*P* value	HR	LCI	UCI	*P* value
Adipsin	Model 1	0.912	0.843	0.988	0.024	0.994	0.989	0.999	0.043	0.907	0.838	0.982	0.017
	Model 2	0.932	0.829	1.047	0.237	0.999	0.991	1.005	0.654	0.931	0.828	1.045	0.227
ADM	Model 1	0.923	0.853	1.000	0.049	0.987	0.979	0.994	0.003	0.911	0.842	0.987	0.022
	Model 2	0.940	0.837	1.056	0.302	0.992	0.983	1.000	0.093	0.933	0.831	1.048	0.243
AGP1	Model 1	0.919	0.849	0.996	0.039	0.989	0.980	0.996	0.011	0.909	0.839	0.984	0.019
	Model 2	0.939	0.836	1.055	0.294	0.989	0.978	0.998	0.043	0.929	0.826	1.044	0.216
B2M	Model 1	0.919	0.849	0.995	0.038	0.988	0.980	0.995	0.008	0.908	0.838	0.983	0.018
	Model 2	0.945	0.841	1.062	0.343	0.991	0.982	0.998	0.053	0.936	0.833	1.052	0.270
CD14	Model 1	0.915	0.845	0.991	0.029	0.990	0.983	0.995	0.008	0.905	0.836	0.981	0.015
	Model 2	0.933	0.831	1.049	0.247	0.994	0.986	0.999	0.090	0.927	0.825	1.042	0.206
CNTN1	Model 1	0.922	0.851	0.998	0.046	0.991	0.983	0.997	0.017	0.913	0.843	0.989	0.025
	Model 2	0.944	0.840	1.061	0.337	0.985	0.974	0.994	0.011	0.930	0.827	1.045	0.223
CRP	Model 1	0.924	0.853	1.001	0.055	0.986	0.977	0.993	0.002	0.911	0.841	0.987	0.023
	Model 2	0.942	0.838	1.059	0.320	0.990	0.980	0.999	0.057	0.933	0.831	1.048	0.246
IGF1	Model 1	0.921	0.850	0.998	0.045	0.994	0.988	0.998	0.029	0.916	0.845	0.992	0.031
	Model 2	0.941	0.837	1.057	0.306	0.994	0.987	0.999	0.076	0.935	0.832	1.050	0.258
MCP1	Model 1	0.916	0.846	0.992	0.031	0.995	0.989	0.998	0.042	0.911	0.841	0.987	0.022
	Model 2	0.936	0.833	1.051	0.266	0.997	0.991	1.000	0.193	0.933	0.830	1.048	0.244
MPO	Model 1	0.916	0.846	0.992	0.031	0.993	0.986	0.998	0.025	0.909	0.839	0.984	0.019
	Model 2	0.940	0.837	1.056	0.298	0.994	0.987	1.000	0.113	0.935	0.832	1.050	0.255
sICAM1	Model 1	0.932	0.860	1.009	0.082	0.989	0.981	0.995	0.004	0.921	0.850	0.997	0.043
	Model 2	0.959	0.853	1.078	0.483	0.992	0.984	0.998	0.051	0.951	0.847	1.069	0.402
UCMGP	Model 1	0.923	0.852	0.999	0.049	0.979	0.969	0.987	1.2E-04	0.903	0.834	0.978	0.012
	Model 2	0.952	0.847	1.069	0.407	0.977	0.963	0.990	0.003	0.930	0.828	1.044	0.220

Linear mixed and mixed effect Cox proportional hazard models was applied. Covariates in Model 1 and 2 were the same as in Table S11. AHEI, Alternate Healthy Eating Index; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; HR, Hazard Ratio; LCI, lower confidence interval; MDS, Mediterranean-style diet score; UCI, upper confidence interval.

Table S13. Mediation analysis of CVD-related proteins in relation to MDS and all-cause mortality in all study participants

Protein	Model	Direct effect				Indirect effect				Total effect			
		HR	LCI	UCI	*P* value	HR	LCI	UCI	*P* value	HR	LCI	UCI	*P* value
AGP1	Model 1	0.898	0.828	0.973	0.009	0.994	0.988	0.998	0.037	0.892	0.823	0.967	0.006
	Model 2	0.891	0.792	1.002	0.055	0.995	0.989	1.000	0.122	0.887	0.788	0.997	0.045
CNTN1	Model 1	0.901	0.831	0.977	0.012	0.992	0.985	0.997	0.019	0.894	0.824	0.969	0.006
	Model 2	0.894	0.795	1.005	0.062	0.988	0.979	0.996	0.021	0.883	0.785	0.994	0.039
CRP	Model 1	0.906	0.835	0.982	0.017	0.989	0.982	0.995	0.007	0.896	0.826	0.972	0.008
	Model 2	0.900	0.800	1.012	0.078	0.993	0.985	0.999	0.095	0.894	0.795	1.005	0.061
IGF1	Model 1	0.904	0.833	0.980	0.015	0.992	0.986	0.997	0.018	0.897	0.826	0.973	0.009
	Model 2	0.897	0.797	1.009	0.070	0.992	0.984	0.998	0.056	0.890	0.791	1.001	0.052
MPO	Model 1	0.897	0.827	0.973	0.009	0.995	0.990	0.999	0.047	0.892	0.823	0.968	0.006
	Model 2	0.896	0.796	1.007	0.066	0.995	0.988	1.000	0.129	0.891	0.793	1.002	0.054
sICAM1	Model 1	0.910	0.839	0.988	0.024	0.992	0.985	0.997	0.021	0.903	0.832	0.980	0.014
	Model 2	0.912	0.810	1.026	0.125	0.994	0.987	1.000	0.104	0.906	0.805	1.020	0.102
UCMGP	Model 1	0.899	0.829	0.975	0.010	0.978	0.968	0.987	9.0E-05	0.879	0.811	0.954	0.002
	Model 2	0.904	0.804	1.016	0.090	0.976	0.961	0.989	0.003	0.882	0.785	0.991	0.035

Linear mixed and mixed effect Cox proportional hazard models was applied. Covariates in Model 1 and 2 were the same as in Table S11. AHEI, Alternate Healthy Eating Index; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; HR, Hazard Ratio; LCI, lower confidence interval; MDS, Mediterranean-style diet score; UCI, upper confidence interval.

Table S14. Median proportion mediated and its 95% confidence interval (CI) of CVD-related proteins in relation to diet scores and all-cause mortality in all study participants

Protein	AHEI			DASH			MDS		
	Median	LCI	UCI	Median	LCI	UCI	Median	LCI	UCI
GDF15	30.1%	16.8%	70.1%						
UCMGP	20.7%	11.2%	43.3%	21.0%	9.4%	82.9%	17.4%	8.8%	47.4%
ADM	10.4%	4.8%	26.0%						
CRP	9.9%	4.2%	24.7%	15.2%	5.2%	74.2%			
B2M	9.9%	4.8%	22.0%						
sICAM1	9.3%	4.1%	24.6%						

Proportion of mediation by a target protein was calculated as the ratio of indirect effect to the sum of both direct and indirect effects.

Table S15. Mediation analysis of CVD-related proteins in relation to AHEI and incdient CVD (fatal and non-fatal) in all study participants

Protein	Model	Direct effect				Indirect effect				Total effect			
		HR	LCI	UCI	*p*-value	HR	LCI	UCI	*p*-value	HR	LCI	UCI	*p*-value
Adipsin	Model 1	0.849	0.768	0.938	0.001	0.993	0.986	1.000	0.088	0.843	0.763	0.931	0.001
	Model 2	0.834	0.730	0.954	0.008	1.000	0.990	1.010	0.958	0.834	0.730	0.954	0.008
ADM	Model 1	0.851	0.770	0.941	0.002	0.993	0.984	1.000	0.094	0.845	0.765	0.934	0.001
	Model 2	0.835	0.729	0.955	0.009	0.994	0.983	1.003	0.221	0.829	0.725	0.949	0.006
AGP1	Model 1	0.851	0.770	0.940	0.001	0.993	0.985	1.001	0.122	0.845	0.765	0.933	0.001
	Model 2	0.834	0.730	0.953	0.008	0.997	0.987	1.005	0.429	0.831	0.727	0.950	0.007
B2M	Model 1	0.843	0.762	0.931	0.001	0.992	0.984	0.999	0.056	0.836	0.757	0.924	4.7E-04
	Model 2	0.833	0.728	0.952	0.007	0.999	0.989	1.009	0.822	0.832	0.728	0.951	7.0E-03
CD14	Model 1	0.850	0.770	0.939	0.001	0.994	0.988	0.999	0.084	0.845	0.766	0.933	0.001
	Model 2	0.832	0.728	0.951	0.007	0.999	0.994	1.003	0.559	0.831	0.727	0.950	0.007
CNTN1	Model 1	0.844	0.763	0.933	0.001	0.996	0.988	1.004	0.313	0.841	0.761	0.929	0.001
	Model 2	0.846	0.738	0.969	0.016	0.987	0.976	0.997	0.031	0.835	0.729	0.956	0.009
CRP	Model 1	0.846	0.765	0.935	0.001	0.997	0.987	1.006	0.463	0.843	0.763	0.932	0.001
	Model 2	0.833	0.728	0.953	0.008	0.998	0.987	1.009	0.742	0.832	0.727	0.951	0.007
Cystatin C	Model 1	0.855	0.774	0.945	0.002	0.994	0.987	0.999	0.066	0.850	0.769	0.939	0.001
	Model 2	0.836	0.732	0.956	0.009	0.997	0.989	1.003	0.352	0.834	0.730	0.953	0.008
GDF15	Model 1	0.866	0.783	0.957	0.005	0.982	0.971	0.991	0.002	0.850	0.769	0.939	0.001
	Model 2	0.845	0.739	0.966	0.014	0.984	0.972	0.994	0.016	0.832	0.728	0.951	0.007
GRN	Model 1	0.849	0.768	0.938	0.001	0.997	0.992	1.002	0.255	0.846	0.766	0.935	0.001
	Model 2	0.835	0.730	0.955	0.009	0.999	0.994	1.005	0.772	0.835	0.730	0.955	0.008
IGF1	Model 1	0.847	0.767	0.937	0.001	0.998	0.991	1.004	0.453	0.845	0.765	0.934	0.001
	Model 2	0.841	0.736	0.962	0.011	0.995	0.986	1.003	0.262	0.837	0.733	0.957	0.009
MMP8	Model 1	0.848	0.767	0.936	0.001	0.995	0.989	1.000	0.102	0.843	0.763	0.932	0.001
	Model 2	0.832	0.728	0.951	0.007	1.000	0.994	1.006	0.977	0.832	0.728	0.951	0.007
MMP9	Model 1	0.849	0.768	0.939	0.001	0.996	0.990	1.001	0.180	0.846	0.765	0.935	0.001
	Model 2	0.829	0.724	0.948	0.006	1.000	0.994	1.006	0.986	0.829	0.724	0.948	0.006
MPO	Model 1	0.848	0.767	0.937	0.001	0.997	0.990	1.004	0.361	0.845	0.765	0.934	0.001
	Model 2	0.831	0.727	0.950	0.007	1.002	0.993	1.011	0.620	0.833	0.728	0.952	0.008
sICAM1	Model 1	0.848	0.766	0.938	0.001	0.992	0.984	0.998	0.043	0.841	0.760	0.930	0.001
	Model 2	0.834	0.729	0.954	0.008	0.993	0.985	1.000	0.107	0.829	0.724	0.948	0.006
UCMGP	Model 1	0.851	0.770	0.941	0.002	0.991	0.975	1.007	0.271	0.843	0.763	0.932	0.001
	Model 2	0.837	0.731	0.958	0.010	0.995	0.973	1.017	0.635	0.833	0.728	0.952	0.008

Linear mixed and mixed effect Cox proportional hazard models was applied. Covariates in model 1 included sex, age, energy intake, smoking status, physical activity score, alcohol intake, body mass index, systolic blood pressure, use of antihypertension medications, high density lipoprotein cholesterol, total cholesterol, and type 2 diabetes. In model 2, we additionally adjusted for education, smoking pack-years, estimated glomerular filtration rate, family history of CVD, aspirin use, and menopausal status, oral contraceptive use and hormone replacement therapy (women only). AHEI, Alternate Healthy Eating Index; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; HR, Hazard Ratio; LCI, lower confidence interval; MDS, Mediterranean-style diet score; UCI, upper confidence interval.

Table S16. Mediation analysis of CVD-related proteins in relation to DASH and incdient CVD (fatal and non-fatal) in all study participants

Protein	Model	Direct effect				Indirect effect				Total effect			
		HR	LCI	UCI	*P* value	HR	LCI	UCI	*P* value	HR	LCI	UCI	*P* value
Adipsin	Model 1	0.864	0.778	0.960	0.006	0.995	0.987	1.001	0.128	0.859	0.774	0.954	0.005
	Model 2	0.864	0.759	0.983	0.027	1.000	0.992	1.008	0.953	0.864	0.759	0.983	0.027
ADM	Model 1	0.867	0.780	0.964	0.008	0.994	0.985	1.000	0.114	0.862	0.776	0.957	0.006
	Model 2	0.871	0.765	0.991	0.037	0.993	0.983	1.003	0.193	0.865	0.760	0.984	0.028
AGP1	Model 1	0.870	0.783	0.966	0.009	0.991	0.980	1.000	0.076	0.862	0.776	0.957	0.005
	Model 2	0.870	0.765	0.990	0.035	0.995	0.984	1.007	0.425	0.866	0.761	0.986	0.029
B2M	Model 1	0.861	0.775	0.956	0.005	0.995	0.988	1.000	0.125	0.856	0.771	0.951	0.004
	Model 2	0.865	0.760	0.985	0.029	0.999	0.992	1.006	0.803	0.865	0.759	0.984	0.028
CD14	Model 1	0.865	0.778	0.960	0.007	0.994	0.986	1.000	0.097	0.859	0.774	0.954	0.005
	Model 2	0.865	0.760	0.985	0.029	0.998	0.991	1.006	0.624	0.864	0.759	0.983	0.027
CNTN1	Model 1	0.863	0.776	0.959	0.006	0.993	0.984	1.002	0.136	0.857	0.771	0.952	0.004
	Model 2	0.874	0.767	0.996	0.043	0.984	0.972	0.995	0.020	0.860	0.755	0.980	0.023
CRP	Model 1	0.861	0.774	0.957	0.005	1.000	0.991	1.009	0.962	0.861	0.775	0.956	0.005
	Model 2	0.867	0.761	0.987	0.031	0.998	0.987	1.009	0.715	0.865	0.760	0.985	0.029
IGF1	Model 1	0.863	0.777	0.960	0.006	0.999	0.994	1.003	0.480	0.862	0.776	0.958	0.006
	Model 2	0.874	0.768	0.995	0.042	0.996	0.989	1.002	0.251	0.871	0.766	0.991	0.037
MCP1	Model 1	0.859	0.773	0.954	0.005	0.998	0.994	1.001	0.289	0.858	0.772	0.953	0.004
	Model 2	0.867	0.762	0.986	0.030	0.999	0.994	1.002	0.407	0.866	0.761	0.985	0.028
MPO	Model 1	0.865	0.779	0.961	0.007	0.995	0.987	1.001	0.154	0.860	0.774	0.955	0.005
	Model 2	0.865	0.760	0.984	0.027	1.002	0.995	1.009	0.574	0.866	0.761	0.985	0.029
sICAM1	Model 1	0.862	0.775	0.959	0.006	0.994	0.986	0.999	0.080	0.856	0.770	0.952	0.004
	Model 2	0.870	0.764	0.990	0.035	0.995	0.987	1.000	0.133	0.865	0.760	0.985	0.029
UCMGP	Model 1	0.868	0.781	0.964	0.008	0.993	0.982	1.004	0.222	0.862	0.776	0.957	0.006
	Model 2	0.871	0.765	0.991	0.037	0.995	0.980	1.010	0.494	0.867	0.761	0.986	0.030

Linear mixed and mixed effect Cox proportional hazard models was applied. Covariates in Model 1 and 2 were the same as in Table S15. AHEI, Alternate Healthy Eating Index; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; HR, Hazard Ratio; LCI, lower confidence interval; MDS, Mediterranean-style diet score; UCI, upper confidence interval.

Table S17. Mediation analysis of CVD-related proteins in relation to MDS and incdient CVD (fatal and non-fatal) in all study participants

Protein	Model	Direct effect				Indirect effect				Total effect			
		HR	LCI	UCI	*P* value	HR	LCI	UCI	*P* value	HR	LCI	UCI	*P* value
AGP1	Model 1	0.927	0.841	1.022	0.128	0.995	0.989	1.000	0.127	0.923	0.837	1.017	0.106
	Model 2	0.915	0.803	1.043	0.183	0.998	0.991	1.003	0.372	0.913	0.801	1.040	0.172
CNTN1	Model 1	0.923	0.837	1.018	0.110	0.996	0.988	1.003	0.242	0.919	0.833	1.014	0.092
	Model 2	0.923	0.808	1.053	0.235	0.988	0.977	0.996	0.030	0.911	0.798	1.040	0.170
CRP	Model 1	0.925	0.839	1.020	0.120	0.997	0.990	1.003	0.346	0.922	0.836	1.017	0.105
	Model 2	0.914	0.801	1.043	0.181	0.998	0.991	1.006	0.638	0.912	0.800	1.041	0.174
IGF1	Model 1	0.924	0.838	1.019	0.115	0.998	0.991	1.003	0.373	0.922	0.836	1.016	0.103
	Model 2	0.920	0.806	1.049	0.215	0.995	0.986	1.002	0.213	0.915	0.803	1.043	0.187
MPO	Model 1	0.925	0.839	1.020	0.118	0.997	0.992	1.002	0.314	0.923	0.837	1.017	0.106
	Model 2	0.915	0.802	1.043	0.184	1.001	0.995	1.008	0.588	0.916	0.803	1.044	0.192
sICAM1	Model 1	0.922	0.835	1.017	0.106	0.994	0.988	0.999	0.073	0.916	0.830	1.011	0.083
	Model 2	0.914	0.801	1.042	0.178	0.996	0.989	1.000	0.183	0.910	0.798	1.038	0.159
UCMGP	Model 1	0.928	0.842	1.023	0.134	0.993	0.982	1.003	0.180	0.921	0.836	1.016	0.100
	Model 2	0.919	0.806	1.048	0.211	0.994	0.978	1.010	0.450	0.914	0.801	1.042	0.179

Linear mixed and mixed effect Cox proportional hazard models was applied. Covariates in Model 1 and 2 were the same as in Table S15. AHEI, Alternate Healthy Eating Index; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; HR, Hazard Ratio; LCI, lower confidence interval; MDS, Mediterranean-style diet score; UCI, upper confidence interval.

Table S18. *P*-values for the mediation effect (indirect effect) of diet-/CVD-related proteins on the associations of diet quality score with all-cause mortality after removal of events occurred in the first 2 years and 5 years after baseline in all study participants

Protein	AHEI		DASH		MDS	
	Exclusion of events during the first 2 years	Exclusion of events during the first 5 years	Exclusion of events during the first 2 years	Exclusion of events during the first 5 years	Exclusion of events during the first 2 years	Exclusion of events during the first 5 years
Adipsin	0.707	0.651	0.669	0.621		
ADM	0.175	0.126	0.148	0.108		
AGP1	0.093	0.167	0.067	0.134	0.152	0.212
B2M	0.034	0.031	0.073	0.069		
CD14	0.234	0.261	0.122	0.152		
CNTN1	0.030	0.034	0.018	0.022	0.033	0.037
CRP	0.098	0.192	0.089	0.171	0.132	0.214
Cystatin C	0.340	0.402				
GDF15	0.003	0.003				
GRN	0.334	0.344				
IGF1	0.058	0.057	0.093	0.093	0.070	0.068
MCP1			0.215	0.228		
MMP8	0.451	0.495				
MMP9	0.069	0.078				
MPO	0.128	0.141	0.146	0.161	0.162	0.176
sICAM1	0.034	0.037	0.055	0.058	0.118	0.123
UCMGP	0.004	0.004	0.005	0.006	0.005	0.005

Linear mixed effect and mixed effect Cox proportional hazard models were applied. Covariates were those adjusted in model 2 mediation analysis for all-cause mortality. AHEI, Alternate Healthy Eating Index; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; MDS, Mediterranean-style diet score.

Table S19. *P*-values for the mediation effect (indirect effect) of diet-/CVD-related proteins on the associations of diet quality score with incident CVD after removal of events occurred in the first 2 years and 5 years after baseline in all study participants

Protein	AHEI		DASH		MDS	
	Exclusion of events during the first 2 years	Exclusion of events during the first 5 years	Exclusion of events during the first 2 years	Exclusion of events during the first 5 years	Exclusion of events during the first 2 years	Exclusion of events during the first 5 years
Adipsin	0.833	0.639	0.835	0.640		
ADM	0.428	0.655	0.379	0.100		
AGP1	0.514	0.202	0.522	0.126	0.438	0.204
B2M	0.837	0.593	0.790	0.069		
CD14	0.803	0.513	0.912	0.160		
CNTN1	0.024	0.030	0.016	0.024	0.026	0.041
CRP	0.918	0.930	0.889	0.173	0.806	0.212
Cystatin C	0.546	0.995				
GDF15	0.027	0.044				
GRN	0.844	0.886				
IGF1	0.271	0.285	0.265	0.095	0.227	0.069
MCP1			0.578	0.222		
MMP8	0.616	0.667				
MMP9	0.593	0.496				
MPO	0.911	0.845	0.867	0.157	0.892	0.169
sICAM1	0.119	0.115	0.147	0.055	0.200	0.119
UCMGP	0.854	0.805	1.000	0.003	0.949	0.002

Linear mixed effect and mixed effect Cox proportional hazard models were applied. Covariates were those adjusted in model 2 mediation analysis for incident CVD. AHEI, Alternate Healthy Eating Index; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; MDS, Mediterranean-style diet score.

Table S20. *P*-values for the mediation effect (indirect effect) by diet-/CVD-related proteins on the associations of diet quality score with all-cause mortality in multi-marker analysis in all study participants

Protein	Correlated proteins for further adjustment	AHEI	DASH	MDS
Adipsin	ADM, AGP1, B2M, CD14, Cystatin C, GDF15, UCMGP	0.697	0.643	
ADM	Adipsin, B2M, Cystatin C, GDF15, UCMGP	0.116	0.116	
AGP1	Adipsin, B2M, CD14, CRP	0.059	0.048	0.125
B2M	Adipsin, ADM, AGP1, CD14, Cystatin C, GDF15, MCP1, sICAM1, UCMGP	0.022	0.048	
CD14	Adipsin, AGP1, B2M	0.175	0.106	
CRP	AGP1	0.073	0.065	0.105
Cystatin C	Adipsin, ADM, B2M, GDF15, MCP1, sICAM1, UCMGP	0.262		
GDF15	Adipsin, ADM, B2M, Cystatin C, MCP1, sICAM1, UCMGP	0.002		
MPO	B2M, Cystatin C, GDF15, sICAM1	0.104	0.093	0.124
sICAM1	B2M, Cystatin C, GDF15, MPO	0.029	0.052	0.110
UCMGP	Adipsin, ADM, B2M, Cystatin C, GDF15	0.002	0.005	0.004

Linear mixed effect and mixed effect Cox proportional hazard models were applied. In addition to correlated proteins, covariates were those adjusted in model 2 mediation analysis for all-cause mortality. AHEI, Alternate Healthy Eating Index; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; MDS, Mediterranean-style diet score.

Table S21. *P*-values for the mediation effect (indirect effect) by diet-/CVD-related proteins on the associations of diet quality score with incident CVD in multi-marker analysis in all study participants

Protein	Correlated proteins for further adjustment	AHEI	DASH	MDS
Adipsin	ADM, AGP1, B2M, CD14, Cystatin C, GDF15, UCMGP	0.926	0.905	
ADM	Adipsin, B2M, Cystatin C, GDF15, UCMGP	0.234	0.222	
AGP1	Adipsin, B2M, CD14, CRP	0.438	0.447	0.384
B2M	Adipsin, ADM, AGP1, CD14, Cystatin C, GDF15, MCP1, sICAM1, UCMGP	0.843	0.769	
CD14	Adipsin, AGP1, B2M	0.597	0.697	
CRP	AGP1	0.792	0.720	0.659
Cystatin C	Adipsin, ADM, B2M, GDF15, MCP1, sICAM1, UCMGP	0.369		
GDF15	Adipsin, ADM, B2M, Cystatin C, MCP1, sICAM1, UCMGP	0.016		
MPO	B2M, Cystatin C, GDF15, sICAM1	0.634	0.655	0.632
sICAM1	B2M, Cystatin C, GDF15, MPO	0.113	0.140	0.194
UCMGP	Adipsin, ADM, B2M, Cystatin C, GDF15	0.670	0.575	0.507

Linear mixed effect and mixed effect Cox proportional hazard models were applied. In addition to correlated proteins, covariates were those adjusted in model 2 mediation analysis for incident CVD. AHEI, Alternate Healthy Eating Index; CVD, cardiovascular disease; DASH, Dietary Approaches to Stop Hypertension; MDS, Mediterranean-style diet score.

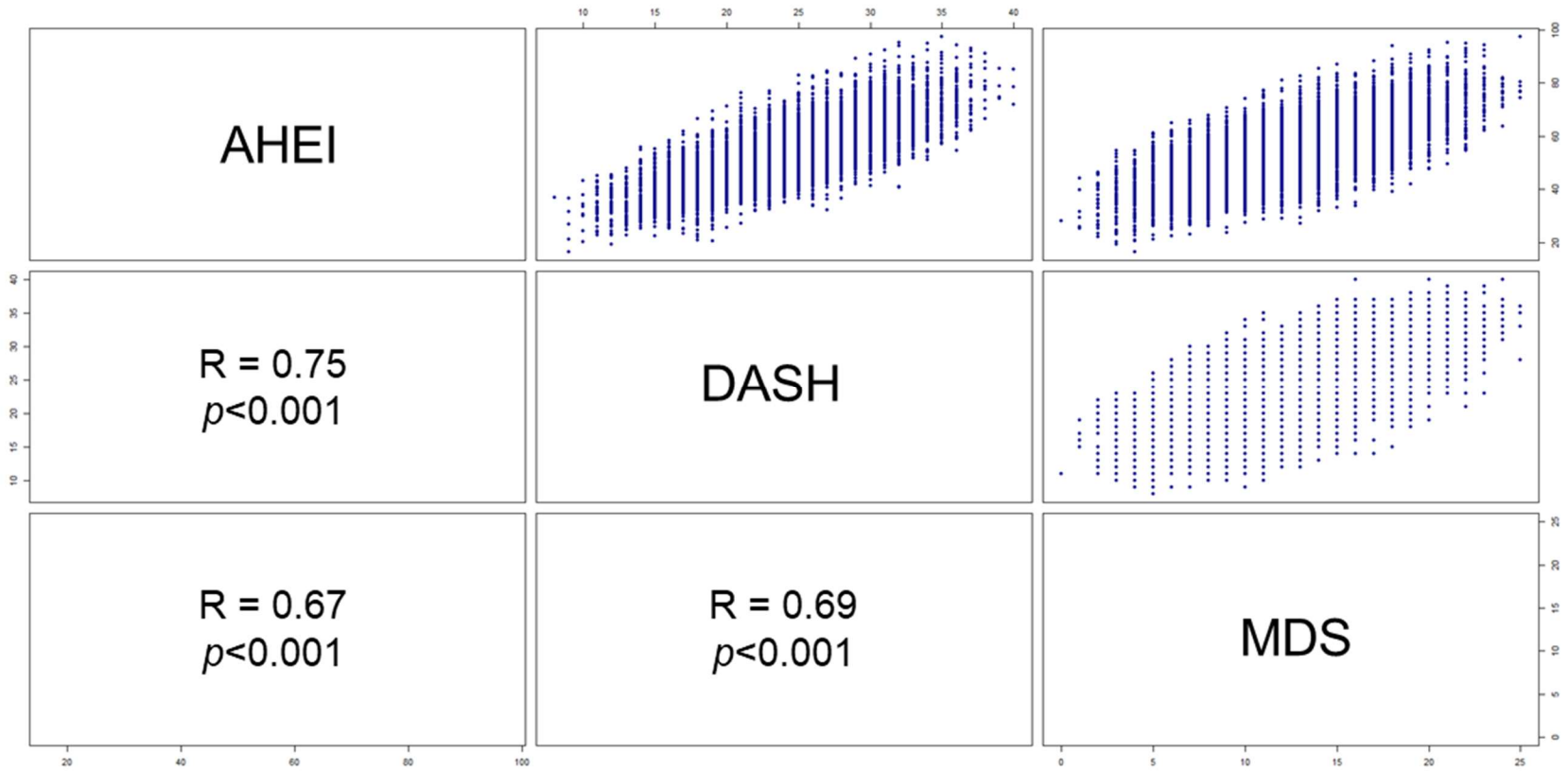

Figure S1. Pearson correlation matrix of diet quality scores. AHEI, Alternative healthy eating index; DASH, Dietary Approaches to Stop Hypertension score; MDS, Mediterranean-style dietary score.

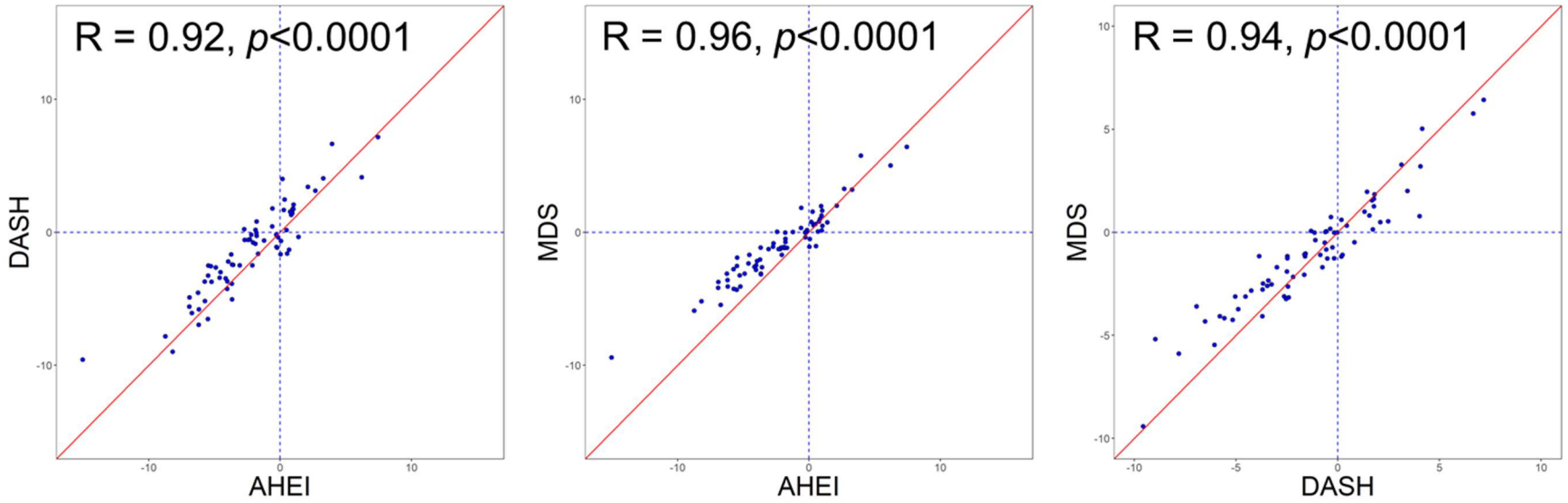

Figure S2. Pearson correlation coefficients of t-statistics between the three diet quality scores. AHEI, Alternative healthy eating index; DASH, Dietary Approaches to Stop Hypertension score; MDS, Mediterranean-style dietary score.

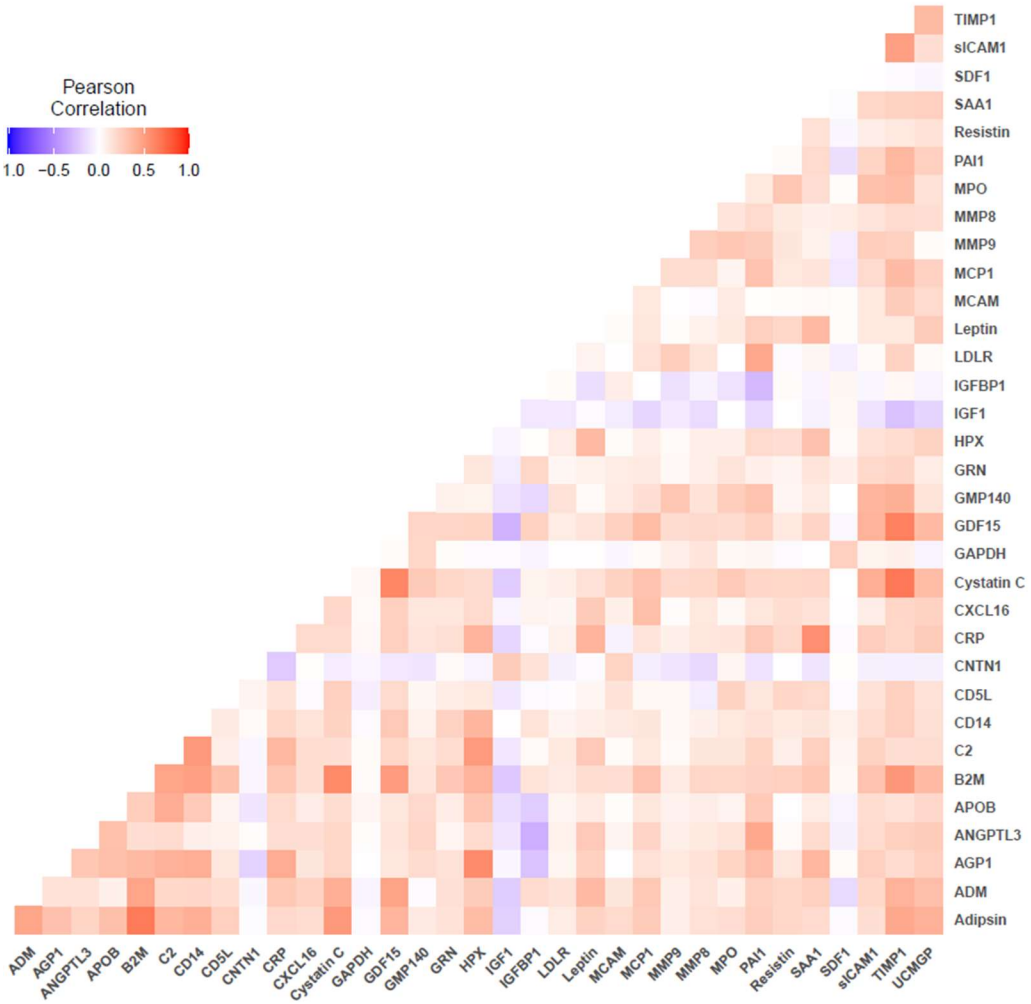

Figure S3. Heatmap of the Pearson's correlation coefficients between the 34 proteins. Positive correlations are shown in blue and negative correlations are in red. Darker color indicates stronger correlation.

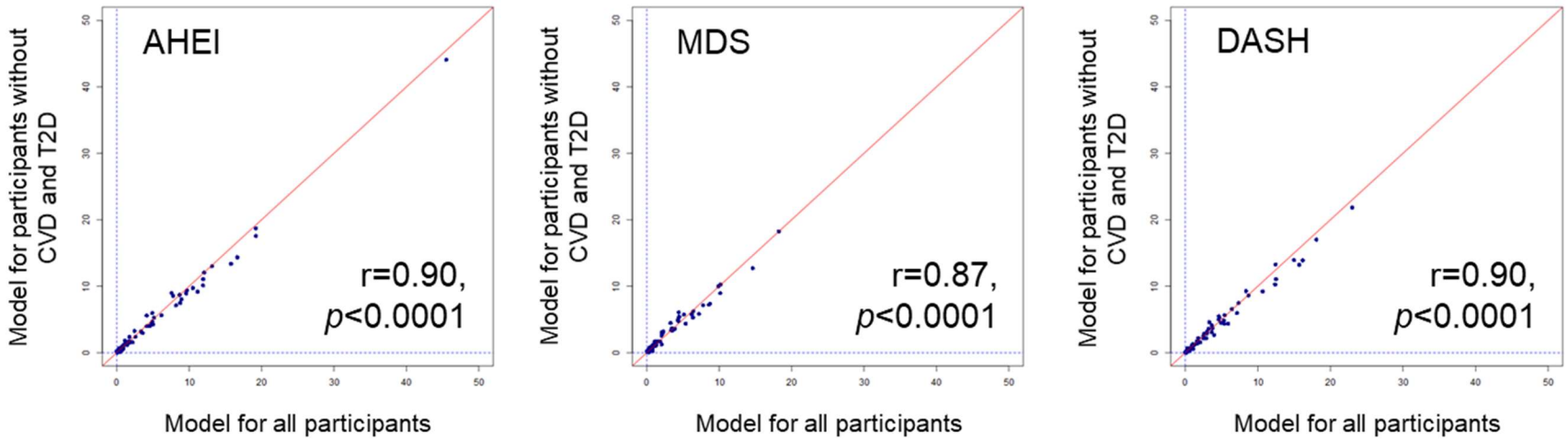

Figure S4. Pearson correlation coefficients between the *p*-values for the associations between three diet quality scores and CVD-related proteins (*n*=71). *P*-values (−log10 [*p* value]) for all participants (*n*=6,360) are shown on the x-axis, and *p*-values (−log10 [*p* value]) for the participants without CVD and type 2 diabetes (*n*=5,687) are shown on the y-axis. The model was adjusted for sex, age, and energy intake, smoking status, physical activity score, alcohol intake, and BMI. AHEI, Alternative healthy eating index; DASH, Dietary Approaches to Stop Hypertension score; MDS, Mediterranean-style dietary score.