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Serum proteins associated with LDL-C and non-HDL-C reduction in response to dietary interventions in the DASH and DASH-Sodium trials

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The Dietary Approaches to Stop Hypertension (DASH) diet reduced low-density lipoprotein (LDL)-cholesterol (C) and non-high-density lipoprotein (HDL)-C levels compared to the control diet. However, the mechanisms underlying the relation between the DASH diet and lipoprotein levels are not fully understood. We identified DASH diet-related proteins that were differently associated with LDL-C and non-HDL-C in two randomized controlled feeding studies comparing a DASH to control diet (DASH and DASH-Sodium trials). Two proteins [collagen triple helix repeat-containing protein 1 (CTHRC1) and growth differentiation factor 8 (MSTN)], and one protein [phytanoyl-CoA hydroxylase-interacting protein-like (PHYHIPL)], were differentially associated with changes in LDL-C and non-HDL-C, respectively, in the DASH and DASH-Sodium trials (P for interaction <0.05 for all tests). All proteins, except MSTN, were higher among those who consumed the DASH *vs.* control diets. All proteins were associated with reductions in lipoprotein levels among those who were provided with the DASH diets (range of β = −3.69 to −1.76) and elevations among those who were provided with the control diets (range of β = 0.20 to 3.47). Proteins involved in the TGF-β pathway and inflammatory processes that were lowered by the DASH diet were associated with reductions in LDL-C and non-HDL-C levels. Clinical Trial registration number: NCT03403166 (DASH trial), NCT00000608 (DASH-Sodium trial).

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Introduction

The Dietary Guidelines for Americans 2020–2025 recommends the Dietary Approaches to Stop Hypertension (DASH) diet for chronic disease risk reduction.[1] The DASH and DASH-Sodium trials, randomized, controlled, feeding studies, tested the efficacy of dietary patterns on blood pressure among individuals with systolic blood pressure <160 mmHg and diastolic blood pressure 80–95 mmHg. Findings from the two feeding studies provided strong evidence for such recommendation.[2,3] Relative to the control diets, which were designed to be similar to a typical US diet of the early 1990s, the DASH diets were high in fruits, vegetables, and low-fat dairy products and low in refined grains, sweets and desserts, and saturated fats.[4,5] The DASH and DASH-Sodium trials found that the DASH *vs.* control diets reduced low-density lipoprotein (LDL)-C.[6,7] Subsequent *meta*-analyses of randomized controlled trials and prospective cohort studies provided additional support for the reduction of LDL-C associated with the DASH diet.[8,9] In our secondary analyses of the DASH and DASH-Sodium trials, DASH *vs.* control diets reduced non-high-density lipoprotein (HDL)-C levels.

To our knowledge, no prior study examined the metabolic mechanism(s) for the favorable effect of the DASH diet on LDL-C and non-HDL-C. Large-scale proteomics, an approach that characterizes thousands of proteins in biofluids, can contribute to enhancing our understanding of the biologic processes underlying the DASH diet and lipoprotein relationship. Previously, our team has identified that 71 out of 7241 relative abundances of serum proteins were significantly different for individuals provided with the DASH *vs.* control diets using data from the DASH and DASH-Sodium feeding studies.[10] Considering the study design (feeding studies where participants received prepared meals), these 71 proteins are promising biomarkers of the DASH diet, and a subset of them may provide valuable information on how the DASH diet reduces lipoproteins.

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Using data from two randomized controlled feeding trials, DASH and DASH-Sodium, the objective of the present study is to identify serum diet-related proteins that were differently associated with changes in LDL-C and non-HDL-C by diet interventions (DASH *vs.* the control diets). Therefore, the present study extends our previous study[10] in that the proteins that were differentially associated by diet interventions may serve as objective biomarkers of diet that can be linked to cardiovascular risk factors and represent key mechanisms through which DASH diet reduces LDL-C and non-HDL-C concentrations. The analyses focused on LDL-C and non-HDL-C, given that these outcomes were inversely associations with the DASH diets in the DASH and DASH-Sodium trials, as well as atherosclerotic cardiovascular disease (ASCVD) risk.[6–9]

Materials and methods

Study design

The DASH and DASH-Sodium trials are multi-center, randomized controlled feeding trials conducted from 1994 and 1996, and from 1997 to 1999, respectively. Details of the two trials have been fully described elsewhere.[4,5] Briefly, the DASH trial compared the effect of 3 dietary patterns on blood pressure. Participants of the DASH trial were randomly assigned to either the (1) DASH diets, (2) fruits and vegetables diet, or (3) control diet for 8 weeks (parallel design). The DASH-Sodium trial tested the combined effect of dietary patterns and sodium intake on blood pressure. Participants of the DASH-Sodium trial were randomly assigned to either the DASH or control diets. Within the two diet interventions, participants were randomized to 3 sodium phases (high, intermediate, low) for 4 weeks each (cross-over design). The sodium interventions were separated by 5 days each. Eligible participants for the two trials were similar: adults (≥22 years) with systolic blood pressure <160 mmHg and diastolic blood pressure 80–95 mmHg who were not taking antihypertensive medication. Study protocols carried out in accordance with the Declaration of Helsinki and were approved by the Institutional Review Boards at each study site. Written informed consent was obtained from all participants. The two trials were registered at https://clinicaltrialsov/ as NCT03403166 (DASH trial) and NCT00000608 (DASH-Sodium trial).

Large-scale proteomics data were obtained using archived blood samples at the end of the following diet interventions: (1) DASH and control diet for the DASH trial and (2) DASH and control diet at the end of the high sodium phases for the DASH-Sodium trial. The highest sodium phases were selected for the DASH-Sodium trial (3600 mmHg), because the sodium level was comparable to the sodium level in the DASH trial (3000 mmHg).

Of the 459 and 412 participants of the DASH and DASH-Sodium trials, respectively, participants who were assigned to the fruits and vegetables diet intervention in the DASH trial were excluded (n_{DASH} = 154). Then, those who did not consent to use of serum specimen or those without a serum specimen (n_{DASH} = 88, $n_{DASH\text{-}Sodium}$ = 16), those with poor serum specimen quality (n_{DASH} = 2, $n_{DASH\text{-}Sodium}$ = 0), and those missing LDL-C and HDL-C at baseline or follow-up (n_{DASH} = 4, $n_{DASH\text{-}Sodium}$ = 9) were excluded. The final analytic sample was 211 for the DASH trial and 387 for the DASH-Sodium trial (SI Fig. S1).

Dietary interventions

In the DASH trial, participants were provided with the control diet for 3 weeks.[4] In the DASH-Sodium trial, participants were provided with the control diet high in sodium for 11–14 days.[5] After these run-in periods, participants consumed either the DASH or control diets. The DASH diets were high in fruits, vegetables, and low-fat dairy; moderate in nuts, seeds, legumes, fish, and poultry; and low in red and processed meats, and snacks and sweets. The control diets were designed to represent typical diets consumed by Americans in early 1990s.

In terms of macronutrient composition, the DASH diets were comprised of 55% energy from carbohydrates, 18% from protein, and 27% from fat.[2,3] The control diets were comprised of 48% of energy from carbohydrates, 15% from protein, and 37% from fat. The DASH diets were much lower in saturated fat compared to the control diets (6% *vs.* 16% of total energy intake). Relative to the control diets, the DASH diets were also higher in fiber, potassium, magnesium, and calcium, and lower in cholesterol. In the DASH diets, the content of potassium, magnesium, and calcium were targeted to be at the 75th percentile of consumption in the US in the 1980s. In the control diets, the content of potassium, magnesium, and calcium were targeted to be at the 25th percentile of consumption in the US. In the control diets, fiber content was similar to the average US consumption. Sodium level was similar in the DASH and control diets in the DASH trial (approximately 3000 mg per day) and the DASH-Sodium trial (approximately 3600 mg day^{-1}).

A seven-day rotating menu was prepared at each study center's research kitchen (4 study centers each for the DASH and DASH-Sodium trials) using a standard protocol. Participants ate at least one meal on site on weekdays. For all other meals, participants received cooked meals to consume off site. Participants' weight was measured on weekdays and total energy intake was adjusted to maintain a weight stable throughout the trial. Adherence to the diets were assessed by monitoring participants' daily food diary and measuring 24 hour urinary excretion of urea nitrogen, sodium, potassium, and phosphorus. Adherence to the diets was high.[2,3]

Large-scale proteomics in the DASH and DASH-Sodium trials

Fasting serum specimens, which were collected at the end of each feeding period, were sent to SomaLogic (SomaLogic Inc, Colorado, Boulder) for large-scale proteomic analyses. Slow off-rate modified aptamers (SOMAmer), modified single strand DNAs, were used as protein-binding reagents to identify >7000 protein targets and protein complexes. This array was reported to have high specificity and sensitivity. Blind duplicates generated from the participants in the DASH and DASH-Sodium

trials showed high correlation coefficients (median r_{DASH} = 0.95; median $r_{DASH\text{-}Sodium}$ = 0.97) and low coefficient of variation (CV_{DASH} = 0.050; $CV_{DASH\text{-}Sodium}$ = 0.044), which were reported in a previous publication.[10]

Of the 7596 protein targets identified using SOMAmers, analyses were conducted in 71 proteins that were significantly different between the DASH and control diets in the DASH and DASH-Sodium trials. All proteins were $\log_2$-transformed to address skewness, and the relative abundances of proteins were winsorized at ±5 standard deviations. Details on the quality control procedures were reported in a previous publication.[10]

Measurements

Trained staff collected twelve-hour fasting blood specimens at baseline and at the end of the feeding period. In the DASH trial, the baseline was end of the screening period (before the run-in period).[6] In the DASH sodium trial, the baseline was the end of run-in period.[7] The present study focused on LDL-C and non-HDL-C, because non-HDL-C was significantly reduced among individuals who were randomized to the DASH *vs.* control diets (SI Table S1).

In the DASH trial, frozen plasma specimens were analyzed at a core laboratory, Oregon Health Sciences University Lipid-Atherosclerosis Laboratory.[6] Enzymatic colorimetric methods and an automated analyzer (Hitachi, Indianapolis, Indiana) were used to measure total cholesterol, and HDL-C.

In the DASH-Sodium trial, frozen serum specimens were analyzed at the Core Laboratory for Clinical Studies at Washington University School of Medicine.[7] Enzymatic kits using the Hitachi 917 automated analyzer were used to determine the amounts of total cholesterol (Miles-Technicon kit), and HDL-C (Miles-Technicon cholesterol kit).

In both trials, lipoprotein measurements were standardized in accordance with the National Heart, Lung, and Blood Institute Lipid Standardization program.[11] LDL-C was estimated using the Friedewald equation.[12] Non-HDL-C levels were estimated by subtracting LDL-C from total cholesterol levels. Change in LDL-C and non-HDL-C levels were calculated as the difference between the end-of-trial measurements and baseline measurements.

Statistical analyses

Baseline characteristics of the study participants in the DASH and DASH-Sodium trials were summarized using means (standard deviations, SD) for continuous variables and proportions for categorical variables. Within each study, participants were stratified by LDL-C <130 mg dL^{-1} and LDL-C ≥130 mg dL^{-1} to assess the associations between the baseline characteristics and the main outcome of interest.

Multivariable linear regression models with cross-product terms between diet and 71 DASH diet-related proteins (per 1-SD higher) were used to identify proteins that were differentially associated by diet interventions (DASH *vs.* control). Models were conducted in each study, and were adjusted for self-reported age, sex, race/ethnicity, and measured baseline body mass index (BMI). Covariates were adjusted to account for the fact that the analytic samples were a subset of the DASH and DASH-Sodium trials, and to increase precision of the estimates. *meta*-Analyses using fixed effects models were conducted across the DASH and DASH-Sodium trials. Fixed effects models were selected because the two trials were similar in terms of study populations, study diets, study procedures, proteomic profiling, and lipid measurements. When heterogeneity across the two trials for the associations between proteins and LDL-C and non-HDL-C were examined using Cochran's *Q* test, only 1 protein (alpha-1*B*-glycoprotein) had Cochran's *Q* test <0.05, suggesting low heterogeneity. Estimates and *P* values were stratified by diets (DASH *vs.* control) for proteins with statistically significant interaction (*P* values for interaction <0.05, nominal significance). The statistical threshold was not adjusted for multiple comparisons given the nature of exploratory analyses which aimed to maximize the ability to discover proteins that were related to a reduction in LDL-C and non-HDL-C.

Then, for proteins with statistically significant interaction, we calculated unadjusted Pearson correlations between relative abundances of the significant proteins measured at the end of each dietary intervention and changes in LDL-C and non-HDL-C levels from baseline to end-of-study, after pooling data from the DASH and DASH-Sodium trials. The correlation matrices were presented separately by diet interventions. Next, we plotted the relative abundances of proteins measured at the end of the dietary interventions against changes in LDL-C or non-HDL-C, separately for DASH and DASH-Sodium trials. Further, we used colored dots and trend lines to visually describe the associations between proteins and lipoprotein associations. Covariates were not adjusted in these scatterplots.

Since adjustment for multiple testing was not conducted, as a secondary analyses, elastic net linear regression models were used to identify a weighted combination of proteins associated with changes in LDL-C and non-HDL-C. As tuning parameters, lambda.1se (sparsity of the model) and α = 0.5 (relative weights of ridge and lasso regression models) were selected. All 71 DASH diet-related proteins were considered for elastic net models.

As a sensitivity analysis, for LDL-C and non-HDL-C, we adjusted for the corresponding baseline values. Scatterplots for two proteins [growth differentiation factor 8 (MSTN) and phytanoyl-CoA hydroxylase-interacting protein-like (PHYHIPL)] were re-evaluated after excluding extreme values which may influence the association with lipoproteins (relative abundance of MSTN >40 for the DASH trial, and MSTN 54 for the DASH-Sodium trial; relative abundance of PHYHIPL >26 for the DASH trial and PHYHIPL >26 for the DASH-Sodium trial).

Results

Baseline characteristics of participants were similar for the DASH and DASH-Sodium trials (Table 1). In both trials, more

Table 1 Participant characteristics of the DASH and DASH-Sodium trials[a]

	DASH (N = 211)	DASH-sodium (N = 387)
Age, %		
≤30 years	26 (12.3%)	12 (3.1%)
31–50 years	115 (54.5%)	226 (58.4%)
≥51 years	70 (33.2%)	149 (38.5%)
Women, %	100 (47.4%)	222 (57.4%)
Race or ethnicity, %		
African American	119 (56.4%)	222 (57.4%)
Income, %		
<$29 999	69 (32.7%)	123 (31.8%)
$30 000–$59 999	94 (44.5%)	141 (36.4%)
≥$60 000	46 (21.8%)	114 (29.5%)
Not answered	2 (0.9%)	9 (2.3%)
Education, %		
High school graduate or less	31 (14.7%)	65 (16.8%)
Some college	77 (36.5%)	141 (36.4%)
College graduate	59 (28.0%)	90 (23.3%)
Post graduate work/degree	44 (20.9%)	90 (23.3%)
Not answered	0 (0%)	1 (0.3%)
Current smoker, %	16 (7.6%)	40 (10.3%)
Weight (kg)	82.7 (14.9)	84.0 (15.3)
Height (cm)	171.0 (9.05)	170.0 (9.22)
Body mass index, kg m^{-2}	28.2 (3.91)	29.2 (4.77)
Systolic blood pressure, mmHg	130.1 (10.5)	135.0 (9.51)
Diastolic blood pressure, mmHg	84.6 (4.44)	85.6 (4.49)
Hypertension,[b] %	96 (45.5%)	175 (45.2%)
Low-density lipoprotein-cholesterol, mg dL^{-1}	119.5 (31.9)	131.3 (30.2)
High-density lipoprotein-cholesterol, mg dL^{-1}	48.8 (15.9)	48.7 (12.9)
Non-high-density lipoprotein-cholesterol, mg dL^{-1}	142.2 (34.7)	152.5 (34.2)
Total cholesterol, mg dL^{-1}	191.0 (35.5)	201.2 (34.6)
Triacylglycerols, mg dL^{-1}	113.5 (56.6)	106.1 (57.4)

[a] Values are mean (standard deviation) for continuous variables and n (proportions) for categorical variables. [b] Hypertension was defined as doctor diagnosis of high blood pressure.

than half of the participants were between 31–50 years of age, women, or African American. Income, education, current smoking status, and BMI were similar. Baseline systolic blood pressure for participants in the DASH-Sodium trial was higher (135 mmHg) compared to those in the DASH trial (130 mmHg). Total cholesterol, HDL-C, and triacylglycerol levels were similar for participants in the DASH and DASH-Sodium trials, but LDL-C and non-HDL-C was on average 10–11 mg dL^{-1} lower among those in the DASH trial. When stratified by LDL-C (<130 or ≥130 mg dL^{-1}) in each study, most characteristics were not significantly different (SI Table S2). In the DASH-Sodium trial, those with LDL-C ≥130 mg dL^{-1} were more likely to be older and have higher triacylglycerols. In both trials, those with LDL-C ≥130 mg dL^{-1} had higher total cholesterol, LDL-C, and non-HDL-C.

At nominal significance, two [collagen triple helix repeat-containing protein 1 (CTHRC1) and MSTN] and one protein (PHYHIPL) were differentially associated with changes in LDL-C and non-HDL-C levels, respectively, in the two trials (Fig. 1 and SI Table S2). All three proteins were associated with reductions in LDL-C or non-HDL-C among those who were provided with the DASH diets, and elevations among those provided with the control diets. Results from unadjusted Pearson correlations between CTHRC1, MSTN, and LDL-C were consistent with the main analysis (SI Fig. S2A and S2B). CTHRC1 and MSTN were correlated with reductions in LDL-C among those who consumed the DASH diets, but elevations among those who consumed the control diets. PHYHIPL showed the same trend with non-HDL-C.

CTHRC1 was associated with a more pronounced elevation in LDL-C among those who received the control diet in the DASH-Sodium trial than those who received the control diet in the DASH trial (Fig. 2). However, for MSTN, the trends for LDL-C were different among those who received the control diets in the DASH (reduction in LDL-C) and DASH-Sodium trial (elevation in LDL-C) for MSTN (Fig. 3). The trends for PHYHIPL and non-HDL-C were similar across the DASH and DASH-Sodium trials (SI Fig. S3).

In secondary analyses of elastic net regression models, three additional proteins [synaptic vesicle membrane protein VAT-1 homolog (VAT1), V-set and immunoglobulin domain-containing protein 4 (VSIG4), and apolipoprotein B (APOB)] predicted changes in LDL-C and non-HDL-C in the DASH-Sodium, but not in the DASH trial (SI Table S3). In the DASH-Sodium trial, VAT1 and APOB were associated with elevations in LDL-C and non-HDL-C. VSIG4 was associated with reductions in LDL-C and non-HDL-C.

As a sensitivity analysis, which adjusted for baseline LDL-C or non-HDL-C, P values for interactions were attenuated for CTHRC1 and MSTN and LDL-C (P for interaction for both tests >0.05) (SI Table S4). However, the direction of the association for CTHRC1 and MSTN was the same as the main analyses. The association PHYHIPL and non-HDL-C did not change substantially. After excluding extreme values for MSTN and PHYHIPL, the associations between proteins and changes in lipoproteins for those who consumed the DASH or control diets did not change.

Discussion

In adults with moderately elevated blood pressure who were randomly assigned to receive the DASH or control diets, two (CTHRC1 and MSTN) and one (PHYHIPL) proteins were associated with reductions in LDL-C and non-HDL-C, respectively, in individuals who consumed the DASH diet, and with elevations in LDL-C and non-HDL-C among those who consumed the control diets at nominal significance. The associations between CTHRC1, MSTN, and LDL-C were modestly different across the DASH and DASH-Sodium trials. In our previous study, except for MSTN, the relative abundances of all proteins were higher among those who were randomly assigned to the DASH *vs.* control diets.[10] In elastic net models, two proteins (VAT1 and APOB) predicted elevations in LDL-C, whereas VSIG4 predicted reductions in LDL-C and non-HDL-C. Our results which found differential associations between proteins and changes in lipoprotein by diet interventions, highlight the

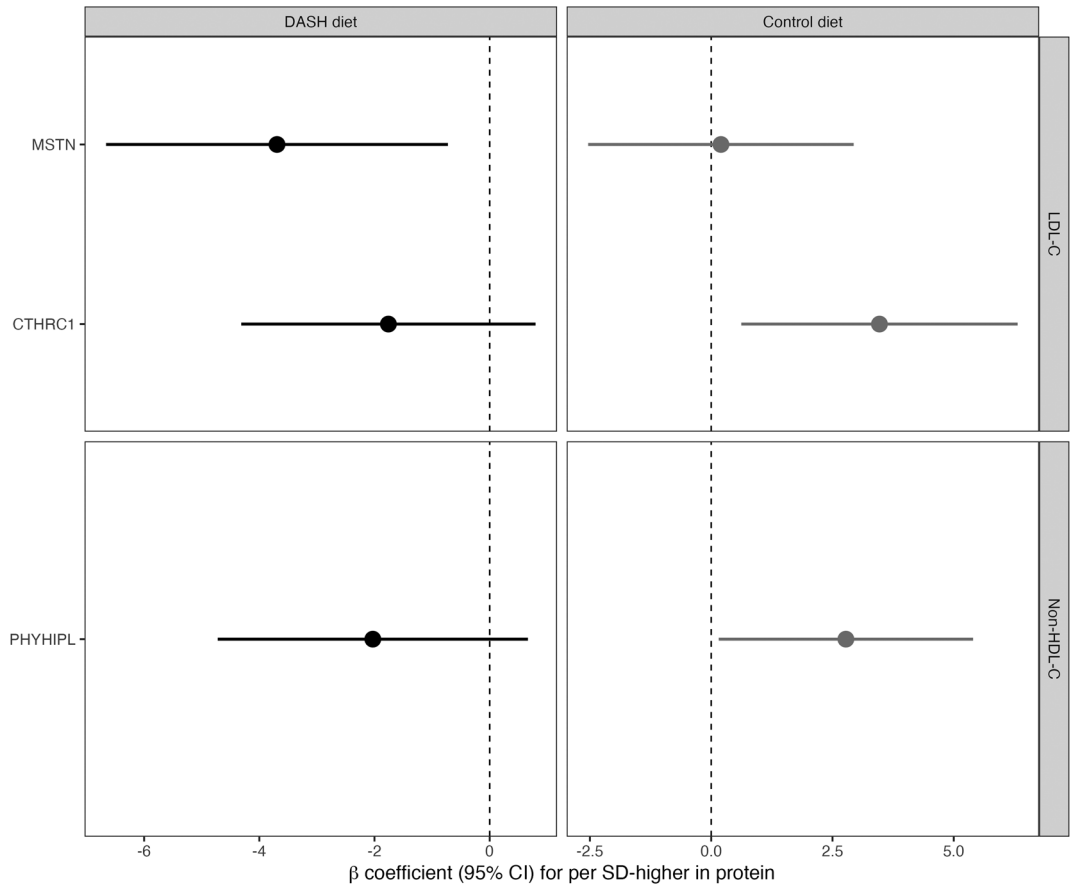

Fig. 1 β coefficients and 95% confidence intervals (CIs) for the association between DASH diet-related proteins and low-density lipoprotein (LDL)-cholesterol (C) and non-high-density lipoprotein (HDL)-C. Estimates were derived from multivariable linear regression models of 71 DASH diet-related proteins (per 1-SD higher) and LDL-C and non-HDL-C for each diet intervention, after adjusting adjusted for age, sex, race/ethnicity, and body mass index (BMI). *meta*-Analyses using fixed effects models were conducted across the DASH and DASH-Sodium trials. Only significant proteins at *P* for interaction <0.05 are shown.

protein targets that are decreased by the DASH diet and increased by the control diet and are associated with lowering of LDL-C and non-HDL-C.

Collagen triple helix repeat-containing protein 1 (CTHRC1) was found to be expressed in injured rat arteries but not in normal arteries.[13] This protein is known to favorably impact vascular remodeling after injury by reducing collagen deposition and increasing cell migration.[14] CTHRC1 is considered an antagonist of the TGF-β pathway, a pathway which controls vascular remodeling and promotes collagen deposition and smooth muscle cell proliferation.[15] Growth differentiation factor 8 (MSTN), known as myostatin, belongs to the TGF-β superfamily. Myostatin is found in skeletal and cardiac muscle, inhibits growth of skeletal muscle and play a role in cardiac development.[16] In mouse models, inhibition of myostatin gene reduced fat accumulation and improved insulin resistance.[17] In LDL receptor null mice (a model used for atherogenesis) fed a Western-style diet, loss of myostatin function led to lower levels of LDL-C, total cholesterol, and very low-density lipoprotein (VLDL), compared to LDL receptor null mice with preserved myostatin function.[18] Serum myostatin was positively correlated with LDL-C in French adults with acute myocardial infarction, consistent with our findings among those who received the control diets.[19] We previously reported that serum levels of CTHRC1 was higher and myostatin were lower among those who were provided with the DASH *vs.* control diets.[10] A major difference in the DASH and control diets was saturated fat content. It is possible that the DASH diet (lower saturated fat intake), influences the TGF-β pathway, and may modify CTHRC1 and myostatin to reduce LDL-C. CTHRC1 and MSTN, preliminary candidate proteins that provide compelling link between consumption of DASH diet and reductions in LDL-C, might be considered for validation in independent populations.

V-set and immunoglobulin domain-containing protein 4 (VSIG4) was associated with a reduction in LDL-C and non-HDL-C in elastic net models. VSIG4, known as complement receptor immunoglobulin (CRIg), is a receptor of complement fragments C3b and iC3b and is expressed in tissue macrophages.[20] CRIg plays an important role in clearing bacteria and viruses, highlighting a role in innate immune response.[21] In response to infection or inflammation, *in vitro* studies have

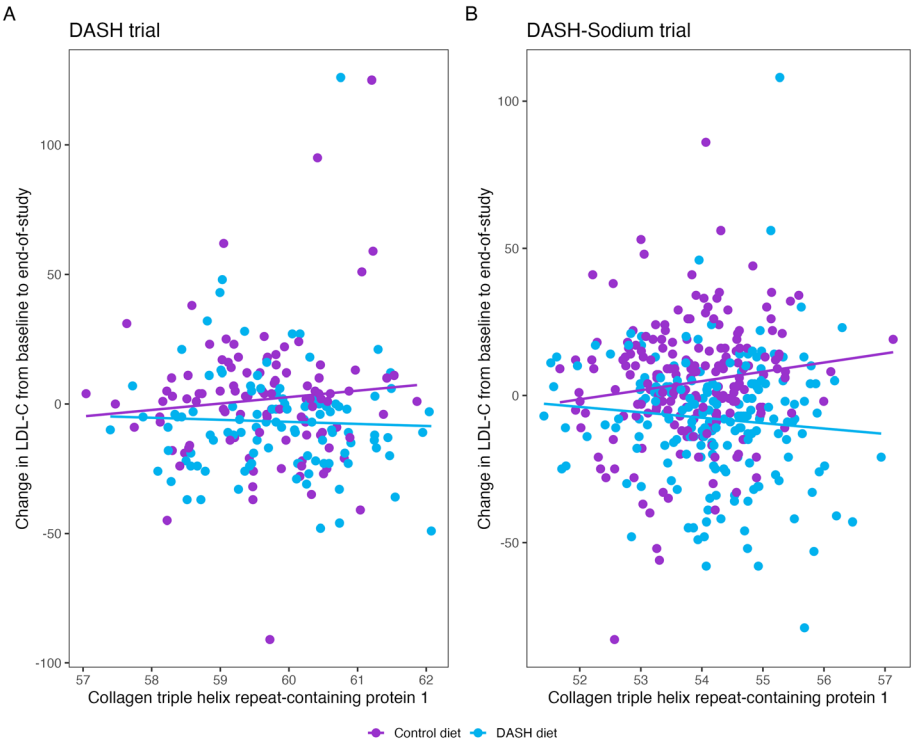

Fig. 2 Scatterplots for the association between relative abundances of collagen triple helix repeat-containing protein 1 (CTHRC1) measured at the end of the diet interventions (DASH or control diets) and change in low-density lipoprotein (LDL)-cholesterol (C) from baseline to end-of-study, stratified by DASH and DASH-Sodium trials. Trend lines were added to visually describe the association between CTHRC1 and change in LDL-C by diet interventions. Covariates were not adjusted.

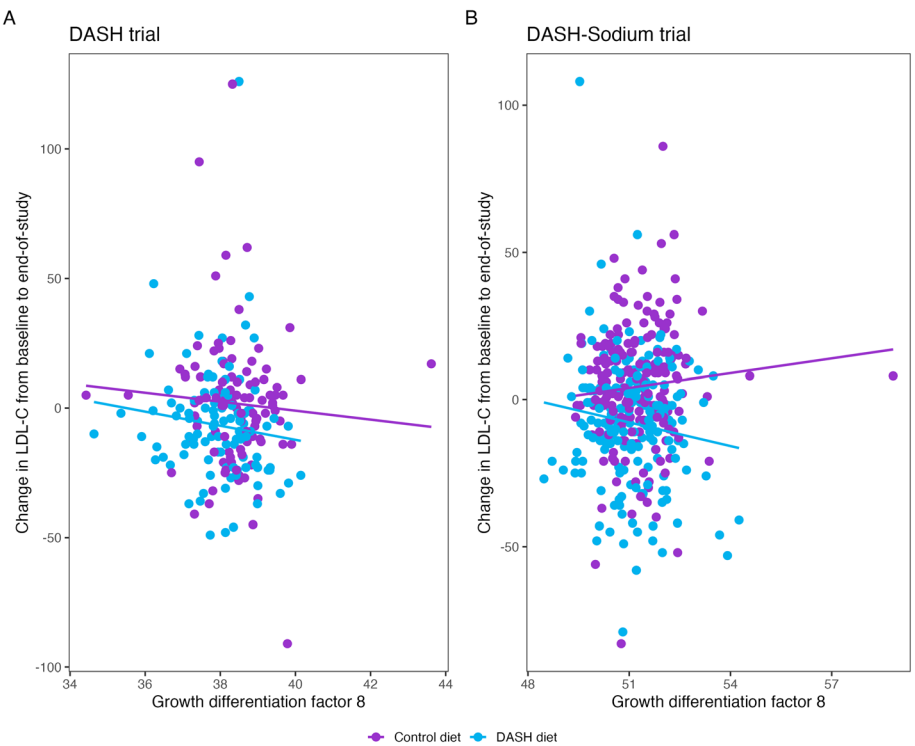

Fig. 3 Scatterplots for the association between relative abundances of growth differentiation factor 8 measured at the end of the diet interventions (DASH or control diets) and low-density lipoprotein (LDL)-cholesterol (C) from baseline to end-of-study, stratified by DASH and DASH-Sodium trials. Trend lines were added to visually describe the association between MSTN and change in LDL-C by diet interventions. Covariates were not adjusted.

reported that VSIG4 reduces T cell proliferation and IL-2 levels, as well as mitochondrial reactive oxygen species and M1 macrophages.[22,23] Additionally, in animal models, inhibition of VSIG4 function has been associated with inflammatory conditions. Among mice which were fed a high fat-diet for 5 weeks, VSIG4 knockout mice gained more body weight and levels of total cholesterol, triglycerides, free fatty acids, and glucose were increased compared to controls.[23] These results suggest that VSIG4 may also have favorable effects on inflammatory conditions, such as reducing LDL-C and non-HDL-C.

We identified a well-known protein positively associated with LDL-C, APOB, and two novel proteins [synaptic vesicle membrane protein VAT1 and PHYHIPL associated with reductions in LDL-C and non-HDL-C]. APOB is an integral protein of LDL-C, VLDL, chylomicrons, and lipoprotein (a).[24] Therefore, it is expected that APOB and LDL-C are positively correlated. VAT1, a membrane protein, stores and releases neurotransmitters in synaptic vesicles.[25] PHYHIPL is hypothesized to be involved in the development of central nervous system and a preliminary report found that PHYHIPL gene expression was down-regulated in human brains with glioblastoma multiforme.[26] No prior study reported an association between VAT1, PHYHIPL, and lipoprotein levels. Of these, PHYHIPL is known to interact with phosphodiesterase 9A (PDE9A),[27] a protein known to hydrolyze cAMP and cGMP.[28] A recent study reported that inhibition of PDE9A stimulated lipolysis and reduced intracellular lipid accumulation,[29] an observation which raises a possibility that PHYHIPL might be also involved in lipid metabolism in an undefined manner. Thus, our findings on PHYHIPL specifically should be interpreted with caution, and future research on the mechanism of this protein is warranted.

Our study has several limitations. First, given that the sample sizes of the two trials were modest, we did not adjust for multiple testing in assessing the statistical significance of the interaction. However, we observed that the direction of the associations for proteins were in the opposite direction for the DASH diets compared to the control diets, lending support for our finding that three proteins were differentially associated with changes in LDL-C and non-HDL-C. To supplement interaction analyses, we conducted elastic net models, which are effective at feature selection in high-dimensional data and reducing overfitting. Findings which emerged from elastic net models provide robust supporting evidence that the blood proteome is involved in dietary intake and lipoprotein relations and offer additional insights underlying diet-disease relations. Second, our results were largely consistent when we adjusted for baseline lipoprotein levels as a covariate. However, the *P*-value for interaction for the association between CTHRC1 and MSTN and LDL-C was attenuated after adjusting for baseline LDL-C. This observation suggests that the association between serum proteins and LDL-C by dietary interventions may partially be contingent on the baseline LDL-C level. In future studies that examine the role of serum proteins in response to dietary intake, it would be important to consider initial lipoprotein values. Third, timing of LDL-C and non-HDL-C at baseline was not consistent across the DASH (before run-in period) and DASH-Sodium trials (after run-in period). However, heterogeneity across the trials was low when we formally tested for heterogeneity using Cochran's *Q*. Fourth, proteomic profiling was measured only at the end of the interventions. By not accounting for baseline protein levels, we may have been unable to detect additional proteins that were associated with change in LDL-C and non-HDL-C. Fifth, given the small number of proteins differentially associated with lipoprotein reduction ($n = 3$) at P for interaction <0.05, the present study could not conduct pathway analysis. In the future, a formal pathway analysis may provide useful information. Lastly, we could not conduct subgroup analysis based on blood pressure levels (high or low blood pressure), because all participants had moderately elevated blood pressure at baseline.

The strengths of our study are randomized study designs, provision of all study diets, high adherence to dietary interventions, rigorous measurement of LDL-C and non-HDL-C in both trials, and results from elastic net models which identified additional proteins associated with changes in LDL-C and non-HDL-C. Further, approximately half of the participants of the DASH and DASH-Sodium trials was women and African Americans. Our study results may be generalizable to different subgroups.

Conclusion

The present study identified three proteins (CTHRC1, MSTN, PHYHIPL) associated with reductions in LDL-C or non-HDL-C among those who received the DASH diets and elevations among those who received the control diets. These findings provide support for the assertion that the DASH diet may reduce LDL-C and non-HDL-C by influencing the TGF-β pathway and inflammatory processes. The results may help provide targeted interventions for lowering of LDL-C and non-HDL-C. Clinical significance has yet to be determined for these proteins. Future studies could follow up on whether measurement and monitoring of these proteins can reduce LDL-C and non-HDL-C levels.

Author contributions

H. K.: data curation, formal analysis, investigation, methodology, writing – original draft. A. H. L., J. C. and L. J. A: writing – review & editing; C. M. R.: conceptualization, funding acquisition, investigation, supervision, resources, project administration, writing – review & editing. The authors thank the participants, staff, and investigators of the DASH and DASH-Sodium trials for their important contributions.

Conflicts of interest

All authors have no conflict of interests to disclose.

Data availability

Study data of the DASH and DASH-Sodium trial, are available through the National Heart, Lung, and Blood Institute Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC). Proteomics data generated from the DASH and DASH-Sodium trials follow the existing data sharing protocols, thus will not be shared publicly. Interested researchers should contact the corresponding author to request access to proteomics data sets.

Supplementary information (SI), including Supplemental Fig. 1–3 and Supplemental Tables 1–4 is available. See DOI: https://doi.org/10.1039/d5fo02593a.

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