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Impact on cardiometabolic risk of a weight loss intervention with higher protein from lean red meat: Combined results of 2 randomized controlled trials in obese middle-aged and older adults

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BACKGROUND: The recognized benefits of a higher protein diet on muscle mass and strength in older adults are tempered by concerns of the potentially negative cardiometabolic impact of dietary sources of animal protein.

OBJECTIVE: The aim of this study was to explore the cardiometabolic impact of 2 weight reduction diets: a higher protein diet, providing balanced portions of lean beef and pork throughout the day, vs. a diet following the Recommended Daily Allowance level of protein in obese middle-aged and older adults.

METHODS: Data from Measuring Eating, Activity and Strength: Understanding the Response-Using Protein and Protein Optimization in Women Enables Results-Using Protein were combined for the present analysis. Subjects were randomly assigned to a 6-month weight loss diet (500 kcal deficit) and prescribed a Recommended Daily Allowance level of protein (0.8 g protein/kg BW), control group, or a higher level of protein (1.2 g protein/kg BW), protein group. For the protein group, lean, high-quality protein was evenly distributed between meals or balanced throughout the day (30 g protein/meal). The following cardiometabolic markers were quantified by nuclear magnetic resonance spectroscopy: lipids, lipoproteins, GlycA, trimethylamine-N-oxide, betaine, branched-chain amino acids, and lipoprotein insulin resistance index scores.

RESULTS: In both groups (control [n = 27] and protein [n = 53]), there were significant ($P \leq .05$) changes from baseline in weight loss (−6.2% and −7.2%), distance walked (+53.1

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and +75.0 meters), and fasting plasma glucose (−7.5 and −6.2 mg/dL), respectively. At endpoint, protein group had significantly ($P \leq .05$) lower triglycerides (−17.3 mg/dL), large very-low-density lipoprotein particle concentration (VLDL-P; −1.2 nmol/L), total low-density lipoprotein particle concentration (LDL-P; −67.8 nmol/L), small LDL-P (−59.4 nmol/L) and lipoprotein insulin resistance index (−5.9), whereas control group had significantly ($P \leq .05$) lower GlycA (−13.1 μmol/L), total VLDL-P (−7.9 nmol/L), and small VLDL-P (−7.0 nmol/L). Differences between groups were observed for small VLDL-P ($P = .02$) and protein intake ($P < .0001$).

CONCLUSIONS: These findings suggest that a hypocaloric diet with either traditional (0.8 g/kg BW/d) or higher protein (1.2 g/kg BW/d; predominantly from lean red meat) content improves risk markers of cardiovascular disease and type II diabetes in obese middle-aged and older adults. Both diets were also associated with improved physical function, and neither had an adverse impact on cardiometabolic outcomes.

Introduction

Dietary intake of high-quality protein that is both ample and balanced has many known benefits for older adults, including promotion of muscle protein synthesis,[1] preservation of lean mass, muscle strength, and function,[2,3] higher bone mineral density,[4,5] and improved markers of glucose metabolism[6]; it may also reduce fracture risk,[7] inflammation, and oxidative stress.[8] This aggregation of benefits may be especially important during obesity interventions for older adults, many of whom have experienced diminished muscle mass and strength. Loss of lean muscle mass and strength in combination with obesity is a multiplier of detrimental health effects in mid- and later-life. It not only markedly reduces physical function,[9,10] but also accelerates the development of type II diabetes and cardiovascular disease (CVD).[11] Effective treatment of obesity in middle-aged and older adults requires preferential reduction of adipose tissue while maintaining lean mass. Improving the quality, quantity, and timing of protein intake during weight reduction may favor these outcomes.[12,13] This conclusion is supported by our findings that high-quality protein, above the Recommended Daily Allowance (RDA) and balanced throughout the day (evenly distributed between meals), benefits physical function (eg, Short Physical Performance Battery) during obesity reduction in functionally limited older adults[14] and by a recent review/meta-analysis[15] showing a protein benefit for preservation of lean mass during weight reduction. An obesity treatment that improves function and preserves lean mass also allows for strong cardiometabolic benefits by improving the ability to be more physically active.

Optimizing both quantity and quality of protein intake in the context of a hypocaloric diet (target of 30 g of high-quality protein at each meal of the day) can be achieved by consuming lean meats and poultry, low-fat dairy protein, and/or other animal foods as the main sources of protein.[16,17] However, the inclusion of higher quantities (above the RDA) of animal proteins, even from lean sources, may raise questions about the potential for negative cardiometabolic effects.[18] Studies of red meat have reached contradictory conclusions regarding the impact on CVD risk, in part because lean meats are often grouped together with processed meats,[19] foods that tend to be much higher in fat content. Recent studies have not associated regular intakes of lean red meat with an increase in markers of cardiometabolic risk.[20–22]

Because higher protein diets can be especially beneficial during weight loss in older adults and recognizing the limited study of red meat in the context of weight reduction in this population to date, the goal of the present study was to explore the cardiometabolic impact of lean and very lean red meat (beef and pork) consumed during weight loss regimens with balanced, higher protein (1.2 g/kg BW/d) vs a control RDA protein (0.8 g protein/kg BW/d) in obese middle-aged and older adults. For this analysis, a cohort of 80 subjects was amassed from 2 previously conducted, similarly designed, randomized controlled clinical trials, with comparable target populations, and both common and novel biomarkers of CVD and type II diabetes risk were measured by nuclear magnetic resonance spectroscopy (NMR). The CVD risk markers included conventional lipids, a comprehensive lipoprotein profile (lipoprotein particle class and subclass numbers and average sizes),[23–26] GlycA (a measure of global inflammatory burden),[27,28] and the gut microbiome–related metabolite trimethylamine-N-oxide (TMAO).[29–31] Markers of type II diabetes risk included lipoprotein insulin resistance index (LP-IR; a high-throughput measure of insulin resistance based on 6 lipoprotein parameters that are altered in metabolic disease),[32–36] branched-chain amino acids (BCAA),[37,38] and fasting blood glucose, all of which have strong positive associations with incident type II diabetes, and betaine (an active metabolite of choline and a component of beets) that is inversely related to type II diabetes risk, CVD, and nonalcoholic fatty liver disease.[39–41] All biomarkers were assessed at baseline and after 6 months of dietary intervention, and an exploratory approach was used with respect to all outcome variables with the effect of higher protein intake with change in the set of cardiometabolic items of primary interest.

Methods

Design of parent trials

Blood samples, dietary intake, body weight, and physical function (6-minute walk test [6MWT]) for the present analyses were collected in 2 randomized controlled trials of weight reduction interventions for middle-aged and older adults of 6-month duration sharing the same experimental design and intervention protocols.[14,17] The first trial, the Measuring Eating, Activity and Strength: Understanding the Response-Using Protein (MEASUR-UP) trial, examined meal-based enhancement of protein intake (30 g high-quality protein per each of 3 meals) during a 6-month weight loss intervention in obese older adults (≥60 years) with functional limitations.[14] The second trial, the Protein Optimization in Women Enables Results-Using Protein (POWR-UP) trial, compared the same diet treatment groups in obese women aged ≥45 years, mean age = 60.0 ± 8.2 years.[17] Each trial included 2 weight loss intervention groups: (1) a control group with RDA-level protein intake (0.8 g/kg BW/d) and (2) a higher protein (protein) group (1.2 g protein kg BW/d) with the protein distributed equally at 3 meals during the day (balanced distribution of protein). Both studies were conducted under "intention-to-treat" criteria. Detailed methods for the interventions and outcome measures[16] and the primary outcomes of each of the 2 studies have already been published.[14,17] Briefly, trial results showed subjects experienced both weight loss and improved physical function when consuming the balanced, higher protein diet and that the improvement in function was either similar to[17] or greater than[14] that in the respective control (RDA-level protein) group. ClinicalTrials.gov identifiers: NCT01715753 and NCT02033655.

Subjects

Subjects in both trials were obese individuals with body mass index (BMI) ≥30 kg/m^2 that were living independently in communities near Durham, NC. Exclusion criteria for both studies included glomerular filtration rate (GFR) <45 mL/min/1.73 m^2, dementia, neurologic conditions causing functional limitations, and unstable or terminal medical conditions. Subjects in the MEASUR-UP trial were men and women aged ≥60 years who were functionally limited as indicated by a Short Physical Performance Battery score of 4 to 10 out of a possible 12. Subjects in the POWR-UP trial were women aged ≥45 years; the population was oversampled to include 38% Black women. Blocking by gender (MEASUR-UP), marital/partner status, and functional status (<550 meters or ≥550 meters on the 6MWT; POWR-UP), eligible subjects in both studies were randomly assigned to either the control or protein group. Subjects were allocated to study groups using a computerized centralized randomization scheme generated by the study statistician. Group allocation was 1:2 for control: protein in both trials, reflecting a greater emphasis on understanding trends within the protein group and the fact that the RDA-level protein control regimen has already been well studied.[42,43]

MEASUR-UP was conducted between September 2012 and December 2014, and POWR-UP was conducted between March 2014 and January 2016. Only those subjects who completed either the MEASUR-UP or POWR-UP studies and provided a blood sample at baseline and at the end of the study were included in this analysis. All outcome measures were conducted at Duke University Center for Living, Durham, NC. Most study outcomes were objectively measured (eg, BodPod output, distance walked in 6 minutes, self-administered questionnaires, etc.), and the data management and analysis were conducted by research staff who were fully blinded to treatment assignment. For the small number of measures that could have a subjective component, for example, nutritional analysis coding of food choices from food records, the analysis was done by staff fully blinded to treatment assignments.

Interventions

All subjects followed a supervised weight loss treatment (hypocaloric; 500 kcal deficit diet) with the goal of 10% weight loss over 6 months under the direction of Research Dietitian Clinicians (hereafter noted as interventionists) and attended a supervised weekly weigh-in and weekly group meetings for counseling and peer support. Control subjects were prescribed a 15% protein, 30% fat, and 55% carbohydrate diet with a protein intake meeting the RDA of 0.8 g/kg BW/d. Protein subjects were prescribed 30% protein, 30% fat, and 40% carbohydrate, with a protein intake of 1.2 g/kg BW/d. Protein group meal plans were structured to include at least 30 g of lean, high-quality protein for each of 3 daily meals. Protein subjects were provided with at least 60 g/d, enough for 2, 30 g meals for each of the 7 days. Lean or very lean beef (ground sirloin, flank steak, and deli roast beef) or pork (tenderloin, pork chops, ground pork, and low-sodium deli ham) was delivered weekly. Other complete proteins (eg, lean meats and poultry, low-fat dairy foods, and eggs) were consumed at the remaining meal (participant's choice) with guidance from the interventionists. Interventionists reviewed subjects' daily food journals each week and adjusted their menus to ensure the target of 30 g protein per meal for breakfast, lunch, and dinner was met, as previously described.[14] Interventionists monitored adherence to the dietary protocol via subjects' weekly food journals, weekly weights, and attendance at group meetings. To assure micronutrient intake adequacy while following the hypocaloric diet and to promote consistent use of supplements, all participants were provided with a low-dose iron-free multivitamin/mineral supplement (Teen Multivitamin for Boys 12-17; GNC Milestones), as well as calcium (400 mg) and vitamin D (600 IU; Citracal; Bayer) and

asked to forego other supplements for the duration of the trial. The choice of these supplements and doses was based on our calculations of micronutrient content as a series of sample menus of the prescribed experimental diets. There was no prescribed exercise intervention; however, subjects were encouraged to be as active as they safely could. A fasting blood sample was collected to evaluate renal function by GFR, determined using the Chronic Kidney Disease Epidemiology Collaboration equation (LabCorp, Burlington, NC).[44] Renal function (GFR; LabCorp) was assessed at baseline, every other month for those with a baseline GFR of 45 to <60 mL/min/1.73 m^2, and at the 6-month endpoint. Adverse events were monitored throughout the trial.[16]

Assessment of protein and energy intakes and physical function

In addition to food journal review by interventionists, energy and protein intakes were assessed using 3-day food records collected at 0 and 6 months. Records were checked for completeness, and subjects contacted for any missing information. Intake of food and beverages was analyzed using Food Processor Nutrition Analysis Software (Version 10.10, 2012; ESHA Research, Salem, OR) to determine daily intakes of calories and macronutrients, as well as protein intakes per meal. Physical function was assessed using the 6MWT, conducted at baseline and 6 months.

NMR measurements

Blood was collected after an 8- to 10-hour fast at 0 and 6 months. NMR LipoProfile, or comprehensive lipoprotein profile, test spectra were collected on a Vantera Clinical Analyzer, a fully automated, high-throughput, ^{1}H-NMR platform, from ethylenediaminetetraacetic acid plasma samples as previously described (LabCorp, Morrisville, NC).[23,24] Briefly, The Vantera is equipped with a 400 MHz (9.4 T) Agilent spectrometer, a 4 mm indirect detection probe and a fixed flow cell that was equilibrated at 47°C via a variable temperature control module. 1D ^{1}H-NMR spectra were collected. The water resonance was attenuated using the WET solvent suppression technique.[24] Each NMR spectrum was acquired for a total of 48 seconds (9024 data points, 4496.4 Hz spectral width, 2.95 s relaxation delay between scans, 12 scans). The free induction decay signal was zero filled to 32,768 points and multiplied by a Gaussian function (for resolution enhancement) before Fourier Transformation. Concentrations for lipoprotein classes (very-low-density lipoprotein [VLDL], low-density lipoprotein [LDL] and high-density lipoprotein [HDL] particle concentration) and subclasses (small, medium, and large) were calculated using the LP3 deconvolution algorithm.[24] Mean VLDL, LDL, and HDL particle sizes are weighted averages derived from the sum of the diameter of each subclass multiplied by its relative mass percentage. The NMR MetaboProfile analysis, which reports results for lipoprotein parameters as well as several key metabolites, was performed using the recently developed LP4 deconvolution algorithm. Linear regression of the NMR subclass signal areas against serum lipid and apolipoprotein levels measured chemically in a large reference range study population (n = 698) provided the conversion factors to generate NMR-derived concentrations of total cholesterol and triglycerides, HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), apolipoprotein A-I, and apolipoprotein B. NMR-derived concentrations of these parameters are highly correlated ($r \geq 0.95$) with those measured by standard methods. Quantification of GlycA,[45] BCAA,[37] TMAO,[30] betaine,[46] and calculation of the LP-IR scores (values 1–100)[32] have been described previously.

Ethics

The 2 studies included in this analysis (MEASUR-UP protocol #0037110 and POWR-UP protocol #00050540) were approved by the Duke University Health System Institutional Review Board, and written informed consent was obtained from all subjects.

Statistical analysis

The MEASUR-UP and POWR-UP trials assessed the effectiveness of meal-based enhancement of protein intake during a weight loss intervention by comparing the treatment arm to a control arm. For both trials, the study analysis was conducted under an "intent to treat" criteria, including all participants, whether compliant to the intervention or not. Secondary outcomes and feasibility factors were also evaluated. Data were double entered with differences adjudicated. Treatment codes were revealed only after the study statistician locked the database at the end of the trial. The main overarching analytical goal was to assess the group differences in change over time for the cardiometabolic items; therefore, a correction for multiple testing was invoked (see next paragraph). Group differences for other measures and within-group changes from baseline to endpoint were considered exploratory and descriptive in nature and were evaluated using an unadjusted *t*-test with the null = 0 (no change). Outcome variables were grouped generally as either (1) Standard outcome (reflected in Table 2), (2) biomarkers (Table 3), or (3) metabolic parameters and insulin resistance (Table 4) and results are presented as mean ± standard deviation. Because of the exploratory approach of the analysis combining 2 trials, no power calculations were done a priori for any of the outcomes. Statistical significance for the 8 variables in Table 2 (body weight, energy intake, fat intake, carbohydrate intake, protein intake, and 6MWT) were set at level alpha = 0.05 (2 tailed), uncorrected for the number of variables tested.

Group effects for changes in all outcomes were tested using a mixed model adjusted for baseline using the following general model: $Y_{endpoint} = Y_{baseline} +$ group

(protein or control). The level for the tests of the 30 cardiometabolic variables was set at level alpha = 0.05 (2 tailed) using the procedure by Holm.[47] The Holm procedure first ranks the 30 *P* values from lowest to highest. The first (lowest) *P* value has to be less than .002 (.05/30) to be statistically significant. The Holm procedure continues sequentially in this fashion using levels of .002 (.05/29), .002 (.05/28), and .05 (.05/1) for the remaining 29 tests, respectively. Statistical analyses were performed using SAS (version 9.4, Cary, NC).

Results

Baseline characteristics of study population

Of the 147 randomized subjects who participated in the 2 diet studies, 89 completed the 6-month interventions (Fig. 1). Of these, 9 subjects were excluded because they did not provide a final blood sample, leaving 80 subjects for the present analysis (MEASUR-UP: control, n = 14, and protein, n = 25; POWR-UP: control, n = 13, and protein, n = 28). Six participants in MEASUR-UP (control, n = 4, and protein, n = 2) completed a 3 rather than a 6-month intervention because of time constraints. Baseline characteristics for the combined study population (Table 1) were compared by group (control, n = 27, and protein, n = 53) before analysis. We found no baseline differences between control and protein groups for age, gender, race, marital status, and education; similarly, body weight, BMI, and reported intakes of protein (g/kg BW/d) and energy did not differ between groups at baseline (Table 1). The study population was largely female (90%) and White (70%), with class II obesity (mean BMI = 37.3 kg/m^2). The relatively high dropout rate for both studies reflected in Figure 1 represents our prior experience with this physically frail and vulnerable population. However, the dropout rate as well as predictors of attrition did not differ between control and protein groups in either trial. In a comparison of subjects who dropped out vs completers, the only association with dropping out in MEASUR-UP was having a lower mean waist circumference ($P < .05$); in POWR-UP, completers were more likely to be White and of higher education level ($P < .05$ for both). In addition, the numbers of subjects with inadequate blood samples were exactly proportionate according to the 2:1 randomization allocation of protein to control subjects.

Changes in body weight, intakes of protein and energy, and physical function

Between baseline and 6-month study completion, both treatment groups reduced energy intake ($P < .0001$) per the prescribed hypocaloric intake, achieved weight loss ($P < .0001$), and experienced a significant and physiologically meaningful increase in 6MWT distances ($P \leq .002$). Compared with the control group, the protein group had greater total protein intake (g/d and % kcal) and protein intake in g/kg BW/d ($P < .0001$), and less carbohydrate intake (% kcal; $P = .001$). There were no between-group differences for changes in weight loss, energy intake, fat intake, and 6MWT distances (Table 2).

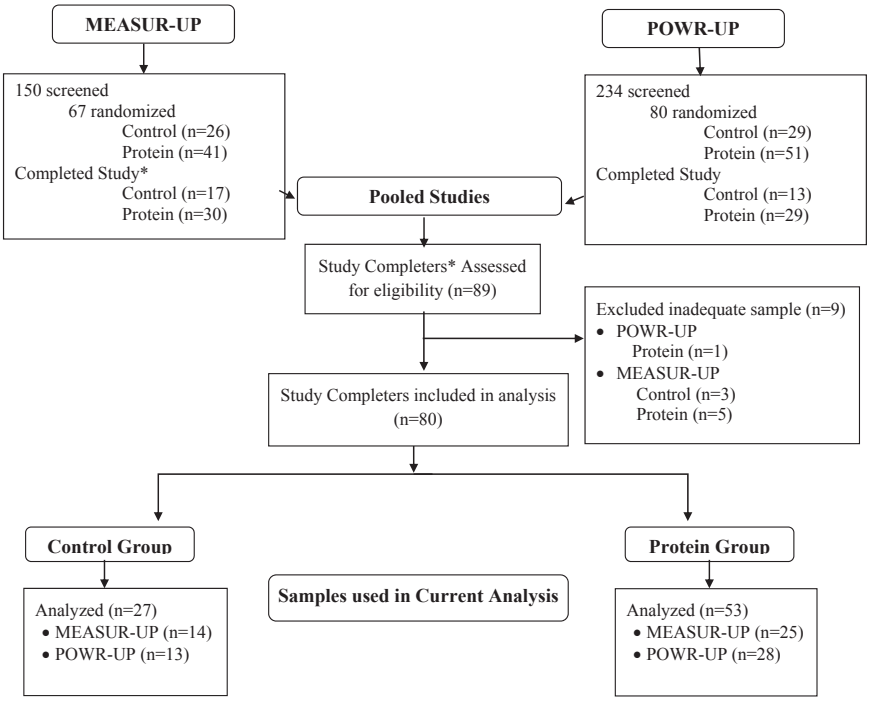

Figure 1 CONSORT flow diagram. CONSORT, Consolidated Standards of Reporting Trials. *Six participants (n = 4 control and n = 2 protein) were enrolled and completed 3 rather than 6 months because of time constraints on study completion.

Table 1 Baseline characteristics of study population by treatment group

	Control (n = 27), mean ± SD or n (%)	Protein (n = 53), mean ± SD or n (%)	Total (n = 80), mean ± SD or n (%)	P value
Age, y				
Mean	66 ± 8	63 ± 8	64 ± 8	.23
Range	46–83	45–78	45–83	
Gender				
Male	3 (11)	5 (9)	8 (10)	.81
Female	24 (89)	48 (91)	72 (90)	
Body weight, kg	102.6 ± 15.3	102.9 ± 22.6	102.8 ± 20.4	.96
BMI, kg/m^2	37.2 ± 5.7	37.3 ± 7.0	37.3 ± 6.6	.92
Race				
Black	7 (26)	17 (32)	24 (30)	.57
White	20 (74)	36 (68)	56 (70)	
Marital status				
Married	5 (18)	8 (15)	13 (16)	.92
Single	14 (52)	29 (55)	43 (54)	
Widow	8 (30)	16 (30)	24 (30)	
Education				
Completed high school	5 (18.5)	7 (14)	12 (16)	.79
Some college	5 (18.5)	12 (24)	17 (22)	
Completed college	17 (63)	31 (62)	48 (62)	
Energy intake, kcal	1966 ± 510	1887 ± 689	1914 ± 632	.60
Protein intake, g/kg BW/d	0.85 ± 0.24	0.86 ± 0.25	0.86 ± 0.24	.89

BMI, body mass index; SD, standard deviation.

Table 2 Body weight, energy, fat, carbohydrate, and protein intake and 6-minute walk test

	Control (n = 27), mean ± SD	P value	Protein (n = 53), mean ± SD	P value	P value, control vs protein
Body weight, kg					
Baseline	102.6 ± 15.3		102.9 ± 22.6		
Change at endpoint	−6.2 ± 5.6	<.0001	−7.2 ± 6.2	<.0001	.48
Body weight, %					
Change at endpoint	−6.2 ± 5.6	<.0001	−7.2 ± 5.6	<.0001	.47
Energy intake, kcal					
Baseline	1966 ± 510		1887 ± 689		
Change at endpoint	−538 ± 531	<.0001	−460 ± 652	<.0001	.67
Fat intake, % kcal					
Baseline	38 ± 7		38 ± 6		
Change at endpoint	−9 ± 9	.004	−11 ± 6	<.0001	.61
Carbohydrate intake, % kcal					
Baseline	45 ± 6		42 ± 8		
Change at endpoint	4 ± 7	.04	−1 ± 8	.54	.001
Protein intake, % kcal					
Baseline	18 ± 3		20 ± 5		
Change at endpoint	6 ± 4	.001	14 ± 7	<.0001	<.0001
Protein intake, g					
Baseline	86.3 ± 20.1		86.2 ± 22.6		
Change at endpoint	−8.6 ± 21.7	.07	27.5 ± 31.9	<.0001	<.0001
Protein intake, g/kg of body weight					
Baseline	0.85 ± 0.24		0.86 ± 0.25		
Change at endpoint	−0.02 ± 0.23	.68	0.37 ± 0.35	<.0001	<.0001
6MWT, m					
Baseline	488.9 ± 63.3		465.2 ± 91.6		
Change at endpoint	53.1 ± 71.4	.002	75.0 ± 70.8	<.0001	.41

6MWT, 6-min walk test; SD, standard deviation.

Table 3 Biomarkers of cardiovascular disease risk by treatment group

	Control (n = 27), mean ± SD	*P* value	Protein (n = 53), mean ± SD	*P* value	*P* value control vs protein
Lipid panel					
TG, mg/dL					
Baseline	85.1 ± 37.4		103.4 ± 66.7		
Change at endpoint	−11.5 ± 34.7	.10	−17.3 ± 50.2	.02	.76
TC, mg/dL					
Baseline	200.2 ± 40.1		202.4 ± 37.0		
Change at endpoint	−5.1 ± 21.8	.24	−4.3 ± 28.5	.30	.78
LDL-C, mg/dL					
Baseline	114.2 ± 30.2		117.3 ± 30.0		
Change at endpoint	−4.8 ± 14.3	.09	−3.3 ± 22.2	.31	.56
HDL-C, mg/dL					
Baseline	63.3 ± 10.6		59.6 ± 13.0		
Change at endpoint	−0.1 ± 8.0	.94	1.3 ± 6.8	.17	.68
Gut microbiome–related metabolite					
TMAO, μM					
Baseline	3.9 ± 3.1		4.6 ± 3.3		
Change at endpoint	0.5 ± 2.8	.41	0.1 ± 4.6	.84	.77
Betaine, μM					
Baseline	34.6 ± 9.5		34.6 ± 5.3		
Change at endpoint	−0.1 ± 8.2	.94	1.2 ± 6.2	.18	.34
Inflammatory biomarker					
GlycA, μmol/L					
Baseline	438.6 ± 44.3		451.8 ± 69.7		
Change at endpoint	−13.1 ± 31.7	.04	−8.2 ± 39.2	.15	.41
Lipoprotein profile					
Total VLDL-P, nmol/L					
Baseline	40.3 ± 15.1		42.4 ± 22.4		
Change at endpoint	−7.9 ± 18.1	.03	−1.8 ± 18.7	.51	.07
VLDL subclasses					
Large VLDL-P, nmol/L					
Baseline	4.8 ± 2.9		5.7 ± 4.9		
Change at endpoint	−0.9 ± 2.7	.08	−1.2 ± 4.1	.05	.64
Medium VLDL-P, nmol/L					
Baseline	12.4 ± 7.4		14.9 ± 11.3		
Change at endpoint	0.1 ± 8.5	.95	0.3 ± 11.1	.87	.56
Small VLDL-P, nmol/L					
Baseline	23.1 ± 12.8		21.8 ± 12.8		
Change at endpoint	−7.0 ± 14.0	.02	−0.9 ± 10.6	.55	.02
Total LDL-P, nmol/L					
Baseline	991.6 ± 277.0		1078.8 ± 272.3		
Change at endpoint	−30.6 ± 194.3	.42	−67.8 ± 211.3	.03	.84
LDL subclasses					
IDL-P, nmol/L					
Baseline	227.0 ± 122.7		219.6 ± 118.8		
Change at endpoint	−18.3 ± 124.1	.45	4.0 ± 108.4	.80	.48
Large LDL-P, nmol/L					
Baseline	289.3 ± 232.8		242.1 ± 202.0		
Change at endpoint	−31.6 ± 136.8	.24	−12.5 ± 153.8	.57	.82
Small LDL-P, nmol/L					
Baseline	475.5 ± 351.1		617.2 ± 329.9		
Change at endpoint	19.2 ± 255.4	.66	−59.4 ± 200.4	.04	.56
Total HDL-P, μmol/L					
Baseline	35.5 ± 5.4		35.8 ± 5.9		
Change at endpoint	−0.4 ± 3.7	.57	0.1 ± 3.9	.91	.51

(*continued on next page*)

Table 3 (*continued*)

	Control (n = 27), mean ± SD	*P* value	Protein (n = 53), mean ± SD	*P* value	*P* value control vs protein
HDL subclasses					
Large HDL-P, μmol/L					
Baseline	9.5 ± 3.1		8.2 ± 3.7		
Change at endpoint	0.3 ± 2.1	.40	0.8 ± 1.8	.003	.71
Medium HDL-P, μmol/L					
Baseline	10.3 ± 6.7		9.5 ± 5.7		
Change at endpoint	−1.0 ± 5.4	.34	0.3 ± 4.1	.58	.27
Small HDL-P, μmol/L					
Baseline	15.7 ± 8.4		18.1 ± 6.7		
Change at endpoint	0.2 ± 5.5	.81	−1.1 ± 4.6	.11	.49
Mean lipoprotein sizes					
VLDL size, nm					
Baseline	52.4 ± 7.0		53.9 ± 9.0		
Change at endpoint	0.2 ± 8.2	.91	−2.2 ± 6.8	.03	.17
LDL size, nm					
Baseline	20.8 ± 0.9		20.6 ± 0.8		
Change at endpoint	−0.1 ± 0.6	.34	0.02 ± 0.6	.77	.56
HDL size, nm					
Baseline	9.8 ± 0.5		9.6 ± 0.5		
Change at endpoint	0.05 ± 0.3	.28	0.13 ± 0.3	.004	.89
Derived apolipoprotein concentrations					
ApoB, mg/dL					
Baseline	87.2 ± 23.4		91.9 ± 22.3		
Change at endpoint	−3.2 ± 11.6	.17	−4.3 ± 16.8	0.08	.93
ApoA-1, mg/dL					
Baseline	162.4 ± 18.8		157.1 ± 24.7		
Change at endpoint	−0.5 ± 18.0	.89	1.4 ± 16.5	.55	.96

ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; HDL-P, high-density lipoprotein particles; LDL-P, low-density lipoprotein particles; SD, standard deviation; TC, total cholesterol; TG, triglycerides; TMAO, trimethylamine-N-oxide; VLDL-P, very-low-density lipoprotein particle.

Changes in markers of CVD risk at 6 months

Compared with baseline, the protein group had improved lipoprotein profiles with greater amounts of large HDL particle concentration ($P = .003$) and larger HDL size ($P = .004$), with reductions in triglycerides ($P = .02$), large VLDL particle concentration (VLDL-P; $P = .05$), total LDL particle concentration (LDL-P; $P = .03$), small LDL-P ($P = .04$), and VLDL size ($P = .03$; Table 3). In the control group, compared with baseline, total VLDL-P ($P = .03$) and small VLDL-P ($P = .02$) were reduced, as were GlycA concentrations ($P = .04$). For both groups, compared with baseline, there were no changes in concentrations of total cholesterol, HDL-C, LDL-C, apolipoprotein AI, apolipoprotein B, TMAO, or betaine (all $P > .05$). Finally, Compared with the protein group, small VLDL-P was significantly reduced in the control arm (control −7.0 ± 14.0 nmol/L; protein −0.9 ± 10.6 nmol/L; $P = .02$); however, when the Holm procedure was applied, the group difference was no longer significant ($P > .002$).

Changes in measures of insulin resistance and amino acid concentrations at 6 months

Both groups showed baseline to 6-month reductions in fasting plasma glucose ($P = .04$; Table 4). In the protein group, compared with baseline, there were 6-month reductions in LP-IR scores ($P = .003$). In the control group, compared with baseline, 6-month isoleucine concentrations were reduced slightly ($P = .03$). Concentrations of total BCAA, leucine, and valine were unchanged in either group ($P > .05$).

Discussion

The article uses the combined data of the MEASUR-UP and POWR-UP trials to evaluate the impact of a weight loss diet with balanced, higher (animal) protein intake vs a weight loss diet with an RDA level of protein intake on markers of cardiometabolic health in obese middle-aged and older adults. Although our findings indicate a hypocaloric diet reduces markers of cardiovascular and type II

Table 4 Metabolic parameters and insulin resistance by treatment group

	Control (n = 27) mean ± SD	*P* value	Protein (n = 53), mean ± SD	*P* value	*P* value control vs protein
Glucose mg/dL					
Baseline	92.7 ± 30.6		92.9 ± 27.7		
Change at endpoint	−7.5 ± 18.1	.04	−6.2 ± 20.6	.04	.63
Total BCAA, μmol/L					
Baseline	419.3 ± 62.4		430.8 ± 68.6		
Change at endpoint	−17.7 ± 62.7	.16	6.6 ± 65.5	.48	.07
Valine, μmol/L					
Baseline	221.7 ± 36.2		228.3 ± 36.4		
Change at endpoint	−4.6 ± 36.1	.51	4.9 ± 34.5	.32	.17
Leucine, μmol/L					
Baseline	155.0 ± 21.0		160.1 ± 28.0		
Change at endpoint	−7.8 ± 24.5	.10	1.8 ± 31.5	.69	.06
Isoleucine, μmol/L					
Baseline	42.6 ± 10.9		42.2 ± 14.2		
Change at endpoint	−5.2 ± 11.7	.03	−0.1 ± 16.4	.97	.16
Lipoprotein insulin resistance index					
Baseline	41.3 ± 18.6		48.1 ± 22.5		
Change at endpoint	−1.6 ± 15.8	.61	−5.9 ± 13.3	.003	.59

BCAA, branched chain amino acids; SD, standard deviation.
The lipoprotein insulin resistance index produces a score from 0 (most insulin sensitive) to 100 (most insulin resistant).

diabetes, the results do not significantly differentiate between diet groups. These data, however, support a recent report linking protein-enriched meals with predominantly favorable effects on cardiometabolic health.[20] Specifically, consuming a diet higher in protein did not adversely impact biomarkers associated with CVD or type II diabetes compared with a lower, RDA level of protein in middle-aged adults.[20]

The most striking finding for CVD risk is that LDL-P—considered a better biomarker for CVD risk than LDL-C in subjects with obesity, metabolic syndrome, and/or type II diabetes[24,26]—was decreased in the protein group. Thus, the association of red meat and saturated fats with increased CVD risk observed in some epidemiologic studies[48,49] was not observed in our clinical trial with a higher protein diet. This could be due, at least in part, to the strict dietary control, such that only lean and very lean red meats and few processed meats were consumed. The positive cardiometabolic responses occurred, although our study population had elevated plasma LDL-P concentrations at baseline. In fact, many subjects had plasma LDL-P concentrations in the high range as the recommended targets for LDL-P levels are <1300 and <1000 nmol/L for patients at moderate and high CVD risk, respectively.[26] Although LDL-P was not reduced in the control group, this may be because of lower subject numbers. There was no between-group difference in LDL-P, suggesting that the control diet may have also had beneficial effects on CVD risk.

GlycA is an NMR-measured systemic inflammatory factor, established as a biomarker of CVD risk.[26,27] The NMR signal named GlycA arises from the N-acetylglucosamine residues within the carbohydrate side-chain of circulating acute phase proteins. As such, GlycA is a composite biomarker that takes into account the increased carbohydrate complexity and secretion of multiple glycosylated acute phase proteins observed during inflammatory processes.[26,27] It has been associated with the presence and extent of coronary artery disease as reported in the secondary prevention CVD cohort CATHeterization GENetics[50] and with incident CVD events in the Women's Health Study,[51] the Prevention of Renal and Vascular End-stage Disease study,[52] the Multi-Ethnic Study of Atherosclerosis,[53] and the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin trial.[54] Not unexpectedly, baseline GlycA concentrations in this study population (mean GlycA = 443 ± 62 μmol/L) were greater than those reported in healthy populations (mean GlycA = 380 ± 61 μmol/L)[44] and similar to the GlycA values in patients with chronic inflammatory diseases, such as rheumatoid arthritis and psoriasis.[55,56] GlycA concentrations are reduced by exercise, reductions in visceral adiposity, and anti-inflammatory drug treatments.[55,57,58] Interestingly, GlycA concentrations were reduced by the intervention in the control group but remained unchanged in the protein group. Currently, there is no obvious explanation for this observation. The weight loss experienced by both groups likely contributed to the reduction or lack of increase in GlycA levels. Certainly, the lack of increase in GlycA—and hence systemic inflammation—in the protein group suggests that consumption of a higher protein diet did not lead to an increase in inflammatory CVD risk.

The gut microbe–derived metabolites TMAO (when elevated) and betaine (when reduced) are associated with

adverse CVD outcomes.[59,60] An elevated TMAO concentration, theorized to be associated with red meat consumption,[61] is associated with primary CVD events and is also a prognostic marker in patients with acute coronary syndrome.[62] Whether or not therapeutic strategies (eg, antibiotics and change in diet composition) can lower TMAO levels remains to be confirmed and continues to be a matter of active study; however, it is possible that this gut-derived metabolite is a "mediator or a bystander in the disease process."[63] Low circulating concentrations of betaine are associated with incident type II diabetes, increased heart failure, and acute myocardial infarction[39,40]; and betaine is responsive to intensive lifestyle interventions.[40] In our subjects, plasma concentrations of TMAO were similar to those in high-risk CVD groups and similar to individuals with type II diabetes.[39] However, neither TMAO nor betaine concentrations were changed by the intervention in either treatment group. Thus, a higher protein diet, when enriched in lean and very lean proteins, did not lead to an increase in gut microbiome–related markers of CVD and/or type II diabetes risk.

To summarize our findings for CVD risk, none of the biomarkers assessed in this analysis showed any adverse changes in either the protein or the control groups. Thus, consuming a diet enriched in lean animal proteins (including lean red meats) to benefit muscle in the context of weight reduction can be advocated without concern for secondary increases in CVD risk. This is consistent with 2 recently published meta-analysis of randomized controlled trials considering intake of red meat. One reported that red meat intake (at ≥0.5 servings per day) did not negatively influence CVD risk factors[21] and the second found that a diet containing red meat had greater improvements from one emphasizing fish or low-quality carbohydrates regarding changes in blood lipids and lipoproteins.[22]

The LP-IR score—based on 6 lipoprotein parameters whose concentrations are abnormal in subjects with insulin resistance—is a measure of insulin resistance. LP-IR scores have correlated directly with the homeostatic model assessment of insulin resistance in the Multi-Ethnic Study of Atherosclerosis study, and inversely with insulin sensitivity, as measured by the glucose disposal rate in a hyperinsulinemic-euglycemic clamp study.[32] Moreover, LP-IR predicts future type II diabetes,[34,35] even in individuals on statin treatment. It is, therefore, a simple, high-throughput means by which to identify patients at increased risk of progressing to type II diabetes.[64] In addition, supporting its utility for monitoring treatments aimed at reducing insulin resistance, lifestyle interventions for weight loss improve LP-IR scores.[65,66] In the present study, the mean postintervention LP-IR score—and hence insulin resistance—was significantly reduced in the protein group. This was mirrored by protein group changes in several of the lipoprotein parameters altered in subjects with insulin resistance (large VLDL-P, large HDL-P, VLDL, and HDL size). There was a nonstatistically significant reduction in the LP-IR scores in the control group, and a nonsignificant between-group difference in insulin resistance. Nevertheless, these findings suggest that a protein-enhanced, hypocaloric diet may elicit a reduction in insulin resistance and a concomitant decrease in the risk of progressing to type II diabetes in obese, older adults. Further study is needed to delineate the mechanism of the insulin sensitivity response of the protein diet, but it could be due, at least in part, to a reduction in carbohydrate intake with the higher protein diet pattern. In addition, by helping to retain muscle mass, the increased protein could, in turn, reduce peripheral insulin resistance and increase muscle glucose uptake. Given the close association of insulin resistance with CVD and the need to preserve lean mass during weight reduction, these findings further support the positive cardiometabolic impact of the protein-enhanced weight loss diet in this population.

This study has several strengths. First, this study provided a unique opportunity to investigate the impact of consuming ~30 g of lean animal protein (including lean red meats) 3 times a day for 6 months on cardiometabolic parameters. Although this study was conducted to evaluate a weight loss diet providing balanced portions of high-quality protein (1.2 g/kg BW/d) rather than the consumption of lean red meat *per se*, its findings underscore the importance of evaluating the health attributes of any food category in the context of the complete diet—in this case, a hypocaloric, low-fat, nutrient-dense diet. The findings further support studies prescribing high animal protein diets for older adults at risk for both functional decline and obesity-related cardiometabolic diseases.

One limitation is the length of the study (6 months). It would be intriguing to see if the changes that were observed in the cardiometabolic risk markers are similar in longer duration dietary interventions. In addition, because the protein group was consuming more protein in a diet equicaloric to the control diet, the proportions of nutrients necessarily shifted so that carbohydrate intake was less in the protein group than in controls. However, the protein diet was not a low-carbohydrate diet: the protein intake in that group was 139 g/d compared with the control group average of 167 g. Considering the exploratory nature of this work and the variety of ways by which cardiovascular risk can be manifested, the most important limitation of this study is the number of metabolic outcomes being reported and the possibility that within-group changes overstate the protein treatment effect. We also acknowledge the study population is predominantly female. However, because obese women outnumber obese men in the older adult population, this is not a major limitation.

In summary, we found that a balanced, higher protein weight loss diet, including lean and very lean beef and pork, promotes weight loss and improves physical function without adversely impacting risk markers of CVD or insulin resistance. These findings indicate that middle-aged and older adults with moderate to severe obesity may achieve improvements in multiple cardiometabolic disease risk factors by prescriptively consuming an energy-

restricted diet with a protein content at or above the RDA level, and that the higher protein intakes may be achieved using protein-rich, lower-fat animal products, including lean red meats.

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Disclosures

M.A.C. is an employee of LabCorp. All other authors report no conflicts of interest. The sponsors had no influence on the protocol design, conduct of the trial, or data analysis.

References

1. Mamerow MM, Mettler JA, English KL, et al. Dietary protein distribution positively influences 24-h muscle protein synthesis in healthy adults. *J Nutr*. 2014;144(6):876–880.
2. Bradlee ML, Mustafa J, Singer MR, Moore LL. High-protein foods and physical activity protect against age-related muscle loss and functional decline. *J Gerontol A Biol Sci Med Sci*. 2017;73:88–94.
3. Mustafa J, Ellison RC, Singer MR, et al. Dietary protein and preservation of physical functioning among middle-aged and older adults in the Framingham Offspring Study. *Am J Epidemiol*. 2018;187:1411–1419.
4. Kim BJ, Lee SH, Isales CM, Koh JM, Hamrick MW. The positive association of total protein intake with femoral neck strength (KNHANES IV). *Osteoporos Int*. 2018;29(6):1397–1405.
5. Langsetmo L, Shikany JM, Burghardt AJ, et al. High dairy protein intake is associated with greater bone strength parameters at the distal radius and tibia in older men: a cross-sectional study. *Osteoporos Int*. 2018;29:69–77.
6. Stentz FB, Brewer A, Wan J, et al. Remission of pre-diabetes to normal glucose tolerance in obese adults with high protein versus high carbohydrate diet: randomized control trial. *BMJ Open Diabetes Res Care*. 2016;4(1):e000258.
7. Langsetmo L, Shikany JM, Cawthon PM, et al. The association between protein intake by source and osteoporotic fracture in older men: a prospective cohort study. *J Bone Miner Res*. 2017;32:592–600.
8. Bauer J, Biolo G, Cederholm T, et al. Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE Study Group. *J Am Med Dir Assoc*. 2013;14:542–559.
9. Prado CM, Wells JC, Smith SR, Stephan BC, Siervo M. Sarcopenic obesity: a critical appraisal of the current evidence. *Clin Nutr*. 2012;31:583–601.
10. Goisser S, Kemmler W, Porzel S, et al. Sarcopenic obesity and complex interventions with nutrition and exercise in community-dwelling older persons–a narrative review. *Clin Interv Aging*. 2015;10:1267–1282.
11. Ma J, Hwang SJ, McMahon GM, et al. Mid-adulthood cardiometabolic risk factor profiles of sarcopenic obesity. *Obesity (Silver Spring)*. 2016;24:526–534.
12. Porter Starr KN, Bales CW. Excessive body weight in older adults. *Clin Geriatr Med*. 2015;31:311–326.
13. Bales CW, Porter Starr KN. Obesity interventions for older adults: diet as a determinant of physical function. *Adv Nutr*. 2018;9:151–159.
14. Porter Starr KN, Pieper CR, Orenduff M, et al. Improved function with enhanced protein intake per meal: a pilot study of weight reduction in frail, obese older adults. *J Gerontol A Biol Sci Med Sci*. 2016;71:1369–1375.
15. Kim JE, O'Connor LE, Sands LP, Slebodnik MB, Campbell WW. Effects of dietary protein intake on body composition changes after weight loss in older adults: a systematic review and meta-analysis. *Nutr Rev*. 2016;74:210–224.
16. McDonald SR, Porter Starr KN, Mauceri L, et al. Meal-based enhancement of protein quality and quantity during weight loss in obese older adults with mobility limitations: rationale and design for the MEASUR-UP trial. *Contemp Clin Trials*. 2015;40:112–123.
17. Bales C, Porter Starr K, Orenduff M, et al. Influence of protein intake, race, and age on responses to a weight reduction intervention in obese women. *Curr Dev Nutr*. 2017;1(5):e000703.
18. van Baak MA, Larsen TM, Jebb SA, et al. Dietary intake of protein from different sources and weight regain, changes in body composition and cardiometabolic risk factors after weight loss: the DIOGenes study. *Nutrients*. 2017;9(12):E1326.
19. Micha R, Michas G, Mozaffarian D. Unprocessed red and processed meats and risk of coronary artery disease and type 2 diabetes–an updated review of the evidence. *Curr Atheroscler Rep*. 2012;14:515–524.
20. O'Connor LE, Paddon-Jones D, Wright AJ, Campbell WW. A Mediterranean-style eating pattern with lean, unprocessed red meat has cardiometabolic benefits for adults who are overweight or obese in a randomized, crossover, controlled feeding trial. *Am J Clin Nutr*. 2018;108:33–40.
21. O'Connor LE, Kim JE, Campbell WW. Total red meat intake of >/=0.5 servings/d does not negatively influence cardiovascular disease risk factors: a systemically searched meta-analysis of randomized controlled trials. *Am J Clin Nutr*. 2017;105:57–69.
22. Guasch-Ferre M, Satija A, Blondin SA, et al. Meta-analysis of randomized controlled trials of red meat consumption in comparison with various comparison diets on cardiovascular risk factors. *Circulation*. 2019;139:1828–1845.
23. Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. *Clin Lab Med*. 2006;26:847–870.
24. Matyus SP, Braun PJ, Wolak-Dinsmore J, et al. NMR measurement of LDL particle number using the Vantera Clinical Analyzer. *Clin Biochem*. 2014;47:203–210.
25. Matyus SP, Braun PJ, Wolak-Dinsmore J, et al. HDL particle number measured on the Vantera(R), the first clinical NMR analyzer. *Clin Biochem*. 2015;48:148–155.
26. Cromwell WCaT DW. Clinical utility of LDL particle number to optimize management of LDL-related cardiovascular risk. *J Fam Pract*. 2016;65:1–8.
27. Connelly MA, Gruppen EG, Otvos JD, Dullaart RPF. Inflammatory glycoproteins in cardiometabolic disorders, autoimmune diseases and cancer. *Clin Chim Acta*. 2016;459:177–186.

28. Connelly MA, Otvos JD, Shalaurova I, Playford MP, Mehta NN. GlycA, a novel biomarker of systemic inflammation and cardiovascular disease risk. *J Transl Med*. 2017;15(1):219.
29. Wang Z, Klipfell E, Bennett BJ, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature*. 2011;472:57–63.
30. Garcia E, Wolak-Dinsmore J, Wang Z, et al. NMR quantification of trimethylamine-N-oxide in human serum and plasma in the clinical laboratory setting. *Clin Biochem*. 2017;50:947–955.
31. Wang Z, Zhao Y. Gut microbiota derived metabolites in cardiovascular health and disease. *Protein Cell*. 2018;9:416–431.
32. Shalaurova I, Connelly MA, Garvey WT, Otvos JD. Lipoprotein insulin resistance index: a lipoprotein particle-derived measure of insulin resistance. *Metab Syndr Relat Disord*. 2014;12:422–429.
33. Dugani SBA AO, Glynn RJ, Ridker PM, Mora S. Lipoprotein particle characteristics and incident diabetes in the JUPITER trial. *Circulation*. 2014;130:A16055.
34. Mackey RH, Mora S, Bertoni AG, et al. Lipoprotein particles and incident type 2 diabetes in the Multi-Ethnic Study of Atherosclerosis. *Diabetes Care*. 2015;38(4):628–636.
35. Harada PHN, Demler OV, Dugani SB, et al. Lipoprotein insulin resistance score and risk of incident diabetes during extended follow-up of 20 years: the Women's Health Study. *J Clin Lipidol*. 2017;11:1257–1267.e2.
36. Flores-Guerrero JL, Connelly MA, Shalaurova I, et al. Lipoprotein insulin resistance index, a high-throughput measure of insulin resistance, is associated with incident type II diabetes mellitus in the Prevention of Renal and Vascular End-Stage Disease study. *J Clin Lipidol*. 2019;13(1):129–137.
37. Wolak-Dinsmore J, Gruppen EG, Shalaurova I, et al. A novel NMR-based assay to measure circulating concentrations of branched-chain amino acids: elevation in subjects with type 2 diabetes mellitus and association with carotid intima media thickness. *Clin Biochem*. 2018;54:92–99.
38. Flores-Guerrero JL, Oste MCJ, Kieneker LM, et al. Plasma branched-chain amino acids and risk of incident type 2 diabetes: results from the PREVEND Prospective Cohort Study. *J Clin Med*. 2018;7(12):E513.
39. Svingen GF, Schartum-Hansen H, Pedersen ER, et al. Prospective associations of systemic and urinary choline metabolites with incident type 2 diabetes. *Clin Chem*. 2016;62:755–765.
40. Walford GA, Ma Y, Clish C, et al. Metabolite profiles of diabetes incidence and intervention response in the diabetes prevention program. *Diabetes*. 2016;65:1424–1433.
41. Sookoian S, Puri P, Castano GO, et al. Nonalcoholic steatohepatitis is associated with a state of betaine-insufficiency. *Liver Int*. 2017;37:611–619.
42. Santanasto AJ, Glynn NW, Newman MA, et al. Impact of weight loss on physical function with changes in strength, muscle mass, and muscle fat infiltration in overweight to moderately obese older adults: a randomized clinical trial. *J Obes*. 2011;2011:516576.
43. Villareal DT, Chode S, Parimi N, et al. Weight loss, exercise, or both and physical function in obese older adults. *N Engl J Med*. 2011;364(13):1218–1229.
44. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604–612.
45. Otvos JD, Shalaurova I, Wolak-Dinsmore J, et al. GlycA: a composite nuclear magnetic resonance biomarker of systemic inflammation. *Clin Chem*. 2015;61:714–723.
46. Dullaart RP, Garcia E, Jeyarajah E, Gruppen EG, Connelly MA. Plasma phospholipid transfer protein activity is inversely associated with betaine in diabetic and non-diabetic subjects. *Lipids Health Dis*. 2016;15:143.
47. Holm S. A simple sequentially reactive multiple test procedure. *Scand J Stat*. 1979;6:65–70.
48. Abdul-Ghani MA, Stern MP, Lyssenko V, Tuomi T, Groop L, Defronzo RA. Minimal contribution of fasting hyperglycemia to the incidence of type 2 diabetes in subjects with normal 2-h plasma glucose. *Diabetes Care*. 2010;33:557–561.
49. Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. *Am Clin Nutr*. 2010;91:535–546.
50. McGarrah RW, Kelly JP, Craig DM, et al. A novel protein glycan-derived inflammation biomarker independently predicts cardiovascular disease and modifies the association of HDL subclasses with mortality. *Clin Chem*. 2017;63:288–296.
51. Akinkuolie AO, Buring JE, Ridker PM, Mora S. A novel protein glycan biomarker and future cardiovascular disease events. *J Am Heart Assoc*. 2014;3:e001221.
52. Gruppen EG, Riphagen IJ, Connelly MA, Otvos JD, Bakker SJ, Dullaart RP. GlycA, a pro-inflammatory glycoprotein biomarker, and incident cardiovascular disease: relationship with c-reactive protein and renal function. *PLoS One*. 2015;10:e0139057.
53. Duprez DA, Otvos J, Sanchez OA, Mackey RH, Tracy R, Jacobs DR Jr. Comparison of the predictive value of GlycA and other biomarkers of inflammation for total death, incident cardiovascular events, noncardiovascular and noncancer inflammatory-related events, and total cancer events. *Clin Chem*. 2016;62:1020–1031.
54. Akinkuolie AO, Glynn RJ, Padmanabhan L, Ridker PM, Mora S. Circulating N-linked glycoprotein side-chain biomarker, rosuvastatin therapy, and incident cardiovascular disease: an analysis from the JUPITER trial. *J Am Heart Assoc*. 2016;5(7):e003822.
55. Joshi AA, Lerman JB, Aberra TM, et al. GlycA is a novel biomarker of inflammation and subclinical cardiovascular disease in psoriasis. *Circ Res*. 2016;119:1242–1253.
56. Bartlett DB, Connelly MA, AbouAssi H, et al. A novel inflammatory biomarker, GlycA, associates with disease activity in rheumatoid arthritis and cardio-metabolic risk in BMI-matched controls. *Arthr Res Ther*. 2016;18:86.
57. Bartlett DB, Slentz CA, Connelly MA, et al. Association of the composite inflammatory biomarker GlycA, with exercise-induced changes in body habitus in men and women with prediabetes. *Oxid Med Cell Longev*. 2017;2017:5608287.
58. Taylor PC, Kremer JM, Emery P, et al. Lipid profile and effect of statin treatment in pooled phase II and phase III baricitinib studies. *Ann Rheum Dis*. 2018;77:988–995.
59. Tang WH, Wang Z, Levison BS, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med*. 2013;368:1575–1584.
60. Wang Z, Tang WH, Buffa JA, et al. Prognostic value of choline and betaine depends on intestinal microbiota-generated metabolite trimethylamine-N-oxide. *Eur Heart J*. 2014;35:904–910.
61. Mendelsohn AR, Larrick JW. Dietary modification of the microbiome affects risk for cardiovascular disease. *Rejuvenation Res*. 2013;16:241–244.
62. Li XS, Obeid S, Klingenberg R, et al. Gut microbiota-dependent trimethylamine N-oxide in acute coronary syndromes: a prognostic marker for incident cardiovascular events beyond traditional risk factors. *Eur Heart J*. 2017;38:814–824.
63. Velasquez MTR A, Manal A, Raj DS. Trimethylamine N-Oxide: the good, the bad and the unknown. *Toxins*. 2016;8:E326.
64. Dugani SB, Akinkuolie AO, Paynter N, Glynn RJ, Ridker PM, Mora S. Association of lipoproteins, insulin resistance, and rosuvastatin with incident type 2 diabetes mellitus: secondary analysis of a randomized clinical trial. *JAMA Cardiol*. 2016;1:136–145.
65. Ellsworth DL, Costantino NS, Blackburn HL, Engler RJ, Kashani M, Vernalis MN. Lifestyle modification interventions differing in intensity and dietary stringency improve insulin resistance through changes in lipoprotein profiles. *Obes Sci Pract*. 2016;2:282–292.
66. Fernandez-Castillejo S, Valls RM, Castaner O, et al. Polyphenol rich olive oils improve lipoprotein particle atherogenic ratios and subclasses profile: a randomized, crossover, controlled trial. *Mol Nutr Food Res*. 2016;60:1544–1554.