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Higher Mediterranean Diet Quality Scores and Lower Body Mass Index Are Associated with a Less-Oxidized Plasma Glutathione and Cysteine Redox Status in Adults

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Abstract

Background: Both systemic redox status and diet quality are associated with risk outcomes in chronic disease. It is not known, however, the extent to which diet quality influences plasma thiol/disulfide redox status.

Objective: The purpose of this study was to investigate the influence of diet, as measured by diet quality scores and other dietary factors, on systemic thiol/disulfide redox status.

Methods: We performed a cross-sectional study of 685 working men and women (ages ≥18 y) in Atlanta, GA. Diet was assessed by 3 diet quality scores: the Alternative Healthy Eating Index (AHEI), Dietary Approaches to Stop Hypertension (DASH), and the Mediterranean Diet Score (MDS). We measured concentrations of plasma glutathione (GSH), cysteine, their associated oxidized forms [glutathione disulfide (GSSG) and cystine (CySS), respectively], and their redox potentials (E_hGSSG and E_hCySS) to determine thiol/disulfide redox status. Linear regression modeling was performed to assess relations between diet and plasma redox after adjustment for age, body mass index (BMI), sex, race, and history of chronic disease.

Results: MDS was positively associated with plasma GSH ($\beta = 0.02$; 95% CI: 0.003, 0.03) and total GSH (GSH + GSSG) ($\beta = 0.02$; 95% CI: 0.003, 0.03), and inversely associated with the CySS:GSH ratio ($\beta = -0.02$; 95% CI: −0.04, −0.004). There were significant independent associations between individual MDS components (dairy, vegetables, fish, and monounsaturated fat intake) and varying plasma redox indexes ($P < 0.05$). AHEI and DASH diet quality indexes and other diet factors of interest were not significantly correlated with plasma thiol and disulfide redox measures.

Conclusion: Adherence to the Mediterranean diet was significantly associated with a favorable plasma thiol/disulfide redox profile, independent of BMI, in a generally healthy working adult population. Although longitudinal studies are warranted, these findings contribute to the feasibility of targeting a Mediterranean diet to improve plasma redox status. *J Nutr* 2018;148:245–253.

Keywords: diet, diet quality, glutathione, Mediterranean diet, oxidative stress, redox status

Introduction

Poor diet quality is a major factor in compromised health status and contributes to the promotion of many chronic diseases, such as cardiovascular disease (CVD), obesity, and type 2 diabetes mellitus. Diet quality can be assessed by categorizing dietary intake components based on a priori dietary recommendations and assigning specific diet indexes or scores. Three dietary patterns that are often used to examine chronic disease risk are the Alternative Healthy Eating Index (AHEI), the Dietary Approaches to Stopping Hypertension (DASH), and the Mediterranean Diet Score (MDS). Adherence to these dietary patterns is associated with a reduced risk of type 2 diabetes mellitus and CVD (1–8). To facilitate the interpretation of dietary patterns in relation to health outcomes, there is a need to understand the mechanistic underpinnings supporting the benefits of such diets and diet quality indexes. Mitigation of oxidative stress, as determined by a disruption of the balance of reversible oxidation-reduction (redox) reactions (9), may provide one such mechanism (10).

Redox balance can be assessed by the measurement of the major intra- and extracellular thiol and disulfide couples,

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glutathione (GSH)/glutathione disulfide (GSSG) and cysteine (Cys)/cystine (CySS), respectively, within the plasma (11). High plasma CySS, low plasma GSH, and a high plasma CySS:GSH ratio are indicative of increased oxidative stress, and are associated with cellular dysfunction, aging, subclinical vascular disease, and an increased risk of death in patients with CVD (12, 13). Total plasma Cys (Cys + CySS) and other plasma thiol redox markers are also positively associated with BMI and obesity risk (12, 14). Dai et al. (10) showed that adherence to the Mediterranean diet is associated with lower plasma GSSG concentrations (the oxidized form of the GSH/GSSG redox couple) and a higher plasma GSH:GSSG concentration ratio. The influence of other dietary patterns on plasma redox has not been studied, and it is not known if intake of specific dietary components, such as meat, fish, and plant-derived foods, contributes to plasma thiol and disulfide redox status.

The aim of this study was to examine the associations of diet, as measured by diet quality scores derived from 3 dietary patterns (AHEI, DASH, and MDS), and other dietary factors, on systemic thiol and disulfide redox status in a large cohort of US adults. We hypothesized that higher scores for all diet quality indexes would be associated with higher plasma GSH and lower CySS concentrations, a lower CySS:GSH ratio, and more reduced redox potentials for GSSG and CySS—all indicative of lower oxidative stress. As BMI is often a major confounder in such studies, a secondary aim was to explore the relations of obesity status with plasma redox and related dietary factors.

Methods

Study population. Participants from the Emory University/Georgia Tech Predictive Health Initiative cohort within the Center for Health Discovery and Well Being (CHDWB) were included in this cross-sectional study (15). The cohort has been previously described (3, 16). In short, the cohort consists of Emory employees and other members of the Emory and Georgia Tech communities. All participants met the inclusion criteria, namely: age ≥18 y, living in the Atlanta area, and being generally healthy and ambulatory. The following criteria excluded participants: hospitalization for acute or chronic disease within the past year; severe psychosocial disorder; addition of new medications to treat a chronic condition within the previous year (with the exception of antihypertensive or antidiabetic agents); history of substance or drug abuse; current active malignant neoplasm; history of malignancy other than localized basal cell cancer during the previous 7 y; uncontrolled or poorly controlled autoimmune, cardiovascular, endocrine, gastrointestinal, hematologic, infectious, inflammatory, musculoskeletal, neurologic, psychiatric, or respiratory disease; and any acute illness in the 12 wk before baseline visits. Participants were enrolled between January 2008 and September 2015. The current study only includes participants for whom food intake data and plasma redox status were available.

Height and weight were measured in light clothing without shoes with a Tanita TBF-215 Total Body Composition Analyzer (Tanita Health Management). BMI was calculated as kg/m^2. Weight status was classified according to WHO guidelines, with BMI <25 as normal weight, BMI 25–29.9 as overweight, and BMI ≥30 as obese (17). Race/ethnicity, education, income, and smoking status were self-reported. Subjects were classified as having a combined history of diabetes, hypertension, and/or hyperlipidemia if they self-reported any of these diseases or were taking medications to control blood pressure or blood glucose or lipid concentrations. Physical activity was measured with the use of the Cross-Cultural Activity Participation Study survey (18). Participants were coded as meeting the 2007 CDC and American College of Sports Medicine recommendations for moderate physical activity or not (19). Blood draws occurred following an overnight fast. All procedures involving human subjects were approved by the Emory University Institutional Review Board. Written informed consent was obtained from all participants.

Dietary intake assessment. Reported dietary intake over the past year was assessed with the 2005 Block FFQ (NutritionQuest). All FFQ data were energy adjusted per 1000 kcal. FFQ participants who reported consuming <500 or >5000 kcal/d were considered outliers by a priori criteria and were excluded from our analysis. Dietary intake patterns were assessed with the use of 3 validated diet quality indexes: AHEI (8), DASH (20, 21) and MDS (22). Components of all diet quality scores are summarized in **Supplemental Tables 1–3**. We defined MDS components by servings per day except for ratio of monounsaturated to saturated fatty acids. Among other dietary components, total sulfur amino acid (SAA), total red meat, and total protein consumption were specifically investigated based on previous research indicating that in humans a short-term increase in dietary SAAs (which are primarily derived from animal protein food sources) intake acutely increases plasma Cys and CySS (23).

Plasma thiol and disulfide redox status. Plasma redox outcomes were measured via HPLC, as detailed by Jones and Liang (11). Briefly, a fasted blood sample was collected and added to a preservation solution consisting of a borate buffer stock solution with iodoacetic acid and γ-glutamylglutamate. The samples were stored at –80°C until ready for analysis, whereupon samples were treated with a dansyl chloride solution for derivatization to allow for quantification of Cys, CySS, GSH, and GSSG with fluorescence detection via HPLC (Waters 2690 HPLC and autosampler system). Total GSH incorporates both GSH and GSSG. The Nernst equation was used to calculate the redox potential (E_h) in mV for the Cys/CySS and GSH/GSSG couples (E_hCySS and E_hGSSG, respectively), which provides a measure of the tendency of redox couples to accept or donate electrons (11). A more negative plasma E_h is indicative of a more reducing redox status and lower oxidative stress. The CySS:GSH ratio was also calculated, where a higher ratio indicates greater oxidative stress (13).

Statistical analysis. Descriptive characteristics were examined for all variables via univariate analysis. Continuous variables were reported as means and SDs for normally distributed variables or medians and IQRs for nonnormally distributed variables. Continuous variables that did not follow a normal distribution (plasma GSH, GSSG, and CySS/GSH) were logarithmically transformed on the natural logarithm scale for modeling. Differences in demographic and biochemical variables by gender were examined with the use of 2-sample *t* tests. We performed ANOVA with Tukey's multiple comparison tests to compare race-specific means of demographic and clinical characteristics as well as BMI category-specific (normal weight, overweight, obese) means of plasma and dietary outcomes. Because of the small sample size of non-white and non-black subjects ($n = 42$), only comparisons of black with white subjects were interpreted. Associations between plasma redox and dietary intake variables were assessed with the use of multiple linear regression, while controlling for age, BMI, sex, race, and history of diabetes, hypertension, or hyperlipidemia. Physical activity, education, income, tobacco use, and presence of hypertriglyceridemia based on fasting triglyceride concentrations were considered as potential confounders. However, controlling for these variables did not influence

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Supplemental Tables 1–7 and Supplemental Figure 1 are available from the "Supplementary data" link in the online posting of the article from the same link in the online table of contents at https://academic.oup.com/jn/.

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Abbreviations used: AHEI, Alternative Healthy Eating Index; CHDWB, Center for Health Discovery and Well Being; CVD, cardiovascular disease; CySS, cystine; DASH, Dietary Approaches to Stop Hypertension; E_h, redox potential; GSH, glutathione; GSSG, glutathione disulfide; MDS, Mediterranean Diet Score; SAA, sulfur amino acid.

associations between dietary measures and plasma redox measures and were not included in the final models. Each model included a single dietary measure and a single plasma measure. Interactions between dietary components and BMI were tested. Covariates were chosen based on inclusion in existing literature and significant associations in bivariate analyses. ANCOVA with Tukey's post-hoc analyses were also performed with the use of dietary intake variables categorized by quartiles. All analyses were performed with the use of SAS v. 9.4 (SAS Institute, Inc.), with a 2-sided $P < 0.05$ used to define statistical significance.

As a complementary analysis and to increase confidence of the multivariate results, bootstrap bagging was used to identify stable and reliable predictors of the diet quality scores (24–26). A dataset was constructed of size equal to the original (685 patients) by random sampling with replacement (bootstrap sampling). On average, approximately one-third of patients were not sampled, whereas some patients were sampled more than once. The bootstrap sample was analyzed with the use of a multiple linear regression model with an automated forward stepwise algorithm with entry criterion of $P < 0.20$. The result was stored. This process of sampling, automated analysis, and storing was repeated 1000 times. The number of times a predictor appeared in these 1000 analyses was taken as a reflection of the reliability (signal). Following Breiman's median rule (devised to balance type I and type II errors), predictors were determined to be reliably associated with the outcome if they appeared in ≥50% of the models (24, 26). The adjusted mean diet quality score and its 95% CI were calculated for each factor in the presence of the others in the final model identified with bootstrap bagging.

Results

Participant characteristics

This analysis included 685 ambulatory adults with available plasma redox and FFQ data. (See **Supplemental Figure 1** for detailed flowchart of study inclusion.) The majority (66%) of the sample was female. The ethnic/racial composition was 72% white, 22% black, and 6% American Indian or Alaska Native, Asian, or Asian Indian. The mean BMI was in the overweight category. Additional demographic and clinical characteristics of subjects overall and by sex are summarized in Table 1. In brief, women's calculated AHEI total scores were,

TABLE 1 Demographic, clinical, and redox characteristics of a cohort of working adults[1]

Characteristic	All	Males	Females	P_{gender}
Subjects, *n* (%)	685 (100)	236 (34)	449 (66)	
Age, y	48.5 ± 10.9	49.91 ± 11.9	47.73 ± 10.2	0.02
Race, *n* (%)				<0.001
White	494 (72)	198 (84)	296 (66)	
Black	151 (22)	19 (8)	132 (29)	
Other	40 (6)	19 (8)	21 (5)	
Weight, kg	79.3 ± 19.7	85.9 ± 14.9	75.9 ± 21.0	<0.001
BMI,[2] kg/m^2	27.8 ± 6.4	27.36 ± 4.1	28.04 ± 7.3	0.12
Reported history of diseases,[3] *n* (%)	219 (32)	76 (34)	135 (31)	0.29
Diabetes	36 (5)	14 (6)	22 (5)	0.62
Hypertension	127 (19)	40 (18)	87 (20)	0.61
Hyperlipidemia	117 (18)	47 (21)	70 (16)	0.08
Currently smoking, *n* (%)	35 (5)	17 (7)	18 (4)	0.11
Meet moderate physical activity guidelines, *n* (%)	170 (25)	53 (22)	117 (26)	0.25
Education completed, y	19 ± 5	21 ± 5	18 ± 4	<0.001
Income ≥$100,000, *n* (%)	388 (57)	163 (74)	225 (52)	<0.001
AHEI score	49.0 ± 10.0	47.4 ± 10.0	49.8 ± 11.3	0.006
DASH score	5.0 ± 1.0	5.2 ± 1.0	5.0 ± 1.0	0.01
MDS score	4.4 ± 1.8	4.4 ± 1.9	4.3 ± 1.8	0.42
Carbohydrates,[4] % energy	47.9 ± 7.6	47.2 ± 8.1	48.3 ± 7.2	0.07
Protein,[4] % energy	16.1 ± 2.9	15.8 ± 2.7	16.2 ± 3.0	0.09
Fat,[4] % energy	35.6 ± 6.1	35.2 ± 6.6	35.8 ± 5.9	0.25
Saturated fat	19.7 ± 9.2	21.8 ± 10.5	18.6 ± 8.2	<0.001
Monounsaturated fat	27.2 ± 11.8	29.6 ± 12.5	25.9 ± 11.2	<0.001
Polyunsaturated fat	16.3 ± 7.3	17.3 ± 7.7	15.7 ± 7.0	0.006
Energy intake, kcal/d	1713 ± 623	1883 ± 656	1624 ± 586	<0.001
Plasma Cys, μmol/L	9.31 ± 2.18	8.94 ± 2.09	9.51 ± 2.20	0.001
Plasma CySS, μmol/L	84.27 ± 18.01	82.85 ± 15.16	85.01 ± 19.32	0.11
Plasma total Cys, μmol/L	180.28 ± 37.20	177.10 ± 31.36	182.00 ± 39.85	0.08
Plasma GSH,[5] μmol/L	1.63 ± 0.58	1.60 ± 0.55	1.64 ± 0.59	0.35
Plasma GSSG,[5] μmol/L	0.049 ± 0.032	0.053 ± 0.033	0.047 ± 0.032	0.05
Plasma total GSH,[5] μmol/L	4.22 ± 1.34	4.28 ± 0.08	4.19 ± 1.38	0.41
Plasma CySS/GSH,[5] μmol/L	50.6 ± 23.1	51.0 ± 21.9	50.4 ± 23.7	0.79
Plasma E_hCySS, mV	−70.0 ± 5.7	−69.1 ± 5.7	−70.4 ± 5.6	0.003
Plasma E_hGSSG, mV	−135.9 ± 9.6	−134.6 ± 10.4	−136.6 ± 9.1	0.01

[1] Values are means ± SDs or *n* (%). AHEI, Alternative Healthy Eating Index; CHDWB, Center for Health Discovery and Well Being; CySS, cystine; DASH, Dietary Approaches to Stop Hypertension; E_h, redox potential; GSH, glutathione; GSSG, glutathione disulfide; MDS, Mediterranean Diet Score.
[2] $n = 683$.
[3] $n = 658$.
[4] Percentage of total calorie intake.
[5] Values were back-transformed from the natural-log values used in analyses and reported as geometric means ± geometric SDs.

TABLE 2 Plasma redox measures and diet intake factors by BMI category in a cohort of working adults[1]

	BMI level			
	BMI < 25 ($n = 238$)	BMI 25–29.9 ($n = 254$)	BMI ≥ 30 ($n = 191$)	P[2]
Plasma redox measures				
Cys, μmol/L	9.15 ± 2.05[a]	9.16 ± 2.14[a]	9.68 ± 2.30[b]	0.02
CySS, μmol/L	78.04 ± 15.26[a]	83.20 ± 16.37[b]	93.53 ± 19.59[c]	<0.001
GSH,[3] μmol/L	1.80 ± 0.58[a]	1.65 ± 0.57[b]	1.41 ± 0.51[c]	<0.001
GSSG,[3] μmol/L	0.050 ± 0.034[a]	0.050 ± 0.032[a]	0.045 ± 0.032[b]	0.05
Total GSH,[3] μmol/L	4.51 ± 1.37[a]	4.31 ± 1.31[a]	3.78 ± 1.22[b]	<0.001
E_hCySS, mV	−70.5 ± 5.5	−69.7 ± 5.8	−69.5 ± 5.6	0.18
E_hGSSG, mV	−137.8 ± 8.5[a]	−136.1 ± 10.0[a]	−133.3 ± 9.9[b]	<0.001
CySS/GSH[3]	42.4 ± 21.9[a]	49.5 ± 21.3[b]	65.1 ± 27.9[c]	<0.001
Diet characteristics				
Mediterranean score	4.7 ± 1.7[a]	4.3 ± 1.9[b]	4.1 ± 2.0[b]	0.002
AHEI score	51.7 ± 10.7[a]	47.9 ± 10.5[b]	46.9 ± 11.0[b]	<0.001
DASH score	5.3 ± 1.1[a]	5.0 ± 1.0[b]	4.7 ± 0.9[c]	<0.001
Protein,[4] g/(d × 1000 kcal)	39.1 ± 6.6[a]	40.6 ± 7.6[b]	41.1 ± 7.4[b]	0.008
Red meat,[4] g/(d × 1000 kcal)	34.4 ± 27.5[a]	44.1 ± 34.9[b]	57.6 ± 39.5[c]	<0.001
Total sulfur amino acids,[4] mg/(d × 1000 kcal)	1.7 ± 0.3[a]	1.8 ± 0.4[b]	1.8 ± 0.4[b]	<0.001

[1] Values are means ± SDs. BMI categories (in kg/m^2) correspond to normal weight (≤25), overweight (25.0–29.9), and obese (≥30). Outcomes within a row that are not connected by a common superscript letter are significantly different ($P < 0.05$). All dietary measures are adjusted for age, BMI, race, sex, and history of diabetes, hypertension, or hyperlipidemia. AHEI, Alternative Healthy Eating Index; CySS, cystine; DASH, Dietary Approaches to Stopping Hypertension; E_h, redox potential. GSH, glutathione; GSSG, glutathione disulfide.
[2] ANOVA was used for the analysis.
[3] Values were back-transformed from the natural-log values used in analyses and reported as geometric means ± SDs.
[4] Diet intake factors adjusted to 1000 kcal with the use of the formula: (food component intake × 1000)/energy intake (kcal).

on average, higher than men's ($P = 0.006$); but men's calculated DASH scores were, on average, higher than women's ($P = 0.01$) (Table 1). Of the plasma redox measures, women had higher Cys ($P = 0.001$); men had higher E_hCySS ($P = 0.003$) and E_hGSSG ($P = 0.01$). **Supplemental Table 4** provides additional details of demographic, clinical, and redox variables stratified by race and sex.

Among all subjects, the MDS was positively associated with the AHEI score ($r = 0.74$, $P < 0.001$) and with the DASH score ($r = 0.51$, $P < 0.001$). The AHEI score was positively associated with the DASH score ($r = 0.46$, $P < 0.001$). The associations between the plasma redox variables are presented in **Supplemental Table 5**.

Associations between plasma thiol and disulfide redox measures and diet characteristics with BMI levels

Among the plasma redox measures, CySS concentrations and CySS:GSH ratio were significantly higher in overweight (BMI 25–29.9) participants compared to normal weight (BMI <25) participants, while GSH concentrations were lower in overweight than in normal weight participants (Table 2). Plasma Cys and CySS concentrations, E_hGSSG, and the CySS:GSH ratio were significantly higher; and GSH and total GSH were lower in obese (BMI ≥ 30) compared to overweight individuals. All diet quality scores were lower among overweight compared to normal weight participants, while reported intake of protein, red meat, and total SAAs were higher among overweight compared to normal weight participants. Compared to overweight participants, those who were obese had a lower DASH score and reported higher intakes of red meat.

Associations between plasma thiol and disulfide redox measures across dietary measures

The independent associations between plasma thiol and disulfide redox measures and dietary measures are shown in Table 3. A 1-point higher MDS was associated with a 1.4% higher plasma GSH ($\beta = 0.02$; 95% CI: 0.003, 0.03; $P = 0.02$) and a 0.1% higher total GSH concentration ($\beta = 0.02$; 95% CI: 0.003, 0.03; $P = 0.02$) and a 0.1% lower CySS:GSH ratio ($\beta = -0.02$; 95% CI: –0.04, – 0.004; $P = 0.01$). Plasma redox outcomes were not associated with AHEI or DASH diet scores. Results were similar when dietary outcomes were categorized based on quartiles (**Supplemental Table 6**). There were no statistically significant interactions between diet quality scores and BMI for any of the plasma redox outcomes.

In bootstrap bagging analyses, plasma redox measures that were reliably and independently associated with MDS were CySS/GSH (52% reliability) and GSSG (58% reliability). Reliability scores for additional potential covariates are shown in **Supplemental Table 7**. In a reduced model including only reliable covariates identified by bootstrap bagging, MDS was significantly inversely associated with the CySS:GSH ratio ($\beta =$ –0.007 ± 0.003; regression coefficient ± SE; $P = 0.02$, Table 4).

Associations between thiol and disulfide redox measures and Mediterranean diet components

Table 5 displays exploratory independent associations between plasma redox measures and the individual food components of the Mediterranean diet. A greater vegetable consumption (1 serving/d) was associated with a 0.44-μM higher plasma Cys concentration (95% CI: 0.08, 0.80 μM; $P = 0.01$) and a 1.18-mV lower plasma E_hCySS redox potential (95% CI: –2.12, −0.24 mV; $P = 0.01$). A greater fish consumption (1 serving/d) was associated with a 3.30-μM lower CySS concentration (95% CI: –5.83, −0.08 μM; $P = 0.01$). A 1-unit higher ratio of monounsaturated to saturated fat was associated with a 0.12-μM higher GSSG concentration (95% CI: 0.01, 0.23 μM; $P = 0.03$). Greater dairy consumption (1 serving/d) was associated with a 0.08-μM (95% CI: 0.02, 0.13 μM) higher plasma GSH, a 0.12-μM (95% CI: 0.01, 0.23 μM) higher GSSG

TABLE 3 Associations between measures of diet quality and plasma redox measures in a cohort of working adults[1]

	Plasma redox measure							
Dietary measure	Cys, μM	CySS, μM	GSH,[2] μM	GSSG,[2] μM	Total GSH,[2] μM	E_hCySS, mV	E_hGSSG, mV	CySS/GSH[2]
Mediterranean score	0.02 ± 0.05	−0.28 ± 0.33	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	−0.08 ± 0.12	−0.11 ± 0.20	−0.02 ± 0.01
	(0.73)	(0.39)	(0.02)*	(0.09)	(0.02)*	(0.53)	(0.58)	(0.01)*
AHEI score	−0.01 ± 0.01	−0.05 ± 0.06	0.001 ± 0.001	0.001 ± 0.002	0.001 ± 0.001	0.02 ± 0.02	−0.02 ± 0.03	−0.002 ± 0.001
	(0.37)	(0.37)	(0.40)	(0.69)	(0.31)	(0.45)	(0.66)	(0.27)
DASH score	−0.07 ± 0.08	−0.23 ± 0.60	0.02 ± 0.01	−0.002 ± 0.03	0.02 ± 0.01	0.18 ± 0.22	−0.64 ± 0.36	−0.03 ± 0.02
	(0.38)	(0.70)	(0.08)	(0.92)	(0.08)	(0.41)	(0.08)	(0.08)
Protein, g/(d × 1000 kcal)[3]	0.003 ± 0.01	−0.04 ± 0.08	0.001 ± 0.002	0.0002 ± 0.004	0.0001 ± 0.002	0.004 ± 0.03	−0.02 ± 0.05	−0.001 ± 0.002
	(0.82)	(0.65)	(0.57)	(0.96)	(0.94)	(0.89)	(0.63)	(0.51)
Red meat, g/(d × 1000 kcal)[3]	0.001 ± 0.003	0.005 ± 0.018	0.0002 ± 0.0004	0.001 ± 0.001	0.0003 ± 0.0004	−0.004 ± 0.007	0.001 ± 0.011	−0.0002 ± 0.0005
	(0.69)	(0.79)	(0.54)	(0.47)	(0.48)	(0.57)	(0.93)	(0.63)
Total sulfur amino acids, mg/(d × 1000 kcal)[3,4]	−0.13 ± 0.23	−0.34 ± 1.65	−0.01 ± 0.04	0.01 ± 0.07	−0.02 ± 0.03	0.32 ± 0.60	0.38 ± 1.00	0.01 ± 0.04
	(0.57)	(0.84)	(0.76)	(0.91)	(0.46)	(0.59)	(0.70)	(0.86)

[1] Data are β ± SE (*P* value). Multiple linear regression was used for the analysis. Statistically significant relations ($P < 0.05$) are denoted with an asterisk. All models are adjusted for age, BMI, race, sex, and history of diabetes, hypertension, or hyperlipidemia. AHEI, Alternative Healthy Eating Index; CySS, cystine; DASH, Dietary Approaches to Stopping Hypertension; E_h, redox potential; GSH, glutathione; GSSG, glutathione disulfide.
[2] Analyses were conducted on natural log-transformed values.
[3] Diet intake factors adjusted to 1000 kcal with the use of the formula: (food component intake × 1000)/energy intake (kcal).
[4] Total sulfur amino acid = dietary CySS + dietary Cys + dietary Met.

concentration, and a 0.09-unit (95% CI: −0.015, −0.02 units) lower CySS:GSH ratio ($P = 0.01$, $P = 0.03$, and $P = 0.01$, respectively). Analyses of different dairy sources (milk, cheese, yogurt) resulted in an inverse relation between yogurt intake (cups) and plasma CySS:GSH ratio ($\beta = -0.27 \pm 0.12$; 95% CI: −0.50, −0.03; $P = 0.02$). Neither milk nor cheese intake were associated with plasma CySS/GSH ($\beta = -0.02 \pm 0.04$, $P = 0.66$ and $\beta = -0.03 \pm 0.06$, $P = 0.69$, respectively). There were no statistically significant interactions between diet components and BMI for any of the plasma redox outcomes.

Discussion

In this study of a working adult population, participants reporting greater adherence to a Mediterranean diet pattern had lower thiol-related oxidative stress. This was shown by a higher plasma GSH concentration and a lower plasma CySS:GSH ratio. Our results are consistent with those of Dai et al. (10) who showed an inverse association between adherence to the Mediterranean diet and oxidative stress, as measured by the GSH:GSSG ratio, in male twin pairs from the Vietnam Era Twin Registry. We have expanded these findings to a more heterogeneous population. Although randomized trials are needed to confirm effects on plasma GSH/GSSG systemic redox, other markers of oxidative stress, such as F2-isoprostane and total antioxidant capacity, were shown to respond to Mediterranean diet-based interventions (27–34). Taken together, these consistent results provide a strong rationale for recommending dietary pattern changes, such as the Mediterranean diet, to improve oxidative stress.

TABLE 4 Correlates of the MDS using multiple linear regression with a bootstrap model selection procedure[1]

Variable	β ± SE[2]	Adjusted mean MDS [95% CI]	*P*	Reliability (%)[3]
Intercept	3.27 ± 0.43			
Currently smoking	0.68 ± 0.33		0.04	79
No		4.2 [3.9, 4.5]		
Yes		3.5 [2.8, 4.1]		
Meet moderate physical activity guidelines	−0.32 ± 0.17		0.05	76
No		3.7 [3.3, 4.1]		
Yes		4.0 [3.5, 4.5]		
Age[4]	0.02 ± 0.01	4.0 [3.4, 4.6]	0.03	63
Diabetes	0.55 ± 0.31		0.08	63
No		4.1 [3.8, 4.5]		
Yes		3.6 [2.9, 4.2]		
Plasma GSSG[5]	3.01 ± 1.59	3.4 [2.6, 4.2]	0.06	58
Income ≥$100,000	−0.32 ± 0.15		0.03	58
No		3.7 [3.3, 4.1]		
Yes		4.0 [3.6, 4.4]		
Plasma CySS/GSH[6]	−0.007 ± 0.003	2.9 [2.1, 3.8]	0.02	52

[1] Covariates with reliability <50% were not included in the final MDS multivariable model. CySS, cystine; GHS, glutathione; GSSG, glutathione disulfide; MDS, Mediterranean Diet Score.
[2] Estimated regression coefficient (β) ± SE for MDS.
[3] Percentage of time each factor appears in 1000 multiple linear regression models. Factors appearing in ≥50% of models are reliable.
[4] Mean = 48.5 y.
[5] Mean = 0.05 μmol/L.
[6] Mean = 50.6.

TABLE 5 Associations between MDS components and plasma redox measures in a cohort of working adults[1]

MDS component	Plasma redox measure							
	Cys, μM	CySS, μM	GSH,[2] μM	GSSG,[2] μM	Total GSH,[2] μM	E_hCySS, mV	E_hGSSG, mV	CySS/GSH[2]
Vegetables, servings/d	0.44 ± 0.18 (0.01)*	0.06 ± 1.33 (0.97)	−0.02 ± 0.03 (0.44)	−0.08 ± 0.06 (0.13)	−0.02 ± 0.03 (0.38)	−1.18 ± 0.48 (0.01)*	−0.52 ± 0.81 (0.52)	0.02 ± 0.03 (0.58)
Legumes, nuts, and soy, servings/d	−0.19 ± 0.18 (0.30)	−0.54 ± 1.32 (0.68)	−0.0004 ± 0.03 (0.99)	0.08 ± 0.06 (0.15)	0.01 ± 0.03 (0.61)	0.39 ± 0.48 (0.42)	1.06 ± 0.80 (0.19)	−0.01 ± 0.03 (0.86)
Fruit, servings/d	−0.22 ± 0.17 (0.21)	−0.48 ± 1.27 (0.71)	0.03 ± 0.03 (0.29)	0.005 ± 0.05 (0.93)	0.03 ± 0.03 (0.23)	0.54 ± 0.46 (0.24)	−0.71 ± 0.77 (0.36)	−0.04 ± 0.03 (0.22)
Total grains, servings/d	−0.22 ± 0.18 (0.24)	0.46 ± 1.33 (0.73)	−0.01 ± 0.03 (0.84)	−0.02 ± 0.06 (0.73)	−0.02 ± 0.03 (0.41)	0.70 ± 0.48 (0.15)	−0.08 ± 0.81 (0.92)	0.01 ± 0.03 (0.67)
Fish, servings/d	−0.08 ± 0.18 (0.66)	−3.30 ± 1.29 (0.01)*	0.02 ± 0.03 (0.46)	0.07 ± 0.05 (0.23)	0.01 ± 0.03 (0.70)	−0.26 ± 0.46 (0.58)	0.33 ± 0.78 (0.67)	−0.06 ± 0.03 (0.07)
Monounsaturated-to-saturated fat ratio	0.20 ± 0.18 (0.27)	2.03 ± 1.32 (0.12)	0.05 ± 0.03 (0.09)	0.12 ± 0.06 (0.03)*	0.05 ± 0.03 (0.06)	−0.19 ± 0.48 (0.68)	0.33 ± 0.80 (0.68)	−0.02 ± 0.03 (0.48)
Alcohol, servings/d	0.11 ± 0.18 (0.54)	0.58 ± 1.31 (0.66)	0.01 ± 0.03 (0.69)	−0.07 ± 0.06 (0.24)	0.01 ± 0.03 (0.62)	−0.29 ± 0.47 (0.54)	−1.15 ± 0.79 (0.15)	0.002 ± 0.03 (0.96)
Dairy, servings/d	0.03 ± 0.19 (0.88)	−0.62 ± 1.35 (0.64)	0.08 ± 0.03 (0.01)*	0.12 ± 0.06 (0.03)*	0.08 ± 0.03 (0.004)*	−0.09 ± 0.49 (0.85)	−0.35 ± 0.82 (0.67)	−0.09 ± 0.03 (0.01)*
Red and white meat, servings/d	0.09 ± 0.18 (0.64)	−0.85 ± 1.34 (0.52)	0.01 ± 0.03 (0.64)	−0.03 ± 0.06 (0.66)	−0.01 ± 0.03 (0.69)	−0.35 ± 0.48 (0.46)	−0.64 ± 0.81 (0.43)	−0.02 ± 0.03 (0.48)

[1] Data are β ± SEs (*P* value). Multiple linear regression was used for the analysis. All models are adjusted for age, BMI, race, sex, and history of diabetes, hypertension, or hyperlipidemia. Statistically significant relations ($P < 0.05$) are denoted with an asterisk. Diet intake factors were adjusted to 1000 kcal with the use of the formula: (food component intake × 1000)/energy intake (kcal). CySS, cystine; E_h, redox potential; GSH, glutathione; GSSG, glutathione disulfide.
[2] Analyses were conducted on natural log-transformed values.

To expand on our findings and those of Dai et al. (10), we performed exploratory analyses to determine which specific components of the MDS were most strongly associated with plasma redox indicators. A novel finding in our cohort was that dairy intake was positively associated with plasma GSH, GSSG, and total GSH concentrations, and inversely associated with CySS:GSH ratio. A previous study by Choi et al. (35) similarly found a positive association between dairy intake and brain GSH concentrations. Intervention studies have also indicated an oxidative stress–lowering effect of high dairy intake (36, 37). Further exploration of our data showed that intake of yogurt, but not cheese and milk, was inversely associated with CySS:GSH ratio. Yogurt intake, specifically, has been shown to inversely correlate with type 2 diabetes risk in the PREDIMED cohort (38, 39). The mechanisms mediating a positive relation between dairy intake and favorable redox outcomes are unknown; however, it is possible that a number of components of dairy foods, such as calcium, vitamin D, vitamin B-12, Cys-rich casein and whey proteins, riboflavin, and probiotics (in the case of yogurt), may contribute (16, 35, 36, 40–43). Whether dairy fat content influences plasma redox remains to be studied. Fish consumption was also associated with lower plasma CySS, a previously unreported finding. Diets rich in ω-3 FAs have been found to decrease oxidation of LDLs in humans (44). Our results are also consistent with existing literature (45), in that vegetable consumption was associated with higher plasma Cys and a lower E_hCySS, indicative of lower oxidative stress.

The dietary factors varied in association with the GSH/GSSG and Cys/CySS redox couples. The GSH and Cys thiol and disulfide systems represent 2 distinct redox pools and are not in equilibrium in the plasma (46). The GSH/GSSG couple is more reflective of tissue redox status, whereas the Cys/CySS couple is more reflective of extracellular oxidation and/or CySS uptake and reduction by systems that are distinct from the GSSG reductase (9, 12). Thus, the nonuniformity of dietary correlations with the redox pools was not unexpected. A novel outcome variable was the CySS:GSH ratio. The CySS:GSH ratio can be considered a mechanistic biomarker that provides a measure of the overall health of the redox networks that influence aging and chronic disease, with CySS serving as an indicator of oxidant burden and GSH reflecting the NADPH-reductive capacity within the redox network (47). This ratio was recently shown to be a stronger predictor of cardiovascular outcomes compared to the individual thiol and disulfide systems (13).

Based on clinical studies showing changes in plasma redox following manipulations in SAA intake (23, 48) and on the high concentrations of SAA in animal proteins (49), we expected to find significant relations between dietary intake of SAAs, total protein, and/or meats with plasma redox biomarkers. In contrast, these individual dietary components were not significantly associated with any plasma redox biomarkers. In a Dutch cohort of older adults, Elshorbagy et al. (50) reported a positive association between animal-derived protein intake and plasma total Cys. The discordance between these 2 cohort studies may stem from differences in diet, age, race, health status, country, or redox methodology. It is possible that dietary components that we did not assess have a greater influence on plasma thiol and disulfide redox in our population. Our study highlights the importance of assessing overall diet quality indexes as a complement to focusing on individual nutrients when investigating contributors to plasma redox status.

The AHEI and DASH scores were not associated with any of the plasma measures of thiol and disulfide redox. Other studies have noted the superiority of the MDS over other diet quality indexes in relation to metabolic risk factors (51, 52). Nonetheless, adherence to the AHEI and DASH diets has been associated with improved health status (2, 3). It is possible that the diets and diet quality scores of participants in this southern US city may not be as diverse or variable enough to observe significant relations with plasma redox. Another possible explanation for the lack of association is that the AHEI and DASH score calculations were based on published cut-points; in contrast, the

MDS was based on median intakes of specific nutrients within our population. Therefore, the MDS may better capture dietary quality in our sample, which may explain the stronger associations observed in our analysis.

In this study, BMI was strongly associated with plasma thiol and disulfide redox, diet quality indexes, and SAA-related dietary outcomes. Our study is the first to our knowledge to report the association between BMI and the CySS:GSH ratio and corresponding redox potentials. Those with higher BMI demonstrated a more-oxidized plasma Cys/CySS redox state and lower plasma GSH. Similar results have been previously reported for the relation of GSH and other oxidative stress markers with obesity (53–55). In vitro data show that an oxidized state of the extracellular Cys/CySS redox environment promotes adipogenesis and expression of pro-adipogenic genes (56). Alternatively, it is possible that high oxidative stress is not a predictor of obesity development, but rather a biomarker for obesity-related disease development (57, 58).

Positive associations of obesity or BMI with protein, red meat (59, 60), and methionine (the precursor of cysteine) intake (57) have been previously reported. A causal relation between adiposity and dietary SAAs in humans has not yet been established; however, animal studies have indicated that dietary Cys/CySS promotes obesity by decreasing energy expenditure (57). Because of the cross-sectional design, our study cannot confirm that this relation is mediated by changes in the plasma redox environment.

Strengths of this study include the large sample size and detailed assessment of the plasma thiol and disulfide redox state. The plasma thiol and disulfide redox systems represent nonfree radical–mediated oxidant mechanisms (11), which are a distinguishing feature of our redox endpoints. Given that interventions targeting free-radical oxidant mechanisms have generally been clinically unsuccessful (61), the plasma redox outcomes we utilized may be relevant biomarker targets for future dietary interventions. A limitation is that no causality of the effect of diet on plasma redox concentrations or obesity can be inferred. Because this was an exploratory analysis, we did not consider the interdependence between endpoints and performed multiple linear regression for each outcome as if they were independent of one another, and a priori correction for multiple testing such as Bonferroni's correction was not performed. To address the issue of multiplicity and balance type I and type II errors, the bootstrap bagging method was employed (24), eliminating the need for additional adjustment and increasing the confidence that the MDS is associated with plasma thiol and disulfide redox. Nevertheless, reproduction of the observed associations and their magnitudes requires further independent studies. Another limitation is that the calculation of dietary index scores is not standardized and may differ based on investigator interpretations. Furthermore, the diet scores were created with the use of self-reported data and, thus, susceptible to recall bias or over- and underreporting. Finally, our population consists primarily of generally healthy university employees with relatively high education and income levels and low smoking levels, limiting the generalizability of our results. However, the implications for simple dietary adjustments and lifestyle changes to influence systemic redox are applicable to all populations.

In summary, a higher MDS was associated with healthier plasma redox status indexes. Dairy, fish, and vegetable intakes were positively associated with a more favorable plasma redox status, while obesity status was highly correlated with a more-oxidized plasma redox state. This study indicates the feasibility of targeting diet to improve systemic redox status and dictates a need for Mediterranean-diet-based interventions. Longitudinal analyses and randomized controlled trials are required to further study the effect of diet, in particular, the Mediterranean diet, on plasma redox status over time and the long-term effects on chronic disease risk.

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