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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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B.S.

Lehigh University

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Abstract

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By: Erika Bettermann

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.
Design: We performed a cross-sectional study of 685 working adults in Atlanta, Georgia. Diet was measured by three 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 (Cys), their associated oxidized forms – glutathione disulfide (GSSG) and cystine (CySS) – and their redox potentials – E_hGSSG and E_hCySS – to determine thiol/disulfide redox status. Linear regression modeling was performed to assess relationships after adjustment for age, body mass index (BMI), race, sex, and history of diabetes, hypertension and/or hyperlipidemia.
Results: MDS was positively associated with plasma GSH (β=0.03, $P<0.05$) and total GSH (GSH + GSSG) (β= 0.03, $P<0.05$), and inversely associated with the CySS/GSH ratio (β=-0.02, $P<0.05$). Additionally, we found correlations between individual Mediterranean diet components (dairy, vegetables, fish, and monounsaturated fat intake) with plasma redox indices. AHEI and DASH diet quality indices and other diet factors of interest were not significantly correlated with plasma thiol/disulfide redox measures.
Conclusion: Mediterranean diet was significantly associated with plasma thiol/disulfide redox systems, adjusted for BMI. These findings contribute to the feasibility of targeting Mediterranean diet to improve plasma redox status.

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Introduction

Poor diet quality is a major contributor of compromised health status and the promotion of many chronic diseases, such as cardiovascular disease (CVD), obesity and type 2 diabetes mellitus (T2DM). Diet quality can be assessed by categorizing dietary intake components based on *a priori* dietary recommendations and assigning specific diet indices 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 Mediterranean Diet Score (MDS). Adherence to these dietary patterns is associated with reduced risk of T2DM 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 indices. 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/disulfide couples, 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 cardiovascular disease (CVD) (12, 13). Plasma CySS is also positively associated with body mass index (BMI) and obesity risk (14). Dai et al 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, a marker of oxidative stress (10). 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/disulfide redox status.

The aim of this study was to examine the associations of diet, as measured by diet quality scores derived from three dietary patterns (AHEI, DASH, and MDS), and other dietary factors, on systemic thiol/disulfide redox status in a large cohort of US adults. We hypothesized that higher scores for all diet quality indices 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 relationships of obesity status with plasma redox and related dietary factors.

Subjects and 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 of: age ≥ 18 years, 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; prescription of medications to treat a chronic condition (with the exception of changes in anti-hypertensive or anti-diabetic agents) within the previous year; history of substance/drug abuse; current active malignant neoplasm; history of malignancy other than localized basal cell cancer during the previous 7 years; uncontrolled or poorly controlled autoimmune, cardiovascular, endocrine, gastrointestinal, hematologic, infectious, inflammatory, musculoskeletal, neurologic, psychiatric or respiratory disease; and any acute illness in the twelve weeks before baseline visits. Participants were enrolled between January 2008 and September 2015. The current study includes 685 CHDWB participants with available food intake data and plasma redox status.

Height (feet) and weight (pounds) were measured in light clothing without shoes with a Tanita Total Body Composition Analyzer (TBF-215 Body Composition Analyzer, *Tanita Health Management*, Arlington Heights, IL). BMI was calculated as kg/m^2. Weight status was classified according to WHO guidelines, with BMI < 25 kg/m^2 as normal weight, BMI 25-29.9 kg/m^2 as overweight, and BMI ≥ 30 kg/m^2 as obese (17). Race/ethnicity, history of chronic disease, education, income and smoking status were self-reported. Physical activity was measured using the Cross-Cultural Activity Participation Study survey (18). Participants were coded as meeting the 2007 Centers for Disease Control and Prevention (CDC) and American College of Sports Medicine (ACSM) recommendations for moderate physical activity or not (19). 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 Food Frequency Questionnaire (FFQ, *NutritionQuest*, Berkeley, CA). All FFQ data were energy-adjusted per 1,000 kcal. FFQ participants who reported consuming <500 kcal or >5,000 kcal per day were considered outliers by *pre hoc* criteria and were excluded from our analysis. Dietary intake patterns were assessed using three validated diet quality indices: AHEI (8), DASH (20, 21) and MDS (22). The nine-component AHEI was developed to improve upon the original HEI (8). High consumption of vegetables, fruits, cereals and grains, beans, nuts, soy, polyunsaturated fats and multivitamins; and low consumption of trans fat, alcohol and red meat are all positive components of the AHEI. The total score ranges from 2.5 (lowest adherence) to 87.5 (highest adherence). The DASH score incorporates high consumption of vegetables, fruit, whole grains, beans, nuts and seeds; and low consumption of sodium, fat, meat and sweets (20). A DASH score of 11 indicates highest adherence. Adherence to the Mediterranean diet is defined by high intake of fruit, vegetables, bread, cereals and other grains, beans, nuts, and seeds; low-to-moderate intake of dairy products, fish, poultry and wine; low intake of red meat; and high dietary ratio of monounsaturated to saturated fatty acids (i.e. high olive oil consumption) (10). We defined MDS components by servings/day except for ratio of monounsaturated to saturated fatty acids. The maximum MDS is nine, which indicates the highest adherence. Components of all diet quality scores are summarized in **Supplemental Tables 1-3**. 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 a short-term increase in dietary SAA (which are primarily derived from animal protein food sources) intake acutely increases plasma Cys and CySS, in humans (23).

Plasma thiol/disulfide redox status

Plasma redox outcomes were measured via high performance liquid chromatography (HPLC), as detailed by Jones and Liang (11). Briefly, a 3 mL blood sample was slowly collected in a syringe with care to avoid hemolysis and added to a preservation solution consisting of a borate buffer stock solution with iodoacetic acid to inhibit autooxidation, as well as γ-glutamylglutamate, as an internal standard. After centrifugation, plasma was transferred to a 10% perchloric acid solution containing boric acid. The samples were stored at -80ºC until ready for analysis, upon which samples were thawed and protein precipitated. The supernatant was treated with a potassium hydroxide/tetraborate solution to adjust the pH to 9.0 ± 0.2. A dansyl chloride solution was added for derivatization to allow for quantification of Cys, CySS, GSH, and GSSG with fluorescence detection via HPLC (Waters 2690 HPLC and autosampler system, Milford, MA). Total GSH incorporates both GSH and GSSG. The Nernst equation was used to calculate the redox potential in millivolts (mV) for the Cys/CySS and GSH/GSSG couples (E_hCySS and E_hGSSG, respectively), which provide a measure of the tendency of redox couples to accept or donate electrons (11). A more negative plasma redox potential 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 SD for normally distributed variables or medians and IQR for non-normally distributed variables. Categorical variables were presented as number of subjects and percentages. 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 using two-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/non-Black subjects (n=42), comparisons were only interpreted for Black versus White subjects. Associations between plasma redox and dietary intake variables were assessed using multiple linear regression, while controlling for age, BMI, sex, race, and history of diabetes, hypertension or hyperlipidemia. Physical activity, education and income were considered as potential confounders. However, controlling for these variables did not influence associations between dietary measures and plasma redox measures and were not included in the final model. Each model included a single dietary measure and a single plasma measure. Covariates were chosen based on inclusion in existing literature and significant associations in bivariate analyses. All dietary measures, diet quality scores, and macronutrient intakes were categorized into quartiles based on the 25%, 50%, and 75% cut-points from the univariate output. ANCOVA with Tukey's post-hoc multiple comparison test was performed to examine associations between dietary intake variables and plasma redox outcomes and dietary quartiles, controlling for age, BMI, sex, race, and history of diabetes, hypertension and/or hyperlipidemia as covariates. All analyses were performed using SAS v 9.4 (SAS Institute, Inc., Cary, NC), with a two-sided $P < 0.05$ used to define statistical significance.

Results

Participant characteristics

Of the total sample of 727 participants with baseline data, a total of 36 participants (5.0%) with missing plasma redox measures and 6 participants (0.1%) with missing FFQ data were excluded from this study. Thus, the final analysis included 685 ambulatory adults (**Supplemental Figure 1**). The majority (65%) of the sample was female. The ethnic/racial composition was 71% White, 22% Black, and 6% American Indian or Alaska Native, Asian, or Asian Indian. The mean BMI (28 kg/m^2) was in the overweight category. Additional demographic and clinical characteristics of subjects are summarized in **Table 1**. We observed significant differences in these characteristics across sex and race categories. Thus, Table 1 presents participant characteristics stratified by sex and race.

Females' calculated AHEI total scores were, on average, higher than males ($P = 0.006$); but males' calculated DASH scores were, on average, higher than females ($P = 0.01$) (**Supplemental Table 4**). Of the plasma redox measures, women had higher Cys ($P = 0.001$); men had higher GSSG ($P = 0.05$), E_hCySS ($P = 0.003$) and E_hGSSG ($P = 0.01$).

Overall, Black participants had a higher BMI (31.4 vs. 27.0 kg/m^2, $P < 0.005$) than White participants. Among females, Whites had a lower BMI (26.7 vs. 31.8 kg/m^2), higher scores for all three diet quality indices, and consumed less carbohydrates and more protein compared to Blacks (Table 1). Among males, Whites had a lower BMI (27.5 vs. 30.0 kg/m^2) and a higher total DASH score (5.2 vs. 4.6) than Blacks. Whites, on average, had more favorable plasma redox measures compared to Blacks: a lower CySS concentration (82.9 vs. 85.5 μM) and a higher GSH concentration (1.6 vs. 1.5 μM), and lower CySS/GSH ratio (49.3 vs. 57.7) (all *P* <0.005); although significant differences were shown only in females (Table 1).

Associations between 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 $\geq$ 25, < 30 kg/m^2) participants compared to normal weight (BMI < 25 kg/m^2) 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 $\geq$ 30 kg/m^2) 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 sulfur amino acids 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 redox measures across dietary measures.

The associations between plasma thiol/disulfide redox measures and dietary measures, adjusted for age, BMI, race, sex and history of diabetes, hypertension and/or hyperlipidemia are shown in **Table 3**. A one-point higher MDS was associated with a 0.02 μM (1.4% and 0.1%, respectively) higher plasma GSH and total GSH concentrations (β=0.02, *P* =0.02) and a 0.02-unit (0.1%) lower CySS/GSH ratio (β=-0.02, *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 5**).

Associations between thiol/disulfide redox measures and Mediterranean Diet components.

Table 4 displays exploratory associations between plasma redox measures and the individual food components of the Mediterranean diet, adjusted for age, BMI, race, sex and history of diabetes, hypertension and/or hyperlipidemia. A greater vegetable consumption (1 serving/day) was associated with a 0.44 μM higher plasma Cys concentration (β = 0.44, *P* =0.02) and a 1.18 mV lower plasma E_hCySS redox potential (β = -1.18, *P* =0.01). A greater fish consumption (1 serving/day) was associated with a 3.30 μM lower CySS concentration (β = -3.30, *P* =0.01). A 1-unit higher ratio of monounsaturated to saturated fat was associated with a 0.12 μM higher GSSG concentration (β = 0.12, *P* =0.03), with a trend towards significance of higher GSH and total GSH. Greater dairy consumption (1 serving/day) was associated with a 0.08 μM higher plasma GSH and a 0.12 μM higher GSSG concentration and a 0.09-unit lower CySS/GSH ratio (β=0.08, *P* =0.01; β=0.12, *P* =0.03 and β=-0.09, *P* =0.01, respectively).

Discussion

In this study of a working adult population in the Atlanta, GA metropolitan area, participants reporting greater adherence to a Mediterranean diet pattern had a higher plasma GSH concentration and a lower plasma CySS/GSH ratio, indicative of lower oxidative stress. Our results are consistent with Dai et al (10) who showed an independent, inverse association between adherence to the Mediterranean diet and oxidative stress, as measured by the GSH/GSSG ratio, in monozygotic and dizygotic male twin pairs from the Vietnam Era Twin Registry. We have expanded these findings to a more heterogeneous population, likely consuming a different type of diet than that cohort. Randomized controlled trials are needed to confirm effects of the Mediterranean diet on changes in plasma GSH/GSSG systemic redox. Other markers for oxidative stress, such as F2-isoprostane and total antioxidant capacity, have been shown to respond to Mediterranean diet-based interventions (24-31). Taken together, these consistent results provide strong rationale for recommending dietary pattern changes, such as the Mediterranean diet, to improve oxidative stress.

To expand upon our findings and that of Dai et al (10), we performed further 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 significantly and positively associated with plasma GSH, GSSG and total GSH concentrations, and inversely associated with CySS/GSH ratio. A previous study by Choi et al similarly found a positive association between dairy intake and brain GSH concentrations (32). Intervention studies have also indicated an oxidative stress-lowering effect of high dairy intake (33, 34). The mechanisms mediating a positive relationship between dairy intake and favorable redox outcomes are unknown; however, it is possible that several components of dairy foods, such as calcium, vitamin D, Cys-rich casein and whey proteins, and riboflavin may contribute (16, 32, 33). Reported fish consumption was also associated with lower plasma CySS, a previously unreported finding. Diets rich in omega-3 fatty acids have been found to decrease oxidation of low-density lipoproteins in humans (35). Our results are also consistent with existing literature, in that vegetable consumption was associated with higher plasma Cys and a lower E_hCySS, indicative of lower oxidative stress (36). A framework for the beneficial effects of the Mediterranean diet, based on our findings, is summarized in **Figure 1.**

Based on previous clinical studies showing changes in plasma redox following manipulations in dietary SAA intake (23, 37) and on the high levels of SAA in animal proteins (38), we expected to find significant relationships between dietary intake of SAA, total protein, and/or meats with plasma redox biomarkers. In contrast, these individual dietary components were not significantly associated with any plasma redox biomarkers. It is possible that other dietary components that we did not assess have a greater influence on plasma thiol/disulfide redox, for example, content of specific amino acids and/or vitamins. Our study highlights the importance of assessing overall diet quality indices 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/disulfide redox, a relationship that has not been previously investigated. Consistent with our results, other studies have noted the superiority of the MDS over other diet quality indices in relationship to metabolic risk factors (39, 40). 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 U.S. city may not be as diverse or variable enough to observe significant relationships 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 stronger associations observed in our analysis.

In this study, BMI was strongly associated with plasma thiol/disulfide redox, diet quality indices, 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. Lower plasma GSH was also associated with elevated BMI. Similar results have been reported for the relationship of GSH and other oxidative stress markers with obesity (41-43). *In vitro* data show that an oxidized state of the extracellular Cys/CySS redox environment promotes adipogenesis and expression of pro-adipogenic genes (44). Alternatively, it is possible that high oxidative stress is not a predictor of obesity development, but rather a biomarker for obesity-related disease development (45, 46).

Positive associations of obesity or BMI with protein, red meat (47, 48), and methionine (the precursor of cysteine) intake (45) have been previously reported. A causal relationship between adiposity and dietary SAA intake in humans has not yet been established; however, animal studies have indicated that dietary Cys/CySS promotes obesity by decreasing energy expenditure (45). Because of the cross-sectional design, our study cannot confirm that this relationship 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/disulfide redox state. The plasma thiol/disulfide redox systems represent non-free 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 (49), the plasma redox outcomes we utilized may be relevant biomarker targets for future dietary interventions. As this was a cross-sectional analysis, no causality of the effect of diet on plasma redox levels or obesity can be inferred. Furthermore, the components of all three diets were created using self-report data from FFQs and, thus, susceptible to recall bias or over- and under-reporting. Another limitation is that the calculation of dietary index scores is not standardized and may differ based on investigator interpretations. Finally, our population consists of full-time Atlanta metropolitan area university employees, who self-enrolled in the CHDWB cohort, generally reported high education levels and were likely health conscious; therefore, our results are not representative of the general US population. However, implications for simple dietary adjustments and lifestyle changes to influence systemic redox may be applicable in other populations, regardless of income or education level. Longitudinal analyses and randomized, controlled trials are required to further study the effect of diet, in particular, the Mediterranean diet, on plasma redox levels over time.

In summary, a higher MDS was associated with healthier plasma redox status indices. Dairy, fish and vegetable intake 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. Future studies should focus on gaining an understanding of how improved diet affects plasma redox status longitudinally and the long-term effects on chronic disease risk.

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Tables

Table 1. Demographic and Clinical Characteristics of the CHDWB Participants at Baseline Stratified by Race and Gender.

Gender	**All**	**Female**			**Male**		
Characteristic		**White**	**Black**	**Other Races**	**White**	**Black**	**Other Races**
No. of subjects (%)	727	310 (43)	137 (19)	23 (3)	208 (29)	22 (3)	19 (3)
Age, y	48.5 (10.8)	48.76 ± 10.28^{a}	46.29 ± 9.31^{b}	43.87 ± 12.55^{b}	50.77 ± 11.78^{a}	45.82 ± 11.04^{a}	45.74 ± 12.00^{a}
Weight, kg^{1}	174.77 ± 43.22	159.98 ± 39.50^{a}	188.91 ± 53.53^{b}	134.79 ± 32.98^{c}	190.81 ± 31.21^{a}	203.80 ± 30.65^{a}	155.13 ± 24.87^{b}
BMI, kg/m^{2}	27.8 (6.4)	26.68 ± 6.41^{a}	31.65 ± 8.23^{b}	23.83 ± 4.47^{a}	27.43 ± 3.95^{a}	29.87 ± 3.50^{b}	23.86 ± 2.27^{c}
Reported History of Any of the Following:^{2}	221 (32)	90 (29)^{a}	51 (37)^{a}	4 (17)^{a}	69 (33)^{a}	11 (50)^{a}	6 (33)^{a}
Diabetes (%)	39 (6)	16 (5)^{a}	10 (7)^{a}	1 (4)^{a}	8 (4)^{a}	4 (20)	2 (11)^{a}
Hypertension (%)	134 (19)	53 (17)^{a}	42 (31)^{b}	1 (4)^{a}	31 (15)^{a}	11 (50)	3 (17)^{a}
Hyperlipidemia (%)	123 (18)	53 (17)^{a}	21 (15)^{a}	2 (9)^{a}	48 (23)^{a}	3 (15)^{a}	1 (6)^{a}
Currently Smoking (%)	36 (5)	22 (7)^{a}	11 (8)^{a}	0 (0)^{a}	15 (7)^{a}	1 (5)^{a}	2 (11)^{a}
Meet Moderate Physical Activity Guidelines (%)	182 (25)	80 (26)^{a}	36 (26)^{a}	4 (17)^{a}	75 (36)^{a}	5 (23)^{a}	4 (21)^{a}
Education completed, y	18.89 ± 4.48	18.74 ± 3.94^{a}	15.99 ± 3.34^{b}	20.78 ± 5.39^{a}	20.68 ± 4.55^{a}	18.41 ± 5.01^{a}	22.21 ± 5.54^{a}
Income ≥ $100,000 (%)	255 (35)	120 (39)^{a}	8 (6)^{a}	7 (30)^{b}	105 (51)^{a}	6 (27)^{b}	8 (37)^{a}
AHEI score	49.21 ± 11.05	47.56 ± 9.94^{a}	47.26 ± 12.39^{a}	49.07 ± 9.52^{a}	47.56 ± 9.94^{a}	46.91 ± 10.94^{a}	49.76 ± 9.10^{a}
DASH score	5.02 ± 1.03	5.19 ± 1.03^{a}	4.53 ± 0.87^{b}	5.33 ± 1.13^{a}	5.19 ± 1.03^{a}	4.59 ± 0.75^{b}	5.58 ± 1.10^{a}
MDS score	4.37 ± 1.85	4.54 ± 1.82^{a}	3.87 ± 1.83^{b}	4.35 ± 1.40^{a}	4.47 ± 1.88^{a}	4.27 ±1.78^{a}	4.89 ± 1.63^{a}
Carbohydrates, %^{3}	47.84 ± 7.49	47.44 ± 7.24^{a}	49.22 ± 6.69^{b}	51.04 ± 8.37^{b}	46.86 ± 8.22^{a}	48.92 ± 6.45^{a}	49.82 ± 6.82^{a}
Protein, %^{3}	16.05 ± 2.90	16.53 ± 2.92^{a}	15.45 ± 2.90	16.64 ± 3.35^{a}	15.81 ± 2.65^{a}	15.95 ± 3.60^{a}	14.86 ± 2.58^{a}
Fat, %^{3}	35.65 ± 6.07	35.54 ± 6.09^{a}	36.92 ± 5.07^{a}	34.13 ± 6.10^{a}	35.03 ± 6.72^{a}	35.33 ± 4.59^{a}	36.71 ± 5.20^{a}
Saturated Fat	19.96 ± 9.70	19.04 ± 7.91^{a}	18.32 ± 8.35^{a}	14.91 ± 8.42^{b}	22.05 ± 10.08^{a}	24.07 ± 13.73^{a}	17.65 ± 7.91^{a}
Monounsaturated Fat	27.60 ± 12.69	25.91 ± 10.42^{a}	26.87 ± 12.89^{a}	21.57 ± 10.94^{a}	35.03 ± 6.72^{a}	35.35 ± 19.33^{a}	29.53 ± 11.89^{a}
Polyunsaturated Fat	16.60 ± 7.87	15.34 ± 6.37^{a}	17.33 ± 8.40	14.07 ± 7.28^{a}	17.15 ± 7.34^{a}	20.10 ± 9.61^{a}	18.82 ± 9.00^{a}
Daily Caloric Intake, kcal	1721.0 ± 626.33	1640.11 ± 553.21^{a}	1632.05 ± 647.23^{a}	1419.25 ± 576.09^{a}	1888.80 ± 615.91^{a}	2156.74 ± 991.17^{a}	1757.09 ± 685.41^{a}

Values are reported as mean ± SD or n (%). Carbohydrates, Protein, Fat and its components were reported as percent of total calorie intake. Outcomes within a row, stratified by gender, that are not connected by the same letter are significantly different (*P* <0.05). Abbreviations: BMI, body mass index; Cys, cysteine; CySS, cystine; GSH, glutathione; GSSG, glutathione disulfide; E_h, redox potential

[1] n=683

[2] n=658

[3] Percent of total calorie intake

[4] Values were back-transformed from the natural-log values used in analyses: mean (SEM).

Table 1. Demographic and Clinical Characteristics of the CHDWB Participants at Baseline Stratified by Race and Gender, cont.

Gender	All	Female			Male		
Characteristic		**White**	**Black**	**Other Races**	**White**	**Black**	**Other Races**
Plasma Cys, µmol/L	9.31 ± 2.17	9.47 ± 2.30^{a}	9.56 ± 1.96^{a}	9.74 ± 2.21^{a}	8.89 ± 1.87^{a}	9.66 ± 3.64^{a}	8.73 ± 2.21^{a}
Plasma CySS, µmol/L	84.32 ± 17.98	83.69 ± 19.28^{a}	89.27 ± 19.31^{b}	76.81 ± 14.84^{a}	82.89 ± 14.83^{a}	85.46 ± 20.98^{a}	79.85 ± 11.72^{a}
Plasma Total Cys, µmol/L	180.39 ± 37.13	179.46 ± 39.71^{a}	190.18 ± 40.15^{b}	165.66 ± 30.72^{a}	177.07 ± 30.66^{a}	183.07 ± 44.10^{a}	171.26 ± 23.07^{a}
Plasma GSH, µmol/L[4]	1.63 (0.022)	1.70 ± 0.03^{a}	1.49 ± 0.05^{b}	1.85 ± 0.16^{a}	1.59 ± 0.04^{a}	1.56 ± 0.15^{a}	1.79 ± 0.13^{a}
Plasma GSSG, µmol/L[4]	0.05 (0.001)	0.05 ± 0.002^{a}	0.05 ± 0.003^{a}	0.05 ± 0.01^{a}	0.05 ± 0.002^{a}	0.07 ± 0.01^{a}	0.06 ± 0.01^{a}
Plasma Total GSH, µmol/L[4]	4.22 (0.051)	4.33 ± 0.08^{a}	3.89 ± 0.12^{b}	4.19 ± 0.29^{a}	4.25 ± 0.09^{a}	4.20 ± 0.26^{a}	4.72 ± 0.27^{a}
Plasma CySS/GSH, µmol/L[4]	50.69 (0.89)	47.98 ± 1.23^{a}	58.38 ± 2.44^{b}	40.76 ± 4.64^{a}	51.41 ± 1.51^{a}	53.40 ± 6.93^{a}	44.24 ± 4.13^{a}
Plasma E_hCySS, mV	-69.95 ± 5.67	-70.49 ± 5.81^{a}	-69.99 ± 5.18^{a}	-72.38 ± 5.19^{a}	-69.01 ± 5.26^{a}	-69.87 ± 8.16^{a}	-68.70 ± 7.55^{a}
Plasma E_hGSSG, mV	-135.94 ± 9.58	-137.50 ± 8.45^{a}	-134.20 ± 9.83^{b}	-139.51 ± 10.41^{a}	-134.87 ± 10.19^{a}	-130.91 ± 13.87^{a}	-135.01 ± 8.06^{a}

Values are reported as mean ± SD or n (%). Carbohydrates, Protein, Fat and its components were reported as percent of total calorie intake. Outcomes within a row, stratified by gender, that are not connected by the same letter are significantly different (*P* <0.05). Abbreviations: BMI, body mass index; Cys, cysteine; CySS, cystine; GSH, glutathione; GSSG, glutathione disulfide; E_h, redox potential

[1] n=683

[2] n=658

[3] Percent of total calorie intake

[4] Values were back-transformed from the natural-log values used in analyses: mean (SEM).

Table 2. Plasma Redox Measures and Diet Intake Factors[1] by Body Mass Index (BMI) Category.

	BMI LEVEL			
	BMI<25 (n=251)	**25≤BMI<30** (n=264)	**BMI≥30** (n=199)	***P* trend**
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, µmol/L[2]	1.80 ± 0.04^{a}	1.65 ± 0.04^{b}	1.41 ± 0.04^{c}	<0.001
GSSG, µmol/L[2]	0.052 ± 0.002^{a}	0.050 ± 0.002^{a}	0.045 ± 0.002^{a}	0.05
Total GSH, µmol/L[2]	4.51 ± 0.09^{a}	4.31 ± 0.08^{a}	3.78 ± 0.09^{b}	<0.001
E_hCySS, mV	-70.48 ± 5.52^{a}	-69.73 ± 5.84^{a}	-69.53 ± 5.58^{a}	0.18
E_hGSSG, mV	-137.83 ± 8.54^{a}	-136.07 ± 9.95^{a}	-133.33 ± 9.91^{b}	<0.001
CySS/GSH[2]	42.41 ± 1.15^{a}	49.46 ± 1.35^{b}	65.08 ± 2.05^{c}	<0.001
Diet Characteristics				
Mediterranean score	4.69 ± 1.66^{a}	4.27 ± 1.86^{b}	4.15 ± 1.95^{b}	0.003
AHEI score	52.03 ± 10.76^{a}	47.97 ± 10.51^{b}	47.25 ± 11.33^{b}	<0.001
DASH score	5.28 ± 1.04^{a}	4.99 ± 1.03^{b}	4.72 ± 0.94^{c}	<0.001
Protein, g/d	39.04 ± 6.72^{a}	40.57 ± 7.47^{b}	41.02 ± 7.52^{b}	0.009
Red Meat, servings/d	1.20 ± 0.96^{a}	1.59 ± 1.29^{b}	1.99 ± 1.38^{c}	<0.001
Total Sulfur Amino Acids, g/d	1.68 ± 0.33^{a}	1.79 ± 0.38^{b}	1.83 ± 0.39^{b}	<0.001

BMI categories correspond to normal weight: (≤ 25 kg/m^{2}), overweight (25.0-29.9 kg/m^{2}), and obese (≥30 kg/m^{2}).

Outcomes within a row that are not connected by the same letter are significantly different (*P* <0.05). All Dietary measures are adjusted for Age; Body Mass Index (BMI); Race; Sex; and History of Diabetes, Hypertension, or Hyperlipidemia. Abbreviations: BMI, body mass index; Cys, cysteine; CySS, cystine; GSH, glutathione; GSSG, glutathione disulfide; E_h, redox potential; AHEI, Alternative Healthy Eating Index; DASH, Dietary Approaches to Stopping Hypertension

[1] Diet intake factors adjusted to 1,000 kcal using the formula: (food component intake x 1,000)/energy intake (kcal)

[2] Values were back-transformed from the natural-log values used in analyses.

Table 3. Regression coefficients (β) for the relationship between measures of diet quality and plasma redox measures.

	Plasma Concentration							
Dietary Measure	**Cys, µM**	**CySS, µM**	**GSH[3], µM**	**GSSG[3], µM**	**Total GSH[3], µM**	**E_hCySS, mV**	**E_hGSSG, mV**	**CySS/GSH[3]**
Mediterranean Score	0.02	-0.28	**0.02**	0.02	**0.02**	-0.08	-0.11	**-0.02**
	(0.73)	(0.39)	**(0.02)**	(0.09)	**(0.02)**	(0.53)	(0.58)	**(0.01)**
AHEI Score	-0.01	-0.05	0.00	0.00	0.00	0.02	-0.02	0.00
	(0.37)	(0.37)	(0.40)	(0.69)	(0.31)	(0.45)	(0.66)	(0.27)
DASH Score	-0.07	-0.23	0.02	0.00	0.02	0.18	-0.64	-0.03
	(0.38)	(0.70)	(0.08)	(0.92)	(0.08)	(0.41)	(0.08)	(0.08)
Protein, g/d[1]	0.00	-0.04	0.00	0.00	0.00	0.00	-0.02	0.00
	(0.82)	(0.65)	(0.57)	(0.96)	(0.94)	(0.89)	(0.63)	(0.51)
Red Meat, ounces/d[1]	0.03	0.14	0.01	0.02	0.01	-0.11	0.03	-0.01
	(0.69)	(0.79)	(0.54)	(0.47)	(0.48)	(0.57)	(0.93)	(0.63)
Total Sulfur Amino Acids, mg/d[2]	-0.13	-0.34	-0.01	0.01	-0.02	0.32	0.38	0.01
	(0.57)	(0.84)	(0.76)	(0.91)	(0.46)	(0.59)	(0.70)	(0.86)

Data reported as β (*P* value). All Dietary measures are adjusted for Age; Body Mass Index (BMI); Race; Sex; and History of Diabetes, Hypertension, or Hyperlipidemia. Abbreviations: Cys, cysteine; CySS, cystine; GSH, glutathione; GSSG, glutathione disulfide; E_h, redox potential; AHEI, Alternative Healthy Eating Index; DASH, Dietary Approaches to Stopping Hypertension.

[1] Diet intake factors adjusted to 1,000 kcal using the formula: (food component intake x 1,000)/energy intake (kcal)

[2] Total Sulfur Amino Acid = Cystine (CySS), Cysteine (Cys), Methionine (Met)

[3] Analyses were conducted on natural log-transformed values

Table 4. Regression coefficients (β) for the relationship between Mediterranean Diet Score (MDS) components^1 and plasma redox measures.

	Plasma Concentration							
MDS Component	**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**	0.06	-0.02	-0.08	-0.02	**-1.18**	-0.52	0.02
	(0.01)	(0.97)	(0.44)	(0.13)	(0.38)	**(0.01)**	(0.52)	(0.58)
Legumes, Nuts, and Soy, servings/d	-0.19	-0.54	0.00	0.08	0.01	0.39	1.06	-0.01
	(0.30)	(0.68)	(0.99)	(0.15)	(0.61)	(0.42)	(0.19)	(0.86)
Fruit, servings/d	-0.22	-0.48	0.03	0.00	0.03	0.54	-0.71	-0.04
	(0.21)	(0.71)	(0.29)	(0.93)	(0.23)	(0.24)	(0.36)	(0.22)
Total Grains, servings/d	-0.22	0.46	-0.01	-0.02	-0.02	0.70	-0.08	0.01
	(0.24)	(0.73)	(0.84)	(0.73)	(0.41)	(0.15)	(0.92)	(0.67)
Fish, servings/d	-0.08	**-3.30**	0.02	0.07	0.01	-0.26	0.33	-0.06
	(0.66)	**(0.01)**	(0.46)	(0.23)	(0.70)	(0.58)	(0.67)	(0.07)
Monounsaturated to Saturated Fat Ratio	0.20	2.03	0.05	**0.12**	0.05	-0.19	0.33	-0.02
	(0.27)	(0.12)	(0.09)	**(0.03)**	(0.06)	(0.68)	(0.68)	(0.48)
Alcohol, servings/d	0.11	0.58	0.01	-0.07	0.01	-0.29	-1.15	0.00
	(0.54)	(0.66)	(0.69)	(0.24)	(0.62)	(0.54)	(0.15)	(0.96)
Dairy, servings/d	0.03	-0.62	**0.08**	**0.12**	**0.08**	-0.09	-0.35	**-0.09**
	(0.88)	(0.64)	**(0.01)**	**(0.03)**	**0.00**	(0.85)	(0.67)	**(0.01)**
Red and White Meat, servings/d	0.09	-0.85	0.01	-0.03	-0.01	-0.35	-0.64	-0.02
	(0.64)	(0.52)	(0.64)	(0.66)	(0.69)	(0.46)	(0.43)	(0.48)

Data reported as β (*P* value). All Dietary measures are adjusted for Age; Body Mass Index (BMI); Race; Sex; and History of Diabetes, Hypertension, or Hyperlipidemia. Abbreviations: Cys, cysteine; CySS, cystine; GSH, glutathione; GSSG, glutathione disulfide; E_h, redox potential

[1] Diet intake factors adjusted to 1,000 kcal using the formula: (food component intake x 1,000)/energy intake (kcal)

[2] Analyses were conducted on natural log-transformed values

Figures

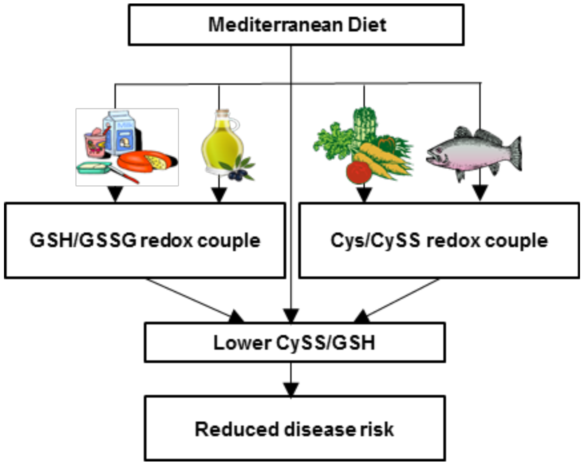


Figure 1. Conceptual framework for the association of Mediterranean diet and individual components with plasma redox measures as a contributor to disease risk. Individual components of the Mediterranean diet influence the plasma redox couples for GSH/GSSG and Cys/CySS towards a less oxidized plasma state, thus lowering the CySS/GSH ratio, which is a predictor of subclinical vascular disease and cardiovascular-related death. In this study, greater dairy intake was significantly associated with plasma GSH and GSSG, and a higher monounsaturated to saturated fat ratio tended to correlate with the plasma GSH/GSSG system. Higher vegetable and fish consumption were significantly associated with more favorable plasma Cys and CySS levels, respectively. Other components of the MDS (legumes, fruits, grains, alcohol, and meats) were not associated with plasma redox biomarkers.

Appendices

Supplemental Table 1. Dietary components used for the Mediterranean Diet Score calculation.

Dietary Component	Foods Included	Criteria for 1 Point		Median[1]	
		Female	Male	Female	Male
Vegetables, servings/d	All vegetables except potatoes	≥ median intake	≥ median intake	2.17	1.98
Legumes, nuts, and soy, servings/d	Peas, beans, nuts, nut butter, and soy products	≥ median intake	≥ median intake	0.98	0.99
Fruits, servings/d	All fruits and juices	≥ median intake	≥ median intake	1.23	1.27
Total Grain, servings/d	Cereals and grains	≥ median intake	≥ median intake	3.95	5.31
Fish, servings/d	All fish	≥ median intake	≥ median intake	0.63	0.68
Ratio of monounsaturated to saturated fatty acids	Whole diet	≥ median intake	≥ median intake	1.39	1.33
Alcohol, servings/d	Wine, beer, and liquor	5-25 g/day	10-50 g/day		
Dairy products, servings/d	All dairy products	< median intake	< median intake	0.97	1.17
Meats, servings/d	Poultry, lunch meat, organ meat, red and white meat	< median intake	< median intake	1.97	2.27

[1] Median intake (servings/d) for dietary components were calculated stratified by gender.

Supplemental Table 2. Dietary components used for the DASH Score calculation.

Dietary Component	Foods Included	Criteria for Points
Vegetables, servings/d	All vegetables	≥ 4 srv/day: 1 point 2-3 srv/day: 0.5 < 2 srv/day: 0
Fruit, servings/d	All fruit and juices	≥ 4 srv/day: 1 point 2-3 srv/day: 0.5 < 2 srv/day: 0
Total Grain, servings/d	All grains	≥ 7 srv/day: 1 point 5-6 srv/day: 0.5 < 5 srv/day: 0
Whole Grain, servings/d	Only whole grains	≥ 2 srv/day: 1 point 1 srv/day: 0.5 < 1 srv/day: 0
Legumes, Nuts, Soy, servings/wk	Peas, beans, nuts, nut butter, and soy products	≥ 4 srv/wk: 1 point 2-3 srv/wk: 0.5 < 2 srv/wk: 0
Dairy products, servings/d	All dairy products	≥ 2 srv/day: 1 point 1 srv/day: 0.5 < 1 srv/day: 0
Meat/Poultry/Fish, servings/d	Poultry, lunch meat, organ meat, red and white meat	≤ 2 srv/day: 1 point 3 srv/day: 0.5 ≥ 4 srv/day: 0
% kcal from fat	Whole diet	≤ 30%: 1 point 31-32%: 0.5 ≥33% : 0
% kcal from saturated fatty acids	Whole diet	≤ 10%: 1 point 11-12%: 0.5 ≥13% : 0
% kcal from sweets	Whole diet	≤ 3.2% = 1 point 3.3 – 5.0% = 0.5 point ≥.5.1% = 0 point
Sodium, mg/d	Whole diet	≤ 1500 mg/day: 1 point 1501 – 2400 mg/day: 0.5 ≥ 2401 mg/day: 0

Supplemental Table 3. Dietary components used for the Alternative Healthy Eating Index (AHEI) Score calculation.

Dietary Component	Foods Included	Criteria for Points	
		Female	**Male**
Vegetables, servings/d	All vegetables	0pts = 0 srv … 10pts = 5 srv	
Fruit, servings/d	All fruits and juices	0pts = 0 srv … 10pts = 4 srv	
Cereal and Grains, g/d	All cereals and grains	0pts = 0 g … 10pts = 15 g	
Legumes, Nuts and Soy, servings/d	Peas, beans, nuts, nut butter, and soy products	0pts = 0 srv … 10pts = 1 srv	
Ratio of white to red meat	Whole diet	0pts = ratio of 0 … 10 pts = ratio of 4	
Alcohol, servings/d	Wine, beer, and liquor	Women: 0 pts = 0, >2.5 srv … 10 pts = 0.5-1.5 srv	Men: 0 pts = 0, >3.5 srv … 10 pts = 1.5-2.5 srv
% kcal from trans fat	Whole diet	0 pts = ≥ 4% energy … 10 pts = ≤ 0.5% energy	
Polyunsaturated to Saturated Fatty Acids Ratio	Whole diet	0 pts = ratio ≤ 0.1 … 10 pts = ratio ≥ 1	
Duration of Multivitamin Use	Prenatal vitamins and multivitamins	2.5 pts = < 5 years … 7.5 pts = ≥ 5 years	

Supplemental Table 4. Demographic and Clinical Characteristics of the CHDWB Participants at Baseline Stratified by Gender.

Characteristic	All	Males	Females	*p* Value
No. of subjects (%)	727	249 (35)	470 (65)	
White (%)	518 (72)	208 (84)	310 (66)	**<0.001**
Age, y	48.5 (10.8)	49.94 ± 11.83	47.80 ± 10.21	**0.02**
Weight, kg[1]	79.32 ± 19.67	85.91 ± 14.91	75.89 ± 20.95	**<0.001**
BMI, kg/m^2	27.8 (6.4)	27.36 ± 3.99	28.00 ± 7.32	0.13
Reported History of Any of the Following:[2]	221 (32)	83 (35)	138 (31)	0.29
Diabetes (%)	39 (6)	15 (6)	24 (5)	0.62
Hypertension (%)	134 (19)	44 (18)	90 (20)	0.61
Hyperlipidemia (%)	123 (18)	51 (21)	72 (16)	0.08
Currently Smoking (%)	36 (5)	17 (7)	19 (4)	0.11
AHEI score	49.21 ± 11.05	47.67 ± 9.95	50.02 ± 11.51	**0.005**
DASH score	5.02 ± 1.03	5.16 ± 1.03	4.94 ± 1.03	**0.005**
MDS score	4.37 ± 1.85	4.49 ± 1.85	4.33 ± 1.83	0.28
Carbohydrates, %[3]	47.84 ± 7.49	47.27 ± 8.01	48.14 ± 7.21	0.14
Protein, %[3]	16.05 ± 2.90	15.75 ± 2.74	16.22 ± 2.97	**0.04**
Fat, %[3]	35.65 ± 6.07	35.18 ± 6.45	35.88 ± 5.85	0.14
Saturated Fat	19.96 ± 9.70	21.89 ± 10.35	18.63 ± 8.10	**<0.001**
Monounsaturated Fat	27.60 ± 12.69	29.94 ± 12.71	25.98 ± 11.25	**<0.001**
Polyunsaturated Fat	16.60 ± 7.87	17.54 ± 7.71	15.86 ± 7.12	**0.004**
Daily Caloric Intake, kcal	1721.0 ± 626.33	1902.40 ± 664.40	1626.90 ± 583.90	**<0.001**
Clinical Features				
Plasma Cys, µmol/L	9.31 ± 2.17	8.94 ± 2.09	9.51 ± 2.20	**0.001**
Plasma CySS, µmol/L	84.32 ± 17.98	82.85 ± 15.16	85.01 ± 19.32	0.11
Plasma Total Cys, µmol/L	180.39 ± 37.13	177.10 ± 31.36	182.00 ± 39.85	0.08
Plasma GSH, µmol/L[4]	1.63 (0.022)	0.64 (0.028)	0.60 (0.036)	0.35
Plasma GSSG, µmol/L[4]	0.05 (0.001)	0.06 (0.002)	0.05 (0.002)	**0.05**
Plasma Total GSH, µmol/L[4]	4.22 (0.051)	4.19 (0.065)	4.28 (0.083)	0.41
Plasma CySS/GSH, µmol/L[4]	50.69 (0.89)	50.40 (1.128)	50.91 (1.443)	0.79
Plasma E_hCySS, mV	-69.95 ± 5.67	-69.05 ± 5.72	-70.43 ± 5.61	**0.003**
Plasma E_hGSSG, mV	-135.94 ± 9.58	-134.60 ± 10.39	-136.60 ± 9.10	**0.01**

Values are reported as mean ± SD or n (%). Statistically significant *P* values are bolded. Abbreviations: BMI, body mass index; Cys, cysteine; CySS, cystine; GSH, glutathione; GSSG, glutathione disulfide; E_h, redox potential

[1] n=683

[2] n=658

[3] Percent of total calorie intake

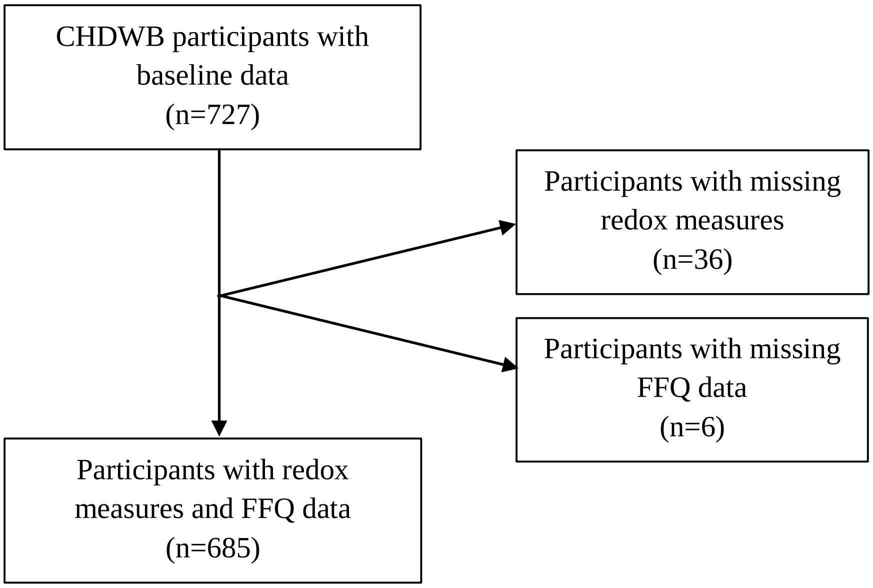
[4] Values were back-transformed from the natural-log values used in analyses: mean (SEM).

Supplemental Table 5. Mean Plasma Redox Measures Across Quartiles of Mediterranean Diet Quality Scores.

	Mediterranean Diet Score					
Variable	0.0-2.0 (n=124)	3.0 (n=105)	4.0-5.0 (n=274)	6.0-9.0 (n=218)	*P* trend	Percent Difference
Cys, µmol/L	9.41 ± 0.20	9.09 ± .22	9.28 ± 0.13	9.44 ± 0.15	0.67	0.32
CySS, µmol/L	85.65 ± 1.45	84.56 ± 1.57	84.93 ± 0.97	83.17 ± 1.10	0.20	-2.90
GSH, µmol/L[1]	0.46 ± 0.03	0.46 ± 0.03	0.47 ± 0.02	0.53 ± 0.02	**0.04**	15.22
GSSG, µmol/L[1]	-3.09 ± 0.06	-3.06 ± 0.07	-3.00 ± 0.04	-2.97 ± 0.05	0.09	-4.19
Total GSH, µmol/L[1]	1.41 ± 0.03	1.40 ± 0.03	1.44 ± 0.02	1.48 ± 0.02	**0.03**	4.96
Eh CySS, mV	-70.13 ± 0.52	-69.19 ± 0.57	-69.75 ± 0.35	-70.54 ± 0.40	0.31	0.58
Eh GSH, mV	-136.23 ± 0.88	-135.77 ± 0.95	-135.50 ± 0.59	-136.55 ± 0.67	0.76	0.23
CySS/GSH[1]	3.97 ± 0.04	3.96 ± 0.04	3.95 ± 0.02	3.86 ± 0.03	**0.01**	-2.77

Data reported as Least-Squares Mean ± Standard Error. All Dietary measures are adjusted for Age; Body Mass Index (BMI); Race; Sex; and History of Diabetes, Hypertension, or Hyperlipidemia. Abbreviations: Cys, cysteine; CySS, cystine; GSH, glutathione; GSSG, glutathione disulfide; E_h, redox potential

[1] Values were back-transformed from the natural-log values used in analyses.



Supplemental Figure 1. Flow chart of the CHDWB participants included in the final analysis.