

Dairy intake is associated with brain glutathione concentration in older adults^{1–3}

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ABSTRACT

Background: A reduction in key antioxidants such as glutathione has been noted in brain tissue undergoing oxidative stress in aging and neurodegeneration. To date, no dietary factor has been linked to a higher glutathione concentration. However, in an earlier pilot study, we showed evidence of a positive association between cerebral glutathione and dairy intake.

Objective: We tested the hypothesis that dairy food consumption is associated with cerebral glutathione concentrations in older adults.

Design: In this observational study, we measured cerebral glutathione concentrations in 60 healthy subjects (mean \pm SD age: 68.7 \pm 6.2 y) whose routine dairy intakes varied. Glutathione concentrations were measured by using a unique, noninvasive magnetic resonance chemical shift imaging technique at 3 T and compared with dairy intakes reported in 7-d food records.

Results: Glutathione concentrations in the frontal [Spearman's rank-order correlation (r_s) = 0.39, P = 0.013], parietal (r_s = 0.50, P = 0.001), and frontoparietal regions (r_s = 0.47, P = 0.003) were correlated with average daily dairy servings. In particular, glutathione concentrations in all 3 regions were positively correlated with milk servings ($P \leq 0.013$), and those in the parietal region were also correlated with cheese servings (P = 0.015) and calcium intake (P = 0.039). Dairy intake was related to sex, fat-free mass, and daily intakes of energy, protein, and carbohydrates. However, when these factors were controlled through a partial correlation, correlations between glutathione concentrations and dairy and milk servings remained significant.

Conclusions: Higher cerebral glutathione concentrations were associated with greater dairy consumption in older adults. One possible explanation for this association is that dairy foods may serve as a good source of substrates for glutathione synthesis in the human brain. *Am J Clin Nutr* 2015;101:287–93.

Keywords aging brain, dairy foods, dietary intake, glutathione, magnetic resonance spectroscopy

INTRODUCTION

The term “oxidative stress” refers to an imbalance between the production of reactive oxygen species (ROS)⁴ and their neutralization by antioxidant defenses (1, 2). The brain is particularly vulnerable to oxidative stress because of its high level of oxidative metabolism, relatively low concentrations of cytosolic antioxidants, and high concentrations of transition metal ions and metals (e.g., iron) as well as PUFAs (3, 4). The in-

tracellular production of ROS is greatly elevated in many neurodegenerative diseases in conjunction with inflammation and mitochondrial dysfunction (5–7). Accordingly, oxidative stress has been implicated in normal aging and many neurodegenerative diseases.

Glutathione is a powerful antioxidant that plays a key role in the brain's capacity for scavenging ROS and free radicals involved in oxidative stress. Protection against oxidative stress is directly afforded through the oxidation of glutathione in mitochondria (8, 9), and decreased concentrations of glutathione were reported in the aging rat brain (10–12). A significantly elevated ROS generation and cellular stress because of mitochondrial dysfunction and inflammation commonly accompany neurodegenerative disorders, and the resulting burden on the brain's antioxidant defenses would likewise be reflected by reductions in concentrations of glutathione (13–15). However, the measurement of glutathione in living tissue is technically challenging, particularly in the human brain, and until recently, the level of oxidative stress could be evaluated only indirectly by using in vitro measures obtained from blood, cerebrospinal fluid, or biopsy tissue samples. By contrast, the work reported here was based on our successful application of magnetic resonance (MR) chemical shift imaging (CSI) to provide regional mapping of in vivo glutathione concentrations in the living human brain (16–18).

Dietary factors may influence the antioxidant capacity of the brain because dietary supplementation was reported to modulate enzyme activities of antioxidants in animal brains (19, 20).

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⁴ Abbreviations used: CSI, chemical shift imaging; HEI, Healthy Eating Index; MR, magnetic resonance; NDSR, Nutrition Data System for Research; ROS, reactive oxygen species.

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Therefore, in one of our early exploratory studies of glutathione in the human brain, we collected data from a food-frequency questionnaire that pertained to the usual dietary intake in a small sample of healthy older adults. The results indicated a positive association between glutathione and the consumption of dairy foods (unpublished data). However, because of the small sample size and large number of correlations examined in the exploratory study, a replication and elaboration on the nature of this relation was warranted. The current study was designed in accordance with these aims by using more-extensive procedures for measuring dietary intake and focusing specifically on the principal dairy foods themselves (milk, cheese, and yogurt) and nutrients obtained in large measure through dairy consumption (e.g., calcium, vitamin D, and riboflavin).

SUBJECTS AND METHODS

Subjects

Between December 2011 and February 2014, we enrolled 60 healthy adults whose ages ranged from 60 to 85 y and who resided in the greater Kansas City metro area in an observational study aimed at investigating the association between dairy food intake and brain glutathione concentrations. The study protocol was approved by the Human Subjects Committee at the University of Kansas Medical Center. Our exclusion criteria included individuals with a history of neurologic disorders, a head injury, claustrophobia, diabetes, unstable medical conditions, malabsorptive syndromes such as gluten and lactose intolerance, or the use of dietary supplements that might alter glutathione concentration such as glutathione or *N*-acetylcysteine. Informed consent was obtained from all study participants. To ensure a broad distribution of dairy intake, we recruited subjects in the following 3 groups on the basis of their initially reported daily intakes of dairy as follows: low (<1 serving/d), moderate (1–2 servings/d), and recommended (≥ 3 servings/d).

Dietary intake assessment

During subject screening, dietary intake was assessed by using 3 standardized, multiple-pass 24-h dietary recalls. These recalls were unannounced and conducted via telephone by trained diet-assessment staff. The information was entered into the Nutrition Data System for Research (NDSR; version 2012), which is commonly used to collect dietary recalls and analyze data from dietary recalls and food records (21) for nutrient- and food-grouping analysis. The resulting data were used to classify subjects initially into the 3 recruitment groups on the basis of their 3-d average intakes of dairy foods (milk, cheese, and yogurt).

To assess dietary intake before the MR scan, 7-d diet records were obtained from all subjects. These records also included all dietary supplements taken by the subject. To facilitate the compiling of these records, subjects were given verbal and written directions on how to accurately record their dietary intakes. Subjects were instructed to stop taking calcium supplements for the 7 d before the scan. On the day of the scan, 7-d diet records were reviewed by a registered dietitian and entered into the NDSR for a nutrient- and food-grouping analysis. The 7-d diet recording period before the MR scan was chosen to provide sufficient time to capture any dietary intake that might influence

glutathione concentrations on the basis of the turnover time of glutathione in the brain. Because of its slower turnover rate compared with that of other metabolites (e.g., aspartate, glutamate, and glutamine), it takes ~ 3 d for the complete turnover of glutathione pools in the rat brain (22). The turnover period was likely to be longer in the human brain on the basis of the general relation between metabolic rates to body mass in mammals (23).

To determine whether there was any difference in the general diet quality in the 3 recruitment groups on the basis of dairy consumption, Healthy Eating Index (HEI)–2010 scores were calculated from 7-d diet records. The HEI-2010 is a measure of diet quality developed by the USDA that assesses conformance to the 2010 Dietary Guidelines for Americans (24). Components considered for daily intake included total fruit, whole fruit, total vegetables, dark green vegetables and beans, refined grains, whole grains, milk, meat and beans, seafood and plant protein, the fatty acid ratio, sodium, and empty calories. Point values on the basis of how well a person met dietary guidelines for each component were calculated by entering the NDSR output into a custom algorithm (25). The sum of these point values was totaled to yield the final HEI score for each day expressed as a percentage per 1000 kcal with 100 points as the maximum score.

Anthropometric measures

Body weight, height, and waist circumference were measured for all subjects before their MR scans. Body weight was measured with a calibrated digital scale (± 0.1 kg; Befour). Height was measured with a wall-mounted stadiometer (model PE-WM-60–84; Perspective Enterprises). Waist circumference was measured in the standing position with measurements obtained midway between the lateral lower rib margin and ileac crest (26). BMI (in kg/m^2) was calculated by using weight and height measurements. Body composition was measured by using a bioelectrical impedance analyzer (Quantum IV; RJL Systems) with the participant in the reclined position. Fat and fat-free muscle mass were determined by using the NHANES III equation set for general populations (27).

MR protocol

All MR scans were performed on a 3-T system (Skyra; Siemens). After positioning the participant supine in the magnet, 3-plane scout MR images were acquired to locate the volume of interest, a 3-cm axial slab positioned above the corpus callosum and also located in the iso-center of the magnet. In vivo mapping of glutathione was performed with a multiple quantum-filtered, CSI technique that used a 2-echo scheme (18, 28, 29). Spectral data of glutathione and creatine were processed with software written in-house in Interactive Data Language 6.3 (RSI) as described previously (17). In brief, glutathione and creatine spectra were first processed with 2 Hz line broadening, and glutathione doublet signals were fitted with 2 Lorentzian peaks separated by 4 Hz at ~ 2.9 ppm. Singlet signals of creatine at 3.03 ppm and choline at 3.21 ppm were fitted by a Lorentzian peak at each respective signal position. Spectral fitting was performed by using the Levenberg-Marquardt algorithm implemented in the MPFIT curve-fitting package (Craig Markwardt, <http://www.physics.wisc.edu/~craigm/ldl/fitting.html>). Concentrations of glutathione were determined by using an internal reference method,

TABLE 1Anthropometric and dietary characteristics for the overall sample and each dairy-consumption group¹

	Overall (<i>n</i> = 60)		Dairy-consumption groups			<i>P</i>
	Value	Range	Low (<i>n</i> = 21)	Moderate (<i>n</i> = 24)	Recommended (<i>n</i> = 15)	
Age, y	68.7 ± 6.2 ²	60–83	69.3 ± 6.4	67.0 ± 5.7	70.5 ± 6.4	0.190
Education, y	17.3 ± 2.7	12–29	17.4 ± 3.8	17.1 ± 2.1	17.6 ± 1.8	0.857
HEI ³	62.5 ± 11.3	34–89	61.2 ± 11.0	61.7 ± 10.2	65.5 ± 13.5	0.490
BMI, kg/m ²	25.4 ± 4.2	17–36	24.9 ± 4.5	26.3 ± 3.5	24.5 ± 4.62	0.327
Fat mass ⁴	22.5 ± 7.5	8–47	22.1 ± 7.3	24.1 ± 8.3	20.5 ± 6.3	0.329
Fat-free mass ⁴	51.3 ± 13.0	29–79	48.1 ± 13.9	50.2 ± 10.7	57.5 ± 13.9	0.087
Dietary intake ⁵						
Energy, kcal	1788 ± 457	791–3172	1637 ± 369	1719 ± 445	2108 ± 455	0.014
Protein, g	75.6 ± 21.1	27–133	69.4 ± 18.8	70.9 ± 19.1	91.7 ± 19.9	0.006
Carbohydrate, g	217.2 ± 74.5	99–481	188.9 ± 62.3	211.5 ± 80.9	266.2 ± 56.9	0.016
Fat, g	67.9 ± 21.7	17–139	65.0 ± 18.8	65.4 ± 21.2	76.2 ± 25.5	0.328
Fruit, servings/d	2.28 ± 1.93	0.2–10.6	2.19 ± 1.77	2.38 ± 2.43	2.26 ± 1.21	0.951
Vegetables, servings/d	3.41 ± 1.68	0.7–9.7	3.53 ± 2.11	3.43 ± 1.28	3.21 ± 1.65	0.910
Grains, servings/d	5.41 ± 2.41	1.8–16.5	4.99 ± 1.97	5.22 ± 2.81	6.31 ± 2.17	0.328
Meats and beans, servings/d	5.28 ± 2.57	0.3–11.8	6.47 ± 2.24	4.42 ± 1.95	5.01 ± 3.31	0.072
Dairy, servings/d	1.83 ± 1.37	0.0–4.6	0.44 ± 0.30	1.85 ± 0.59	3.74 ± 0.61	<0.001
Milk, servings/d	1.08 ± 1.15	0.0–3.8	0.09 ± 0.11	0.94 ± 0.63	2.70 ± 0.75	<0.001
Cheese, servings/d	0.56 ± 0.49	0.0–2.3	0.26 ± 0.22	0.70 ± 0.60	0.77 ± 0.38	0.004
Yogurt, servings/d	0.19 ± 0.21	0.0–0.9	0.09 ± 0.11	0.21 ± 0.21	0.29 ± 0.27	0.058
Calcium, mg	1090 ± 501	308–2662	752 ± 352	999 ± 276	1708 ± 401	<0.001
Riboflavin, mg	5.3 ± 12.1	1.0–87.8	5.9 ± 18.8	5.9 ± 8.1	3.6 ± 1.0	0.910
Vitamin C, mg	300 ± 794	21–6062	208 ± 231	435 ± 1218	217 ± 301	0.635
Vitamin D, µg	24.1 ± 28.1	1.3–135.9	27.4 ± 38.2	20.2 ± 21.4	25.7 ± 21.0	0.800
Vitamin E, mg	31.7 ± 51.4	2.2–214.7	56.7 ± 72.8	19.3 ± 34.7	16.4 ± 12.2	0.042
Selenium, µg	106.8 ± 40.1	33–278	106.6 ± 38.2	97.5 ± 36.2	122.1 ± 49.0	0.309

¹Final dairy-consumption groups on the basis of 7-d diet records. *P* values were based on a 1-factor ANOVA comparing the 3 dairy-consumption groups. *P* values for dietary intake variables were adjusted for multiple comparisons on the basis of the false-discovery rate (30).

²Mean ± SD (all such values).

³HEI, Healthy Eating Index.

⁴On the basis of bioelectrical impedance.

⁵On the basis of a 7-d diet record (per day).

which employed the creatine signals acquired simultaneously with those of glutathione taking into account the relaxation times, volume definition, and editing efficiency. The use of creatine as an internal concentration reference provided an automatic correction for brain atrophy (17, 28, 29).

Glutathione concentrations were calculated from the central region ($5 \times 5 \times 3 \text{ cm}^3$) of CSI data, where the static magnetic field (B_0) was the most adjusted for the conservative estimation of glutathione. The total area spanned portions of both frontal and parietal lobes and was labeled frontoparietal. In addition, glutathione concentrations were measured from the anterior one-half ($2.5 \times 5 \text{ cm}^2$) and the posterior one-half ($2.5 \times 5 \text{ cm}^2$) and labeled mostly frontal and mostly parietal, respectively (17). Glutathione concentrations from all 3 areas were obtained independently from each specified area, and the glutathione concentration from the frontoparietal area was not merely the arithmetic average of the 2 regional values from the mostly frontal and mostly parietal areas.

Statistical analysis

On the basis of our earlier exploratory study, the primary focus of the current study was to investigate the relation between glutathione concentrations and daily consumption of dairy foods. The reason for recruiting subjects from 3 dairy-consumption groups

(low, moderate, or recommended intakes) was to ensure diversity in dairy consumption across the sample. From the outset, the intention was to treat dairy consumption as a continuous variable rather than structuring the data analysis around the 3 groups. Therefore, primary analyses consisted of bivariate correlations between glutathione concentrations and dairy consumption.

This more-informed focus of the study also dictated the other variables included in secondary analyses. Most of these variables were selected on the basis of their possible utility for illuminating the relation between dairy consumption and glutathione and, therefore, consisted of the principal dairy foods themselves (milk, cheese, and yogurt) and nutrients obtained largely through dairy consumption such as calcium, vitamin D, and riboflavin. Other variables were selected to complete each subject's overall dietary profile in terms of the major nutritional components (carbohydrates, protein, and fat). Finally, the 3 specific nutrients vitamin C, vitamin E, and selenium were examined because they are known to have major antioxidant properties.

Distributions of all variables were tested for normality by using the Kolmogorov-Smirnov test. Glutathione concentrations in all 3 areas (frontal, parietal, and frontoparietal) were distributed normally. However, many of the dietary variables including daily servings of dairy were positively skewed to a significant degree. Therefore, the bivariate relations reported throughout this article

are nonparametric correlations on the basis of Spearman’s rank-order coefficients. Because of the large number of dietary variables (i.e., 14) examined in this study, *P* values for all analyses involving these variables were adjusted by using the false-discovery rate (30) to correct for the expanded possibility of a type 1 error resulting from multiple comparisons.

RESULTS

A total of 60 participants (21 men and 39 women) who ranged in age from 60 to 83 y (mean ± SD: 68.7 ± 6.2 y) completed the study. The initial classification into 3 recruitment groups was reassessed on the basis of subjects’ 7-d diet records. The final classification was changed for 17 of 60 subjects. Of the participants, 12 subjects were changed to a lower category (recommended to moderate: *n* = 7; moderate to low: *n* = 5), and 5 subjects were changed to a higher category (low to moderate: *n* = 3; moderate to recommended: *n* = 2). Anthropometric and dietary intake characteristics of the sample are listed in **Table 1**.

Subjects in the final classification of low, moderate, and recommended dairy-consumption groups did not differ significantly in age, years of education, BMI, or overall quality of their diets as measured by using the HEI-2010 (Table 1). However, subjects in the recommended dairy group showed significantly higher energy, protein, and carbohydrate intakes than did the other groups. Also, there was a greater percentage of women in the recommended dairy group (60%) than low group (24%) or middle group (26%; chi-square = 6.1, df = 2, *P* = 0.046).

Glutathione CSI data were acquired from a slab spanning both frontal and parietal regions of the human brain shown as the rectangular volume of interest on T1-weighted sagittal and coronal MR images (**Figure 1A**). Figure 1B shows the mapping of glutathione CSI signals (Figure 1B, left) and simultaneously acquired creatine (Figure 1B, right), and both CSI spectra were overlaid on the anatomical MR images in the middle section of the CSI slab. Glutathione signals from protons of cysteine β-CH₂ near 3 ppm were clearly visible in all CSI voxels. Consistent line shapes of glutathione and creatine signals showed a good quality of CSI data, which enabled a reliable quantification of glutathione concentrations. **Table 2** presents glutathione concentrations in the frontal, parietal, and frontoparietal areas of the brain for each dairy-consumption group.

Spearman’s rank-order correlations (*r*_s) were computed between each of the dietary variables and glutathione concentrations. As shown in **Table 3**, variables that reflected subjects’ general dietary intakes (i.e., energy, protein, carbohydrates, and fat) or their intakes of antioxidants (i.e., vitamin C, vitamin E,

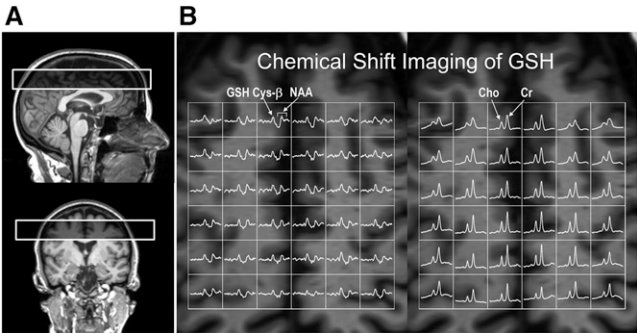


FIGURE 1 GSH CSI and Cr CSI data overlaid on MRI. Location of the GSH CSI slab overlaid on T1-weighted sagittal and coronal MRI of the brain of a subject (A). The CSI slab (rectangles) was positioned superior to the corpus callosum in the frontoparietal regions of the brain. Partial views of GSH CSI (left) and simultaneously acquired Cr CSI (right) of a subject overlaid on T1-weighted transverse MRI corresponding to the middle slice of the CSI slab (B). Data-acquisition variables were slice thickness of 3 cm, matrix size of 8 × 8, nominal voxel size after one-time zero padding of 1.25 × 1.25 × 3 cm³, field of view of 20 cm, TE of 115 ms, TR of 1500 ms, and scan time of 16 min. Spectra of GSH CSI and Cr CSI were processed with 2-Hz line broadening. Cho, choline; Cr, creatine; CSI, chemical shift imaging; GSH, glutathione; NAA, N-acetylaspartate; TE, echo time; TR, repetition time.

and selenium) obtained through diet and supplements were not associated with glutathione concentrations. Significant correlations with glutathione were confined to daily servings of dairy foods or the daily intake of calcium. Servings of dairy and milk were positively related to glutathione concentrations in all 3 brain areas, and daily intake of calcium was positively correlated with parietal and frontoparietal glutathione.

Daily servings of dairy and milk showed the strongest associations with glutathione concentrations (**Figure 2**) with correlation coefficients that ranged between 0.394 and 0.500 (all *P* ≤ 0.013; Table 3). Neither dairy nor milk servings were related to age, BMI, fat mass, fat intake, or total scores on the HEI. However, both dairy and milk servings were positively related to intakes of fat-free mass (dairy: *r*_s = 0.326, *P* = 0.011; milk: *r*_s = 0.360, *P* = 0.005), energy (dairy: *r*_s = 0.342, *P* = 0.007; milk: *r*_s = 0.394, *P* = 0.002), protein (dairy: *r*_s = 0.401, *P* = 0.002; milk: *r*_s = 0.403, *P* = 0.001), and carbohydrate (dairy: *r*_s = 0.375, *P* = 0.003; milk: *r*_s = 0.458, *P* < 0.001). Also, women consumed more servings of dairy (*t* = 2.313, *P* = 0.024) and milk (*t* = 2.202, *P* = 0.032) than did men. Therefore, partial correlations were calculated to determine whether relations between glutathione and dairy or milk servings might be accounted for by these other variables. Even after controlling for fat-free mass, energy, protein, carbohydrates and sex, partial correlations (*r*_p) between glutathione

TABLE 2
Glutathione concentrations overall and for each dairy-consumption group¹

Brain regions for glutathione	Overall (<i>n</i> = 60)		Dairy-consumption groups			<i>P</i>
	Value	Range	Low (<i>n</i> = 21)	Moderate (<i>n</i> = 24)	Recommended (<i>n</i> = 15)	
Frontal	1.27 ± 0.32 ²	0.43–1.92	1.09 ± 0.30	1.30 ± 0.32	1.42 ± 0.22	0.006
Parietal	1.28 ± 0.27	0.66–1.94	1.14 ± 0.23	1.31 ± 0.27	1.45 ± 0.22	0.002
Frontoparietal	1.29 ± 0.28	0.53–1.93	1.14 ± 0.24	1.30 ± 0.29	1.43 ± 0.22	0.004

¹Final dairy-consumption groups on the basis of 7-d diet records. *P* values were based on 1-factor ANOVA comparing the 3 dairy-consumption groups.

²Mean ± SD (all such values).

TABLE 3
Relations between cerebral glutathione concentrations and dietary intake¹

	Frontal glutathione		Parietal glutathione		Frontoparietal glutathione	
	<i>r_s</i>	<i>P</i>	<i>r_s</i>	<i>P</i>	<i>r_s</i>	<i>P</i>
Energy, ² kcal	-0.057	0.795	-0.028	0.921	-0.011	0.936
Protein, ² g	0.054	0.795	0.079	0.813	0.093	0.821
Carbohydrate, ² g	0.001	0.994	-0.043	0.921	-0.012	0.936
Fat, ² g	-0.197	0.370	-0.101	0.813	-0.083	0.821
Dairy, servings/d	0.394	0.013	0.500	0.001	0.465	0.003
Milk, servings/d	0.402	0.013	0.437	0.003	0.437	0.003
Cheese, servings/d	0.165	0.483	0.374	0.015	0.275	0.098
Yogurt, servings/d	0.006	0.994	0.024	0.921	-0.025	0.936
Calcium, ² mg/d	0.280	0.145	0.328	0.039	0.328	0.047
Riboflavin, ² mg/d	0.245	0.207	0.230	0.216	0.273	0.098
Vitamin C, ² mg/d	0.072	0.795	0.096	0.813	0.040	0.936
Vitamin D, ² µg/d	0.135	0.534	0.187	0.355	0.177	0.408
Vitamin E, ² mg/d	-0.147	0.524	-0.013	0.921	-0.047	0.936
Selenium, ² µg/d	0.081	0.795	0.073	0.813	0.106	0.821

¹Spearman's rank-order correlations with *P* values adjusted for multiple comparisons on the basis of the false-discovery rate (30).

²Total daily intake from food and supplements.

and dairy servings (frontal: $r_p = 0.385$, $P = 0.004$; parietal: $r_p = 0.509$, $P < 0.001$; frontoparietal: $r_p = 0.427$, $P = 0.001$) or milk servings (frontal: $r_p = 0.399$, $P = 0.003$; parietal: $r_p = 0.498$, $P < 0.001$; frontoparietal: $r_p = 0.446$, $P = 0.001$) remained significant.

DISCUSSION

The most-robust associations with brain glutathione concentrations in this sample of healthy older adults involved daily servings of dairy and milk. Furthermore, all other significant relations with brain glutathione concentrations involved nutrients that were largely obtained through dairy, particularly calcium. The consumption of dairy differed by sex and was positively related to fat-free mass and energy, protein, and carbohydrate intakes, but partial correlation analyses revealed that, collectively, these variables did not account for relations between glutathione and servings of either dairy or milk. These results confirmed the findings of our earlier exploratory study (data not shown). They were also consistent with the results of a recent study that used the Dietary Approaches to Stop Hypertension diet, which is rich in dairy food. Individuals randomly assigned to consume the Dietary Approaches to Stop Hypertension (DASH) diet were shown to have higher plasma glutathione concentrations (31).

The mechanism by which dairy may influence glutathione concentrations in the brain or elsewhere has yet to be determined; however, several possibilities were suggested in the literature. Dairy is a significant source of both calcium and riboflavin, both of which have been implicated in the maintenance of glutathione. The relation between calcium and glutathione has been investigated extensively, and studies indicated that cells depleted of calcium have reduced concentrations of glutathione (32, 33). The importance of riboflavin likely derives from the fact that the enzymatic activity of glutathione reductase requires riboflavin. Studies of riboflavin-deficient animals have reported lower glutathione concentrations in the liver (34) and eye lenses (35). The only comparable human study failed to find significant

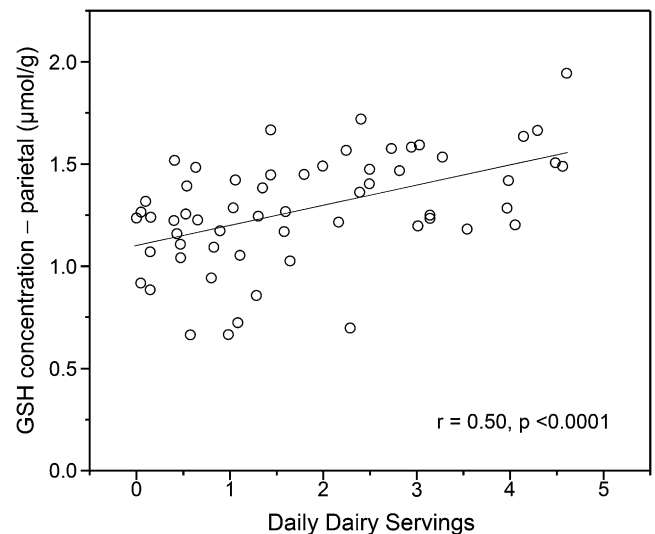


FIGURE 2 Correlations between dairy servings and GSH concentrations in the parietal region. The GSH concentration in the mostly parietal region showed a significant correlation with dairy servings per day ($r = 0.50$, $P = 0.001$, $n = 60$). Values of daily dairy servings were based on 7-d diet records obtained from each subject immediately before GSH measurement. The line indicates the best fit of data. Each data point represents a subject. GSH, glutathione.

differences in erythrocyte glutathione concentrations in blood samples of 28 individuals classified into either riboflavin-adequate or -deficient subgroups (36). Nevertheless, a subsequent study reported an increase in glutathione reductase activity after riboflavin supplementation in women who were marginally deficient in this vitamin. In the current study, correlations between riboflavin and cerebral glutathione concentrations were positive (ranging between 0.230 and 0.273) but were NS.

Dairy foods are also rich in cysteine, which is a semiessential amino acid, that serves as the rate-limiting substrate for glutathione synthesis (37). Particularly, the whey protein fraction of milk is plentiful in cysteine, and several studies in animals (38–41) and humans (42–45) involving supplementation with whey protein or whey protein isolates have documented increases in plasma and tissue glutathione concentrations along with reductions in oxidative stress. For example, Zavorsky et al. (45) reported a dose response in lymphocyte glutathione concentrations in healthy young adults who consumed bars that contained increasing amounts of whey protein. A study of older adults with recent incidences of ischemic stroke reported increases in serum glutathione concentrations in those who consumed a whey-based formula, but no changes in those who consumed a casein-based formula (42). However, to our knowledge, the effect of these supplementations on the human brain has not been previously shown.

Lower concentrations of glutathione in plasma, erythrocytes, lymphocytes and gastric mucosa have been observed in older compared with younger adults (46–51). It has commonly been thought that both lower rates of glutathione synthesis and higher levels of oxidative stress in older adults were responsible for these differences. However, Sekhar et al. (52) concluded that lower glutathione in the elderly was attributable to inadequate precursors rather than diminished enzyme efficiency on the basis of their finding that no differences in glutathione were observed between older and younger subjects when the older group

received cysteine and glycine supplementation. This finding raises the possibility that participants in the current study who were consuming less than recommended daily servings of dairy may have lacked sufficient precursor amino acids to support the adequate synthesis of glutathione. In line with this possibility, note that subjects in the recommended dairy-consumption group had significantly higher protein intake than in the other groups. In contrast, glutathione concentrations were not correlated with protein intake (Table 3), and analyses that used partial correlation did not support the notion that protein intakes accounted for the relation between dairy and glutathione. Because of the crucial role played by cysteine in the synthesis of glutathione, it would be interesting to examine the relation of both dairy servings and glutathione concentrations to cysteine. Unfortunately, different cysteine intakes are not provided through the NDSR database and, therefore, were unavailable for consideration in this study. An additional investigation is required to determine whether cysteine might serve as a vital nutrient linking dairy and brain glutathione concentrations.

In addition to the absence of data concerning dietary intakes of cysteine, another limitation of the current study was that all dietary intake data were based on self-reported methods, which are known to result in the underreporting of some dietary components (e.g., energy consumption) and overreporting of others (e.g., fruit and vegetables). It is also possible that the overreporting of dairy consumption resulted from transparency regarding the study's focus because recruitment clearly centered on the dairy consumption of individuals. However, note that energy and macronutrient intakes as well as HEI-2010 scores for this sample concurred with national data by the NHANES 2009–2010 (http://www.cdc.gov/nchs/nhanes/nhanes_questionnaires.htm) and the HEI reference (25).

The recruitment of older adults who met the recommendation for adequate dairy consumption consisting of ≥ 3 servings/d proved to be a difficult task. Our experience was consistent with the findings of national surveys in which only 23% of older adults in the United States appeared to meet dietary recommendations for dairy (53). Therefore, previous studies that reported lower concentrations of glutathione in peripheral samples obtained from older adults may have been influenced by low dairy intake. Future studies are needed to confirm that the consumption of dairy at recommended intakes improves brain glutathione concentrations in aging. If this effect is confirmed, obstacles to obtaining adequate dairy consumption in older adults should be examined and approaches toward circumventing such obstacles explored.

In conclusion, in this study the *in vivo* measurement of glutathione in the brains of older adults was achieved through MR spectroscopy by using a selective multiple quantum-filtered CSI technique developed in our laboratory. Brain glutathione concentrations were significantly related to adults' reported consumption of dairy foods and calcium, which was obtained in large measure through dairy consumption. Research capitalizing on this approach for evaluating the effect of diet on brain antioxidants could lead to the development of new recommendations toward strengthening cerebral antioxidant defenses and, thereby, improving brain health in the aging population.

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