

**THE RELATIONSHIP BETWEEN DIETARY INTAKE AND GLUTATHIONE IN OLDER
ADULTS**

By

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**THE RELATIONSHIP BETWEEN DIETARY INTAKE, GLUTATHIONE AND ALZHEIMER'S
DISEASE**

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ABSTRACT

Glutathione is an important cellular antioxidant. This study evaluated the association of diet intake and brain glutathione levels in living subjects. A Block food frequency questionnaire (FFQ) and Magnetic Resonance Imaging were used to measure dietary intake and brain glutathione levels for two groups of elderly subjects, one group with early stage Alzheimer's disease (n=23) and a second age-matched control group (n=21). The average age was 73.4 ± 5.5 years and 63% male. There was no significant difference in brain glutathione levels between the two groups. Stepwise regression analysis of diet intake and brain glutathione levels showed a direct relationship between specific nutrients: positive relationship with potato and dairy servings, cysteine, retinol and vitamin B12; negative relationships with meat servings, riboflavin, vitamin D and calcium supplements. This study investigating diet and brain glutathione levels in living subjects support previous study results showing specific nutrients are associated with brain glutathione levels.

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Chapter 1: Introduction

Justification

Glutathione is a tripeptide made from nonessential dietary amino acids: glutamate, cysteine and glycine and plays many important roles in the body. A primary role for glutathione is acting as a potent cellular antioxidant to prevent oxidative damage from reactive oxygen species (ROS) released during normal metabolism and increased reactive oxygen species production under stress conditions (1). Glutathione is also a storage form for cysteine (1). Cysteine is the rate limiting substrate amino acid and is found oxidized to cystine outside the cell (1). Cells vary in their capacity to use cystine as a substrate for glutathione synthesis and many cell types, including brain neurons, rely on extracellular glutathione to supply substrate cysteine (1).

Dietary intakes of amino acids influence tissue levels of glutathione. Studies investigating the effect of protein intake on glutathione levels under oxidative stress conditions like inflammation, infection, malnutrition and ageing show improved glutathione status (2-8). Intakes of other nutrients also influence glutathione levels. Studies have shown increased intakes of saturated fat and omega 6 fatty acids reduce glutathione levels in neurons while intakes of omega 3 and unsaturated fatty acids increase glutathione levels in neurons (9-12).

Other research suggests the ageing process is associated with declining levels of glutathione and increasing levels of oxidative stress (1,13-15). Studies investigating progression of mild cognitive impairment and early Alzheimer's disease show evidence of increasing oxidative damage with disease progression (16).

Researchers have documented a relationship between dietary intake and glutathione levels in different human tissues (2-12). Research also suggests a relationship between glutathione levels and oxidative damage in different tissues (1, 13-15). Studies investigating glutathione levels and cognitive decline associated with Alzheimer's disease have shown serum levels of glutathione do correlate with disease progression (16,17). Further, brain cellular glutathione levels in postmortem subjects is also indicative for cognitive decline status (16,17).

There has been very little research investigating brain glutathione levels, oxidative damage and progression of cognitive decline in living patients. Research is needed to establish the relationship between dietary intake, brain cellular glutathione levels and progression of cognitive decline in living subjects. Previous research indicates brain glutathione levels are predictive for cognitive decline status and diet can improve glutathione levels in many tissues under stress conditions (2-8,16,17). The correlation of brain glutathione levels with both diet and cognitive decline in living subjects could offer a new way to identify cognitive decline in early stages and potential to use dietary intake to prevent, treat or slow disease progression.

Dietary intake could affect brain levels of glutathione, a powerful cellular antioxidant, which may prevent oxidative damage associated with ageing, cognitive decline and the progression of Alzheimer's disease.

Statement of Purpose

The purpose of this study is to determine the relationship between dietary intake and brain glutathione levels in elderly individuals with and without cognitive decline.

Research Questions

Primary Question

1. Does dietary intake influence brain glutathione concentrations within elderly individuals with early Alzheimer's disease?

Secondary Question

2. Do brain levels of glutathione differ between groups of individuals with and without Alzheimer's Disease?

Chapter 2: Review of Literature

Brain Antioxidant Requirements

Human brain cells make up a very small portion of our total body weight but use 20% of the body's total oxygen intake (1,17). High levels of oxygen used for oxidative phosphorylation by brain mitochondria lead to increased generation of superoxide, a reactive oxygen species (1,17,18). Research predicts up to 4% of the oxygen used in brain tissue is converted to superoxide (1,18). Superoxide dismutase converts superoxide to hydrogen peroxide which is converted to water and oxygen by glutathione, glutathione peroxidase and catalase (1,9,17)(appendix A&B). The activity of these enzymes is lower in the brain than in other organs, including the liver or kidney (1). Lower levels of superoxide dismutase, catalase and glutathione peroxidase activity coupled with increased levels of reactive oxygen species increase the potential for oxidative damage to the high lipid content of the brain (1,4,15,17). Brain levels of antioxidants are also important because some areas of the brain contain increased levels of iron (14,15,17,19,20). Brain tissues under oxidative stress can release iron creating hydroxyl radicals which accelerate tissue damage (14,15,17,19,20). Antioxidant levels are also important because the brain synthesizes large amounts of nitric oxide (1). Nitric oxide has many functions in the brain including acting as a neural transmitter (1). Under oxidative stress, nitric oxide can react with superoxide to produce peroxynitrate, further increasing oxidative damage (1,9,17). Brain cells also have varied capacity to use antioxidant substrate amino acids (1,21). Supply of substrate amino acids is important because brain tissues have a limited capacity to use methionine as a precursor to cysteine and rely heavily on extracellular sources of cysteine and glutathione to supply the rate limiting substrate cysteine (1,21).

Glutathione: Roles and Functions

As an antioxidant, glutathione acts directly on free radicals and reactive oxygen species and is oxidized to glutathione disulfide which is recycled by glutathione reductase back to glutathione (1,4,9,12,17,22). Glutathione acts indirectly with the enzymes glutathione peroxidase and glutathione-S-transferase (1,9). Glutathione peroxidase reacts with glutathione and hydrogen peroxide reducing it to water and oxygen while forming glutathione disulfide, which is then recycled back to glutathione (1,4,9). Glutathione-S-transferase reacts with glutathione on electrophilic compounds and xenobiotics to detoxify cells by forming disulfides which are transported out of the cell (1,4,9,23). Glutathione provides a storage and transport mechanism for cysteine which is most often oxidized to cystine outside the cell (1,4,9,17). Glutathione forms S-nitrosoglutathione with nitric oxide in the brain to control oxidative stress (1,4,24). Glutathione prevents oxidative stress from damaging proteins by reversibly binding to proteins preventing oxidation (1). Glutathione is also important in recycling other antioxidants, cell growth and immune functions (1,4,9,14,25,26).

Cellular Regulation and Synthesis of Glutathione

Glutathione levels are regulated by the availability of the substrate amino acids and enzymes γ -glutamylcysteine synthetase (γ -GCS) and glutathione synthetase (GS) (14,27). γ -GCS is the rate limiting enzyme for synthesis of glutathione (14,27). Oxidants generally increase synthesis of γ -GCS while low protein, phosphorylation and nitrosation of γ -GCS decrease synthesis (9,10,27).

Glutathione levels are controlled by feedback of glutathione which competes with glutamate in binding to γ -GCS in the first step of glutathione synthesis (9,27). High levels of glutathione inhibit synthesis while low levels of glutathione increase synthesis of glutathione (14).

Cysteine is the rate limiting substrate amino acid (9,27). Cellular levels of cysteine are much lower than levels of glutamate or glycine (27). Cysteine is a nonessential amino acid which becomes conditionally essential under stress conditions (9,27). Outside the cell, cysteine is primarily found oxidized as cystine (9). Brain cells can differ in their capacity to transport cystine (1). Astroglial cells use both cysteine and cystine in glutathione synthesis while neurons can only use cysteine (1). Cells without a transport mechanism for cystine rely on extracellular glutathione to serve as a source for cysteine in glutathione synthesis (1,17). Brain cells with cystine transport system xc- exchange cystine (imported) for glutamate (exported) (1,17). High levels of extracellular glutamine associated with disease conditions can limit this transport mechanism (1,6,9,27,28).

Cellular Glutathione Levels

Cellular glutathione levels normally reflect a balance between synthesis and loss. Synthesis is based on the activity of γ -GCS, the supply of amino acid substrates and glutathione's negative feedback on γ -GCS (27). Balance is dependent on regeneration of glutathione from glutathione disulfide by glutathione reductase which maintains cellular glutathione levels (14). Most cells also release or transport glutathione out of the cell leading to reduced cellular levels (27,29).

Glutathione levels are also affected by cellular reactions leading to loss. Cellular levels of glutathione can decrease with increased levels of glutathione disulfide related to high levels of oxidative stress (27). High levels of glutathione disulfide can lead to its transport out of the cell, lowering the glutathione pool (17,27,30). Glutathione-S-transferase and glutathione act on electrophilic compounds and xenobiotics to detoxify cells (1,4,9,23). Conjugates formed using glutathione-S-transferase result in conversion to mercapturic acid which is excreted from the body, thus lowering glutathione levels (17,27).

Brain Tissue Glutathione Levels

Brain levels of glutathione can vary based on cell type (15,17). Astrocytes (glial cells) contain most brain glutathione and provide the glutathione substrate reservoir for cysteine, which is required for neural glutathione synthesis (1,31,32,33). Neurons cannot transport extracellular cystine and rely upon glutathione released from astrocytes for synthesis of neural glutathione (1,32,33). Neurons also contain lower levels of glutathione compared to astrocytes but contain higher levels of other antioxidants including ascorbate (17,27,30).

Diet and Glutathione Levels

Studies investigating the effect of dietary protein and specific amino acid intakes show improved glutathione levels in study participants under oxidative stress conditions (2-8,14,34). Four studies (1,35-37) investigated the potential of whey protein to increase cellular glutathione levels.

Whey protein increased glutathione levels in the heart and liver of ageing mice along with improving longevity (35). Whey protein isolate was shown to improve glutathione levels in human prostate epithelial cells (1). Commercial whey protein products Immunocal and Protectamin improved plasma glutathione levels in HIV patients (36,37). Researchers believe whey protein and whey protein supplements, which contain high levels of cysteine, improve glutathione levels by removing the substrate rate limiting step in glutathione synthesis (2,8,35-37). Supplementation of N-acetylcysteine (NAC) also works like whey protein to provide a source for cysteine. NAC was shown to increase glutathione levels under stress conditions in the erythrocytes of malnourished children and in prostate epithelial cells (2,8).

New research is associating long term dietary intakes, and nutrition related conditions like diabetes, with increased risk for neurodegenerative diseases (38,49). Two studies concluded type II diabetes substantially increases risk for developing Alzheimer's disease (AD) (38,39). The mechanism linking type II diabetes to AD is related to control of blood sugar and high carbohydrate, low fat diets (38). High levels of glucose or fructose lead to formation of advanced glycation end products (AGE) (38). AGE damage to LDL and LDL receptor proteins prevent astrocytes from receiving LDL lipids and cholesterol (38). Because astrocytes supply fats and cholesterol to neurons both types of cells become depleted disrupting proper neural function (38). Researchers propose astrocytes then increase cholesterol synthesis to meet neural demands (38). This process requires higher energy production and over time increases the oxidative stress damage to astrocytes (38). Intakes of other nutrients also influence glutathione levels.

According to Wu et al. (9,12), “glucosamine, taurine, n-3 polyunsaturated fatty acids, phytoestrogens, polyphenols, carotenoids, and zinc” preserve glutathione levels while “high-fat diet, saturated long-chain fatty acids, low density lipoproteins, linoleic acid, and iron” lower glutathione levels (9,12). The first group maintains glutathione levels by decreasing nitric oxide production while the second group lowers glutathione levels by increasing nitric oxide production (9,12). This is important for brain tissue because the brain produces large amounts of nitric oxide and increased production in neurons inhibits γ -GCS activity lowering glutathione levels (9,10,11).

New research suggests specific diets can be used to treat neurological disorders. Jarrett et al. (40) investigated the use of a ketogenic diet to prevent epileptic seizures. Researchers found the high fat, low CHO diet increased γ -GCS activity improving brain mitochondria glutathione levels in rats (40). The ketogenic diet also helped improve mitochondrial levels of the antioxidant lipoic acid (40). The ketogenic diet improved these antioxidant levels while decreasing levels of hydrogen peroxide produced by the mitochondria (40). The study concluded the improved redox state of the brain mitochondria is the mechanism by which the ketogenic diet helps control epileptic seizures (40). Based on this research, dietary changes to improve glutathione levels may be important in treating neurological disorders (40).

Glutathione and Ageing

Evolutional physiology describes the ageing process based on the body’s ability to adapt and survive to reach reproductive age.

The body's evolved defenses and response to disease, microbes, infection and virus using inflammatory/oxidative responses to cellular challenges may impact longevity in favor of reproductive survival (14). The long term effect of oxidative stress, diet and lifestyle along with individual genetic regulation may result in the conditions associated with ageing (3,14).

Recent animal and human studies report glutathione levels or glutathione/glutathione disulfide ratios decline during the ageing process after the age of 45 (14,41). Reduced levels of glutathione may be related to decreased synthesis and/or increased oxidation (13). Decreased synthesis can be related to substrate levels, enzyme activity, receptor activity, energy levels and genetic regulation, all of which can be effected by oxidative damage, nutrient intakes and disease conditions (1,13,27).

Studies reviewed here report improved glutathione levels or oxidative conditions in participants with stress conditions using dietary proteins; results indicate potential long term dietary intakes and/or deficiencies may be related to increasing oxidative conditions. The ageing process is generally associated with decreased lean muscle, lower levels of activity and lower energy requirements (42). These changes in combination with an increasing oxidative state may contribute to oxidative dysfunction (42).

Studies investigating protein and safe amino acid levels associate specific sulfur amino acids with improvements in glutathione levels under stress conditions or low protein intakes (34,42,43). A study investigating protein intakes and nitrogen balance in healthy adults found glutathione levels were reduced and oxidation increased when protein levels decreased (34).

The researchers reported increased oxidative stress during the period needed to re-establish nitrogen balance may indicate protein or specific amino acid levels may not be sufficient to protect subjects under normal daily stress or stress associated with ageing or disease conditions (34). These studies also reported general recommendations for increasing protein intakes and specific sulfur amino acids is complex because of the role they play in methylation reactions which include gene regulation (34,42).

Glutathione and Alzheimer's Disease

Oxidative damage in neural tissue has been associated with the ageing process, cognitive decline and Alzheimer's disease (AD) (13,16). Research shows ageing is associated with declining glutathione levels relative to increasing oxidation in the brain (1,13-16,19,44). Increased oxidation and decreased levels of glutathione lead to increasing damage to brain tissues (1). High levels of oxygen consumption in the brain lead to increased formation of superoxides (1). Superoxides normally react with super oxide dismutase to form hydrogen peroxide which is acted on by glutathione peroxidase and catalase to produce water and oxygen (1,17). Decreased levels of glutathione limit this conversion causing increased cellular levels of superoxide and hydrogen peroxide increasing oxidative stress (1).

The brain also synthesizes large amounts of nitric oxide (1,45,46). Glutathione depletion allows superoxide to react with nitric oxide forming peroxynitrite (9,10,17). Formation of peroxynitrite leads to oxidation of cellular proteins and lipids (1). Peroxynitrite inhibits glutathione peroxidase and enzyme activity related to energy production (1,46).

Decreased energy production limits synthesis of glutathione by inactivating ATP dependent enzymes γ -GCS and GS reducing cellular glutathione levels (1,15,16,19,27,47).

Peroxidation of lipids leads to conversion of PUFA's to 4-hydroxynonenal (4-HNE) (1,48).

4-HNE is a toxic aldehyde which increases the demand for glutathione-S-transferase and lowers glutathione levels when conjugates are formed and exported out of the cell

(1,13,1,30). Increasing levels of oxidative damage can also release iron from brain tissue leading to the lipid peroxidation associated with the β -amyloid protein in AD (15,16).

Research investigating the earliest effects of oxidative damage propose damage to transcription factors for γ -GCS and to cellular transport and receptor proteins initiate the dysfunction and decline of the cellular redox state (1,2,9,14-17,27).

Glutathione Levels and Oxidative Stress

The ageing process is thought to be associated with declining levels of glutathione and increasing levels of antioxidant stress (1,13-15). Lang et al. (49) reported erythrocyte glutathione levels are reduced in participants with chronic disease associated with ageing but not in healthy ageing participants without disease (49). Based on study findings erythrocyte glutathione levels may indicate level or progression of disease conditions (49). Data also suggest decreased levels of glutathione are related to a reduction in synthesis, not increased production of glutathione disulfide (49).

Research has shown brain tissue glutathione levels may indicate cellular oxidative stress status (16). Antioxidant levels have been correlated with changes in cognitive status and progression of cognitive decline (16,44).

Ansari et al. (16) and Padurariu et al. (44) reported antioxidant levels, including glutathione, were indicative for cognitive status based on the Mini Mental State Examination (MMSE) (16,44). The study's compared levels of antioxidants to MMSE scores for study groups including: noncognitively impaired, mild cognitive impairment, mild Alzheimer's disease and Alzheimer's disease (16,44). Both studies report a decline in glutathione levels is associated with the earliest stages of cognitive decline and corresponds with disease progression (16,44).

Conclusion

The brain demands high levels of oxygen for energy production which increases levels of reactive oxygen species (1,17). The high lipid content of the brain makes it highly susceptible to oxidative damage (1,17). Glutathione is protective against reactive oxygen species and under normal conditions, levels are regulated by the demand for antioxidants (9,14,27). Previous research has shown glutathione levels decline after reaching maturity as a part of the ageing process (14,41). Glutathione levels are thought to decline due to decreased synthesis or increased levels of oxidants (14,41). New research suggests glutathione levels may or may not decline with ageing and decreases are associated with progression of conditions associated with ageing (49). Research related to cognitive decline and Alzheimer's disease show declining glutathione levels are associated with early stages of disease progression (16,44). Based on these findings it is thought glutathione levels may be used to indicate progression of disease conditions (16,44).

Researchers investigating oxidative damage propose stress induced increases of oxidation damage the synthetic pathways associated with glutathione metabolism thus increasing cellular dysfunction (1,2,9,14-17,27).

Studies reviewed within this paper report glutathione levels in several different type cells under stress conditions in both human and animal subjects. Results indicate the status of glutathione related to stress conditions and some report the effect of diet on those glutathione levels. No studies reviewed assessed glutathione levels in brain tissues of living subjects. Studies which investigated cognitive decline in living subjects have shown serum levels of antioxidants are correlated with disease progression (17).

Sneff et al. (38) reported subjects with type II diabetes are up to five times more likely to develop Alzheimer's disease (38). This also indicates overall status of glutathione may be associated with systemic oxidative stress contributing to progression of many conditions including cognitive decline. Research investigating the association between dietary intakes and glutathione levels show under stress conditions diet can influence cellular levels. Further, dietary intakes of whey protein and NAC are shown to improve cellular levels of glutathione in subjects under stress conditions (2-8). Dietary intakes of fatty acids also are shown to influence glutathione levels via their effect on metabolism and nitric oxide production which can inhibit γ -GCS in neurons (6,9,40).

Currently, in the United States 4.5 million people have Alzheimer's or related dementias (50). The number of Americans with Alzheimer's is increasing and could reach 16 million by the year 2050 (50). Alzheimer's disease is now the sixth-leading killer and medical care for Alzheimer's patients is estimated to cost more than a trillion dollars a year (50).

The United States government recently released a draft of its new Alzheimer's plan which calls for new ways to treat and or prevent Alzheimer's disease by 2025 (50).

Research has been conducted to show a relationship between dietary intake and glutathione levels in different human tissues (2-12). Research has also shown a relationship between and glutathione levels and oxidative damage in different tissues (1,13-15). Studies investigating glutathione levels and the cognitive decline associated with Alzheimer's disease have shown serum levels of glutathione do correlate with disease progression (16,17). Further, brain cellular glutathione levels in postmortem subjects is also indicative of cognitive decline status (16,17). There are few studies investigating brain cell glutathione levels, oxidative damage and progression of cognitive decline in living patients.

Research is needed to establish the relationship between dietary intake, brain cellular glutathione levels and progression of cognitive decline in living subjects. Previous research indicates glutathione levels are predictive for cognitive decline and diet intake can improve glutathione levels in many tissues under stress conditions (2-8, 16,17). The correlation of brain glutathione levels with both diet and cognitive decline in living subjects could offer a new way to identify cognitive decline in early stages and a way to prevent or at least slow disease progression. Research suggests increasing oxidative damage is associated with progression of cognitive decline and under stress conditions dietary intake can help to improve glutathione levels.

Chapter 3: Methods

Overview

The purpose of this thesis is to determine the effect dietary intake has on brain glutathione levels which have been associated with the cognitive decline of Alzheimer's disease. This cross-sectional study collected data for dietary intake and brain glutathione levels in elderly people (n=44) with early Alzheimer's (n=23), as well as, elderly individuals without Alzheimer's (n=21).

Sample

Study participants were recruited using University of Kansas Medical Center emails and fliers, the Grayhawk Registry and the KU Brain Aging Project Uniform Dataset Registry. To be eligible for this study participants had to be between 60 and 85 years of age, education of more than ten years, no significant psychiatric disorders, ability to perform neuropsychological testing, and general good health. Participants with a clinical dementia rating of mild to very mild Alzheimer's were required to have a study partner available to provide information about the participant's daily activities. Exclusion criteria were: presence of neurological disease with the exception of Alzheimer's disease which affects cognition; diabetes mellitus; abnormal vitamin B₁₂; rapid plasma regain or thyroid function lab values; use of psychoactive medications; use of specific antioxidant therapy or special dietary regimens and specific MRI contraindications such as metal plates in their body.

Research Setting

The data were collected from subjects at the Hoglund Brain Imaging Center at the University of Kansas Medical Center. Interested subjects from the Grayhawk Registry, the KU Brain Aging Project Uniform Dataset Registry and respondents from KUMC email and flyers were first screened by telephone to determine eligibility using self reported height, weight and brief medical history. The Grayhawk Registry is a database of Kansas City area residents greater than 60 years of age who join the registry making them potential study participants for University of Kansas Medical Center research projects. The Uniform Dataset Registry operated by the University of Kansas Alzheimer and Memory Program recruits age matched healthy and Alzheimer's diagnosed study participants for aging and Alzheimer's disease research. Eligible subjects who passed the initial screening were screened for metal in their body following a MRI safety protocol.

Ethics

The procedures for this thesis project were approved by the University of Kansas Medical Center Human Subjects Committee. All participants signed and received a consent form. Study participants who completed the diet history questionnaire and the magnetic resonance scan will received fifty dollars for participation in the study.

Data Collection

At the initial study visit diet research staff collected demographic information, anthropometric measurements and administered a food frequency questionnaire outlined in the instrumentation section below. Upon completing the initial study visit, the participants returned approximately one week later for magnetic resonance testing (MRI).

Instrumentation

On the initial study visit research staff conducted the following:

Demographics Questionnaire

Demographic information (age, gender, race/ethnicity) were collected to characterize the sample population used in the study.

Food Frequency Questionnaire

The Block Food Frequency Questionnaire was used to estimate participants' normal dietary intake over the last year (51-56). This tool was originally developed by Gladis Block and has been validated and used in numerous research studies. (51-56). Food frequency data are recommended for estimating dietary intake in elderly and Alzheimer's participants who may suffer short term memory loss (51-56). Participants completed the questionnaire and submitted it to a research dietitian to review for completeness.

Individuals with early AD completed the questionnaire the help of their study partner.

Magnetic Resonance Imaging

After the initial study visit, approximately one week later, participants returned to the University of Kansas Hoglund Brain Imaging Center for magnetic resonance imaging. The imaging took approximately one hour and assessed glutathione levels in the frontal and parietal areas of the brain using a chemical shift technique to detect glutathione.

Analysis of Data

Data from questionnaires, diet analysis, and brain imaging were entered into a data system managed using SAS (statistical analysis system). Data were verified by dual entry and then exported for analysis. Descriptive statistics and were used to describe the characteristics of the study population.

Descriptive statistics, t-test and a stepwise regression model were used for dietary intake and brain glutathione data analysis comparing the two groups separately and both groups combined. Statistical significance was set at $p < 0.05$.

Chapter 4: Results

This section includes the results for the participant demographics, dietary intake, brain glutathione concentrations and correlations between diet and brain glutathione.

Demographics

Study participants (n=44) were recruited from University of Kansas Medical Center emails and fliers, the Grayhawk Registry and the KU Brain Aging Project Uniform Dataset Registry. The participants were Caucasian males and females between 60 and 82 years of age. The demographic data are depicted in Table 1.

Table 1: Participant Characteristics

	All (n = 44)	Alzheimer's (n = 23)	Controls (n = 21)
Age (years)	73.4 ± 5.5	73.7 ± 6.5	74.6 ± 6.1
Gender (%)	63% Male 36% Female	69% Male 30% Female	57% Male 42% Female

Table 2 displays the brain glutathione concentrations in the participants. There was no statistically significant difference in brain glutathione concentration between the Alzheimer's and control group.

Table 2: Brain Glutathione Concentration

	All (n = 44)	Alzheimer's (n = 23)	Controls (n = 21)
Glutathione (μmol/g)	0.99 ± 0.154	0.966 ± 0.149	1.01 ± 0.159

(p = 0.31)

Dietary Intake

The average energy intake of the study population was 1665 ± 776 kcal. The macronutrient distribution was 49 ± 7 % carbohydrate 15 ± 3 % protein and 34 ± 6 % fat. Table 3 lists the energy and selected nutrient intake of the population. There was no difference in energy or macronutrient intake between the two groups. Table 4 lists the micronutrient intake of the population and Table 5 displays the servings consumed from various food groups.

Table 3. Energy and selected nutrient intake for the population.

Macronutrient	All (n = 44)	Alzheimer's (n = 23)	Controls (n = 21)	p value
Energy (kcal)	1665 ± 776	1697 ± 873	1518 ± 626	0.83
Carbohydrate (g)	203 ± 78	197 ± 88	193 ± 84	0.97
Protein (g)	64 ± 34	64 ± 39	59 ± 27	0.89
Fat (g)	65 ± 43	70 ± 46	56 ± 25	0.74
Fiber (g)	17 ± 8.6	16 ± 8.4	17 ± 9.6	0.92
% Carbohydrate	49 ± 6.7	47 ± 5.8	50 ± 7.7	0.70
% Protein	15 ± 2.9	14 ± 2.0	15 ± 3.5	0.75
% Fat	34 ± 6.4	36 ± 6.9	33 ± 6.0	0.68

Table 4. Micronutrient intake for the study population.

Micronutrient	All (n = 44)	Alzheimer's (n = 23)	Controls (n = 21)
Vitamin A (μg)	465 \pm 268	410 \pm 237	473 \pm 265
Vitamin C (mg)	100 \pm 53	90 \pm 53	117 \pm 57
Vitamin D (μg)	159 \pm 108	132 \pm 99	179 \pm 114
Vitamin E (mg)	7.2 \pm 3.5	6.9 \pm 3.5	7.3 \pm 3.7
Folic Acid (μg)	127 \pm 83	138 \pm 99	122 \pm 80
Folate (food) (μg)	271 \pm 123	258 \pm 129	284 \pm 141
Vitamin B12 (μg)	5.05 \pm 2.62	4.9 \pm 2.76	4.9 \pm 2.45
Calcium (mg)	851 \pm 441	781 \pm 505	884 \pm 402
Iron (mg)	12.82 \pm 6.23	12.6 \pm 6.34	12.4 \pm 6.37
Selenium (μg)	83 \pm 46	83 \pm 53	73 \pm 34
Zinc (mg)	10.2 \pm 5.54	10.2 \pm 6.28	9.5 \pm 4.6

Table 5. Food Servings for Specific Food Groups.

Food Group	All (n = 44)	Alzheimer's (n = 23)	Controls (n = 21)
Fruit servings	1.5 \pm 1.11	1.3 \pm 1.10	1.7 \pm 1.11
Vegetable servings	3.1 \pm 2.11	2.8 \pm 2.10	3.2 \pm 1.97
Dairy servings	1.3 \pm 1.21	1.1 \pm 1.15	1.6 \pm 1.16
Grain servings	4.7 \pm 2.90	4.8 \pm 3.06	3.7 \pm 2.02
Meat servings	1.9 \pm 1.33	2.1 \pm 1.45	1.6 \pm 1.09
Fat servings	3.6 \pm 1.58	3.7 \pm 1.54	3.3 \pm 1.63

There was no significant difference between groups in brain glutathione concentrations or dietary intake and thus the sample was combined for further analysis. Stepwise regression analysis results in Table 6 show the variables which were found to have a linear relationship with brain glutathione concentration in Alzheimer's and control subjects. The analysis found a positive association between brain glutathione levels and the following dietary intake variables: percentage of servings vegetable potato, percentage of servings orange vegetables, dietary cysteine, dairy servings, dietary retinol and vitamin B12. The analysis found a negative association between brain glutathione levels and the following dietary intake variables: percentage of servings of meat, fish, poultry, dietary riboflavin, dietary vitamin D and supplemental calcium.

Table 6. Variables linearly related to brain glutathione concentration

X	Δ in GSH (y) per unit Δ of X	p - value
Percentage Servings Meat, Fish, Poultry	-0.050	.001
Percentage Servings Vegetable Potato	0.235	.001
Dietary Cysteine (mg)	0.242	.001
Dietary Riboflavin (mg)	-0.196	.001
Dairy Servings	0.159	.001
Dietary Retinol (mcg)	.00001	.001
Dietary Vitamin B-12 (mcg)	0.047	.011
Dietary Vitamin D (mcg)	-0.001	.013
Percentage Servings Orange Vegetable	0.242	.031
Supplemental Calcium (mg)	-6.465	.053

Table 7 below displays the mean and standard deviation for the dietary variables found to be significant in the model and not included in the original descriptive dietary intake data (Tables 3-5).

Table 7. Descriptive statistics for significant dependent variables.

X	Mean	Std. Deviation
Percentage Servings Meat, Fish, Poultry	3.18	2.471
Percentage Servings Vegetable Potato	.23	.565
Dietary Cysteine (mg)	.80	.509
Dietary Riboflavin (mg)	2.11	.868
Dairy Servings	1.34	1.219
Dietary Retinol (mcg)	465.20	268.376
Dietary Vitamin B-12 (mcg)	5.05	2.623
Dietary Vitamin D (mcg)	159.45	108.574
Percentage Servings Orange Vegetable	.02	.151
Supplemental Calcium (mg)	434.23	488.168

Chapter 5: Discussion

The aim of this study was to determine the influence diet has on human brain levels of glutathione in vivo. The results of this study show an association between certain nutrition components and brain levels of glutathione.

These results confirm previous research showing dietary intake does influence glutathione levels in different tissues under disease/stress conditions. In the current study dietary cysteine and dairy servings were positively correlated with brain glutathione levels in AD and control subjects. Dairy products, including whey protein, contain high levels of cysteine which have been shown to improve glutathione levels (1,35-37). These results agree with previous study results showing whey protein and whey protein isolates improve glutathione levels in human and animal cells under stress conditions (1,35-37). Whey protein, whey protein isolates, dairy intake and dietary cysteine provide the rate limiting substrate amino acid cysteine for glutathione synthesis and other essential amino acids (2,8,35-37).

Meat servings were negatively correlated with brain glutathione levels in AD and control subjects. Previous research has shown dietary intake of protein improved glutathione levels in different tissues under stress conditions and in malnourished children (2,8,34-37,42,43). These studies investigated safe protein intake levels, nitrogen balance, whey protein and N-acetyl cysteine (NAC) supplements and found improvements in cellular glutathione levels under stress conditions. The researchers concluded improvements were likely related to improving protein/amino acid intake, improving methionine intake and supplying substrate cysteine for glutathione synthesis (2, 8,35-37). The population in the current study differed as the participants were not malnourished.

The negative correlation shown in this study between meat servings and brain glutathione levels in AD and control subjects could be related to variables associated with meat consumption not accounted for in the diet history. Variables associated with dietary meat intake could include: cooking of meat can affect the quality/amount of oxidized saturated and unsaturated fatty acids which have been shown to affect glutathione status, and meat quality including the amount of fat and the animals feed content can contribute to meat containing varying levels of omega 3, omega 6 and saturated fats which have been shown to affect glutathione levels (9,12). Another potential variable contributing to the negative correlation between meat servings and brain glutathione levels may be that intake of protein does not necessarily represent absorption and utilization of the protein. With the study population average age of 73 it is possible some study subjects had reduced ability to denature and absorb protein properly (57).

This study also found servings of vegetable/potato were positively correlated with brain glutathione levels in AD and control subjects (table 6). The Block Food Frequency Questionnaire includes the following categories for potato/vegetable consumption: French and home fries, hash browns, tater tots, yams, sweet potato, mashed potato, boiled and baked potato and potato salad. Without knowing the nutritional content of the potato consumed in this category it is difficult to conclude how vegetable/potato servings contribute to improved brain glutathione levels.

Dietary Riboflavin/Vitamin B2 and Vitamin D intake were found to have an inverse relationship with brain glutathione levels in AD and control subjects. Riboflavin is a coenzyme involved in oxidation reduction reactions and is important for maintaining cellular redox and for energy production (58).

It was unexpected to find the inverse association with brain glutathione because riboflavin is a coenzyme in reactions with glutathione reductase and glutathione peroxidase (58). As a coenzyme in these reactions riboflavin helps to regenerate glutathione from glutathione disulfide, thus maintaining the cells glutathione pool (12). Common dietary sources of riboflavin and Vitamin D are eggs, meats, milk, milk products, enriched grain products and cereals (58). The inverse relationship shown between dietary riboflavin, Vitamin D and glutathione may be comparable to the negative correlation between meat intake and glutathione levels. Because these nutrients are found in many animal products it is possible increased dietary levels represent a diet higher in fats including saturated fats, processed foods and lower in antioxidant intake. Previous research has shown the potential for this type of diet to lower the cellular glutathione pool (9,12).

Dietary retinol and percentage servings orange vegetables were shown to have a positive linear relationship with brain glutathione levels (table 6). While previous research has not shown a direct relationship between brain glutathione levels and orange vegetable intake, research has shown a diet rich in vegetable intake can provide phytonutrients and antioxidants which have been shown to preserve glutathione levels (5, 9,12). It is also probable this type of diet, which contains higher vegetable intake, may also contain lower levels of animal products and damaged fats which have been shown to influence glutathione levels (9,12). Dietary retinol comes largely from animal products. With the positive correlation shown between dairy servings and glutathione levels it is possible dairy products which contain and are fortified with Vitamin A share the positive linear relationship with brain glutathione levels.

Dietary Vitamin B12 intake was shown to have a positive linear relationship with brain glutathione levels (table 6). Research reviewed here has not previously shown this direct relationship between B12 and tissue glutathione or brain glutathione levels. Proper cellular function is supported by the methylation pathway where Vitamin B12 is required to regenerate methionine from homocysteine. Systemic glutathione levels are supported by methionine which can be used to supply the rate limiting substrate cysteine for glutathione synthesis. While this mechanism is less active in the brain than in other tissues, support of the methyl cycle could increase brain glutathione levels when other tissues release glutathione into circulation (1,21). Glutathione released from cells acts as a reservoir for cysteine which can be imported into brain astrocytes for glutathione synthesis (1,21).

Brian Glutathione Levels

These study findings did not support previous research associating lower brain glutathione levels with the cognitive decline of Alzheimer's Disease (16,44). Previous research has shown cognitive decline to be associated with decreasing levels of brain antioxidants, including glutathione (16,44). In this study brain levels of glutathione were not found significantly different when comparing controls to Alzheimer's subjects. This study investigated brain glutathione levels in subjects with a clinical dementia rating of very mild or mild Alzheimer's disease. It is possible significant differences in brain glutathione levels detectable by MRI in living subjects may become apparent with more disease progression. The significance of the results found in this study is the association of brain levels of glutathione with dietary intake in living human subjects.

Conclusion

This study is one of the first to establish a relationship between dietary intake and brain glutathione levels in living subjects. The correlation of brain glutathione levels with dietary intake in living subjects could offer a new way to identify cognitive decline in early stages and the potential for nutrition therapy to treat or slow disease progression.

Research presented here indicates dietary intake does influence brain glutathione levels which have been shown in previous research to be associated with early stages and progression of cognitive decline.

Limitation/Future Research

More research is needed to define the role specific nutrients and dietary variables play in the progression of oxidative damage and cognitive decline. In this study a diet history questionnaire was used to establish the relationship nutrients have with measured brain glutathione levels in living subjects. Diet history questionnaires are useful to identify eating patterns and types of foods eaten over periods of time. More specific diet and nutrient intake data will be needed to establish nutritional mechanisms which might be useful for preventing, treating or controlling oxidative damage associated with aging and cognitive decline. Other study limitations would include limited sample size and other possible confounding variables associated with development and progression of Alzheimer's Disease which may play a role in diet and food preferences. This study investigated glutathione levels in subjects with a CDR of very mild to mild Alzheimer's in living subjects. It is possible future research using MRI assessment of brain glutathione levels in living subjects may find significant differences in brain glutathione concentration levels where progression of cognitive decline is more advanced.

Nutrition is shown here to be a primary environmental influence on brain glutathione levels. Further research will be needed to identify other environmental variables involved in disease progression. Inherited genetics and polymorphisms can influence metabolism and physiological function. Further, new omic's sciences are showing environmental influences of epigenetics, including nutrition, lifestyle and toxicity, can modify cellular processes and regulate genetic expression. These environmental and genetic influences can affect specific nutrition requirements needed to optimize health in each individual over the lifespan and play a role in aging or disease progression.

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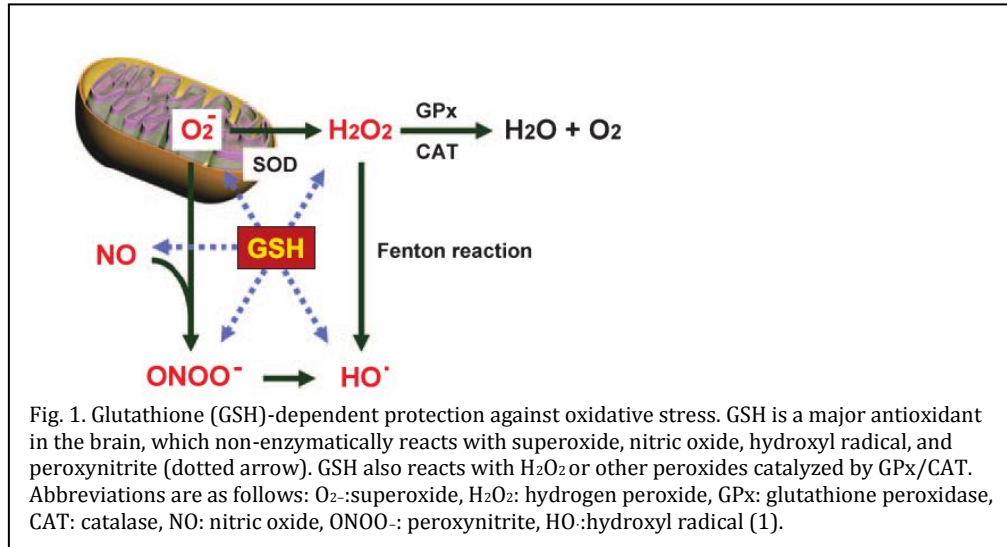
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APPENDIX A



Reference (1)

APPENDIX B

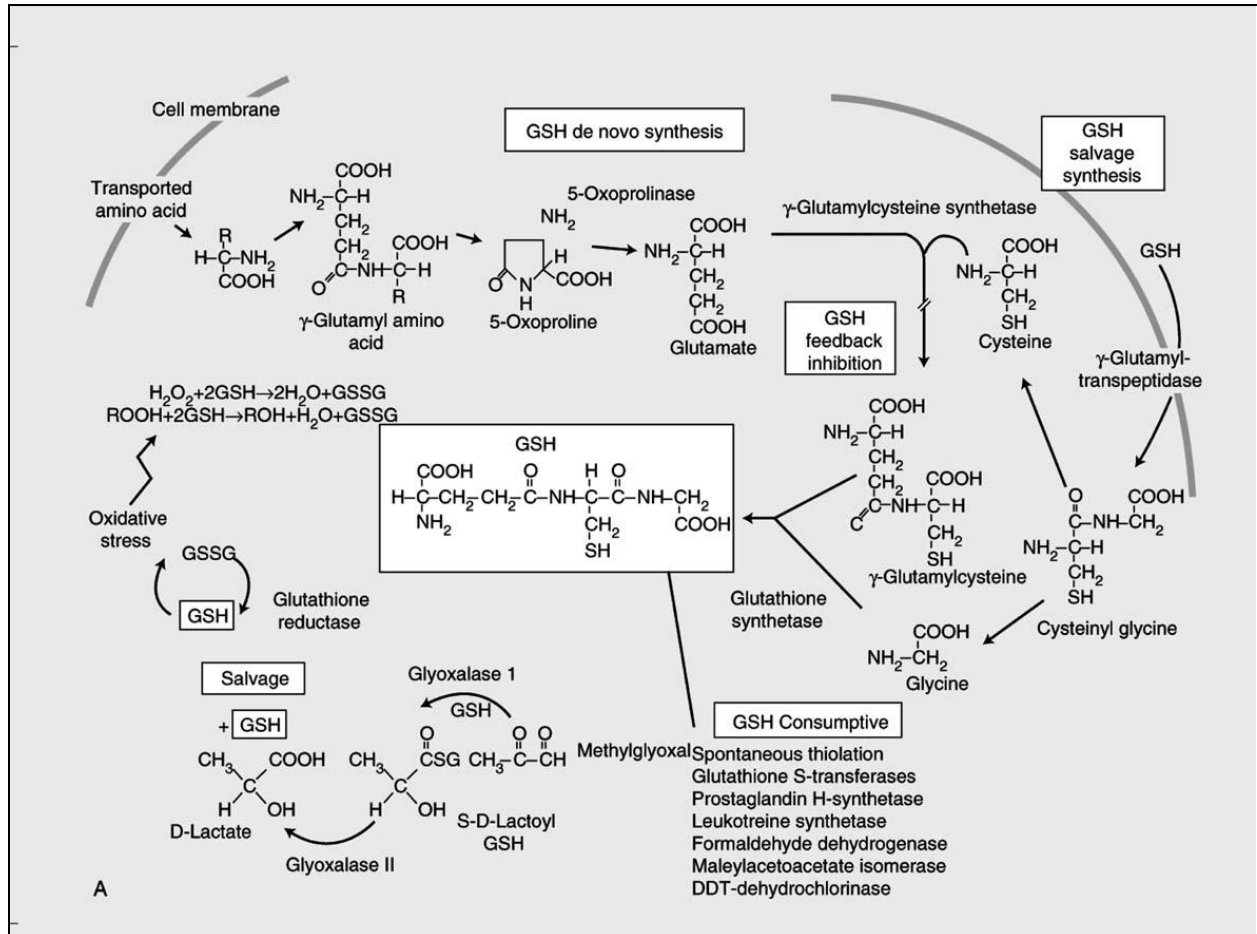


Fig. 1. Homeostasis of glutathione is maintained intracellularly through a de novo and salvage synthesis pathway. Tight regulation of the GSH:GSSG ratio is maintained by glutathione reductase (14).

Reference (14)