DIETARY GLUTATHIONE INTAKE AND THE RISK OF LARYNGEAL CANCER: A POPULATION-BASED CASE-CONTROL STUDY IN NORTHERN CALIFORNIA

by

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CERTIFICATION OF APPROVAL

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DEDICATION

To my grandfather who passed away from cancer, 1919-1992.

ABSTRACT

Glutathione (GSH) is a tripeptide with well known endogenous antioxidant properties. It is also found in a variety of foods such as fruits, vegetables and meat products. Diets high in GSH may confer additional antioxidative protection as exogenously administered GSH has been shown to increase plasma levels of GSH. The association between dietary GSH intake and risk of laryngeal cancer was investigated in a secondary analysis of a large population-based case-control study of environment and laryngeal cancer, conducted in northern California from July 1988 through April 1993. A total of 889 cases (679 males and 210 females) and 1463 controls (805 males and 631 females) were available in this study. A food frequency questionnaire consisting of 92 questions was used to estimate GSH intake by quartiles. Specifically, this study hypothesized that dietary GSH is protective for laryngeal cancer. Because data were also available on subsite distribution, it was hypothesized that GSH would be more protective for the glottic subsite than the supraglottic subsite based on the presumption that the higher incidence of glottic tumors are due to smoking cigarettes and alcohol consumption.

This study found no association between total dietary GSH intake and laryngeal cancer risk. Further, associations varied by food source lending further support that GSH was not associated with laryngeal cancer risk. A significant inverse association was found between GSH derived from raw fruits and laryngeal cancer risk [p trend <.001 and estimated relative risk of .44 for the highest quartile of GSH in raw fruits (95% confidence interval .31-.63)]. Similarly for salad, an inverse association was also found [p trend <.001, estimated relative risk of .42 for the highest quartile of GSH from salad

(95% CI = .30 - .59)]. These findings are supported by literature suggesting raw fruits and vegetable consumption is protective for laryngeal cancer. Contrarily, meat consumption was a risk factor with an estimated relative risk of 1.59 for the highest quartile (95% CI = 1.13 – 2.25) with p trend = .01. The increased risk associated with meat is likely attributable to a reciprocal interrelationship between fruits and meat, common to nutrient analysis by food source as well as the independent risk of meat. Risk of laryngeal cancer differed by race with GSH being most protective for the Hispanic population and least for the Black population. Underlying genetic differences and other unadjusted factors may explain differential risk among the various race groups or this may simply be a chance finding. Finally, laryngeal cancer risk was not found to be significantly associated with any particular subsite. Further nutritional epidemiological studies will need to conducted and more studies will need to better assess to what extent dietary GSH is bioavailable in humans.

PRECIS

Topics about nutrition and health throughout my public health training have always interested me. I was initially intrigued by the many ecological migrant studies looking at the relationship between nutrition and cancer. For most cancers, populations migrating from an area with its own cancer incidence rates acquired the rates of the new location to which they migrated. These studies suggested genetic factors could not fully account for differences in incidence rates. Further, they suggested there were likely environmental factors strongly influencing risks of acquiring cancer. Many studies since then have discovered nutritional components, which modify risk of acquiring cancer.

Currently, there is much interest in antioxidants and their potential for modifying cancer risk. Glutathione, a tripeptide well known for its endogenous antioxidant properties, is found in many food items. It is not known whether exogenous intake of glutathione is associated with a decreased risk of cancer. Dr. Don Austin was generous enough to allow me to use primary data from a case-control study on laryngeal cancer risk factors. This study also had a food frequency questionnaire, which became the focus of my study.

I quickly learned the difficulties in doing a secondary analysis in nutritional epidemiology. Dr. Walter Willett stated, "Although epidemiology is logically equipped to address the dietary causes of disease, the complex nature of diet poses an unusually difficult challenge to this discipline." This was an understatement. What seemed like a relatively straight forward project, blossomed into a quite a few unanticipated problems. Perhaps this is the experience of every novice; perhaps this is the experience of every researcher.

This project began 5 years ago, prior to my matriculation into medical school. I started and stopped the project multiple times due to the demands of medical school. At times, I did not think I would finish this project. I am now grateful to have finished, as I have gained just enough insight into the workings of research to perhaps attempt this again someday.

INTRODUCTION

In the United States, cancers of the larynx account for approximately 1% of all new cancer diagnoses with over 90% classified as squamous cell carcinomas.[24] Detection of laryngeal carcinomas occurs relatively early because they alter phonatory and airway functions of the larynx (particularly at the more common glottic subsite).[24] Prognosis is relatively good with a 5-year survival rate of approximately 66%, though this has remained relatively unchanged for the past 25 years.[23]

The overall U.S. male incidence rate for laryngeal cancer is 8.2 per 100,000 and 1.7 per 100,000 for females--nearly a five-fold difference.[23] There are also peculiar differences in cancer subsite distribution. Males are typically diagnosed with more glottic tumors (64% glottic, 31% supraglottic) while females are diagnosed with more supraglottic tumors (54% supraglottic, 40% glottic).[23] Incidence rates are also 50% higher among black than among white males. White male incidence rates are twice as high when compared to Hispanic or Asian incidence rates in the United States.

To date, the predominant environmental risk factors established for laryngeal cancer are tobacco and alcohol use. Relative risks range from approximately 3 to 40 for tobacco use, and 1 to 30 for alcohol use.[1, 2, 23, 24] Risks vary by type and quantity of tobacco and alcohol. Dose-response relationships have also been demonstrated.[1, 2, 25] In addition, interactions between tobacco and alcohol seem to have a multiplicative effect on risk estimates.[21, 26]

An increasing body of literature suggests dietary factors may play a significant role in influencing laryngeal cancer risk. A common finding in many studies is a reduced risk associated with fruit and vegetable consumption.[1-5, 25] Fruits and vegetables contain various nutrients, which may individually or in combination, elicit a protective effect. Of particular interest are the antioxidants/anticarcinogens commonly found in fruits and vegetables. Carotenoids, vitamin C, and vitamin E, for example, have been shown to have anticarcinogenic effects for various cancers. [6-8]

Glutathione (GSH), a sulfhydryl tripeptide, is well established as an endogenous antioxidant and anticarcinogen through detoxification of reactive oxygen intermediates and toxic electrophilic metabolites of xenobiotics. [9,10] In other words, glutathione plays an important role as an antioxidant, protecting against cellular mutagens. GSH is present and synthesized in mammalian tissue, and also functions in cellular transportation, metabolism, and storage.[11]

Interest in dietary or orally administered GSH has risen over the years through the recognition that several disease states are associated with GSH deficiency. Theoretically, GSH could be given therapeutically to increase plasma levels of GSH thereby protecting against xenobiotic toxicity and oxidation. However, research in this area is both limited and inconclusive. Research using animal models provides ample evidence to suggest a plausible biological mechanism whereby GSH can be absorbed intact through the intestinal wall and subsequently increase plasma GSH levels. For example, animal studies demonstrate the intact absorption of exogenous GSH through the intestinal tract of rats. [13-15] In addition, animal studies have shown dietary GSH to increase plasma GSH levels by absorption through rat intestinal epithelial cells.[16-18] Furthermore, Trickler et al. demonstrated the first finding of orally administered glutathione's ability to inhibit oral carcinogenesis in the buccal pouches of hamsters.[19] However, intestinal γ-

glutamyltransferase and hepatic γ-glutamyltransferase both degrade dietary GSH and amino acid constituents such as cysteine and glutamate may be more important factors.[35]

Human research on orally administered GSH availability, however, is less clear. Hagen and Jones in a pilot study found a 2.5-fold increase in plasma GSH one to three hours after GSH administration. [27] However, Witschi et al. did not find an increase in plasma GSH levels, or their constituent amino acids, after orally administering the same dose of GSH as Hagen and Jones. [28] Flagg et al. found an inverse relationship between self-reported GSH intake and plasma levels of GSH in those individuals, though the results were not significant. [29] Human dietary GSH absorption and utilization remain unresolved.

To date only one epidemiological study has explored dietary GSH and cancer risk. Flagg et al. demonstrated a significant association between dietary glutathione and a reduced risk for oral/pharyngeal cancer .[20] However, lowered risk in this study was limited to raw vegetables and fruits rather than meat or cooked vegetables. Because a protective effect was limited to fruits and vegetables, GSH may be a surrogate for another protective nutrient or combination of nutrients.

Because there is so little human research on GSH, many questions remain unresolved. Because GSH is also synthesized endogenously, more needs to be understood about what portion of plasma GSH is under homeostatic control and what portion of dietary GSH is actually utilized. Flagg et al showed Vitamin C intake actually made the correlation between dietary GSH and plasma GSH more inversely associated.[29] Clearly dietary GSH availability is more complicated than a linear relationship where more dietary GSH necessarily results in a greater plasma concentration of GSH. Also as

Hagen and Jones study illustrated, the increase in plasma GSH as a result of dietary GSH was short-lived. [27] Unsustainable increases in plasma GSH may not be protective.

Despite the lack of data to clearly show GSH as a sustainable bioavailable exogenous nutrient, there were clearly studies suggesting GSH may be bioavailable, as mentioned above. Therefore, it would be useful to study the association between dietary glutathione and risk of laryngeal cancer. To my knowledge, this would be the first study to do so. To accomplish this, a secondary analysis of data from a large case-control study in Northern California will be done. This study investigated potential risk factors for laryngeal cancer with data on laryngeal cancer subsites, cigarette smoking, alcohol use, dietary intake, occupational risk factors, and various host factors (i.e. exposure to human papilloma virus). Significant findings included alcohol and smoking as risk factors independently and combined, with the latter resulting in a multiplicative risk. Also, method of food preparation was found to have differential risk. For example, raw and lightly cooked fruits and vegetables were more protective for laryngeal cancer than well cooked foods.[21]

Data from Austin and Reynolds [21] include many features which make a secondary analysis of laryngeal cancer and glutathione ideal. It is a large population-based case-control study with a large sample of cases and a control population that is representative of the cases. Data were also collected on subsite, which makes GSH analysis by subsite possible. Third, a comprehensive food frequency questionnaire will allow for the estimation of glutathione. Glutathione is fairly ubiquitous in the diet (particularly in fruits, vegetables and meat products) and a comprehensive FFQ is desirable in order to assure important sources of GSH are not excluded. Finally, important data on confounders such as alcohol and cigarette smoking were collected and can be adjusted for. Risk of laryngeal cancer will be assessed at various quartiles of reported GSH intake for various races, gender, subsites and different food sources.

The specific hypotheses to be tested are as follows:

1. Dietary GSH intake is hypothesized to be protective for laryngeal cancer. If true, a significant negative inverse association should be observed between total GSH intake and laryngeal cancer risk. Secondly, this effect should not vary by food source. Variation of risk by food source would suggest GSH is not itself protective.
2. Males acquire more glottic tumors than supraglottic tumors presumably due to greater intake of alcohol and tobacco than females. A sub-hypothesis of this study is GSH will be more protective for males and glottic subsite. If true, GSH may exhibit stronger inverse associations between males and laryngeal cancer risk than females. Likewise, stronger inverse associations between glottic subsite and laryngeal cancer risk would be expected if the hypothesis were true.

If GSH was found to be protective for laryngeal cancer, possible explanations might include: 1) Dietary GSH, absorbed whole and intact, could be responsible for the protective effect. 2) Intestinal γ-glutamyltransferase and hepatic γ-glutamyltransferase both degrade dietary GSH. Perhaps the amino acid constituents cysteine and glutamate are protective for laryngeal cancer rather than GSH itself. 3) Dietary GSH may be a surrogate for some other protective factor or synergistic effect of several dietary nutrients. 4) GSH may be a surrogate for broader protective behavioral factors.

If an association between dietary GSH intake and a reduced risk for laryngeal cancer can be demonstrated, there are significant public health implications. Changing dietary practices to include foods with more GSH may reduce laryngeal cancer risk and risk for other cancers. Also there is mounting commercial interest in GSH, especially as a dietary supplement. Dietary GSH or variations in the delivery of orally administered GSH may also potentially play a role in future therapeutic interventions.

METHODS

This population-based case-control study investigates the association between reported dietary glutathione intake and risk of laryngeal cancer. This was accomplished by conducting a secondary analysis using data collected for a population-based case control study of environment and laryngeal cancer, conducted in northern California from July 1988 through April 1993, supported by the California Tobacco-Related Disease Research Program, grant # 1 RT-0139.[21] The original study hypothesized that the greater prevalence of smoking in males and a greater consumption of alcohol might account for the predominance of glottic cancer in males. Secondly, it was hypothesized that raw and lightly cooked fruits and vegetables were more protective against laryngeal cancer than those well cooked. Data were collected on various potential risk factors including smoking, alcohol, diet, occupational exposures, host factors (human papilloma virus infection, etc.), subsite, and demographic data.

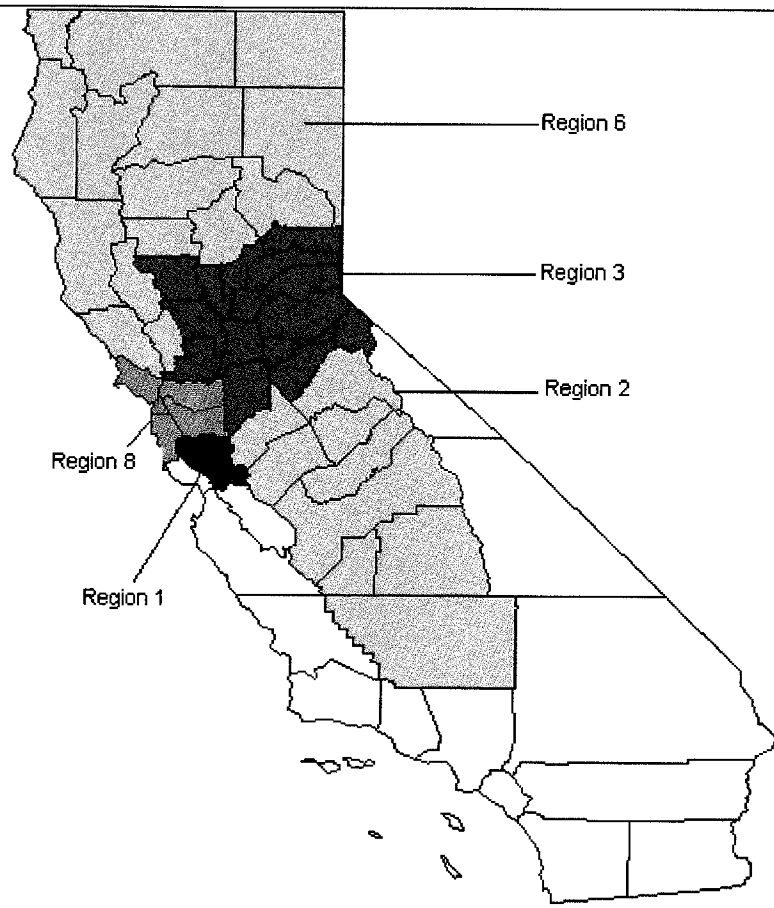
Population Sample

The study population spanned 44 counties in Northern California with a population of approximately 10.5 million residents across five cancer reporting regions (Figure 1): region 1 (only Santa Clara county), region 2 (all nine counties), region 3 (all thirteen counties), region 6 (all sixteen counties) and region 8 (all five counties).

Cases were identified from regional tumor registries and by rapid case ascertainment from July 1988 to April 1992. Cases selected were patients less than 75 years old with newly diagnosed laryngeal cancer, totaling 889 cases (679 males and 210 females) after excluding persons with previously unknown exclusion criteria. If cases were too ill or deceased at the time of the interview, proxy respondents next of kin were used. Thirty-nine percent of cases were interviewed by proxy. Cases were also identified by subsite and histological type of cancer.

Controls were acquired either through random digit dialing (Waksberg method[36]) or sampling by HCFA (Health Care Financing Administration) of Medicare files for those over the age of 64. A total of 1463 controls were analyzed (805 males and 631 females). Cases were frequency matched to controls by age, sex and race, but not by region. Control matching ratios were 1:1 for males and 3:1 for females.

Figure 1. Five Region Study Area, Northern California.



Region 1 (Santa Clara):	Santa Clara
Region 2 (Central Valley):	Fresno, Kern, Kings, Madera, Mariposa, Merced, Stanislaus, Tulare, Tuolumne
Region 3 (Sacramento):	Alpine, Amador, Yuba, Calveras, El Dorado, Nevada, Placer, Yolo, Sacramento, Sutter, Sierra, Solano, SanJoaquin
Region 6 (North):	Butte, Colusa, Del Norte, Glenn, Lake, Lassen, Humboldt, Mendocino, Siskiyou, Modoc, Napa, Plumas, Shasta, Sonoma, Tehama, Trinity
Region 8 (Bay Area):	Alameda, Contra Costa, Marin, San Francisco, San Mateo

Dietary Survey

The dietary portion of the questionnaire contained 92 questions (see Appendix B). Questions attempted to estimate the usual frequency of consumption of various beverages and food items by asking about the frequency and the number of times a food item was eaten during a given time period. For cases, the time frame was 2 years prior to their diagnosis. For the controls, the time frame was approximately the same time period.

The dietary history section (Section E) contained 71 questions in 5 major categories: fruits, vegetables, meat products, dairy products and grain products. Sixty-two questions were used for this study. The nine questions omitted from the study either did not contribute to GSH estimation or the question posed difficulties in GSH estimation (i.e. open ended questions). In addition to the type of food, preparation methods were ascertained, such as "prepared from raw or fresh frozen" or "prepared from canned".

The beverage history section (Section D) contained 21 questions, of which 6 were used. These included commonly consumed beverages such as milk and various fruit juices. The remaining 15 questions inquired about alcohol use. Though alcohol consumption was used to control for confounding in the analysis, alcohol was left out of the GSH calculations. Certain types of alcohol did contain GSH, and because alcohol was also a major risk factor, it was felt that adding GSH from alcohol into the GSH totals would not add any additional benefit in interpretation. Because alcohol is a well established risk factor, if GSH were protective, the two variables would be in opposition. At low alcohol doses, one would expect the GSH effect to predominate while at very high doses the elevated risk effect would predominate because there would be other accompanying deficiencies. If GSH were protective, the fact that alcohol is a risk factor at low to moderate alcohol use levels, would suggest that GSH in alcohol would not contribute a net protective effect. Therefore exclusion of GSH data from alcohol should not be problematic.

For each item on the dietary questionnaire, an attempt was made to approximate reported glutathione intake by assigning GSH values from an article by Jones et al, "Glutathione in Foods Listed in the National Cancer Institute's Health Habits and History Food Frequency Questionnaire"[12] (see Appendix B). This study provided the only published database for GSH in foods, using the 98 most commonly reported food items in the NHANES II (found to comprise 90% or more of calories, dietary fiber, and 18 major nutrients in the US diet). High-performance liquid chromatography techniques with correction methods for losses during sample preparation, were used in the GSH analysis.[37]

Because little is known about the bioavailability of GSH in foods, GSH content in foods prior to consumption was used to approximate GSH exposure in the study by Jones. This study also quantified two glutathione forms, reduced (GSH) and disulfide forms (GSSG). Experimental studies have shown reduced GSH to be the actual form absorbed[12]. However, reductive mechanisms in the small intestine can convert disulfide forms to reduced GSH (oxidized GSH or GSSG, disulfides of GSH, proteins and other thiol-containing compounds). Therefore, both reduced (GSH) and total dietary GSH ($GSH + GSSG = GSH_t$) will be used in this study.

Food and drink items in this study without corresponding GSH values from the Jones et al study, were assigned values of zero. In questions inquiring about multiple food items simultaneously, an average of items with available GSH data were used. For example, item E12 f (see Appendix A and corresponding item in Appendix B) asks about the frequency and interval "Canned luncheon meats, cold cuts, hot dogs, polish sausage and bologna" was consumed. The Jones DP et al article only reports GSH content for 1 of these items. Therefore the one value reported was the value used for all the items in this question (Please see Appendix B for all calculations).

Two questionnaire items warrant special attention. Question E-5 asked what items were eaten in salad (tomatoes, carrots, etc.) and how often (often, sometimes, or rarely). Because of the difficulty in quantifying the responses "often, sometimes and rarely" into actual GSH values, this part of question E-5 was eliminated from the analysis and only the number of times green salad was eaten was used in the analysis (E4). Question E-6 asked about vegetables eaten raw, outside of salad. This was an open-ended question allowing for 3 responses, but only 1 related question about number of times these items were eaten and with what frequency. All responses with corresponding food items from the Jones article were assigned values accordingly. Those open-ended responses without corresponding items in the Jones article (i.e. no corresponding GSH value) were simply counted as zero. An average was then taken of the 1-3 items reported and multiplied by the number of times/frequency the item was eaten. This variable was eliminated from the final analysis due to a large amount of missing data (no responses) and concern about misclassification.

Data Management:

The original data were stored as SAS-transport files. These were converted to tab-delimited files and initially imported into Microsoft Access due to the large number of variables. GSH values per serving size were obtained and were then multiplied by an interval and the number of times consumed during that interval to arrive at GSH consumed per day per food item. All food and beverage items were then summed to arrive at GSH totals per individual. Participants who refused to answer dietary questions, who had greater than one missing value, or had erroneous responses were excluded from the study. Erroneous responses included errors in coding, and improbable frequencies of consumption (i.e. 512 times/day). Of the original cases, 603/889 cases were available for analysis.

Next, conversion of 'interval','number of times' a food item was eaten, and GSH/GSH_t values were converted to GSH/GSH_t consumed per day per food item. Subtotals were then created for the various food categories such as vegetables, fruits, meats, grains, and dairy products. Grand totals for GSH/GSH_t per individual per day were finally calculated.

The totals and subtotals along with corresponding participant identification numbers were imported into SPSS version 10.1 for Windows statistical package. Individuals were divided into computer generated quartiles of GSH/GSH_t exposure. Other variables of interest were also imported from the original data set and those variables used in the multiple logistic regression model are listed in Table 1.

Table 1: Predictor Variables and Covariates in multiple logistic regression.

Predictor	Type
Education	Categorical
Race (white, black, Hispanic)	Categorical
Gender	Dichotomous
Age	Continuous
Region	Categorical
Polyps	Dichotomous
Smoking/Alcohol use	Categorical
Reduced GSH (GSH)	Categorical
Total GSH (GSH_t)	Categorical

Many of these variables were already formatted as categorical variables from the original study. For example, the combined smoking/alcohol categorical variable had 9 categories of exposure based on the distribution of the total study population. Categories ranged from low tobacco/low alcohol to high tobacco/high alcohol exposure. Tobacco and alcohol exposure were combined into a categorical variable with 9 levels of exposure from the original study and were also employed in this study. The alcohol and tobacco exposure variables resulted in nearly equal tertile divisions:

Table 2: Categorical variable of combined tobacco and alcohol exposure used logistic regression models.

	Low Alcohol 0-3 drinks/wk	**Moderate** Alcohol 3-20 drinks/wk	**High** Alcohol >20 drinks/wk
Low Tobacco (<0-10 packyears)	**1** Low Tobacco/ Low Alcohol	**2** Low Tobacco/ Moderate Alcohol	**3** Low Tobacco/ High Alcohol
Moderate Tobacco (10-50 packyears)	**4** Moderate Tobacco Low Alcohol	**5** Moderate Tobacco Moderate Alcohol	**6** Moderate Tobacco High Alcohol
High Tobacco (> 50 packyears)	**7** High Tobacco Low Alcohol	**8** High Tobacco Moderate Alcohol	**9** High Tobacco High Alcohol

Cigarette exposure was divided into low (none to less than 10 packyears), moderate (10 to 50 packyears) and high (greater than 50 packyears). Alcohol was divided into low (none to less than 3 alcoholic beverages per week) to moderate (3 to less than 20 alcoholic beverages per week) and high (greater than 20 alcoholic beverages per week). The 3 divisions of alcohol use and tobacco use resulted in nearly equal divisions (tertiles) for each variable.

Region was entered into the regression model because cases and controls were found to differ significantly with respect to region of residence in the primary analysis.[21] Specifically, region 6 was inadvertently oversampled in cases and controls. Therefore region of residence will be used as an adjusting variable. Case-control differences also existed in education, racial distribution, cigarette exposure, alcohol exposure, and history of laryngeal polyps and were included in logistic models.

Aside from adjusting for the well known risk factors alcohol and tobacco, there were many other potential confounders which unfortunately were not adjusted for due to unavailability, and questionable benefit to this analysis. Total caloric intake, amino acid precursor consumption (methionine and cystine), vitamins A, C and E, β-carotene and fiber all have reported associations and ideally could have been adjusted for. Methionine and cystine are precursors to endogenously produced GSH and adjusting for these may have helped to delineate the effects of exogenous GSH and endogenously produced GSH from the precursors. As suggested earlier, Vitamins A, C, E and carotenoids are well known for their antioxidant effects and they have been shown to have some protective effect. Fiber is protective for many types of cancers and may itself be protective or may be a surrogate for some other protective factor. However, adjusting for these additional nutrients may not provide any additional analytical benefit because nutritional analysis is always confounded by multicollineariety. Total caloric intake could not be assessed from this food frequency questionnaire.

Data Analysis:

Univariate analyses were performed on variables GSH and GSH_t as well as tests to assess the normality of GSH and GSH_t distribution in this population. Histograms/stem-leaf plots of GSH and GSH_t revealed normal bell-shaped distributions.

Associations between risk of laryngeal cancer and reported dietary intake of GSH and GSH_t were estimated by computing crude and adjusted odds ratios from logistic regression analysis using SPSS Version 10.1 for Windows. Individuals were divided into roughly equal quartiles of GSH and GSH_t and indicator variables were used to estimate risk for each quartile of GSH and GSH_t intake compared to the reference group with lowest quartile of GSH intake.

Variable selection for the logistic regression models was ultimately based on clinical relevance rather than selecting variables using Hosmer and Lemeshow criteria or using other variable selection methods (stepwise selection, etc).[38] Variables were entered into SPSS using the block method. The first block included covariates education, race, gender, age, region and polyps. Block 2 included the quartiles of GSH and GSH_t and block 3 included a combined categorical smoking and alcohol variable. This sequence was performed in order to test for the confounding effects of alcohol and smoking. All variables were categorical except for age, which was left as a continuous variable.

Tests for trend in the logistic regression coefficients across the quartiles were conducted using the polynomial contrast method. Ninety-five percent confidence intervals for odds ratios were calculated as part of the logistic regression model procedures.

Similar logistic regression models were created for analysis by food category. The block method was also used here and confidence intervals and trend were calculated in a similar manner. However, instead of using GSH and GSH_t totals for all foods, GSH and GSH_t subtotals were used for each food subcategory (i.e. meats, vegetables, fruits, etc).

The analysis was stratified by race using by entering GSH/GSH_t as an interaction term into the models. The expB from the interaction term was then multiplied by the GSH values from the same output to arrive at the odds ratios for the individual races. Confidence intervals were obtained by entering only the interaction terms into the model. Unfortunately, this method of obtaining odds ratios for substrata does not allow for computation of trend using SPSS. Logistic regression models for subsite were done in a similar fashion to that described above.

Odds ratios were adjusted for age, race, gender, education, region, polyps and tobacco and alcohol use. Potential confounding was assessed by calculating separate logistic regression models that included and excluded the adjustment term for alcohol/tobacco exposure. Possible bias from use of data from surrogate respondents was evaluated by excluding data from surrogate respondents and comparing this to data with surrogate respondents. The Hosmer-Lemeshow goodness of fit statistic was used to assess how well the logistic regression models fit the observed data.

RESULTS

Table 3 presents the distribution of demographic variables by case-control status. There were a total of 889 cases and 1436 controls available for analysis (679 males cases, 805 male controls, 210 females cases and 631 female controls). Females had a matching ratio of cases to controls of 1:3 while males had a matching ration of 1:1. Distribution of cases and controls by region were roughly similar among cases and controls, except in region 6 where controls were inadvertently sampled twice as often as needed. This was subsequently adjusted for in the regression modeling. Distribution of cases and controls by race was different with more representation of non-white race among the cases. Finally, education level varied by case-control status with controls tending to be more educated. Race and education were also adjusted for in the logistic analyses.

Tables 4 and 5 reveal the food items that comprised greater than 99% of reported intake of GSH/GSH_t in cases and controls, respectively. Nine of ten food items contributing the most to GSH intake were similar in cases and controls. These nine items were: hamburger, white potatoes, watermelon, orange juice, chicken/turkey, grapefruit, pork chops/ribs, bananas and asparagus. This accounted for roughly 70% of the cases' total GSH and 65% of the controls' total GSH. Overall, controls had more food items contributing to GSH totals than cases (38 vs. 31, respectively), suggesting controls had a more varied diet. Most of the controls' more variable diet was due to an increase in fruit consumption. Controls had approximately 29% of total GSH intake contributed by fruits. In contrast, cases had only 21% (Figure 2). Contribution of vegetables toward total GSH intake was similar between cases and controls (14% and 13% respectively). Meat products comprised approximately 36% of total GSH for cases and only 28% for controls.

Table 6 displays the distribution of cases and controls by quartile of GSH and GSH_t. Risk of laryngeal cancer was not related to GSH or GSH_t when adjusted for age, race, gender, education, region, polyps, tobacco and alcohol use. Total caloric intake, a likely significant confounder, was not adjusted for in this analysis. Risk of laryngeal cancer appeared to be most related to GSH_t intake among females (p trend .09) though not significant. Trend for GSH consumption showed similar decreasing odds ratios associated with increasing quartile in females, but again was not significant (p trend .23). The regression model fit well with p= .892 from the Hosmer-Lemeshow goodness-of-fit test.

Laryngeal cancer risk appeared to vary depending upon the food source (Tables 7 and 8). GSH and GSH_t from raw or fresh frozen fruits was strongly associated with a decreased risk for laryngeal cancer. Cooked or canned fruits were not associated with decreased risk of laryngeal cancer. GSH/GSH_t derived from vegetables raw or fresh frozen or cooked/canned preparations were not associated with a decreased risk. However, GSH/GSH_t derived from only salad were associated with a decreased risk of laryngeal cancer (p trend <.001). The contribution of salad to total GSH/GSH_t is small, likely explaining why the association disappears when combined with all vegetables. Finally, GSH/GSH_t derived from meat or meat products was associated with an increased risk of laryngeal cancer (Table 7, Table 8).

Table 9 presents the distribution of cases and controls by quartile of GSH stratified by race (analyses for GSH by race and subsite were done for both GSH and

GSH_t. Because of the high degree of correlation between GSH and GSH_t only results for GSH are described in the results. Estimated relative risks for virtually every analysis was nearly identical. Of probable significance is the association between GSH and reduced laryngeal cancer risk among Hispanics. There appears to be downward trending odds ratios in this group (p trend = .02). Other races were not found to have significant associations between GSH and laryngeal cancer risk.

Adjusted odds ratios for GSH quartiles by subsite are found in table 10. Data were further stratified by gender. Three subsites were analyzed: glottic, supraglottic and subglottic. No significant association was found between subsite and risk for laryngeal cancer. Females with glottic tumors were closest to being significant (p trend = .13). GSH_t values are not reported here because of the high correlation between GSH and GSH_t in all previous analyses.

No differences were found when comparing adjusted OR's with and without proxy respondents (OR's for quartile of GSH exposure without proxy respondents were 1 (reference), 1.18 (CI .79 – 1.77), .99 (.65 – 1.5), and .95 (.63 – 1.42) for quartiles 1 through 4 respectively. These values are similar to values found in table 6 which includes both proxy and non-proxy respondents.

DISCUSSION

This study found no association between dietary GSH intake and risk of laryngeal cancer. Special consideration must be given to this null assocation, especially in the context of nutritional epidemiology. Because of the limited range of variation in diet and inevitable errors in measuring intake, the estimated relative risks will be low. Therefore small errors due to methodological bias could lead to incorrect associations. There are many potential sources of error which make the interpretation of this null association limited. Selection bias is one such potential source of error. Controls were selected from the same geographic area cases and during the same time period. Therefore, the controls should be sampling the population from which the cases were derived and they should be representative of the individuals who would have been in the case group had they developed the disease of interest.

Another potential source of error is non-response bias. Because diet is significantly associated with the level of health consciousness, diets of those who participate are likely to differ from those who do not. This may have resulted in the inflated health of the control group and created a false association if non-response rates were different among cases and controls. However, the non-response rates were approximately equal at 70% in both cases and controls, making this an unlikely source of bias.

Recall bias is also a source of error which was minimized by asking participants to recall usual diet from the past. Although one's actual diet is probably best assessed by asking about past diet, there are strong influences of current diet on the recollection of past diet. This is of concern in case-control studies where the diets of cases, but not

Finally, biological complexity of nutrient interactions could compound error because the effect of one nutrient may depend on the level of another. These interactions could vary from study to study and may account for inconsistent findings from study to study.

In addition to a null association between GSH and laryngeal cancer, the effects of GSH varied by food source. An inverse association between dietary glutathione intake and risk of laryngeal cancer was found when derived from raw or fresh frozen fruits, (p trend <.001 for both GSH and GSH_t in Tables 7, 8) while glutathione derived from meats was a significant risk factor (p trend = .01 for GSH and < .002 for GSH_t). This is further evidence that GSH is not associated with laryngeal cancer risk. Unfortunately, a drawback to looking at GSH by food groups is the tendency for reciprocal interrelationships to emerge. In other words, fruit eaters tend not to be meat eaters and vice versa. A classic example of a reciprocal interrelationship is that potato chips are inversely associated with blood carotene levels. This is not because potato chips cause any direct decrease in carotene levels, but because potato chip eaters tend to avoid vegetables and fruits. Therefore, potato chips become associated with an adverse outcome when "in fact" it is really the absence of a protective factor that is responsible.

To test whether a reciprocal interrelationship existed between fruits and meats, a pearson's correlation was performed and found the two were not correlated (.007 with two-tailed sig .75). This suggested that the observed risk factor of meat consumption may not be solely explained by a reciprocal interrelationship. Meat consumption may be an independent risk factor for laryngeal cancer as supported by the literature.[39] The protective effect of GSH derived from fruits is likely representative of the general protective effect of fruits for laryngeal cancer. This is supported by literature which suggests fruits [1-5, 25] are protective for laryngeal cancer. Flagg et al. similarly found a protective effect of glutathione derived from fruits consumed raw, though the association with fruits diminished after controlling for fruit fiber. Another possibility is that GSH from raw fruits and vegetables may be a surrogate for some other factor or combination of factors in fruits and vegetables which is protective. There are many protective micronutrients reported in the literature: carotenoids, vitamins C and A have all been reported as having protective effects.[34] Unfortunately, the effects of individual nutrients would be difficult to discern due to the confounding by multicollinearity.

Other limitations of the study which could have contributed to a significant source of error was the lack of validation. Unfortunately, the FFQ used was not validated for the purpose of micronutrient analysis. Validity is an important concept in nutritional epidemiology where the accuracy of measurement is compared to other techniques of dietary assessment. The use of validated instruments becomes important when looking at nutritional epidemiology data where the dietary effects anticipated are usually small. Even small sources of error can create results that can be uninterpretable. Because there is no 'true' external reference measure for dietary instruments, FFQ's are often compared to other methods such as diet records. However, this becomes a difficult task in epidemiological studies where the main purpose is to study the relationship of disease to diet from the past. In other words, the validating instrument would need to measure dietary intake at the same time the FFQ asks about dietary intake. This leaves many investigators faced with doing studies without validation. Interpretation from this study therefore must remain guarded. Though the FFQ from this study was not formerly validated, consultations were obtained such that this FFQ would mimic other validated FFQs.

One important source of potential confounding was not adequately controlled for and could have either diminished or created an association. Total energy intake, unfortunately, was not controlled for in the analysis because it was not available with the FFQ data. Controlling for total energy intake is important for 3 reasons:[30] 1) The level of energy intake may be a primary determinant of disease 2) Individual differences in total energy intake produce variation in intake of specific nutrients unrelated to dietary composition as the consumption of most nutrients is positively correlated with total energy intake. This added variation may be extraneous, and thus a source of error, in many analyses. 3) When energy intake is associated with disease but is not a direct cause, the effects of specific nutrients may be distorted, that is confounded, by total energy intake. The effect of not having adjusted for energy intake would vary by the relationship between energy intake, GSH and laryngeal cancer. One reason for adjusting is to reduce extraneous variation caused by differences in body size and energy expenditure. More importantly, if total caloric intake were associated with risk for laryngeal cancer, then a potentially important confounder would not have been adjusted for. The effect of confounding is difficult to predict without knowing its relationship to the laryngeal cancer. Flagg et al. implicated total caloric intake as one of two principal confounders in their study of GSH and oropharyngeal cancers.[20] They found that adjusting for total caloric intake changed the association between GHS and oral cancer to an inverse association after adjusting for total caloric intake. If the same effect were to hold true for this data set, the association would be accentuated rather than diminished. Further, adjusting for caloric intake would have created less extraneous error.

This study found no association between glutathione and risk of laryngeal cancer when GSH was derived from "vegetables prepared from raw/fresh frozen" (which includes cooked vegetables). However, when salad was analyzed alone, an inverse association was found between GSH derived from salad and laryngeal cancer (p trend < .001). The most likely explanation for the lack of association when salad was combined with "vegetables prepared from raw/fresh frozen" is due to the relatively small contribution of salad to the total vegetables. Essentially the effect was diluted when combined with "vegetables prepared from raw/fresh frozen". This inverse association is consistent with current literature which suggests vegetables consumed raw are protective.[1-5, 25] Cooking method may also influence risk with lightly cooked vegetables being more protective than well-cooked vegetables.[21]

GSH derived from other food sources were not found to be protective. Interpretation must remain guarded with respect to the various food categories because the quartiles of GSH exposure varied widely from one food source to another. For example, category "cooked/canned vegetables" largest quartile is only up to 6.3 mg GSH/day while the smallest quartile for raw or fresh frozen vegetables is up to 24.4 mg GSH/day. The food categories with small ranges for GSH may have had too little variation to detect any trend.

Other findings from this study include an inverse association between dietary GSH intake and risk of laryngeal cancer in the Hispanic group. As quartile of GSH exposure increases risk of laryngeal cancer decreases (p trend = .02). This finding is consistent with the literature on US incidence rates among Hispanics, which appear to be among the lowest when compared to other races.

The association between GSH and laryngeal cancer risk did appear to vary by race, which suggests other risk factors for laryngeal cancer that were not accounted for. Though speculative, Hispanic populations may have a very different diet than other populations even though GSH exposure may have been the same. GSH is a fairly ubiquitous micronutrient and depending on the composition of the diet, one might arrive at the same GSH total with very different food items. Hispanic populations may have a diet, for example, which is composed of more raw fruits and vegetables and black populations could have more meat as commonly reported in the literature. This in part could be contributing to the differences in odds ratios. There is always the possibility of genetic differences or structural anatomical differences in the larynx between races that in part account for these differences. Finally, it is also possible that these findings are simply due to chance, especially with the degree of misclassification in this study.

No inverse association was discovered between GSH exposure and laryngeal cancer by subsite (Table 10). It was originally hypothesized that if GSH was more protective for individuals with glottic tumors that supraglottic tumors (due to the antioxidant effects of GSH on epithelial cells of glottic tumors and not the columnar epithelium of supraglottic tumors), than GSH may have more of a protective effect in individuals with glottic tumors than supraglottic tumors.

The data did not support this hypothesis as none of the odds ratio trends were statistically significant. The only subgroup coming close to significance was the female glottic subsite group (p trend = .13). This finding is difficult to interpret considering the trend in males is not quite as prominent. This could reflect a female diet with GSH coming from more fruits and vegetables, while the male diet may have more GSH from meat products. Females for the supraglottic and subglottic subsites did not exhibit the same degree of trend as did the females in the glottic subsite. This could be consistent with the hypothesis that raw fruit and vegetable consumption is more protective for the glottic subsite than the other subsites. However, interpretation should be guarded here, as none of the odds ratio trends were significant.

The finding that glutathione derived from raw fruits and salad was protective for laryngeal cancer lends support to literature suggesting there are protective effects from raw fruits and vegetables per se. This may be a surrogate for some other nutrient or combination of nutrients or this may be a surrogate for behavioral factors, which are not accounted for. Antioxidants are proposed in many studies to be protective against squamous cell cancers of the head, neck and respiratory tract.[7] Glutathione is a well known endogenous antioxidant in humans. Exogenous GSH obtained from the diet may be protective for laryngeal cancer as research in the literature suggests this is biologically plausible. Animal studies in rats, for example, have shown dietary GSH increases plasma GSH levels. Human studies also have shown orally administered GSH to increase plasma levels of GSH 1 hour after administration. However, Witschi et al did not find an increase in plasma levels after administering the same doses as Hagen and Jones.[28] Further it is not clear if these plasma levels are sustainable and to what degree they contribute to epithelial cell GSH levels where the actual protective effect is thought to exist. There are studies, however, which show exogenous GSH is absorbed by epithelial cells both in intestinal cells and alveolar type II cells.[7]

In conclusion, this study found no association between GSH and laryngeal cancer risk. Lack of causality is supported by the effect of GSH varying by food source. An inverse association between GSH from raw fruits and vegetables and risk of laryngeal cancer was found. This is likely because fruits are known to be protective for laryngeal cancer or perhaps fruits are a surrogate for some broader behavioral aspect uncontrolled for. Even though there was no demonstrated statistical relationship, it is possible that part of the increased risk with meat consumption is due to a reciprocal relationship. However, an inverse correlation did not exist between fruits and vegetables suggesting meat may be an independent risk factor for laryngeal cancer.

Because this is only the first study to look at GSH and laryngeal cancer specifically, more epidemiological studies need to be conducted as the strength of case-control studies comes from multiple studies. Further studies are required in multiple areas to better understand the effects of exogenous GSH intake. More studies need to address whether orally administered GSH is absorbed intact by human intestinal cells, whether it results in sustainable increases in plasma and uptake by epithelial cells and how it is metabolized.

Table 3: Distribution of Variables by Case-Control Status

Category		Cases	Controls
Total		889	1436
Males		679 (45.8%)	805 (54.2%)
Females		210 (25.0%)	631 (75.0%)
Region Distribution:			
Males	Region 2:	105 (15.5%)	131 (16.3%)
	Region 3:	192 (28.3%)	194 (24.1%)
	Region 6:	74 (10.9%)	158 (19.6%)
	Region 1,8:	308 (45.4%)	322 (40.0%)
Females	Region 2:	27 (12.9%)	120 (19.0%)
	Region 3:	67 (31.9%)	136 (21.6%)
	Region 6:	17 (8.1%)	103 (16.3%)
	Region 1,8:	99 (47.1%)	272 (43.1%)
Race			
Males	White non-Hispanic	82.2%	84.7%
	African American	7.4%	5.0%
	Hispanic	6.3%	4.1%
	Other	3.7%	6.1%
	(Missing)	0.4%	0.1%
Females	White non-Hispanic	82.2%	85.4%
	African American	7.6%	4.4%
	Hispanic	2.9%	5.4%
	Other	3.8%	4.4%
	(Missing)	0.5%	0.3%
Education			
Males	< 12 years	27.4%	11.4%
	12 years	28.3%	17.8%
	Post HS and Some College	26.9%	33.8%
	College Grad and Post Grad	17.4%	37.0%
	Missing	0.0%	0.0%
Females	<12 years	28.1%	13.3%
	12 years	35.7%	24.3%
	Post HS and Some College	25.7%	39.5%
	College Grad and Post Grad	10.5%	22.9%
	Missing	0.0%	0.2%

TABLE 4. Daily contribution of foods to reported dietary intake of total glutathione (GSHt) and reduced glutathione (GSH) among cases in a laryngeal cancer case-control study in Northern California.

		Cases	
Food	n[a]	GSH%*[b]	GSHt%*[c]
1. Hamburger, steak	855	19.95	17.67
2. Cooked, white potatoes	848	15.50	14.41
3. Watermelon	831	8.23	7.52
4. Orange juice	855	6.52	6.77
5. Chicken, turkey	852	5.76	6.14
6. Grapefruit, orange	829	5.53	5.02
7. Pork-chops/ribs	844	5.33	4.62
8. Bananas	832	4.21	3.57
9. Pasta with tomato sauce	851	2.77	2.30
10. Cooked asparagus (R/F)[d]	836	2.61	2.37
11. Bacon, pork, sausage or ham	845	2.13	2.54
12. Apples or pears (R/F)	833	1.89	2.23
13. Cooked summer squash/zucchini (R/F)	839	1.77	0.90
14. Salad, green	855	1.63	2.70
15. Raw vegetables (carrots, celery, broccoli, etc)	762	1.49	1.52
16. Cooked carrots (R/F)	841	1.39	1.17
17. Luncheon meats, polish sausage	844	1.22	2.20
18. Cooked broccoli, cauliflower, brussel sprouts (R/F)	842	1.19	1.50
19. Fresh or frozen fish	845	1.11	0.92
20. Tomato/V-8 juice	844	1.08	0.93
21. Hot cereal or grits	837	1.06	1.15
22. Cooked okra or artichoke (R/F)	836	1.06	0.78
23. Peaches, plums, nectarines, apricots, cherries	830	0.93	0.95
24. Liver, including chicken liver	835	0.84	0.81
25. Cooked pumpkin, winter, butternut, acorn squash	830	0.79	0.58
26. Cooked peas (R/F)	838	0.77	0.96
27. Cooked peas (Canned)	826	0.73	0.55
28. Cooked rice	845	0.71	1.00
29. Cooked spinach or other greens (R/F)	836	0.70	0.69
30. Strawberries or blueberries (R/F)	836	0.63	0.65
31. Cooked onions (R/F)	838	0.43	4.02
Cumulative	**603**	**99.97**	**99.17**

* Percentages reflect the mean of the GSH/GSHt individual sums for each food item (grams), divided by the mean of the grand total GSH/GSHt individual sums. Each percentage approximates the contribution of each food item's GSH/GSHt to the overall GSH/GSHt for the entire population.

[a] The number of subjects per food item varied because of missing data or errors. Data may be missing because of refusal to answer questions or when answers to questions were unknown. Errors excluded were food quantities considered inappropriate and coding errors.

[b] GSH represents reduced forms of glutathione in food items.

[c] GSHt represents both reduced and oxidized forms of glutathione (total GSH) in food items.

[d] Denotes food item was prepared from a raw or fresh frozen source.

TABLE 5. Daily contribution of foods to reported dietary intake of total glutathione (GSHt) and reduced glutathione (GSH) among controls in a laryngeal cancer case-control study in Northern California.

Food	n [a]	Controls	
		GSH% [*b]	GSHt% [*c]
1.Hamburger, steak	1428	13.61	11.96
2.Cooked, white potatoes	1430	12.43	11.47
3.Watermelon	1425	8.11	7.36
4.Orange juice	1433	7.90	8.14
5.Grapefruit, orange	1425	7.52	6.78
6.Chicken, turkey	1428	6.84	7.22
7.Bananas	1427	5.10	4.30
8.Pork-chops/ribs	1428	3.38	2.90
9.Apples or pears (R/F) [d]	1425	3.34	3.91
10.Cooked asparagus (R/F)	1428	3.06	2.72
11.Pasta with tomato sauce	1431	2.45	2.02
12.Raw vegetables (carrots, celery, broccoli, etc)	1433	2.44	2.44
13.Cooked summer squash/zucchini (R/F)	1430	2.34	1.18
14.Salad, green	1432	2.00	3.30
15.Peaches, nectarines, cherries (R/F)	1423	1.56	1.59
16.Cooked Carrots (R/F)	1432	1.52	1.27
17.Cooked broccoli, cauliflower, brussel sprouts (R/F)	1430	1.51	1.88
18.Bacon, pork, sausage or ham	1428	1.32	1.55
19.Cooked Okra or artichoke	1431	1.19	0.87
20.Fresh or frozen fish	1428	1.15	0.94
21.Hot cereal or grits	1428	1.10	1.18
22.Strawberries or blueberries (R/F)	1428	1.04	1.07
23.Cooked pumpkin, winter, butternut, acorn squash	1431	0.97	0.71
24.Luncheon meats, polish sausage	1428	0.87	1.56
25.Cooked peas (R/F)	1429	0.86	1.07
26.Grapes (R/F)	1428	0.76	0.57
27.Tomato/V-8 juice	1432	0.70	0.60
28.Cooked spinach or other greens (R/F)	1429	0.69	0.68
29.Cooked rice	1431	0.69	0.95
30.Cooked peas (canned)	1427	0.53	0.40
31.Liver, including chicken liver	1428	0.52	0.49
32.Mango, papaya (R/F)	1424	0.42	0.36
33.Cooked onions (R/F)	1430	0.41	3.76
34.Pinapple (canned)	1422	0.39	0.33
35.Stew/chili made w/beef/chicken or beans	1428	0.32	0.23
36.Peaches, apricots, (canned)	1422	0.32	0.36
37.Cooked beets (canned)	1431	0.10	0.56
38.Whole grain breads	1431	0.00	0.47
Cumulative	**1331**	**99.14**	**99.15**

[*] Percentages reflect the mean of the GSH/GSHt individual sums for each food item (grams), divided by the mean of the grand total GSH/GSHt individual sums. Each percentage approximates the contribution of each food item's GSH/GSHt to the overall GSH/GSHt for the entire population.

[a] The number of subjects (n) per food item varied because of missing data or errors. Data may be missing because of refusal to answer questions or answers to questions were unknown. Errors included were food quantities considered abnormally high or inappropriate and coding errors.

[b] GSH represents reduced forms of glutathione in food items.

[c] GSHt represents both reduced and oxidized forms of glutathione (total GSH) in food items.

[d] Denotes food item was prepared from a raw or fresh frozen source.

Figure 2: Percentage of Food Categories Contribution to Total GSH Among Cases and Controls

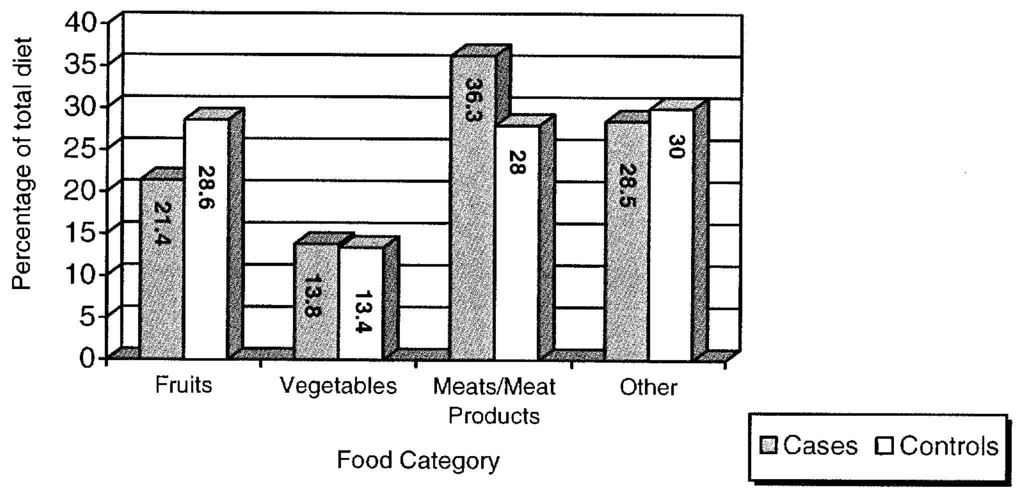


TABLE 6. Crude and adjusted OR's for GSH and GSHt.[a]

Quartile	n total	cases	controls	Crude OR	Adjusted OR	95% CI
GSHt						
All Subjects	1799	517	1282			
1 (0 – 35.71)[b]	447	140	207	1	1	-
2 (37.72 – 44.80)	448	140	308	0.98	1.13	(.79 – 1.62)
3 (44.81 – 56.02)	456	117	339	0.69	0.93	(.65 – 1.33)
4 (56.03 – 202.0)	448	120	328	0.75	0.89	(.62 – 1.27)
p trend				0.01	0.33	
Male[c]	1126	399	727			
1 (low)	300	104	196	1	1	-
2	270	103	167	1.16	1.25	(.82 – 1.92)
3	273	96	177	0.87	1.22	(.80 – 1.87)
4 (high)	283	96	187	0.89	1	(.65 – 1.53)
p trend				-	0.97	
Female	673	118	555			
1 (low)	147	36	111	1	1	-
2	178	37	141	0.80	0.90	(.46 – 1.77)
3	183	21	162	0.39	0.48	(.23 – 0.97)
4 (high)	165	24	141	0.46	0.64	(.31 – 1.31)
p trend				-	0.09	
GSH						
All Subjects	1799	517	1282			
1 (0 – 24.37)	448	138	310	1	1	-
2 (24.38 – 30.69)	449	141	308	1.04	1.13	(.79 – 1.61)
3 (30.69 – 38.42)	453	117	336	0.73	0.95	(.66 – 1.37)
4 (38.43 – 146.0)	449	121	328	0.78	0.88	(.61 – 1.26)
p trend				0.01	0.33	
Male	1126	399	727			
1 (low)	296	105	191	1	1	-
2	264	102	162	1.16	1.20	(.78 – 1.85)
3	280	95	185	0.87	1.09	(.71 – 1.67)
4 (high)	286	97	189	0.89	0.95	(.62 – 1.45)
p trend				-	0.72	
Female	673	118	555			
1 (low)	152	33	119	1	1	-
2	185	39	146	0.93	0.97	(.50 – 1.88)
3	173	22	151	0.50	0.73	(.35 – 1.51)
4 (high)	163	24	139	0.56	0.69	(.34 – 1.43)
p trend				-	0.23	

[a] All quartile units are mg/day.
[b] OR's are adjusted for age, race, gender, education, region, polyps, tobacco and alcohol use.
[c] Male and female OR's for both GSH and GSHt are adjusted for age, race, education, region, polyps, tobacco and alcohol use.

TABLE 7. Adjusted OR's for GSHt from specific food sources.[a]

GSHt Quartile	n	Adjusted OR	(95% CI)
Raw or fresh frozen fruits	1946		
1 (0 – 4.59)[b]	467	1	-
2 (4.59 – 8.77)	494	0.69	(0.50 – 0.96)
3 (8.78 – 15.08)	495	0.75	(0.53 – 1.05)
4 (15.08 – 176)	490	0.44	(0.31 – 0.63)
p trend		<.001	
Cooked/canned fruits	1968		
1 (0 – .014)	515	1	-
2 (.014 – .158)	479	0.81	(0.58 – 1.13)
3 (.158 – .411)	485	0.76	(0.54 – 1.06)
4 (.412 – 7.7)	489	0.89	(0.63 – 1.25)
p trend		0.45	
Meat/Meat products	1966		
1 (0 – 9.8)	503	1	-
2 (9.8 – 13.71)	504	0.93	(0.65 – 1.34)
3 (13.71 – 18.17)	482	1.34	(0.94 – 1.89)
4 (18.17 – 112)	477	1.56	(1.11 – 2.20)
p trend		.002	
Raw or fresh frozen vegetables[c]	1913		
1 (0 – 35.71)	482	1	-
2 (35.72 – 44.8)	488	0.96	(0.67 – 1.36)
3 (44.8 – 56.02)	474	1.12	(0.79 – 1.59)
4 (56.02 – 202)	469	1.05	(0.74 – 1.49)
p trend		0.58	
Cooked/Canned Vegetables	1936		
1 (0 – .092)	425	1	-
2 (.092 – .38)	507	0.74	(0.51 – 1.06)
3 (.38 – .936)	507	0.88	(0.62 – 1.26)
4 (.936 – 10.2)	497	0.96	(0.68 – 1.37)
p trend		0.92	
Grains	1956		
1 (0 – 1.12)	490	1	-
2 (1.12 – 1.85)	485	0.79	(0.56 – 1.10)
3 (1.85 – 2.88)	497	0.82	(0.58 – 1.15)
4 (2.88 – 21.7)	484	0.95	(0.67 – 1.35)
p trend		0.83	

[a] OR's are adjusted for age, race, gender, education, region, polyps, tobacco and alcohol use.
[b] All quartiles represent milligrams GSHt/day.
[c] Raw or fresh frozen vegetables does not include item, 'vegetables outside of salad'.

TABLE 8. Adjusted OR's for GSH from specific food sources. [a]

GSH Quartile	n	Adjusted OR	(95% CI)
Raw or fresh frozen fruits	1946		
1 (0 – 3.41) [b]	470	1	-
2 (3.41 – 6.47)	494	0.71	(0.51 – 0.99)
3 (6.47 – 11.16)	493	0.76	(0.54 – 1.06)
4 (11.16 – 133)	489	0.47	(0.33 – 0.67)
p trend		<0.001	
Cooked/canned fruits	1968		
1 (0 – .01)	515	1	-
2 (.01 – .10)	467	0.82	(0.59 – 1.16)
3 (.10 – .285)	492	0.74	(0.53 – 1.04)
4 (.286 – 5.8)	494	0.89	(0.63 – 1.25)
p trend		0.90	
Meat/Meat products	1966		
1 (0 – 6.9)	503	1	-
2 (6.9 – 9.81)	501	0.83	(0.58 – 1.19)
3 (9.81 – 13.12)	488	1.34	(0.95 – 1.89)
4 (13.13 – 85)	474	1.59	(1.13 – 2.25)
p trend		0.01	
Raw or fresh frozen vegetables [c]	1913		
1 (0 – 24.37)	474	1	-
2 (24.38 – 30.69)	490	1.07	(0.76 – 1.52)
3 (30.69 – 38.42)	475	1.11	(0.78 – 1.58)
4 (38.43 – 146)	474	1.22	(0.86 – 1.73)
p trend		0.28	
Cooked/Canned Vegetables	1936		
1 (0 – .027)	428	1	-
2 (.027 – .151)	505	0.70	(0.48 – 1.01)
3 (.151 – .446)	506	1	(0.70 – 1.42)
4 (.446 – 6.3)	497	0.94	(0.66 – 1.33)
p trend		0.79	
Grains	1956		
1 (0 – .636)	489	1	-
2 (.636 – 1.2)	485	0.83	(0.59 – 1.17)
3 (1.2 – 1.79)	500	0.75	(0.53 – 1.07)
4 (1.79 – 13.75)	482	1.04	(0.74 – 1.48)
p trend		0.96	

[a] OR's are adjusted for age, race, gender, education, region, polyps, tobacco and alcohol use.
[b] All quartiles represent milligrams GSH/day.
[c] Raw or fresh frozen vegetables does not include item, 'vegetables outside of salad'.

TABLE 9. Adjusted OR's for GSH by race.*

GSH Quartile	n	Case	Control	Adjusted OR	(95% CI)
White, non-Hispanic	1541	440	1101		
1 (low)	375	115	260	1	-
2	387	118	269	1.08	(0.72 – 1.60)
3	399	102	297	0.91	(0.61 – 1.36)
4 (high)	380	105	275	0.92	(0.62 – 1.36)
p trend				0.51	
Black, non-Hispanic	93	32	61		
1 (low)	35	13	22	1	-
2	17	7	10	1.64	(0.28 – 9.7)
3	17	4	13	0.85	(0.14 – 5.3)
4 (high)	24	8	16	1.06	(0.24 – 4.7)
p trend				0.85	
Hispanic	83	28	55		
1 (low)	17	7	10	1	-
2	25	11	14	1.03	(0.16 – 6.51)
3	17	6	11	0.74	(0.09 – 5.87)
4 (high)	24	4	20	0.04	(0.001 – .57)
p trend				0.02	
Other	82	17	65		
1 (low)	21	3	18	1	-
2	20	5	15	1.42	(0.17 – 11.9)
3	20	5	15	1.54	(0.18 – 12.8)
4 (high)	21	4	17	0.46	(0.05 – 4.64)
p trend				0.54	

* OR's are adjusted for age, gender, education, region, polyps, tobacco and alcohol use. Only GSH and not GSHt is reported here due to the high degree of correlation between GSH and GSHt.

TABLE 10. Adjusted OR's for GSH by subsite.*

GSH Quartile	n	Case	Control	Adjusted OR	(95% CI)
Site 1, Glottic					
All Subjects	1589	307	1282		
1 (low)	393	83	310	1	-
2	391	83	308	1.13	(0.75 – 1.73)
3	407	71	336	0.97	(0.63 – 1.48)
4 (high)	398	70	328	0.81	(0.53 – 1.24)
p trend				0.25	
Male [a]	991	264	727		
1 (low)	263	72	191	1	-
2	226	64	162	1.03	(0.64 – 1.67)
3	250	65	185	1.13	(0.70 – 1.81)
4	252	63	189	0.86	(0.53 – 1.78)
p trend				0.63	
Female [a]	598	43	555		
1 (low)	130	11	119	1	-
2	165	19	146	1.68	(0.66 – 4.24)
3	157	6	151	0.60	(0.19 – 1.91)
4	146	7	139	0.56	(0.18 – 1.74)
p trend				0.13	
Site 2, Supraglottic					
All Subjects	1446	164	1282		
1 (low)	351	41	310	1	-
2	354	46	308	1.13	(0.66 – 1.93)
3	375	39	336	1.17	(0.67 – 2.05)
4 (high)	366	38	328	1.04	(0.60 – 1.80)
p trend				0.87	
Male	828	101	727		
1 (low)	214	23	191	1	-
2	191	29	162	1.77	(0.86 – 3.64)
3	209	24	185	1.40	(0.66 – 2.97)
4	214	25	189	1.43	(0.68 – 3.00)
p trend				0.48	
Female	618	63	555		
1 (low)	137	18	119	1	-
62	163	17	146	0.72	(0.30 – 1.76)
3	166	15	151	1.23	(0.50 – 3.06)
4	152	13	139	0.72	(0.72 – 1.84)
p trend				0.77	
Site 3, Subglottic					
All Subjects	1328	46	1282		
1 (low)	324	14	310	1	-
2	320	12	308	0.83	(0.34 – 2.01)
3	343	7	336	0.55	(0.20 – 1.51)
4 (high)	341	13	328	0.90	(0.38 – 2.12)
p trend				0.60	
Male	761	34	727		
1 (low)	201	10	191	1	-
2	171	9	162	1.18	(0.40 – 3.43)
3	191	6	185	0.62	(0.18 – 2.09)
4	198	9	189	0.92	(0.31 – 2.69)
p trend				0.61	
Female	567	12	555		
1 (low)	123	4	119	1	-
2	149	3	146	0.33	(0.04 – 2.85)
3	152	1	151	0.18	(0.01 – 2.96)
4	143	4	139	1.34	(0.21 – 8.49)
p trend				0.93	

*OR's are adjusted for age, race, gender, education, region, polyps, tobacco and alcohol use.
[a] Male/Female OR's are adjusted for age, race, education, region, polyps, tobacco and alcohol use.

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APPENDIX A

Excerpt from Study of Environment and Laryngeal Cancer Survey used for this study, Sections D and E. Italicized portions were not used in this study.

SECTION D

BEVERAGE HISTORY

I'd like to ask you about the foods and beverages (you/your ______) ate or drank during (your/his/her) adult life. I am interested in (your/his/her) usual diet as an adult. If (your/your ______'s) changed (your/his/her) diet over the last year or two, only tell me about (your/your _______'s) usual patterns over the adult years.

First I will be asking about beverages. As I ask about each one, please tell me the usual amounts consumed per day, week, month, or year, whichever is easiest for you to answer. Although I would like you to be as accurate as possible, if you don't remember exactly please give me your best guess as that information would be better than no information at all.

D-1. Thinking of a serving as an ordinary 8-oz. water glass, over most of (your, your _____'s) adult life before two years ago, how many times per day, week, month, or year did (you/your _________) usually drink a glass, or serving of (LIQUID)?

a. Milk........................

	DAY..............1
__________	WEEK............2
TIMES	MONTH..........3
	YEAR............4
NONE.............................00	

b. Orange Juice.............

	DAY..............1
__________	WEEK............2
TIMES	MONTH..........3
	YEAR............4
NONE.............................00	

c. Grapefruit Juice..........

	DAY..............1
__________	WEEK............2
TIMES	MONTH..........3
	YEAR............4
NONE.............................00	

d. Tomato or V-8 juice.....

		DAY..............1
________ TIMES		WEEK............2
		MONTH..........3
		YEAR.............4
NONE...............................00		

e. Other fruit juices, such as apple juice..............

		DAY..............1
________ TIMES		WEEK............2
		MONTH..........3
		YEAR.............4
NONE...............................00		

f. Fruit drinks such as Hi-C, Tang, or Hawaiian Punch

		DAY..............1
________ TIMES		WEEK............2
		MONTH..........3
		YEAR.............4
NONE...............................00		

SECTION E

DIET HISTORY

Now I will ask about foods (you, your_____) might have eaten during the time period from 1984 through 1998. I will ask separately about those foods that may be prepared or eaten in several different ways, such as raw, cooked from fresh or fresh frozen, prepared from canned or preserved foods, or prepared from dried foods. Please give me the usual eating pattern during that period, for each preparation method asked.

Before asking about the first food, I would like to ask about cooked vegetables prepared from fresh or fresh frozen vegetables and the usual cooking style for preparing those vegetables.

E-1. When eating cooked vegetables that are prepared from fresh or frozen vegetables, not canned, during the period from 1984 through 1988, were (your/your______'s) vegetables usually:

a. very well cooked and soft............................. .1
b. moderately cooked, or....................................2
c. very lightly cooked and somewhat firm............3
d. DK..8

E2. During that period, how many servings of vegetables did (you/your_______) eat per day, week or month, not counting green salad or potatoes? _______ per _______
d/w/m

0 = never (SKIP to E-4)

E-3. Thinking of all the vegetables (you/your _________) ate during 1984 through 1988, how frequently did (you/your __________) eat canned vegetables? By that I mean, how many servings of canned vegetables did (you/your __________) eat per day, week or month? _______ per _______
d/w/m

0 = never

E-4. During 1984 through 1988, how often did you eat green salad? How many servings per day or week or month? _______ per _______
d/w/m

0 = never (SKIP to E-6)

E-5. When (you/your_______) ate green salad during that period, how often did (you/your________) have the following raw vegetables in it? Tell me "often", "sometimes", or "rarely".

a. Lettuce	___ o	___ s	___ r	g. Green peppers	___ o	___ s	___ r
b. Spinach	___ o	___ s	___ r	h. Red peppers	___ o	___ s	___ r
c. Tomatoes	___ o	___ s	___ r	i. Onions	___ o	___ s	___ r
d. Broccoli	___ o	___ s	___ r	j. Cucumbers	___ o	___ s	___ r
e. Cauliflower	___ o	___ s	___ r	k. Radishes	___ o	___ s	___ r
f. Carrots	___ o	___ s	___ r	l. Other (Specify) ___________	___ o	___ s	___ r

E-6. Not Counting in salad, how often did (you/your _______) eat vegetables raw, such as carrot sticks, zucchini sticks, broccoli or cauliflower alone or with dips, etc. during that period? Remember, this is not including salad.

_______ per _______ 0 = never
d/w/m

(IF > 1/WK) Which three did (you/your __________) eat the most often?

a. _______________
b. _______________
c. _______________

E-7

During 1984 through 1988 how many times per day, week, month or year did (you/ your ______) usually eat (FOOD)?

A= PREPARED FROM RAW OR FRESH FROZEN B= PREPARED FROM CANNED
(INTERVAL: 0 = NEVER, 1 = DAY, 2 = WEEK, 3 = MONTH, 4 = YEAR)

	A PREPARED FROM RAW OR FRESH FROZEN		B PREPARED FROM CANNED	
a. Cooked peas (A, B)	______ #	______ INT	______ #	______ INT
b. Cooked onions (A, B)	______ #	______ INT	______ #	______ INT
c. String beans, green beans, or waxed beans (A, B)	______ #	______ INT	______ #	______ INT
d. Other beans, such as lima, pinto, kidney beans or black eyed peas or lentils (A, B)	______ #	______ INT	______ #	______ INT
e. Cooked cabbage or sauerkraut (A, B)	______ #	______ INT	______ #	______ INT
f. Cooked green pepper (A, B)	______ #	______ INT		
g. Cooked carrots (A, B)	______ #	______ INT	______ #	______ INT
h. Stewed tomatoes (A, B)	______ #	______ INT	______ #	______ INT
i. Cooked broccoli, cauliflower or Brussels sprouts (A, B)	______ #	______ INT	______ #	______ INT
j. Cooked yams, sweet potatoes (B)	______ #	______ INT	______ #	______ INT
k. Cooked beets (A, B)	______ #	______ INT	______ #	______ INT
l. Cooked spinach or other greens (A, B)	______ #	______ INT	______ #	______ INT

E-7

During 1984 through 1988 how many times per day, week, month or year did (you/ your ______) usually eat (FOOD)?

A= PREPARED FROM RAW OR FRESH FROZEN B= PREPARED FROM CANNED
(INTERVAL: 0 = NEVER, 1 = DAY, 2 = WEEK, 3 = MONTH, 4 = YEAR)

	A PREPARED FROM RAW OR FRESH FROZEN		B PREPARED FROM CANNED	
m. Cooked corn or hominy (not grits) (A, B)	______ #	______ INT	______ #	______ INT
n. Cooked okra or artichoke (A, B)	______ #	______ INT	______ #	______ INT
o. Cooked asparagus (A, B)	______ #	______ INT	______ #	______ INT
p. Cooked turnips, rutabagas, parsnips (A, B)	______ #	______ INT	______ #	______ INT
q. Cooked summer squash/zucchini (A, B)	______ #	______ INT	______ #	______ INT
r. Cooked pumpkin, winter squash, butternut or acorn squash (A, B)	______ #	______ INT	______ #	______ INT
s. Cooked white potatoes cooked any way (A, B)	______ #	______ INT	______ #	______ INT
t. Other vegetables often eaten (A, B) *If yes specify* ______________	______ *#*	______ *INT*	______ *#*	______ *INT*
u. ______________	______ *#*	______ *INT*	______ *#*	______ *INT*
v. ______________	______ *#*	______ *INT*	______ *#*	______ *INT*

E-8. During the period 1984 – 1988, how many servings of fresh fruit did (you/your ________) eat per day, week or month? ________ *per* ________ *0 = never*
 # d/w/m

E-9. During that period, about how often did (you/your ________) eat dried fruit such as dried apricots, apples, prunes or raisins? ________ *per* ________ *(IF > 1 WK)*
 # d/w/m

Which did you/your ________) eat most often? ______________ *0 = never*

E-10. About how often did (you/your ________) eat canned fruit?

________ *per* ________ *0 = never*
 # d/w/m

a. ______________ *b.* ______________ *c.* ______________

Now I'd like to ask you about some specific fruits.

E-11

During 1984 through 1988 how many times per day, week, month or year did (you/ your ______) usually eat (FOOD)?

A= RAW OR FRESH FROZEN B= COOKED OR CANNED
(INTERVAL: 0 = NEVER, 1 = DAY, 2 = WEEK, 3 = MONTH, 4 = YEAR)

	A RAW OR FRESH FROZEN		B COOKED / CANNED	
a. Peaches, plums, nectarines, apricots or cherries (A, B)	______ #	______ INT	______ #	______ INT
b. Apples or pears prepared anyway (A, B)	______ #	______ INT	______ #	______ INT
c. Berries; strawberries, blackberries, blue or raspberries, incl. pie (A, B)	______ #	______ INT	______ #	______ INT
d. Tropical fruits:pinapple,mango papaya, passion fruit (A, B)	______ #	______ INT	______ #	______ INT
e. Stewed prunes (A, B)	______ #	______ INT	______ #	______ INT
f. Grapefruit, oranges, mandarin oranges, tangerines (A, B)	______ #	______ INT	______ #	______ INT
g. Grapes (A, B)	______ #	______ INT	______ #	______ INT
h. Bananas (A, B)	______ #	______ INT	______ #	______ INT
i. Watermelon, cantaloupe, or other melons such as honeydew or casaba (A)	______ #	______ INT	______ #	______ INT
j. Fruit cocktail (B)	______ #	______ INT	______ #	______ INT
k. Mixed fruit salad (A)	______ #	______ INT	______ #	______ INT
l. Any other fruit often eaten? (A, B)	______ *#*	______ *INT*	______ *#*	______ *INT*
IF YES Specify ______________	______ *#*	______ *INT*	______ *#*	______ *INT*
m. ______________	______ *#*	______ *INT*	______ *#*	______ *INT*
n. ______________	______ *#*	______ *INT*	______ *#*	______ *INT*
o. ______________	______ *#*	______ *INT*	______ *#*	______ *INT*

Now I'm going to ask about foods including meats and meat products. I'm going to ask if the food was eaten and, if so, how often.

E-12

During 1984 through 1988 how many times per day, week, month or year did (you/ your ______) usually eat (FOOD)?

(INTERVAL: 0 = NEVER, 1 = DAY, 2 = WEEK, 3 = MONTH, 4 = YEAR)

a. Stew or chili made with beef chicken or lamb	______ # ______ INT	
b. Other beef, including steak, hamburger, meatballs, meatloaf, roast or ribs	______ # ______ INT	
c. Chicken or turkey	______ # ______ INT	
d. Liver, including chicken liver	______ # ______ INT	
e. Liverwurst, liver cheese or liver sausage	______ # ______ INT	
f. Canned luncheon meats, cold cuts or prepared meats, such as hotdogs, polish sausage or bologna	______ # ______ INT	
g. Bacon ,pork sausage or ham	______ # ______ INT	
h. Any other pork including pork chops or ribs	______ # ______ INT	
i. Fresh or frozen fish	______ # ______ INT	
j. Canned fish, such as tuna, salmon and sardines	______ # ______ INT	
k. Shellfish, such as shrimp, oysters or crab	______ # ______ INT	
l. Eggs: boiled, fried, scrambled or poached	______ # ______ INT	

Now I'm going to ask about some milk-based foods. I'm going to ask if the food was eaten and, if so, how often.

E-13 During 1984 through 1988 how many times per day, week, month or year did (you/ your _______) usually eat (FOOD)? (INTERVAL: 0 = NEVER, 1 = DAY, 2 = WEEK, 3 = MONTH, 4 = YEAR)		
a. Cottage cheese or yogurt	______ \#	______ INT
b. Other cheese, served by itself or in a sandwich	______ \#	______ INT
c. Butter	______ \#	______ INT
d. Margarine	______ \#	______ INT
e. Ice cream or ice milk	______ \#	______ INT

Finally, I'm going to ask about foods made of grains. I will ask if the food was eaten and, if so, how often.

E-14 During 1984 through 1988 how many times per day, week, month or year did (you/ your _______) usually eat (FOOD)? (INTERVAL: 0 = NEVER, 1 = DAY, 2 = WEEK, 3 = MONTH, 4 = YEAR)		
a. White bread, rolls, biscuits, or muffins	______ \#	______ INT
b. Whole grain breads including whole wheat, rye, pumpernickel or whole grain rolls or muffins	______ \#	______ INT
c. Cornbread, corn tortillas, or anything made with corn meal	______ \#	______ INT
d. Fortified cold breakfast cereals, such as Total, Product 19, or More	______ \#	______ INT
e. Other cold breakfast cereals, such as Cheerios, corn flakes, shredded wheat or Rice Krispies	______ \#	______ INT
f. Hot cereals or grits	______ \#	______ INT
g. Hot cakes or waffles	______ \#	______ INT
h. Cooked rice	______ \#	______ INT
i. Spaghetti or other pasta with tomato sauce	______ \#	______ INT
j. Macaroni and cheese, other pasta with cheese sauce	______ \#	______ INT
k. Other pasta dishes	______ \#	______ INT

E-15. During the period 1984 through 1998, how many meals per day did (you/your __________) usually eat?

____

MEALS PER DAY

E-16. During that period, how many times per day, week, month or year did (you/your __________) eat meat or fish that had been smoked or that had a strong smoky taste?

_______________ *TIMES*

DAY....................................1
WEEK..................................2
MONTH..............................3
YEAR..................................4
NEVER................................00

APPENDIX B: GSH and total GSH values for items in the food frequency questionnaire, derived from Jones DP, et al. *

FOOD ITEM	SERVING SIZE	GSH matching items from GSH in foods listed in the NCI's HHHFFQ	GSH_T mg/ 100g	GSH mg/ 100g	GSH_T/ serving size	GSH/ serving size
Milk	245g	Milk, cow fluid, whole Milk, cow fluid, 2% Milk, cow fluid, 1%	0.0	0.0	0.0	0.0
Orange Juice	199 g	Orange juice, reconst., from conc.	4.2	2.8	8.4	5.6
Grapefruit Juice	198 g	Grapefruit juice, canned	0.0	0.0	0.0	0.0
Tomato or V-8 Juice	195 g	Tomato juice, bottled	1.6	1.3	3.1	2.5
Other Fruit Juices, Apple	199 g	Apple juice, bottled	0.0	0.0	0.0	0.0
Fruit Drinks-Hi-C, Tang, Hawaiian Punch	199 g	Orange drink from powder (Tang and Start)	0.0	0.0	0.0	0.0
Alcoholic Beverages: Beer Wine Hard Liquor	357 g 180 g 180 g 180 g 42 g	Beer Table Wine 12.2% alcohol Table Wine, red Table Wine, white 80 Proof, 33.4% alcohol Average:	1.2 2.3 1.6 2.9 0.0 1.6	1.1 1.5 0.7 2.3 0.0 1.1	4.3 4.1 2.9 5.2 0.0 3.3	3.9 2.7 1.3 4.1 0.0 2.4
Salad items in salad: Lettuce Spinach Tomatoes Broccoli Cauliflower Carrots GreenPeppers Red Peppers Onions Cucumbers Radishes	 83 g 28 g 100 g 44 g 50 g 55 g 50 g 50 g 80 g 52 g 58 g	 green salad spinach, raw tomatoes, raw broccoli spears, fresh, cooked cauliflower, fresh, cooked carrots, raw pepper, green, bell, raw pepper, red, bell, raw onions, cooked cucumbers, raw, pared ?	 2.6 12.2 9.0 9.1 9.1 7.9 5.5 5.0 6.4 4.3 ?	 1.1 11.4 7.5 5.5 4.0 5.9 3.4 5.5 0.5 3.5 ?	 2.2 3.4 9.0 4.0 4.6 4.3 2.8 2.5 5.1 2.2 ?	 0.9 3.2 7.5 2.4 2.0 3.2 1.7 2.8 0.4 1.8 ?

Not counting salad, vegetables eaten raw, such as carrot sticks, zucchini sticks, broccoli, etc. (take 3 most common vegetables/ take average)	assuming ½ cup serving Carrots-55g Zucchini =65g Broccoli =44g	Carrots, raw Squash, zucchini, fresh, cooked Broccoli spears, fresh, cooked Average:	C=7.9 Z=6.2 B=9.1 7.7	C=5.9 Z=8.4 B=5.5 6.6	C=4.3 Z=4.0 B=4.0 4.1	C=3.2 Z=5.5 B=2.4 3.7
Cooked Peas Raw/Fresh Frozen (R/F)	1/2 cup, 80 grams	Peas & carrots, frozen, cooked	5.9	3.2	4.7	2.6
Cooked Peas, Canned (C)	½ cup, 85 g	Peas, green, canned, heated	5.6	5.2	4.8	4.4
Cooked Onions (R/F)	½ cup,105 g	Onions, cooked	6.4	0.5	6.7	0.5
String Beans, green beans, or wax beans (R/F)	68 g	?	?	?	?	?
String Beans, green beans, or wax beans (C)	68 g	Beans, green, canned, heated	0.0	0.0	0.0	0.0
Other beans, such as lima Pinto Kidney beans black eyed peas lentils (R/F)	90 g 85 g 90 g 83 g 100 g	? ? ? ? ?	? ? ? ? ?	? ? ? ? ?	? ? ? ? ?	? ? ? ? ?
Other beans– lima, pinto, kidney beans or black eyed peas or lentils (C)	150 g 150 g ¾ cup	Beans, pinto, canned, heated Blackeyed peas, canned, heated Average:	0.6 2.1 1.7	0.5 1.3 1.2	0.9 3.2 2.1	0.8 2.0 1.4
Cooked cabbage or sauerkraut (R/F)	75 g	Cabbage, fresh, cooked	4.7	2.1	3.5	1.6

Cooked cabbage or sauerkraut (C)	118 g	?	?	?	?	?
Cooked greenpepper (R/F)	68 g	?	?	?	?	?
Cooked carrots (R/F)	78 g	Carrots, fresh, cooked	5.8	4.7	4.5	3.7
Cooked carrots (C)	73 g	Carrots, canned, heated	0.0	0.0	0.0	0.0
Stewed tomatoes (R/F)	120 g	?	?	?	?	?
Stewed tomatoes (C)	127 g	?	?	?	?	?
Cooked broccoli, cauliflower, or brussels sprouts (R/F)	92g 78 g 78 g	Broccoli spears, fresh, cooked Cauliflower, fresh, cooked Brussels sprouts, frozen, cooked Average:	9.1 9.1 2.5 6.9	5.5 4.0 1.9 3.8	8.4 7.1 2.0 5.8	5.1 3.1 1.5 3.2
Cooked broccoli, cauliflower, or brussels sprouts (C)	78g/ for all items	?	?	?	?	?
Cooked yams, sweet potatoes (R/F)	130 g	Sweet potatoes, fresh, baked	2.9	1.9	3.8	2.5
Cooked yams, sweet potatoes (C)	98 g	Sweet potatoes, canned, heated	3.8	1.4	3.7	1.4
Cooked beets (R/F)	85 g	?	?	?	?	?

Cooked beets (C)	85g	Beets, canned	7.9	0.9	6.7	0.8
Cooked spinach or other greens (R/F)	90 g 72 g	Spinach, fresh, cooked Turnip greens, fresh, cooked Average:	7.2 3.2 5.2	5.7 1.5 3.6	6.5 2.3 4.4	5.1 1.1 3.1
Cooked spinach or other greens (C)	95 g	Spinach, canned, heated	2.2	1.4	2.1	1.3
Cooked corn or hominy (R/F)	85 g 80 g	? ?	? ?	? ?	? ?	? ?
Cooked corn or hominy (C)	83 g	Corn, sweet, canned, heated	1.7	0.6	1.4	0.5
Cooked okra or artichoke (R/F)	80 g 84 g	Okra, fresh, cooked Artichoke	12.0 ?	11.3 ?	9.6 ?	9.0 ?
Cooked asparagus (R/F)	93 g	Asparagus, fresh, cooked	28.3	21.8	26.3	20.3
Cooked asparagus (C)	120 g	?	?	?	?	?
Cooked turnips Rutabagas Parsnips (R/F)	80 g 85 g 79 g	? ? ?	? ? ?	? ? ?	? ? ?	? ? ?
Cooked turnips, rutabagas, parsnips (C)	N/A	?	?	?	?	?
Cooked summer squash/ zucchini (R/F)	90 g	Squash, zucchini, fresh, cooked	6.2	8.4	5.6	7.6

Cooked summer squash/ zucchini (C)	115 g	?	?	?	?	?
Cooked pumpkin or winter squash, butternut or acorn squash (R/F)	123 g/ for all items	Squash, winter, acorn, baked pumpkin, butternut	11.7	11.0	14.4	13.5
Cooked pumpkin or winter squash,butter nut or acorn squash (C)	123 g	?	?	?	?	?
Cooked white potatoes, cooked anyway (R/F)	80g 120 g	Potatoes, boiled with skin Potatoes, french fries, fast food Average:	13.6 14.3 14.0	11.0 10.2 10.6	10.9 17.2 14.1	8.8 12.2 10.5
Cooked white potatoes, cooked anyway (C)	90 g	?	?	?	?	?
Dried fruit: apricots, Apples Prunes Raisins)	65 g 43 g 80 g 80 g	? ? ? ?	? ? ? ?	? ? ? ?	? ? ? ?	? ? ? ?
Peaches, plums, nectarines, apricots or cherries (R/F)	100 g	Peaches, unsweetened, raw Nectarines, raw, pared Sour cherries, raw Sweet cherries, raw	7.4 7.4 0.0 0.0	5.0 4.9 0.0 0.0	7.4 7.4 0.0 0.0	5.0 4.9 0.0 0.0
Peaches, plums, nectarines, apricots or cherries (C)	125g/ for all items	Peaches, sweetened, canned Apricots, canned Plums, nectarines, cherries? Average (not including plums, nectarines, or cherries):	1.9 1.9 ? 1.9	1.2 1.2 ? 1.9	2.4 2.4 ? 2.4	1.5 1.5 ? 1.5

Apples or pears prepared any way (R/F)	1 medium or 1/2 cup, 138g	Apples, raw, not pared Pears, raw, not pared Average:	3.3 5.0 4.2	1.5 3.3 2.4	4.6 6.9 5.8	2.1 4.6 3.4
Apples or pears prepared any way (C)	100g/apples 125g/pears	?	?	?	?	?
Strawberries Blackberries Blueberries Raspberries (R/F)	120 g 75 g ? ? ?	Strawberries, frozen Strawberries, raw (in season) ? Blueberries, frozen ?	9.9 7.1 ? 0.0 ?	5.1 6.9 ? 0.0 ?	11.9 5.3 ? 0.0 ?	6.1 5.2 ? 0.0 ?
Strawberries Blackberries Blueberries Raspberries (C)	125 g 130 g 130 g 125 g	? ? ? ?	? ? ? ?	? ? ? ?	? ? ? ?	? ? ? ?
Tropical fruits: pineapple, papaya, mango, passion fruit (R/F)	? 70 g 105 g ?	? Papaya, raw, pared Mangoes, raw, pared ?	? 6.4 5.8 ?	? 5.8 4.3 ?	? 4.5 6.1 ?	? 4.1 4.5 ?
Pineapple, papaya, mango, passion fruit (C)	? ?	pineapple, canned	0.0 ?	0.0 ?	0.0 ?	0.0 ?
Stewed Prunes (C)	120 g	?	?	?	?	?
Grapefruit Orange Mandarin orange, tangerine (R/F)	1/2 medium 1 medium ?	Grapefruit, white, raw, pared Orange, raw, pared ?	7.9 7.3 ?	6.5 4.8 ?	14.6 10.6 ?	12.1 7.0 ?

Grapefruit Oranges Mandarin oranges, tangerines (C)	125 g 105 g 125 g 125 g	? Fruit cocktail, sweetened, canned	? 0.0	? 0.0	? 0.0	? 0.0
Grapes (R/F)	80 g	Grapes, raw	3.0	2.7	2.4	2.2
Grapes (C)	130 g	?	?	?	?	?
Bananas(R/F)	120 g	Bananas, raw, common	4.1	3.3	4.9	4.0
Watermelon Cantaloupe Honeydew Casaba (R/F)	500g 80 g 85 g 85 g	Watermelon, raw ? ? ?	6.6 ? ? ?	5.0 ? ? ?	33.0 ? ? ?	25.0 ? ? ?
Watermelon, cantaloupe, or other melons such as honeydew or casaba ? (C)	?	?	?	?	?	?
Fruit cocktail (R/F)	90 g	?	?	?	?	?
Fruit cocktail (C)	120 g	Fruit cocktail, sweetened, canned	0.0	0.0	0.0	0.0
Mixed fruit salad (R/F)	90 g	?	?	?	?	?
Mixed fruit salad (C)	120 g	?	?	?	?	?
Stew made w/beef Stew made w/chicken Chili made w/beef Chile made w/beans	¾ cup=190g 1 cup=240 g ¾ cup=195g 1 cup=240 g ¾ cup=200g 1 cup=250 g ? ?	Stew, beef, canned, heated " " ? ? Chili con carne, canned " " Chili w/ beans? Veg. Chili " "	1.2 1.2 ? ? 1.1 1.1 0.0 0.0	1.3 1.3 ? ? 0.9 0.9 0.0 0.0	2.3 2.9 ? ? 2.2 2.8 0.0 0.0	2.5 3.1 ? ? 1.8 2.3 0.0 0.0

Other beef: hamburger meatballs meatloaf steak	115 g 115 g 110 g 110 g	Hamburger, pan fried ? ? Steak, beef, pan fried Average:	17.5 ? ? 13.4 15.5	11.8 ? ? 12.3 12.1	20.1 ? ? 14.7 17.4	13.6 ? ? 13.5 13.6
Chicken or turkey	113 g 113 g	Fried Chicken,incl.fried breast Other chicken/turkey, roasted Average:	13.1 8.7 10.9	6.5 7.7 7.1	14.8 9.8 12.3	7.3 8.7 8.0
Liver, including chicken liver	113 g 113 g	Liver, beef, pan fried Liver, chicken, pan fried Average:	2.5 18.8 10.7	0.8 14.5 7.7	2.8 21.2 12.0	0.9 16.4 8.7
Liverwurst, liver cheese or liver sausage	55 g 55 g	Liver, including chicken livers or liverwurst	9.1	7.0	5.0	3.9
Canned luncheon meats cold cuts hot dogs polish sausage bologna	85 g 45 g 75 g 85 g	? ? ? Polish-style sausage ?	? ? ? 6.2 ?	? ? ? 2.4 ?	? ? ? 4.7 ?	? ? ? 1.8 ?
Bacon, pork sausage or ham	13 g 30 g 55 g	Bacon, pan fried Pork sausage Boiled Ham Average:	5.0 6.2 23.3 11.5	2.2 2.4 13.7 6.1	0.7 1.9 12.8 4.8	0.3 0.7 7.5 2.8
Any other pork including pork chops or ribs	88 g	Pork chop, lean, pan fried Ribs, others	23.6	18.9	20.8	16.6
Fresh or frozen fish	85 g	Fish (pollock), deep fried Fish (cod and perch), pan fried	2.6 6.0	1.5 5.7	2.2 5.1	1.3 4.8
Canned fish, such as tuna, salmon and sardines	80 g	Tuna fish, canned salmon or sardines	1.6	1.1	1.3	0.9

Shellfish: shrimp oysters crab	115 g 115 g 70 g	Shrimp, canned ? ?	1.3 ? ?	1.0 ? ?	1.5 ? ?	1.2 ? ?
Eggs: boiled, fried, scrambled or poached	1 egg, 50 g	Chicken eggs, pan fried	0.0	0.0	0.0	0.0
Cottage cheese or yogurt	½ cup, 115 g 1 cup, 240 g	Cottage cheese Yogurt, plain, low fat Flavored yogurt	0.0 0.0 0.0	0.0 0.0 0.0	0.0 0.0 0.0	0.0 0.0 0.0
Other cheese, served by itself or in a sandwich	2 slices or 2 oz., 55 g	Cheese, American	0.0	0.0	0.0	0.0
Butter	2 pats, 20 g	Butter	0.0	0.0	0.0	0.0
Margarine	2 pats, 20 g	Margarine	0.0	0.0	0.0	0.0
Ice cream or ice milk	1 scoop or 1/2 cup,65 g	ice cream, 10% fat	0.0	0.0	0.0	0.0
White bread, rolls, biscuits, or muffins	2 slices, 60g 1med piece, 30 g	bread, white, enriched biscuits, muffins	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0
Macaroni & Cheese, other Pasta with cheese sauce	240 g	Macaroni and Cheese	0.0	0.0	0.0	0.0
Whole grain breads including whole wheat, rye and pumpernickel or whole grain rolls or muffins	2 slices, 60 g 1 med piece 30g	bread, whole wheat biscuits, muffins average:	1.2 0.0 0.6	0.0 0.0 0.0	0.7 0.0 0.4	0.0 0.0 0.0

Cornbread, corn tortillas, or anything made w/ corn meal	70 g 30 g	Cornbread corn tortillas, others?	0.0	0.0	0.0	0.0
Fortified cold breakfast cereals, such as Total, Product 19 or More	45 g	Fortified corn cereal Bran Flakes Average:	0.6 1.2 0.9	0.4 0.5 0.5	0.3 0.5 0.4	0.2 0.2 0.2
Other cold breakfast cereals, such as: Cheerios, corn flakes, shredded wheat, rice krispies	35 g 43 g 65 g 43 g	? Corn flakes ? ?	? 0.0 ? ?	? 0.0 ? ?	? 0.0 ? ?	? 0.0 ? ?
Hot cereal or grits	150 g	Oatmeal or rolled oats, cooked	2.4	1.5	3.6	2.3
Hot cakes or waffles	3 pancakes =115 g 1 large waffle=35g	Waffles, pancakes	0.0	0.0	0.0	0.0
Cooked rice	125 g	Rice, white enriched, cooked	1.6	0.8	2.0	1.0
Spaghetti or other pasta w/ tomato sauce	250 grams	Spaghetti and meat sauce, restaurant	3.6	3.0	9.0	7.5