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ABSTRACT

THE RELATIONSHIP BETWEEN UNMETABOLIZED FOLIC ACID AND SERUM FOLATE CONCENTRATIONS AND CANCER RISK IN OLDER US ADULTS

by

Regine L. Baldauff

Importance Several studies have reported an increase in serum and unmetabolized folic acid levels since the implementation of folic acid fortification (January 1, 1998). However, the literature published during the post-folic acid fortification period is controversial with regards to the safety and potential risk for cancer in non-target populations.

Objective To study the association between unmetabolized folic acid and serum folate and cancer in older US adults.

Design, Setting, and Participants This is a cross sectional study using data from National Health and Nutrition Examination Surveys. Among 700 participants with identified unmetabolized folic acid, 147 cases were reported a history of having cancer from 1999-2002. Within the 7,981 subjects who had a recorded value for serum folate from 1999-2008; 1,459 reported a history of all cancer. Among the 4,007 women who had a recorded value for serum folate between 1999-2008; 288 reported a history of breast cancer.

Main Outcome Measures Associations of unmetabolized folic acid and serum folate with all cancer and breast cancer was evaluated using a multivariable logistic regression analysis controlling for demographic and dietary intakes.

Results Men and women without unmetabolized folic acid were 0.7 times less likely to develop cancer. Those over the age of sixty with the highest concentration of serum folate were 1.4 times more likely to have cancer than participants with lower serum folate concentrations. Women over the age of sixty with the highest concentration of serum folate were 1.8 times more likely to have breast cancer compared to women with lower serum folate concentrations.

Conclusions and Relevance The presence of unmetabolized folic acid and high serum folate concentrations were related to an increased prevalence of cancer. Further research is warranted to investigate the cause and effect relationship.

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by
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ABBREVIATIONS

US	United States
NHANES	National Health and Nutrition Examination Survey
UMFA	Unmetabolized Folic Acid
dUMP	Deoxyuridine Monophosphate
dTMP	Thymidine Monophosphate
SAM	S - adenosylmethionine
DNA	Deoxyribonucleic Acid
ER	Estrogen Receptor
ER+	Estrogen Receptor Positive
ER-	Estrogen Receptor Negative
UL	Upper intake Level
HPLP	High Performance Liquid Chromatography
CAPI	Computer Assisted Personal Interviewing
MEC	Medical Examination Center
CDC	Center for Disease Control and Prevention
NCHS	National Center for Health Statistics
nmol/L	Nano-mole per Liter
pmol/ml	Pico-mole per milliliter
µg/g	Micro-gram per gram

SE	Standard Error of the geometric mean
UnAdj OR	Unadjusted odds ratio
Adj OR	Adjusted odds ratio
$ER\beta^-$	Estrogen receptor beta negative
$ER\alpha^+ \beta^-$	Estrogen receptor alpha positive beta negative
MTHFR C677T	Methylenetetrahydrofolate reductase C677T polymorphism
MTR 2756GC	Methionine synthase GG polymorphism

CHAPTER I

THE RELATIONSHIP BETWEEN UNMETABOLIZED FOLIC ACID AND SERUM FOLATE CONCENTRATIONS AND CANCER RISK IN OLDER US ADULTS

INTRODUCTION

Folate, chemically known as polyglutamate, is found in dark green leafy vegetables, i.e. spinach and kale. Folic acid also known as monoglutamate is the synthetic form of folate and is found in all fortified foods and supplements containing folate in the United States (US)[1]. The Food and Drug Administration (FDA) implemented folic acid fortification in January of 1998 with the intent of lowering the prevalence of neural tube defects in infants born in the US[2]. Folate is the major methyl donor in one-carbon metabolic pathways throughout the body; and plays a key role in the development of precursors for the building of DNA, repair, and DNA methylation[3]. Over the past decade during the post-fortification era, the research community has become concerned that the non-targeted population (i.e. men, children, and postmenopausal women) might be getting more folic acid than they need. Additionally, studies have been implemented to address any potential consequences of excess folic acid intake by non-target populations. Most recently the focus has been on the increased consumption of folic acid and the occurrence of cancer.

Recently, researchers have raised the question of whether or not folate and unmetabolized folic acid plays a dual role in cancer, as both a cancer preventer and promoter[1,4]. Due to the function folate plays in cell division and cellular growth, and stemming from its role as a cofactor in the synthesis of purines and thymidylate, and

nucleic acid synthesis, researchers have begun to question its involvement in cancer, helpful or harmful[4]? When examining cancer cells and the accelerated rate at which they replicate and divide in comparison to healthy cells found in the body, removing folate sources and preventing folate metabolism has shown to inhibit tumor growth[4]. This finding has created a platform for the use of antifolate drugs in cancer chemotherapy treatments[4].

Multiple studies have been conducted in various fields of cancer development and treatment, and although the results are still controversial many have demonstrated a positive association between increased levels of serum folate and cancer incidences. Most notably, researchers have illustrated a relationship between the folic acid fortification era and a spike in colorectal cancer incidence in the US[4,5]. The evidence from several recent clinical trials regarding colorectal cancer incidence rates, has shown a relationship between folic acid and cancer prevalence and pre-malignant lesions[4]. A study conducted with postmenopausal women showed a relationship between high dietary folate intake and increased risks for breast cancer[3]. The study also illuminated the need for a large population based study to assess the relationship between the folic acid fortification and breast cancer prevalence[3].

The National Health and Nutrition Examination Survey (NHANES), was used in the current study to collect a nationally representative data set for serum folate concentrations, unmetabolized folic acid concentrations, and cancer prevalence. NHANES, monitored and carried out by the Center for Disease Control and Prevention (CDC) is a complex survey design used to collect nationally representative data for the following areas: demographics, socioeconomic, dietary, medical history, and laboratory

values from a diverse population, with the intent of monitoring the overall health status of the US popualtion[6]. The current investigation is the first study to conduct a large-scale study using NHANES data to establish an association between folic acid supplementation and cancer risk in both men and women over the age of 60.

Objective

The purpose of this study was to assess the relationship between the folate status and cancer in older US adults using a nationally representative sample survey. The 3 specific aims of the study are to examine: 1) the relationship of unmetabolized folic acid with the prevalence of all cancer in older adults over the age of 60, 2) the effect of serum folate on all cancer in both men and women over the age of 60, and 3) the relationship between serum folate concentrations and breast in women over the age of 60 years in the US.

Hypothesis

We hypothesize is that serum folate status is related to an increased prevalence of both breast cancer and all cancer rates in both men and women over the age of sixty; additionally the presence of unmetabolized folic acid is related to an increased prevalence of cancer in older US adults.

CHAPTER II

LITERATURE REVIEW

Folate (polyglutamate), the natural vitamer is commonly found in dark green leafy vegetables, citrus fruits, legumes, shellfish, yeast, and liver[7,8]. Folic acid (monoglutamate), is found in supplements and fortified foods such as grains, flours, and cereals[9]. Folic acid chemically differs from its natural form, folate, existing in an oxidized state with only one conjugated glutamate residue[4,7]. The differences between the chemical structures of folic acid and folate allows folic acid to have a higher bioavailability[10] and absorb more rapidly across the intestines[4,10]. Folate is part of the water-soluble B vitamin family, known as B9[7,8,9,11].

In 1998, the Federal Food and Drug Administration (FDA) mandated fortification of all enriched flour[2], grain, and cereal products with 140µg/100g of folic acid [2,7,12]. The mandatory fortification followed an announcement from the US Public Health Services recommending that all pregnant women and women of the child bearing age should consume 400µg of folic acid per day[13], with an upper intake level (UL) of 1000µg/day for all populations[7,13,14]. This recommendation was directed towards reducing neural tube defects in newborns[13]. However, since folic acid fortification began it has been reported that the average folic acid intake is greater than 800µg/day, particularly in individuals consuming both fortified foods and daily supplements[7].

Although the FDA has set an UL for folic acid intake[13], there is not a bench mark for a safe upper limit regarding serum folate[4,13]. Many have agreed within the scientific and medical communities that a serum folate concentrations above 45 nmol/L is considered significantly higher than standard physiological levels[4]. Recently it has been reported that 23% of Americans have a serum folate concentration >45nmol/L since the implementation of folic acid fortification[4]. Additionally, mandated folic acid fortification has lead to the tripling of the average serum folate level in populations across the US[15]. Mandatory folic acid fortification lead to an extremely high increase in folic acid consumption[16] far beyond the intended target population[4] and could potentially have adverse effects[4,16].

Over the past several years multiple studies have been conducted to evaluate the amount of folic acid intake after the fortification of folic acid began, and the effect folic acid fortification has had on the general population. One of the more recent concerns is the finding of unmetabolized folic acid in an individual's blood serum. Unmetabolized folic acid accumulates in an individuals blood serum upon a person consuming high amounts of folic acid; as their cellular metabolism becomes saturated with the vitamin it is unable to process and convert all of the ingested folic acid back to its reduced state, known as folate, to be utilized by one-carbon transfer reactions and DNA methylation[1,17,18,]. The result is an excess of circulating folic acid that ultimately remains in its oxidized form in the blood[1,17]. Unfortunately researchers only understand pieces regarding unmetabolized folic acid and its effects on the population[1,19]. Many researchers believe the growing level of unmetabolized folic acid is a safety concern and could be contributing to several biological illnesses, such as cancer[1,17].

Folate is a required co-factor in cellular metabolism and plays a critical role in one-carbon transfers, DNA synthesis, repair and maintenance[4,7,15,20]. One carbon transfers are vital for the building of nucleotides and methylation reactions[7,20,21]. Most notably, folate is a required cofactor for the thymidylate synthesis reaction[7]; in this reaction deoxyuridine monophosphate (dUMP) is converted to thymidine monophosphate (dTMP) through a one carbon transfer to ultimately produce purines and thymidine nucleotides to be used in DNA synthesis[7,9,11,15,16,20,21,22,23]. Furthermore, folate is vital for the remethylation of homocsysteine back to methionine; the carbon donor for S-adenosylmethionine (SAM) a critical methyl donor for physiological methylation reactions, i.e. DNA methylation[4,7,11,15,20,24]. The involvement of folate in DNA synthesis makes it an essential factor in DNA replication and cell division[20]. Due to the importance of folate as a key player in cellular metabolism, and DNA synthesis[7], current research suggests consuming high amounts of synthetic folate may enhance the proliferation of cancer cells and malignant tumors[1].

Among researchers there is a debate over the belief of whether folic acid prevents the onset of diseases such as cancer or if it fuels the progression of the disease. Many scientists believe folic acid insufficiency is cancer causing, because the depletion of folic acid within the body leads to a cascade of hindered one-carbon transfer reactions, and ultimately cellular metabolic processes become compromised, escalating to more severe outcomes: DNA instability, altered DNA methylation, and the inability to repair DNA[11,25,26]. According to Jennings[26], folic acid is a cancer preventer, emphasizing that defected DNA segments extracted from animal and cell experiments where folic acid was with-held, resembles the DNA found in cancer cells. Research has also illustrated, when

cells are denied and depleted of folic acid the outcome results in DNA damage[26]. In studies using rats, DNA damage from inadequate folic acid intake was indicated by DNA strand breakages within the rats white blood cells[26].

Researchers have illustrated folate deficiency leads to the break down of chromosomes due to the incorporation of uracil in the place of thymine during DNA synthesis[8,15,22,27,28]. Additionally decreased methylation caused by a depletion of folate stores causing the over methylation of the promoter region in tumor suppressor genes leading to gene silencing and unregulated cancer cell development[8,15,25,27,28]. Ultimately, the argument for folic acid being a cancer preventative lies in the following scientific postulate. When an individual is folic acid deficient they deplete their folate stores, the biosynthetic pathways vital for DNA integrity are unable to function successfully, leading to an increased risk for tumor development[25,29].

The discussion regarding folate as a tumor cell and cancer promoter is usually avoided due to the controversy created with the public health sector[12]. According to Kim[12], cancer cells grow at an accelerated rate, due to enhanced DNA replication and cell division. When considering the pathways and essentialness of folate in the biosynthesis of DNA, enhanced folate status acts as an accelerant for tumor cell growth[12]. Research has demonstrated when folate is removed hindering its pathways, DNA replication is reduced leading to a decrease in cancer cell proliferation[12]. In experimental trials the depletion of folate has shown to prevent further development of pre-established malignant cells[12]. Both animal based studies and clinical trials have illustrated that folate supplementation enhances cancer risk via fueling cancer progression when large doses of the vitamin are ingested after the development of malignant cells[12]. In a study conducted

on mice inoculated with leukemia cells, excess folic acid given through diet expedited the cancer progression causing an increased mortality rate compared to the control group[15]. According to Baggott et. al.[16], folic acid supplementation supports cancer development by enhancing the pool of nucleotides needed for DNA synthesis in abnormal cells. Furthermore, the current research supports the "acceleration phenomenon" which occurs when folic acid feeds pre-existing undiagnosed tumor cells[5,12]. The danger associated with these findings from a public health prospective, is that it is essentially impossible to determine the onset of abnormal cell development[12]. For individuals who are consuming large doses of folic acid, malignant and abnormal cells will flourish in the presence of high levels of folate available for their cells cellular metabolism[12].

Since the early 20th century scientists have studied and linked folate with malignant formations and cancer related diseases[14]. Baggott et. al.[16] stated, "antifolates are the only antivitamins that are effective in cancer development." In the 1940's, scientists hypothesized folate deficiency was the cause of acute leukemia, unfortunately treatment with folate failed causing an excitatory effect in the malignant cells among the patients[14,15]. Following this discovery, researchers Heinle and Welch[30] induced folate deficiency within their patients, ultimately inhibiting the progression of the malignant leukemia cancer cells[14,15]. In 1947, Farber developed the first antifolate drug aminopterin; treatment with aminopterin lead to remission among children with leukemia[14]. Since Farber's discovery the field of antifolates as a chemotherapeutic agent has expanded and has become a primary treatment protocol for cancer diagnosis which now encompasses multiple classes of antifolates [14,31].

Researchers have hypothesized that the damaging effects from folate (i.e. the proliferation of cancer cells) occurs most often in cases of high consumption of folic acid from both the intake of fortified foods and supplements[32]. From a study conducted by Mason et. al.[5], folic acid fortification was hypothesized to be a potential key factor in the reversal of the decrease in the number of cases seen in colorectal cancer in both the US and Canada[5,24]. The results from their study indicated a 10% increase in colorectal cancer since the implementation of folic acid supplementation in the two countries[24,31]. A recent study examining the relationship between prostate cancer prevalence and folic acid fortification illustrated the presence of prostate cancer in patients with higher levels of serum folate[24]. Bistulfi et. al.[31], reported on a study examining prostate cancer in men over the age of 50, and concluded that the occurrence of cancer in this subset of men was high while attributing the results to folic acid fortification and its accelerant effect on malignant cell growth.

According to recent data, breast cancer is the leading cause of cancer related mortalities in women living in the US[33]. In light of these statistics, understanding and identifying major risk factors for breast cancer is crucial for both the prevention and treatment of breast cancer consumption[33]. Scientists have reported a connection between breast cancer and high folic acid consumption[34]. A link between folate and the estrogen receptor (ER) genes has been reported, indicating folate as a key participant in silencing the ER genes, leading to malignant cell formation[34]. The literature indicates folic acid may provide a protective effect for premenopausal women, but could pose a potential threat to postmenopausal women because they have decreased folate needs compared to

premenopausal women[35]. The current data examining the association between folic acid and breast cancer is still inconclusive due to the number of conflicting results[36].

Upon examining the literature there is a substantial concern regarding the folic acid fortification public health policy and its overall effect on the non-targeted population. Currently the data conclusively indicates a substantially larger increase in folic acid intake in ”non-targeted” populations, which includes men and women past the “child-bearing” age. In light of the controversial data currently available regarding folic acid and the development of cancer in the non-targeted populations, further research is warranted to further investigate the true underlying relationship between folic acid fortification and health risks for the general public.

CHAPTER III

METHODS

Study description and survey design

The National Health and Nutrition Examination Survey (NHANES) is conducted by the National Center for Health Statistics (NCHS), and an integral part of the Center for Disease Control and Prevention (CDC)[6]. The NHANES program conducts surveys, interviews, and medical examinations to report critical health statistics for all populations in the United States on a yearly cycle[6]. NHANES utilized a stratified, multistage probability sample survey design to assess the US population health status[37]. NHANES, surveyed and interviewed a nationally representative sample of individuals from a diverse collection of counties across the country, 15 of the same counties are sampled each year[6]. Individuals over the age of sixty, African Americans, and Hispanic were over sampled as per the design of the survey[6]. All individuals who participated were requested to be surveyed, medically examined by a physician, and participate in the health questionnaires conducted by a team of trained interviewers[6]. All health and survey questionnaires conducted during the initial part of the survey were carried out in the participants' homes as part of the "house-hold" interview[6]. A physician examined all individuals who participated in the surveys, and his or her body measurements were recorded in the NHANES Mobile Examination Centers (MECs)[6].

In the current study, data from 2 NHANES cycles: NHANES 1999-2000[38] and 2001-2002[39] were examined to evaluate the relationship between unmetabolized folic

acid and cancer risk (all cancer and breast cancer). The detailed description for both of the 2 surveys methodologies and analytical guidelines are reported elsewhere[40,41]. To examine the relationship between serum folate and both outcomes, all cancer and breast cancer, 5 NHANES cycles were analyzed: 1999-2000[38], 2001-2002[39], 2003-2004[42], 2005-2006[43], 2007- 2008[44], to create a master data set 1999-2008. The NHANES 1999-2000 cycle was conducted between March 1999 and December 2000; during this time period a total of (12,160) individuals were screened, within the screened population (9,965) individuals were interviewed in their home, and (9,282) individuals were examined in the MEC[45]. The NHANES 2001-2002 cycle was conducted between January 2001 and December 2002; during this time frame a total of (13,156) individuals were screened, of the screened individuals (11,039) individuals were interviewed in their homes, and (10,477) participants were examined in the MEC[46].

The NHANES 2003-2004 cycle was conducted between January 2003 and December 2004; during this time frame a total of (12,761) individuals were screened, of the screened population (10,122) were interviewed in their homes, and (9,643) individuals were examined in the MEC[47]. The NHANES 2005-2006 cycle was conducted between January 2005 and December 2006; a total of (12,862) individuals were screened, of the screened population (10,348) participants were interviewed in their homes, and (9,950) individuals were examined in the MEC[48]. The NHANES 2007-2008 cycle was conducted between January 2007 and December 2008; a total of (12,943) individuals were screened, of the screened population (10,149) participants were interviewed in their homes, and (9,762) individuals were examined in the MEC[49].

The response rate for the 1999-2000 NHANES cycle in home interview was (82%), the response rate for the medical exam was (76%)[45]. The response rate for the 2001-2002 NHANES cycle in home interview was (84%), the response rate for the medical exam was (80%)[46]. For the 2003-2004 NHANES cycle the in home interview response rate was (79%), and the medical exam response rate was (76%)[47]. The in home response rate for the 2005-2006 NHANES cycle was (80.45%), and the medical exam response rate was (77.36%)[48]. The response rate for the 2007-2008 NHANES cycle in home interview was (78.4%), the medical exam response rate was (75.4%)[49].

Current study methodology

The current study utilized the NHANES data for unmetabolized folic acid from the cycles 1999-2000, 2001-2002 to combine one large database 1999-2002. The researcher focused on subjects 60 years or older in the analysis who had both a recorded unmetabloized folic acid concentration and had answered the medical condition questionnaire data concerning the presence of cancer for all cancers. Serum folate concentration data was gathered from the NHANES cycles 1999-2000, 2001-2002, 2003-2004, 2005-2006, 2007-2008, to create on large data file 1999-2008. Subjects from this database were separated into 2 cohorts, all cancer and breast cancer risk. The researchers used the medical condition questionnaire data concerning the presence for all cancer and breast cancer to analyze the association between serum folate concentrations, all cancer risk, breast cancer risk in US adults 60 years of age or older.

Study sample

We excluded individuals from the analysis with missing values for sex, age, race-ethnicity, unmetabolized folic acid concentrations, serum folate concentrations, and a

missing or "refused" to respond answers on the medical questionnaire for both, "all cancer" and "breast" cancer. After the above exclusion criteria was applied the final study sample consisted of (2,682) individuals in the unmetabolized folic acid cohort (men =1320; women =1362), (7,981) subjects in the serum folate all cancer cohort (men = 3974; women = 4007), and (4,007) women in the serum folate breast cancer cohort.

For the purpose of the data analysis age was classified into 2 categories (60-79 years and ≥ 80 years). Race-ethnicity was classified into 4 categories: (non-Hispanic white, non-Hispanic black, Hispanic, other). Unmetabolized folic acid was analyzed for 3 separate markers: methytetrahydrofolate (pmol/mL), folic acid (pmol/mL), and total folate (pmol/mL) for both "all cancer", and "breast" cancer. Serum folate was divided into 4 quartiles; the participants were categorized into one of the 4 quartiles based on their serum folate level (i.e. individuals with the lowest concentration of serum folate were categorized into the 1st quartile, and individuals with the highest serum folate concentrations were categorized into the 4th quartile).

Measurements

Unmetabolized Folic Acid

Unmetabolized folic acid concentrations were determined using the affinity/HPLC method and the electrochemical detection method[50]. These methods were developed by NHANES at the Human Nutrition Research Center at Tufts University[50]. Total folate ascertained during this procedure is highly correlated ($r^2 = 0.93$, $p < 0.001$) with total folate obtained by microbial assay[50]. A detailed description of the laboratory methods is given elsewhere[50,51].

Serum Folate

Serum folate was ascertained by NHANES on participants aged 1 years of age older who were eligible for screening[52]. NHANES used two separate methods during the studies time period: 1999-2008. During the 4 NHANES data cycles 1999-2000, 2001-2002, 2003-2004, and 2005-2006 the following laboratory methodology was utilized. The Bio-Rad Laboratories "Quantaphase II folate/vitamin B12" radioassy kit was used to quantify serum folate in all samples obtianed[52]. During the NHANES 2007-2008 cycle participant's serum folate was measured using the microbiologic assay[53]. The assay was calibrated with 5-methlytetrahydrofolic acid from Epro[53]. A detailed description of the laboratory methodology was reported elsewhere by NHANES[51].

Medical Conditions

The medical conditions questionnaire data file provided personal interview data regarding an individual's medical condition, for both their current and past medical history[54]. Participant's medical conditions were collected and reported using a questionnaire modeled after the US National Health Interview Survey[54]. The medical conditions questionnaire used by NHANES collects a wide range of health related statistics for both children and adults[54]. The survey was conducted during the in-home interview portion of the survey[54]. The interviewers administer the questionnaire using the Computer Assisted Personal Interviewing – CAPI system[55]. The information regarding the data processing and editing participants responses has been previously reported elsewhere by NHANES[55,56].

Statistical analysis

To account for the complex survey design of NHANES the SUDAAN statistical software Windows (version 8.0.2: Research triangle Institute, Research Triangle Park, NC) was used[57]. According to the NHANES analytical and reporting guidelines sample weights, stratification, and clustering of the design was accounted for in the analysis to allow proper estimates and standard errors of the estimates to be obtained and prevent bias[58]. SAS statistical software for Windows (version 9.1; SAS Institute Inc., Cary, NC) was also used in conjunction with SUDAAN to extract and analyze the original NHANES data files[57]. Due to the unevenness of the distribution of the population sample across the examined categories, geometric means and standard errors of the geometric means were estimated using the Taylor Series Linearization method. The geometric means and standard errors were used to describe the unmetabolized folic acid in both men and women based on age (60-79, ≥80), sex, and race (non-Hispanic White (NHW), non-Hispanic Black (NHB), Mexican American/ Hispanic (MA/H) and others) for the study population. The geometric means and standard errors of the geometric means are presented as means ± SE.

To determine the risk of all cancer based on metabolized folic acid and serum folate concentrations, the prevalence rates (%) for individuals positive for cancer were calculated, and a logistic regression model was completed to estimate the odds ratio (OR), confidence interval (CI), and associated p-values to establish significance. A t-test was conducted to determine the significance between the geometric means of unmetabolized folic acid in individuals positive for cancer and individuals who were cancer free. To determine the risk for breast cancer among women >60, the prevalence

rate (%) of cancer for individuals who replied "yes" to the medical survey question "history of breast cancer" were calculated, and a logistic regression model was designed to estimate the odds ratio (OR), confidence (CI), and associated p-values to establish significance. All p-values were set at $p \leq 0.05$ for significance. The researchers considered age, sex, race-ethnicity, poverty income ratio, and alcohol consumption as potential confounders. The analysis models were adjusted for the confounders to ascertain a more precise relationship between the exposures (unmetabolized folic acid and serum folate) and the outcomes of interest (all cancer and breast cancer).

CHAPTER IV

RESULTS

Characteristics of the Study Population

The sample size for unmetabolized folic acid, all cancer, and breast cancer cohorts by gender, race-ethnicity, and age are presented in Table 1. In the unmetabolized folic acid cohort 43.5% men and 56.5% women had measurable unmetabolized folic acid. The highest percentage of unmetabolized folic acid for the ethnic category was in the non-Hispanic white accounting for 82% of the sample size, while non-Hispanic black, Hispanic, and Other were by and large similar in sample size. The subjects in the age group 60-79 years old represented the majority of the individuals with unmetabolized folic acid, 83.4%.

For the subjects in the all cancer cohort category, 44.2% were men and 55.8% were women. The ethnic groups non-Hispanic black, Hispanic, and Other accounted for the smallest fraction of the sample population. Non-Hispanic whites, represented the largest ethnic group in the study population, 81.8%. The majority of the individuals classified by age were between 60-79 years old making up 82.5% of the cohort.

In the breast cancer cohort, non-Hispanic whites (81.3%) and subjects aged between 60-79 years old (80%) represented the largest proportion of the subjects. While other ethnic groups and subjects ≥ 80 years old represented the smallest percentage of individuals analyzed.

Unmetabloized Folic Acid, Total Serum Folate, and All cancer Risk in the US

Data on unmetabolized folic acid and total serum folate concentrations in the US are presented in Table 2. The mean unmetabolized folic acid concentrations between cancer free and cancer cohorts are not statistically significant. Similarly, no significant difference was observed between cancer-free and cancer cohorts in serum folate and unmetabolized folic acid concentrations.

The likelihood of subjects with unmetabolized folic acid having cancer relative to individuals without unmetabolized folic acid is presented in Table 3. In subjects with unmetabolized folic acid, the prevalence of cancer was 24% compared to 19.2% in subjects without unmetabolized folic acid. In the unadjusted analysis individuals without the presence of unmetabolized folic acid were 0.76 times less likely to develop cancer compared to subjects with circulating unmetabolized folic acid ($p < 0.045$). In the multivariate adjusted analysis, similar findings were observed ($P = 0.05$; OR: 0.76, 95% CI: 0.58, 1.00)

Serum Folate Concentrations, All Cancer Risk, and Breast Cancer in the US

For the risk of all cancer and breast cancer according to serum folate concentrations; unadjusted ORs, multivariate adjusted ORs, and 95% CI are presented in Table 4 and Table 5, respectively. The prevalence of all cancer was highest in serum folate quartile 4 at 24.7%. In the unadjusted analysis, subjects in serum folate quartile 4 are 1.62 times more likely to have cancer compared to quartile 1 ($p < 0.000$, 95% CI: 1.30, 2.03). Similar trends are seen in quartile 2 (OR: 1.34, 95% CI: 1.12, 1.60, $p < 0.002$), but were not statistically significant for quartile 3. In the multivariate adjusted analysis, subjects in quartile 4 were 1.42 times more likely to have cancer than

individuals in quartile 1 ($p < 0.003$, 95% CI: 1.13, 1.78). A similar finding was noticed in quartile 2 (OR: 1.30, 95% CI: 1.08, 1.57, $p < 0.006$). However, the results for quartile 3 were not statistically significant when compared with the reference group, quartile 1 (Table 4).

Subjects with the highest prevalence of breast cancer were reported in serum folate quartile 4 at 22.2%. In the unadjusted analysis, individuals in serum folate quartile 4 are 1.53 times more likely to have breast cancer compared to subjects in quartile 1 ($p < 0.004$, 95% CI: 1.15, 2.02). Similarly, in the multivariate adjusted analysis, participants in serum folate quartile 4 were 1.86 times more likely to have cancer compared to the subjects in quartile 1 ($p < 0.027$, 95% CI: 1.08, 3.23). However, no significant difference was found between serum folate quartile 2 or quartile 3 when compared to serum folate quartile 1.

Table 1. Baseline Characteristics for All Subjects Analyzed in The NHANES Cycles 1999-2008[1]

	Sample size (n)[2]	(%)
Unmetabolized Folic Acid[3]		
Sex		
Men	1320	43.5%
Women	1362	56.5%
Race - ethnicity		
Non-Hispanic white	1514	81.2%
Non-Hispanic black	436	7.6%
Hispanic	678	8.4%
Other	54	2.8%
Age		
60-79	2086	83.4%
≥ 79	596	16.6%
	Sample size (n)[4]	(%)
All Cancer[5]		
Sex		
Men	3974	44.2%
Women	4007	55.8%
Race - ethnicity		
Non-Hispanic white	4631	81.8%
Non-Hispanic black	1358	7.9%
Hispanic	1788	6.7%
Other	204	3.5%
Age		
60-79	6222	82.5%
≥ 79	1759	17.5%
	Sample size (n)[6]	(%)
Breast Cancer[7]		
Sex		
Women	4007	
Race - ethnicity		
Non-Hispanic white	2277	81.3%
Non-Hispanic black	691	8.4%
Hispanic	932	6.8%
Other	107	3.5%
Age		
60-79	3072	80.0%
≥ 79	935	20.0%

[1] NHANES cycles 1999-2000, 2001-2002, 2003-2004, 2005-2006, 2007-2008 were used to obtain information regarding unmetabolized folic acid, serum folate concentrations, all cancer, and breast cancer.

[2] Total sample size for unmetabolized folic acid study population (n = 2682).

[3] NHANES cycles 1999-2000 and 2001-2002 were combined into one large database to analyze the association between unmetabolized folic acid and all cancer risk US.

[4] Total sample size for the All cancer study population (n = 7981).

[5] NHANES cycles 1999-2000, 2001-2002, 2003-2004, 2005-2006, 2007-2008 were combined into one large database to analyze the association between serum folate concentrations and all cancer risk in the US.

[6] Total sample size for the breast cancer study population (n = 4007).

[7] NHANES cycles 1999-2000, 2001-2002, 2003-2004, 2005-2006, 2007-2008 were combined into one large database to analyze the association between serum folate concentrations and breast cancer risk in the US.

Table 2. Unmetabolized Folic Acid (UMFA)[1] and Total Serum Folate Concentration for All Cancer Risk in The US[2]

	Sample size[3]	**Mean ± SE[4]**	**P-Value**
Unmetabolized Folic Acid pmol/L			
Total Cases[5]	2682	1.9 ± 0.17	
Cancer[6]	474	2.15 ± 0.36	0.70
Cancer – Free[7]	2210	1.83 ± 0.16	
Total Folate pmol/L[8]			
Total Cases	2682	42.41 ± 0.81	
Cancer	474	43.16 ± 1.42	0.45
Cancer - Free	2210	42.22 ± 0.80	

[1] UMFA references unmetabolized folic acid.

[2] NHANES cycles 1999-2000 and 2001-2002 were combined to create one large database to analyze the association between unmetabolized folic acid, serum folate concentrations and all cancer risk.

[3] The total sample size for the study population (n = 2682).

[4] Geometric mean ± standard error (SE) of the geometric mean were used to account for the unevenness of the population distribution.

[5] Total cases include subjects from both the cancer group and cancer – free group.

[6] Cancer signifies the study population who reported having cancer.

[7] Cancer – Free signifies the study population who reported not having cancer.

[8] Total Folate pmol/mL includes both circulating unmetabolized folic acid and methyltetrahydrofolate (enzyme form of circulating folate).

Table 3. Unmetabolized Folic Acid (UMFA)[1] and All Cancer Risk in The US[2]

	UMFA – Positive[3]	**UMFA – Negative[4]**
Total (n)[5]	700	1982
Cancer Yes (n)[6]	147	327
Prevalence (%)	21	16.5
UNADJ OR (95% CI)[7]	1.00	0.76 (0.57, 0.99)
P-Value	-	0.045
ADJ OR (95% CI)[8]	1.00	0.76 (0.58, 1.00)
P-Value	-	0.051

[1] UMFA references unmetabolized folic acid.

[2] NHANES cycles 1999-2000 and 2001-2002 were combined to create one large database to analyze the association between unmetabolized folic acid and all cancer risk in the US (n = 2682).

[3] UMFA – Positive indicates individuals who have measurable levels of unmetabolized folic acid.

[4] UMFA – Negative indicates individuals who do not have measurable levels of unmetabolized folic acid.

[5] Total (n) indicates the total sample size for both of the groups UMFA – Positive (n = 700), and UMFA – Negative (n = 1982).

[6]Cancer Yes indicates subjects in the study population who reported having cancer.

[7] UNADJ OR (95% CI) reports the unadjusted logistic regression odds ratio and corresponding confidence interval at a 95% significance level.

[8] ADJ OR (95% CI) reports the adjusted multivariate logistic regression odds ratio and corresponding confidence interval at a 95% significance level, the data was adjusted for the confounders: sex, age, poverty income ratio, alcohol and race-ethnicity.

Table 4. Serum Folate Concentrations and All Cancer Risk in the US[1]

	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**
Total (n)[2]	2288	2146	1810	1737
Cancer Yes (n)[3]	320	390	341	408
Prevalence (%)	14	18.2	18.8	23.5
UADJ OR (95% CI)[4]	1.00	1.34 (1.12, 1.60)	1.29 (1.06, 1.56)	1.42 (1.13, 2.03)
P-Value	-	0.002	0.011	<0.000
ADJ OR (95% CI)[5]	1.00	1.30 (1.08, 1.57)	1.19 (0.98, 1.46)	1.42 (1.13, 1.78)
P-Value	-	0.006	0.08	0.003

[1] NHANES cycles 1999-2000, 2001-2002, 2003-2004, 2005-2006, 2007-2008 were used to create one large database to analyze the association between serum folate concentrations and all cancer risk in the US.

[2] The total sample size for the study population (n = 7981).

[3] Cancer Yes indicates the subjects in the study population who have cancer.

[4] UNADJ OR (95% CI) reports the unadjusted logistic regression odds ratio and corresponding confidence interval at a 95% significance level.

[5] ADJ OR (95% CI) reports the adjusted multivariate logistic regression odds ratio and corresponding confidence interval at a 95% significance level, the data was adjusted for the confounders: sex, age, poverty income ratio, alcohol and race-ethnicity.

Table 5. Serum Folate Concentrations and Breast Cancer Risk in the US[1]

	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**
Total (n)[2]	933	1054	954	1006
Cancer Yes (n)[3]	36	55	57	80
Prevalence (%)	3.6	5.2	6	8
UADJ OR (95% CI)[4]	1.00	1.26 (0.98, 1.62)	1.27 (0.97, 1.65)	1.53 (1.15, 2.02)
P-Value	-	0.08	0.08	0.004
ADJ OR (95% CI)[5]	1.00	1.48 (0.96, 2.53)	1.56 (0.96, 2.53)	1.86 (1.08, 3.23)
P-Value	-	0.10	0.07	0.027

[1] NHANES cycles 1999-2000, 2001-2002, 2003-2004, 2005-2006, 2007-2008 were used to create one large database to analyze the association between serum folate concentrations and breast cancer risk in the US.

[2] The total sample size for the study population (n = 4007).

[3] Cancer Yes indicates the subjects in the study population who have cancer.

[4] UNADJ OR (95% CI) reports the unadjusted logistic regression odds ratio and corresponding confidence interval at a 95% significance level.

[5] ADJ OR (95% CI) reports the adjusted multivariate logistic regression odds ratio and corresponding confidence interval at a 95% significance level the data was adjusted for the confounders: sex, age, poverty income ratio, alcohol and race-ethnicity.

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CHAPTER V

DISSCUSSION

In this report, we present the first data on the relationship between unmetabolized folic acid and serum folate concentrations on the prevalence of overall cancer and breast cancer in the US using data from NHANES 1999-2008. When examining the 1999-2002, data, individuals without reported unmetabolized folic acid had a 24% reduction in the risk for developing all cancer ($p = 0.05$), compared to the subjects with reported unmetabolized folic acid, in both men and women. The results for serum folate and all cancer risk indicated that subjects in the highest quartile are 1.4 times more likely to have cancer compared to the individuals in the lower quartiles ($p<0.003$). Similar results were obtained for the relationship between breast cancer and serum folate concentrations. Women in the highest quartile reported being 1.9 times more likely to have breast cancer compared to the subjects in the lower quartiles ($p<0.027$).

Multiple studies have reported the presence of unmetabolized folic acid after the mandate to fortify cereal and unprocessed grains with folic acid in January 1998. It has been reported that 78% of postmenopausal women in the US have unmetabolized folic acid, and it accounts for approximately 16% of all folate found in blood plasma[4,23]. The data suggests that high unmetabolized folic acid causes a decrease in natural killer cytotoxicity leading to an increase in cancer by blocking the human body's natural immune response to destroy the malignant cells, leading to the propagation of tumors[16,23]. According to Troen et al., there is a strong relationship between postmenopausal

women, unmetabolized folic acid and decreased natural killer cytotoxicity[23]. The researchers postulate that the connection is significant because as individuals age there immune systems become weaker more susceptible to change, ultimately leading to diseases such as cancer[23].

The body of evidence relating folic acid with cancer has reported conflicting results regarding folate status and all cancer risk. Although the results are still debated, the research community agrees that the role of folate in cancer development depends strongly on the timing of an individual's onset for folate consumption and the presence of malignant cell formation [4]. Scientists using animal research studies to examine colorectal cancer have illustrated that when cancer cells are introduced to high levels of folic acid, tumor cells grow at an increased rate[29]. However during times of folate deficiency cellular metabolism is hindered, reducing the rate of DNA synthesis and replication, thereby decreasing the growth of tumors[29]. Mason et al[5], reported that the key role of folate in DNA synthesis is the same factor that makes it a growth factor for malignant cells. The results from the current study supports the finding made by Mason et al.[5], contributing to the spike in colorectal cancer seen in both the US and Canada in the late 1990's and early 2000's to the introduction of folic acid fortification.

The findings from 3 studies examining colorectal cancer cells in rats further confirm the finding that high levels of folic acid lead to cancer cell proliferation. All of the experiments used rat models to explore the relationship between folate deficiency and cancer cell growth; their results indicated that in rats fed a folate deficient diet intestinal, colon, and rectal malignant growths were significantly decreased[59], while rats fed average levels of folate (2.5 g/kg)[60], had enhanced tumor growth[60], showing a positive

relationship between folate intake and cancer outcomes[59,61,60]. The researchers hypothesized that the role of folate in modifying DNA methylation is a possible factor for folate enhancing colorectal malignant growths[59]. These findings further support the data suggesting high levels of serum folate concentrations pose a potential threat to older population cohorts[4].

Studies based on high serum folate concentrations and the prevalence of prostate cancer in older men further support the finding of all cancer. In a study by Tomaszewski et al.[61], it was reported that high serum folate concentrations were detected in males diagnosed with prostate cancer, they concluded that male patients with high blood plasma folate concentrations often tested positive for the Ki67 maker for prostate cancer. According to a randomized control trial, subjects given folic acid had a 9.7% increased chance of developing prostate cancer over the follow-up period of 10 years, compared to the control group, these findings are in agreement with reports stating that the increase in prostate cancer in men over the age of 50 is fueled by folic acid supplementation[62,31].

The findings related to breast cancer risk studies in postmenopausal women are consistent with the literature, however the cited literature also explored several potential factors that could affect the relationship between folic acid intake and breast cancer. The data from 2 large trails confers our findings; the cancer prevention study II reported women consuming the largest amount of folate showed a 12% higher prevalence of breast cancer when compared with the lowest intake group[3]. The ovarian cancer screening trial reported that women consuming folic acid had a 20% increased incident of cancer, and individuals consuming the highest levels of total folate intake were associated with a 32% prevalence of breast cancer; they concluded that women who consume high

amounts of folate have also been found to have higher folic acid concentrations in their breast tissue and that this could trigger the production of the folate receptor α, causing an increased growth rate of malignant cells compared to women consuming less folic acid[63,64,65].

According to a meta-analysis, a recent study reported the relationship between increased plasma folate concentrations and the development of estrogen receptor beta negative ($ER\beta^-$) and estrogen receptor alpha positive beta negative ($ER\alpha^+ \beta^-$) malignant breast formations[7]. A second study concluding a possible association between increased folate consumption, breast cancer, and estrogen receptor positive (ER+)[66] malignancies, but the same was not found in estrogen receptor negative (ER-)[66,67,68] tumor cells[7,66]. It has also been suggested that high folate status may be a contributing factor for cancer in women with a family history for breast cancer[7]. Finally, two studies have reported a possible connection between folate receptor polymorphisms (MTHFR C677T) and (MTR2756GG) with increased folate intake and prevalence of breast cancer[69,65,8].

The current study has several strengths; first we used multiple cycles of NHANES to yield a larger sample size so that the results can be generalized to the population at large. Second, laboratory values were used to report subject's level of serum folate and unmetabolized folic acid eliminating any bias that could be present when using dietary intake data. Finally, using serum folate concentrations allowed the researcher to establish a clearer understanding of the connection between folate intake and cancer risk.

However there are several limitations within the study design that need to be discussed. Because of the cross-sectional design used in this study the data shall not be looked at in terms of a cause and effect relationship. Secondly, due to the nature of the

secondary data used the researcher was unable to further investigate the association between the breast cancer status reported by the subjects (either ER+ or ER-) "not available" and folate intake.

Conclusion

Antifolate drugs have been used since the 1940's to combat the progression of cancer cells and block the cell receptors from binding with folate. The main finding of folate is the proliferation in one-carbon transfer reactions to support DNA synthesis, repair, methylation and replication. Individual in-vitro studies have shown the effect that folate deficiency has on hindering the growth and proliferation of established malignant tumor cells. In 1948, Faber discovered the "acceleration phenomenon" when treating leukemia with folic acid, causing the cancer cells to grow at an extremely enhanced speed[15,5]. Both the results from the current study and the literature support the concerns regarding subjects with undetectable malignant cells and the intake of folic acid. Individuals who are not part of the "target population" for the mandated folic acid fortification need to take special consideration with the amounts of folic acid they consume. Recommendations to decrease and avoid excess folic acid intake should be considered for patients who have a history of conditions that are risk factors for cancer, especially, diseases of the intestines, colon, and prostate, previous history of cancer, or are currently undergoing cancer treatment. Overall the literature supports the findings in the study; that older individuals in the US who have high folate status and unmetabolized folic acid are at a higher risk for all cancer, and specifically breast cancer.

REFERENCES

1. Bailey RL, Mills JL, Yetley EA, et al. Unmetabolized serum folic acid and its relation to folic acid intake from diet and supplements in a nationally representative sample of adults aged > or =60 y in the United States. *Am. J. Clin. Nutr.* 2010;92(2):383–389.

2. Dietrich M, Brown CJP, Block G. The effect of folate fortification of cereal-grain products on blood folate status, dietary folate intake, and dietary folate sources among adult non-supplement users in the United States. *J Am Coll Nutr*. 2005;24(4):266–274.

3. Stevens VL, McCullough ML, Sun J, Gapstur SM. Folate and other one-carbon metabolism-related nutrients and risk of postmenopausal breast cancer in the Cancer Prevention Study II Nutrition Cohort. *Am. J. Clin. Nutr.* 2010;91(6):1708–1715.

4. Smith AD, Kim Y-I, Refsum H. Is folic acid good for everyone? *Am. J. Clin. Nutr.* 2008;87(3):517–533.

5. Mason JB, Dickstein A, Jacques PF, et al. A temporal association between folic acid fortification and an increase in colorectal cancer rates may be illuminating important biological principles: a hypothesis. *Cancer Epidemiol. Biomarkers Prev.* 2007;16(7):1325–1329.

6. National Center for Health Statistics. National Health and Nutrition Examination Survey - About the National Health and Nutrition Examination Survey. *Centers for Disease Control and Prevention Homepage, NHANES*. Available at: http://www.cdc.gov/nchs/nhanes/about_nhanes.htm. Accessed March 7, 2013.

7. Kotsopoulos J, Kim Y-I, Narod SA. Folate and breast cancer: what about high-risk women? *Cancer Causes Control*. 2012;23(9):1405–1420.

8. Ericson UC, Ivarsson MIL, Sonestedt E, et al. Increased breast cancer risk at high plasma folate concentrations among women with the MTHFR 677T allele. *Am. J. Clin. Nutr.* 2009;90(5):1380–1389.

9. Keijer J, Bekkenkamp-Grovenstein M, Venema D, Dommels YEM. Bioactive food components, cancer cell growth limitation and reversal of glycolytic metabolism. *Biochim. Biophys. Acta*. 2011;1807(6):697–706.

10. Clapin HF, Fritschi L, Iacopetta B, Heyworth JS. Dietary and supplemental folate and the risk of left- and right-sided colorectal cancer. *Nutr Cancer*. 2012;64(7):937–945.

11. Kim Y-I. 5,10-Methylenetetrahydrofolate reductase polymorphisms and pharmacogenetics: a new role of single nucleotide polymorphisms in the folate metabolic pathway in human health and disease. *Nutr. Rev.* 2005;63(11):398–407.

12. Kim Y-I. Will mandatory folic acid fortification prevent or promote cancer? *Am. J. Clin. Nutr.* 2004;80(5):1123–1128.

13. Ganji V, Kafai MR. Trends in serum folate, RBC folate, and circulating total homocysteine concentrations in the United States: analysis of data from National Health and Nutrition Examination Surveys, 1988-1994, 1999-2000, and 2001-2002. *J. Nutr.* 2006;136(1):153–158.

14. Robien K. Folate during antifolate chemotherapy: what we know... and do not know. *Nutr Clin Pract*. 2005;20(4):411–422.

15. Tisman G, Garcia A. Control of prostate cancer associated with withdrawal of a supplement containing folic acid, L-methyltetrahydrofolate and vitamin B12: a case report. *J Med Case Rep*. 2011;5:413.

16. Baggott JE, Oster RA, Tamura T. Meta-analysis of cancer risk in folic acid supplementation trials. *Cancer Epidemiol*. 2012;36(1):78–81.

17. Kelly P, McPartlin J, Goggins M, Weir DG, Scott JM. Unmetabolized folic acid in serum: acute studies in subjects consuming fortified food and supplements. *Am. J. Clin. Nutr.* 1997;65(6):1790–1795.

18. Lee JE, Willett WC, Fuchs CS, et al. Folate intake and risk of colorectal cancer and adenoma: modification by time. *Am. J. Clin. Nutr.* 2011;93(4):817–825.

19. Bailey RL, Mills JL, Yetley EA, et al. Serum unmetabolized folic acid in a nationally representative sample of adults ≥60 years in the United States, 2001-2002. *Food Nutr Res*. 2012;56.

20. Duffy CM, Assaf A, Cyr M, et al. Alcohol and folate intake and breast cancer risk in the WHI Observational Study. *Breast Cancer Res. Treat.* 2009;116(3):551–562.

21. Nagothu KK, Rishi AK, Jaszewski R, Kucuk O, Majumdar APN. Folic acid-mediated inhibition of serum-induced activation of EGFR promoter in colon cancer cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2004;287(3):G541–546.

22. Marian C, Tao M, Mason JB, et al. Single nucleotide polymorphisms in uracil-processing genes, intake of one-carbon nutrients and breast cancer risk. *Eur J Clin Nutr*. 2011;65(6):683–689.

23. Troen AM, Mitchell B, Sorensen B, et al. Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. *J. Nutr.* 2006;136(1):189–194.

24. Shabbeer S, Williams SA, Simons BW, Herman JG, Carducci MA. Progression of prostate carcinogenesis and dietary methyl donors: temporal dependence. *Cancer Prev Res (Phila)*. 2012;5(2):229–239.

25. Milner JA, McDonald SS, Anderson DE, Greenwald P. Molecular targets for nutrients involved with cancer prevention. *Nutr Cancer*. 2001;41(1-2):1–16.

26. Jennings E. Folic acid as a cancer-preventing agent. *Med. Hypotheses*. 1995;45(3):297–303.

27. Duthie SJ. Folic-acid-mediated inhibition of human colon-cancer cell growth. *Nutrition*. 2001;17(9):736–737.

28. Chapkin RS, Kamen BA, Callaway ES, et al. Use of a novel genetic mouse model to investigate the role of folate in colitis-associated colon cancer. *J. Nutr. Biochem.* 2009;20(8):649–655.

29. Harris HR, Bergkvist L, Wolk A. Folate intake and breast cancer mortality in a cohort of Swedish women. *Breast Cancer Res. Treat.* 2012;132(1):243–250.

30. HEINLE RW, WELCH AD. Experiments with pteroylglutamic acid and pteroylglutamic acid deficiency in human leukemia. *J. Clin. Invest.* 1948;27(4):539.

31. Bistulfi G, Foster BA, Karasik E, et al. Dietary folate deficiency blocks prostate cancer progression in the TRAMP model. *Cancer Prev Res (Phila)*. 2011;4(11):1825–1834.

32. Nina Roswall. Folate and Lung Cancer Risk. *ElSEVIER - Lung Cancer*. 2009;(67):380–381.

33. Inoue-Choi M, Ward MH, Cerhan JR, Weyer PJ, Anderson KE, Robien K. Interaction of nitrate and folate on the risk of breast cancer among postmenopausal women. *Nutr Cancer*. 2012;64(5):685–694.

34. Ericson U, Borgquist S, Ivarsson MIL, et al. Plasma folate concentrations are positively associated with risk of estrogen receptor beta negative breast cancer in a Swedish nested case control study. *J. Nutr.* 2010;140(9):1661–1668.

35. Shrubsole MJ, Shu XO, Li H-L, et al. Dietary B vitamin and methionine intakes and breast cancer risk among Chinese women. *Am. J. Epidemiol.* 2011;173(10):1171–1182.

36. Lee Y, Lee S-A, Choi J-Y, et al. Prognosis of breast cancer is associated with one-carbon metabolism related nutrients among Korean women. *Nutrition Journal*. 2012;11(1):59.

37. National Center for Health Statistics. National Health and Nutrition Examination Survey 2001-2002 Public Data General Release File Documentation. *Centers for Disease Control and Prevention Homepage, NHANES*. Available at: http://www.cdc.gov/nchs/data/nhanes/nhanes_01_02/general_data_release_doc.pdf. Accessed April 1, 2013.

38. National Center for Health Statistics. National Health and Nutrition Examination Survey 1999-2000. *Centers for Disease Control and Prevention Homepage, NHANES*. Available at: http://wwwn.cdc.gov/nchs/nhanes/search/nhanes99_00.aspx. Accessed April 10, 2013.

39. National Center for Health Statistics. National Health and Nutrition Examination Survey 2001-2002. *Centers for Disease Control and Prevention Homepage, NHANES*. Available at: http://wwwn.cdc.gov/nchs/nhanes/search/nhanes01_02.aspx. Accessed April 11, 2013.

40. National Center for Health Statistics. National Health and Nutrition Examination Survey 1999-2000 - Manuals, Brochures, and Consent Documents. *Center for Disease Control and*

Prevention Homepage, NHANES. Available at:
http://www.cdc.gov/nchs/nhanes/nhanes1999-2000/current_nhanes_99_00.htm.
Accessed April 11, 2013.

41. National Center for Health Statistics. National Health and Nutrition Examination Survey 2001-2002 - Manuals, Brochures, and Consent Documents. *Centers for Disease Control and Prevention Homepage, NHANES*. Available at:
http://www.cdc.gov/nchs/nhanes/nhanes2001-2002/current_nhanes_01_02.htm.
Accessed April 11, 2013.

42. National Center for Health Statistics. National Health and Nutrition Examination Survey 2003-2004. *Centers for Disease Control and Prevention Homepage, NHANES*. Available at:
http://wwwn.cdc.gov/nchs/nhanes/search/nhanes03_04.aspx. Accessed May 7, 2013.

43. National Center for Health Statistics. National Health and Nutrition Examination Survey 2005-2006. *Centers for Disease Control and Prevention Homepage, NHANES*. Available at:
http://wwwn.cdc.gov/nchs/nhanes/search/nhanes05_06.aspx. Accessed May 7, 2013.

44. National Center for Health Statistics. National Health and Nutrition Examination Surevy 2007-2008. *Centers for Disease Control and Prevention Homepage, NHANES*. Available at:
http://wwwn.cdc.gov/nchs/nhanes/search/nhanes07_08.aspx. Accessed May 7, 2013.

45. National Center for Health Statistics. National Health and Nutrition Examination 1999-2000 Unweighted response rates by age and gender. *Centers for Disease Control and Prevention Homepage, NHANES*. Available at:
http://www.cdc.gov/nchs/nhanes/response_rates_CPS.htm. Accessed April 9, 2013.

46. National Center for Health Statistics. National Health and Nutrition Examination Survey 2001-2002 Unweighted Response Rates by age and gender. *Centers for Disease Control and Prevention Homepage, NHANES*. Available at:
http://www.cdc.gov/nchs/nhanes/response_rates_CPS.htm. Accessed April 9, 2013.

47. National Center for Health Statistics. National Health and Nutrition Examination Survey 2003-2004 Unweighted response rates by age and gender. *Centers for Disease Control and Prevention Homepage, NHANES*. Available at:
http://www.cdc.gov/nchs/nhanes/response_rates_CPS.htm. Accessed April 9, 2013.

48. National Center for Health Statistics. National Health and Nutrition Examination Survey 2005-2006 Unweighted Response Rates by age and gender. *Centers for Disease Control and Prevention Homepage, NHANES*. Available at:
http://www.cdc.gov/nchs/nhanes/response_rates_CPS.htm. Accessed April 9, 2013.

49. National Center for Health Statistics. National Health and Nutrition Examination Survey 2007-2008 Unweighted Response Rates by age and gender. *Centers for Disease Control and Prevention Homepage, NHANES*. Available at:
http://www.cdc.gov/nchs/nhanes/response_rates_CPS.htm. Accessed April 9, 2013.

50. National Center for Health Statistics. National Health and Nutrition Examination Survey 1999 - 2000: Unmetabolized Folic Acid (Surplus Sera) Data Documentation, Codebook, and Frequencies. *Centers for Disease Control and Prevention Homepage, NHANES*. Available at:

http://www.cdc.gov/nchs/nhanes/nhanes1999-2000/SSFOL_A.htm. Accessed April 10, 2013.

51. National Center for Health Statistics. National Health and Nutrition Examination Survey Laboratory Procedure Manual: Folate/Vitamin B12, serum and Whole Blood - Bio-Rad Laboratories "Quantaphase II Folate/Vitamin B12" radioassay kit. *Centers for Disease Control and Prevention Homepage, NHANES*. Available at: http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/l06_c_met_folates%20B12.pdf. Accessed February 20, 2013.

52. National Center for Health Statistics. National Health and Nutrition Examination Survey 2001 - 2002: Blood Lead and Cadmium, Ferritin, Serum Folate, RBC Folate, Vitamin B12, Homocysteine, Total Mercury, Methylmalonic acid, Cotinine (Second Day) Data Documentation, Codebook, and Frequencies. *Centers for Disease Control and Prevention Homepage, NHANES*. Available at: http://www.cdc.gov/nchs/nhanes/nhanes2001-2002/L06_2_B.htm. Accessed April 10, 2013.

53. National Center for Health Statistics. National Health and Nutrition Examination Survey 2007 - 2008: RBC Folate and Serum Folate Data Documentation, Codebook, and Frequencies. *Centers for Disease Control and Prevention Homepage, NHANES*. Available at: http://www.cdc.gov/nchs/nhanes/nhanes2007-2008/FOLATE_E.htm. Accessed May 5, 2013.

54. National Center for Health Statistics. National Health and Nutrition Examination Survey 2001 - 2002: Medical Conditions Data Documentation, Codebook, and Frequencies. *Centers for Disease Control and Prevention Homepage, NHANES*. Available at: http://www.cdc.gov/nchs/nhanes/nhanes2001-2002/MCQ_B.htm. Accessed April 10, 2013.

55. National Center for Health Statistics. National Health and Nutrition Examination Survey 2005 - 2006: Medical Conditions Data Documentation, Codebook, and Frequencies. *Centers for Disease Control and Prevention Homepage, NHANES*. Available at: http://www.cdc.gov/nchs/nhanes/nhanes2005-2006/MCQ_D.htm. Accessed April 10, 2013.

56. National Center for Health Statistics. National Health and Nutrition Examination Survey 2001-2002 - Survey Questionnaire and Exam Components. *Centers for Disease Control and Prevention Homepage, NHANES*. Available at: http://www.cdc.gov/nchs/nhanes/nhanes2001-2002/questexam01_02.htm. Accessed April 30, 2013.

57. Ganji V, Kafai MR. Hemoglobin and hematocrit values are higher and prevalence of anemia is lower in the post–folic acid fortification period than in the pre–folic acid fortification period in US adults. *Am J Clin Nutr*. 2009;89(1):363–371.

58. National Center for Health Statistics; CDC. Analytic & reporting Guidelines [The National Health & Nutrition Examination Survey (NHANES)]. *Centers for Disease Control and Prevention*. Available at: http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/nhanes_analytic_guidelines_dec_2005.pdf. Accessed April 10, 2013.

59. Le Leu RK, Young GP, McIntosh GH. Folate deficiency reduces the development of colorectal cancer in rats. *Carcinogenesis*. 2000;21(12):2261–2265.

60. Le Leu RK, Young GP, McIntosh GH. Folate deficiency diminishes the occurrence of aberrant crypt foci in the rat colon but does not alter global DNA methylation status. *J. Gastroenterol. Hepatol.* 2000;15(10):1158–1164.

61. Tomaszewski JJ, Cummings JL, Parwani AV, et al. Increased cancer cell proliferation in prostate cancer patients with high levels of serum folate. *Prostate*. 2011;71(12):1287–1293.

62. Figueiredo JC, Grau MV, Haile RW, et al. Folic acid and risk of prostate cancer: results from a randomized clinical trial. *J. Natl. Cancer Inst.* 2009;101(6):432–435.

63. Stolzenberg-Solomon RZ, Chang S-C, Leitzmann MF, et al. Folate intake, alcohol use, and postmenopausal breast cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *Am. J. Clin. Nutr.* 2006;83(4):895–904.

64. Kim Y-I. Does a high folate intake increase the risk of breast cancer? *Nutr. Rev.* 2006;64(10 Pt 1):468–475.

65. Ma E, Iwasaki M, Junko I, et al. Dietary intake of folate, vitamin B6, and vitamin B12, genetic polymorphism of related enzymes, and risk of breast cancer: a case-control study in Brazilian women. *BMC Cancer*. 2009;9:122.

66. Zhang SM, Hankinson SE, Hunter DJ, Giovannucci EL, Colditz GA, Willett WC. Folate intake and risk of breast cancer characterized by hormone receptor status. *Cancer Epidemiol. Biomarkers Prev.* 2005;14(8):2004–2008.

67. Sellers TA, Vierkant RA, Cerhan JR, et al. Interaction of dietary folate intake, alcohol, and risk of hormone receptor-defined breast cancer in a prospective study of postmenopausal women. *Cancer Epidemiol. Biomarkers Prev.* 2002;11(10 Pt 1):1104–1107.

68. Maruti SS, Ulrich CM, White E. Folate and one-carbon metabolism nutrients from supplements and diet in relation to breast cancer risk. *Am. J. Clin. Nutr.* 2009;89(2):624–633.

69. Kotsopoulos J, Sohn K-J, Martin R, et al. Dietary folate deficiency suppresses N-methyl-N-nitrosourea-induced mammary tumorigenesis in rats. *Carcinogenesis*. 2003;24(5):937–944.