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Maternal Nutrition and Risk of Leukemia in Children

By

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ABSTRACT

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Acute leukemia is the most common type of cancer that occurs in children. Because many cases of childhood leukemia originate *in utero,* it has been hypothesized that maternal nutrition before or during pregnancy may influence the development of leukemia through its role in fetal development. Findings from studies examining the association of childhood leukemia with maternal diet before and during pregnancy have been inconsistent. Because dietary factors are known to influence the genetic and epigenetic processes involved in the development of childhood cancer, and because diet is a modifiable risk factor, maternal prenatal nutrition continues to be of interest in the etiology of childhood leukemia.

This study examined the association between maternal diet and vitamin supplement use before and during pregnancy as assessed by a food frequency questionnaire in a large and ethnically-diverse population-based case-control study, the California Childhood Leukemia Study (CCLS). Analyses were conducted with original dietary data, and employed principal components (PC) analysis to account for the high correlations between nutrients. Higher maternal intake of one-carbon metabolism nutrients from food and supplements was associated with a reduced risk of acute lymphoblastic leukemia (ALL) (odds ratio for the principal component (OR_{PC}) = 0.91, confidence interval (CI) 0.84-0.99) and possibly acute myeloid leukemia (AML) (OR_{PC} = 0.83, CI 0.66-1.04). The association of ALL with nutrient intake from food only was similar to the association of total nutrient intake from food and supplements, both in the study population overall and within racial/ethnic groups. However, intake of B vitamins from supplements (any versus none) was associated with a reduced risk of ALL in children of Hispanic women (OR=0.51, CI 0.28-0.94), but not in children of non-Hispanic white women (OR = 0.91, CI 0.64-1.31) or Asian women (OR = 2.24, CI 0.91-5.51).

The possible influence of exposure misclassification and selection bias on the associations observed between maternal prenatal use of vitamin supplements and risk of childhood leukemia was qualitatively and quantitatively assessed. Case and control participation and exposure assessment was reviewed in twelve published studies examining the association of childhood leukemia with maternal vitamin supplement use. The review found that most studies had low control participation, and controls generally had higher socioeconomic status than participating cases. Additionally, half of the included studies examined broad categories of vitamin supplements (e.g. prenatal vitamins) and few asked about brand or frequency of consumption. The quantitative bias analysis conducted in the CCLS data indicated that, under the assumed bias

parameters, selection bias and exposure misclassification were unlikely to account for the association of vitamin supplement use and ALL observed in CCLS Hispanic women. The measures of association corrected for these systematic errors among non-Hispanic white women varied widely. However, all exposure misclassification corrections for higher sensitivity in cases (hypothesized to be the more common scenario in case-control studies) produced negative associations further away from the null value than the uncorrected estimate. In conclusion, relatively modest differences in systematic errors between Hispanic and white women could account for the heterogeneity observed in the association between vitamin supplement use and ALL by maternal ethnicity.

In addition, examination of the relationship between overall maternal diet quality, summarized by a diet quality index, and risk of childhood leukemia found that higher maternal diet quality score was associated with a reduced risk of childhood ALL (OR = 0.88, CI 0.78-0.98 for each five point increase), with a more pronounced reduction in risk observed among younger children and children of women who did not use vitamin supplements before pregnancy. There was a similar reduced risk of AML with increasing maternal diet quality score, although the confidence interval include 1.0 (OR = 0.76, CI 0.52-1.11). No single diet quality index component (i.e. food group or nutrient) appeared to account for the results, suggesting that the quality of the whole diet and the cumulative effects of many dietary components may be important in influencing childhood leukemia risk.

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Chapter 1:

Introduction

INTRODUCTION

Acute leukemia, the most common type of cancer occurring in children, is an aggressive malignancy originating from lymphoid and myeloid progenitor cells in the bone marrow [1, 2]. The annual incidence of childhood leukemia is 3 to 4 cases per 100,000 children, with around 2,200 cases diagnosed each year in the United States [3, 4]. Acute leukemia is a heterogeneous disease, and marked variation in the incidence of leukemia subtypes by age and race/ethnicity suggests distinct etiologies for specific forms of the disease [5]. Most cases of childhood leukemia are lymphocytic and peak in incidence from 2-6 years of age, although leukemias of myeloid and T-cell lineage and other subtypes also occur less frequently among children [1]. The age-adjusted incidence rate of childhood leukemia is highest in children of Hispanic ethnicity, and Surveillance, Epidemiology and End Results (SEER) registry data from 1992-2011 suggest that incidence rates have been rising in this population in particular [6]. Enormous advances have been made in the treatment of childhood leukemia, with survival now reaching approximately 90%, although certain subtypes have poorer outcomes [7].

Extensive epidemiologic research on childhood leukemia over the past several decades has led to the identification of several risk factors for disease, including ionizing radiation [4], parental exposure to pesticides [8-10], pre-conception paternal smoking [11, 12], and high birth weight [13-15]. The evidence regarding many other environmental risk factors of interest, such as exposure to electromagnetic fields, is inconclusive [7]. Advances have also been made in identifying inherited susceptibility to childhood leukemia, with candidate-gene association studies finding associations of acute lymphoblastic leukemia (ALL) with genes involved in folate metabolism, xenobiotic transfer and metabolism, DNA repair, and immune response [16]. One prominent etiologic hypothesis for ALL is that an aberrant immune response to infection serves as the postnatal oncogenic "hit" that induces development of disease: specifically, the decrease in or absence of infections in early life in more affluent, hygienic societies may result in a pathological immune response to common bacterial or viral infections experienced later in childhood [17]. Epidemiologic research indicating a protective effect of daycare attendance (known to increase exposure to common infections early in life), later birth order, and breastfeeding have supported this hypothesis [17-21].

Research has increasingly pointed to the crucial role of the intrauterine environment in determining risk of disease later in life [22, 23]. The "developmental origins of health and disease" hypothesis posits that nutritional and environmental exposures *in utero* permanently alter gene expression and the physical development of the fetus through a process called "programming" [24, 25]. There are strong reasons to believe that these developmental processes may influence the risk of cancer in children. Many of the genetic abnormalities involved in childhood leukemia are initiated *in utero*, as indicated by the identification of leukemia-related chromosomal alterations in newborn blood spots [26, 27]. These pre-leukemic chromosomal translocations are insufficient for the development of acute leukemia in all cases, indicating that additional prenatal or postnatal oncogenic events are often required for the development of disease [26, 27]. The relatively low concordance of leukemia in monozygotic twins, estimated to be around five percent, also points to the importance of environmental factors in determining disease risk [27].

Genetic variation and epigenetic modifications may play a role in the additional oncogenic events that instigate the development of leukemia among children with pre-leukemic clones (i.e. a population of cells with early mutations that accumulate in a single cell lineage and may ultimately become cancer) [28]. Epigenetics refers to heritable changes in gene expression that are independent of changes in the primary DNA sequence, occurring through the mechanisms of DNA methylation, histone modification, and the expression of non-coding RNAs (miRNAs) [29]. Fetal establishment and developmental programming of the epigenome occurs during embryogenesis and is modified in response to external factors such as environmental exposures and internal factors such as maternal characteristics [30, 31]. While genetic abnormalities have traditionally been recognized as central to cancer progression, it is now understood that epigenetic modifications are also common to many cancers [29]. A cancer epigenome is characterized by genome-wide hypomethylation, which increases genomic instability and can activate growth-promoting genes (i.e. proto-oncogenes), as well as site-specific hypermethylation, which can silence tumor suppressor and DNA repair genes [29]. A common epigenetic "signature" has been identified for ALL, with distinct DNA methylation signatures further characterizing ALL subtypes [32, 33].

Maternal Diet and Childhood Leukemia

Diet is strongly linked to risk of a variety of cancers and is considered an important lifestyle factor that can be modified to reduce cancer risk [34, 35]. Diet may increase cancer risk through exposure to dietary mutagens or mutagenesis due to nutrient deficiencies [36]. Micronutrients and other dietary components may also protect against the development of cancer by supporting cellular integrity, reducing inflammation and improving immune response [36-38]. A growing body of research suggests that another mechanism by which diet influences cancer risk is through the influence of particular nutrients on epigenetic processes [35, 39, 40] .

Maternal diet during pregnancy may inhibit or encourage the development of leukemia through these mechanisms and its influence on fetal development. Many studies have examined the association between maternal diet during pregnancy and risk of childhood leukemia. Maternal dietary exposures of primary interest have included inhibitors of topoisomerase II (a nuclear enzyme involved in DNA replication), coffee and tea, alcohol, food groups (e.g. fruits, vegetables, protein), and specific micronutrients such as folate.

Topoisomerase II Inhibitors

The use of topoisomerase II inhibitors in chemotherapy treatment of malignancies such as non-Hodgkin's lymphoma and neuroblastoma is associated with common *MLL* gene translocations associated with therapy-related acute myeloid leukemias [41, 42]. Consequently, it was hypothesized that topoisomerase inhibitors may be involved in the etiology of infant leukemia, which commonly involves the same *MLL* gene translocation identified in these therapy-related leukemias [43]. Topoisomerase inhibitors are found in diverse sources including chemotherapeutic agents, herbal medicines, quinolone antibiotics, certain types of laxatives, and pesticides [44]. Certain dietary sources also contain topoisomerase inhibitors including, but not limited to, tea, coffee, wine, and certain fruits and vegetables [44]. A small exploratory study examining maternal exposure to topoisomerase inhibitors during pregnancy and risk of childhood leukemia found an increased risk of acute myeloid leukemia (AML) with increasing

topoisomerase II inhibitor exposure (N=29 cases, OR= 10.2 (95% CI 1.1-96.4) for high exposure), but no increased risk for ALL (N=82, OR= 1.1 (95% CI 0.5-2.3) [43]. Subsequent studies have found no association between dietary intake of topoisomerase II inhibitors and risk of ALL [45] or infant AML, with the exception of the AML(MLL+) subtype [46]. Recent laboratory evidence indicates that dietary flavonoids have various types and degrees of topo II interference [47, 48], and that the flavonols found in tea, wine and cocoa (which account for the majority of dietary flavonoid intake) do not inhibit topoisomerase activity *in vitro* and thus are unlikely to increase the risk of translocations related to topo II inhibitors [48].

Coffee or Tea

Some studies have examined the effect of coffee and tea consumption during pregnancy on risk of childhood leukemia. An early case-control study of 280 cases and 288 hospitalized controls found an increased risk of ALL among children of mothers reporting coffee consumption greater than four cups a day during pregnancy (OR = 2.4, 95% CI 1.3-4.7 for 4-8 cups, and OR = 3.1, 95% CI 1.0-9.5 for >8 cups), with similar ORs observed for acute non-lymphoblastic leukemia (ANLL) that did not reach statistical significance [49]. Some subsequent case-control studies have found maternal consumption of coffee during pregnancy to be associated with an increased risk of ALL [11, 50], AML [50], and possibly infant leukemia [43], while others have failed to find an association [51-53]. There is some evidence from these studies that the increased risk of leukemia with maternal coffee consumption may be more pronounced among children born to non-smoking mothers [50, 52]. Several studies failed to find an association of childhood leukemia with maternal tea consumption during pregnancy, although most ORs were less than 1.0 [11, 49, 50, 52].

Alcohol

Maternal alcohol intake before or during pregnancy has been hypothesized to influence childhood leukemia risk by altering immune function or by teratogenic effects on cell differentiation [54]. Alcohol is also an antagonist to folate metabolism and methionine synthase and may modify DNA methylation status in interaction with folate levels [55, 56]. A systematic review and meta-analysis of 21 case-control studies found that alcohol intake during pregnancy was associated with AML (summary OR=1.56, 95% CI 1.13-2.15 produced from 9 studies comprising 731 cases) but not with ALL (summary OR=1.10, 95% CI 0.93-1.29 produced from 11 studies comprising 5,108 cases) [56]. Heterogeneity between studies was explained in part by some studies [53, 57] that demonstrated a negative association of childhood leukemia with maternal alcohol consumption during pregnancy. Sub-group analyses in the meta-analysis indicated an increased risk of AML with reported consumption of wine but not beer or spirits, providing some support for the topo II hypothesis [56]. For ALL, there was an association between maternal consumption of spirits during pregnancy, but not beer or wine [56]. One subsequent study supported the finding of an increased risk of AML with maternal alcohol consumption during pregnancy [11] while another did not find an association [50]; neither found a relationship between maternal alcohol consumption and ALL. In contrast, two recent studies found that maternal consumption of alcohol during pregnancy was associated with a decreased risk of ALL [58] and of infant leukemia [59].

Food Groups

Fruits and vegetables contain a variety of vitamins and minerals that have anti-cancer, anti-proliferative, and anti-inflammatory effects [35], and negative associations of fruit and vegetable consumption with risk of various types of cancer have been observed [34]. Some food groups, in particular fruits and vegetables, have been associated with childhood leukemia risk in several studies. Research has found statistically significant negative associations between maternal consumption of fruits and vegetables and risk of childhood ALL [45, 60, 61], and one study found significant or near-significant inverse linear trends associated with fresh fruit and vegetable consumption and risk of infant leukemia, especially specific ALL subtypes [46]. Negative associations have also been observed for childhood leukemia and maternal consumption of other food groups, specifically protein sources such as fish and seafood [60] and beans and beef [45, 61]. One study demonstrated an increased risk of ALL with increased maternal consumption of meat or meat products and sugars or syrups [60], and a recent study found an increased risk of ALL with maternal consumption of cola beverages during pregnancy [50].

Folate and Other One-Carbon Metabolism Nutrients

Folate and other B vitamins are critical cofactors in the one-carbon metabolism cycle that influences DNA synthesis and epigenetic processes [62], and maternal folic acid supplementation during pregnancy has been demonstrated to influence DNA methylation in children [55, 63]. Maternal folic acid supplementation is also associated with the development of childhood diseases, such as neural tube defects [64]. High folate intake has been associated with reduced risk of breast [65] and colorectal [66] cancer and increased risk of prostate cancer [67] among adults, while other meta-analyses have indicated no effect of folic acid supplementation on adult cancer incidence [67-69].

A small, exploratory case-control study of 83 ALL cases and 166 controls in Western Australia found a strong negative association of common ALL with maternal use of iron or folic acid supplements during pregnancy (OR = 0.37, 95% CI 0.21-0.65) [70]. Subsequent observational studies examining maternal folic acid intake through vitamin supplements and risk of childhood leukemia have demonstrated mixed findings, with some finding negative associations and others finding no relationship [71]. An analysis by the Childhood Leukemia International Consortium (CLIC) pooled findings from individual studies and found that maternal use of folic acid supplements was associated with a reduced risk of ALL (OR = 0.80, 95% CI 0.71-0.89) and AML (OR = 0.68, 95% CI 0.48-0.96), after adjustment for study center and other covariates [71]. Fewer studies have examined the influence of maternal folate and B vitamin intake from foods. Three studies from the California Childhood Leukemia Study (CCLS) examining the total intake of folate, vitamin B6 or vitamin B12 from both diet and supplements found no associations with ALL [45, 61, 72]. A case-control study in Australia found some evidence that higher dietary intakes of folate and B12 from food in the last six months of pregnancy were associated with a decreased risk of ALL, whereas higher dietary intakes of vitamin B6 were unexpectedly associated with an increased risk of ALL [73].

A CCLS analysis of 313 incident ALL cases, 44 incident AML cases, and 405 matched population-based controls found that blood folate concentration at birth, as measured in archived dried bloodspots and thought to be reflective of the child's folate exposure at the end of pregnancy as well as the child's folate metabolism, was not associated with risk of childhood

leukemia [74]. Newborn and child serum nutrient levels are influenced by many factors, including maternal and child genetic polymorphisms [75-77]. Many studies have found significant associations between single nucleotide polymorphisms (SNPs) in folate-related genes and childhood leukemia, but there are inconsistencies in the specific SNPs that have been identified across studies [72, 78-82]. A CCLS analysis of genetic variation in case and control children found statistically significant associations between SNPs in genes in the folate pathway (i.e. *CBS*, *MTRR*, and *TYMS/ENOFS*) and childhood ALL, and levels of maternal folate intake and child Hispanic ethnicity were found to modify some of these associations [72]. Other studies have not found evidence that the associations of folate pathway SNPs and childhood leukemia are modified by maternal folic acid supplementation [79, 83, 84].

In conclusion, there remains substantial uncertainty regarding the relationship between maternal diet during pregnancy and the risk of childhood leukemia, with a substantial body of research reporting largely inconsistent findings. The incidence of childhood leukemia differs among racial and ethnic groups, with the highest incidence of ALL observed among Hispanics [85], and the effect of various exposures in etiologic studies of childhood leukemia has also been found to differ by ethnicity [86]. However, despite ethnic differences in leukemia risk and distinct dietary patterns among ethnic groups [87, 88], no studies have explicitly examined the potential modifying effect of ethnicity on the relationship between maternal diet or vitamin supplement intake and risk of childhood leukemia. Furthermore, most of the evidence to date on the role of folate in childhood leukemia risk is based on studies examining the use of periconceptional vitamin supplements which usually contain a wide variety of nutrients, thus limiting the ability to attribute an effect to one specific nutrient. Few studies have examined intake of folate and other B vitamins from food, and these studies have been limited by small sample sizes. In addition, there has been relatively limited consideration of the role of maternal diet on risk of AML and other leukemia subtypes. Finally, measures of overall diet quality may better represent nutritional status and the complex biological interaction of multiple nutrients, but these measures have not been used to examine the influence of maternal diet on childhood leukemia.

Evidence of the strong effect of maternal diet on other early life outcomes suggests that this is a research area of continued importance. In particular, the proposed importance of epigenetics and immune regulation in the development of leukemia suggests that maternal consumption of dietary components known to influence these processes may be of particular consequence. Further research is warranted to better understand the relationship between these dietary factors and childhood leukemia risk because diet is a modifiable risk factor and targeted interventions can be developed, as in the case of neural tube defects.

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Chapter 2:

Maternal prenatal intake of one-carbon metabolism nutrients and risk of childhood leukemia

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INTRODUCTION

Acute leukemia, an aggressive malignancy originating from lymphoid and myeloid progenitor cells in the bone marrow, is the most common cancer in children, comprising around a third of all pediatric cases [1, 2]. Around 80% of childhood leukemia cases are lymphocytic, with a distinctive peak in incidence from 2-6 years of age, while leukemias of myeloid lineage and other subtypes occur less frequently among children [2]. Observations of concordant leukemia in monozygotic twins and identification of leukemia-related chromosomal alterations in neonatal blood spots provide strong evidence that many of the genetic abnormalities involved in childhood leukemia are initiated *in utero* [3, 4]. However, pre-leukemic chromosomal translocations occur around 100 times more frequently than the development of acute leukemia, indicating that in order for these initiating genetic abnormalities to result in disease, additional prenatal or postnatal oncogenic events are required [3, 4].

Maternal nutrition during pregnancy may be related to both the occurrence of primary genetic abnormalities and to additional oncogenic events that lead to the development of childhood leukemia. One-carbon metabolism refers to three interrelated metabolic cycles in the cytosol of cells that are responsible for critical cellular processes, including the synthesis of nucleotides required for DNA and RNA, the conversion of homocysteine to methionine, and the generation of s-adenosylmethionine (SAM), the primary methyl donor for DNA, RNA, proteins and lipids [5, 6]. One-carbon metabolism has been described as an "integrator of nutrient status" [5] due to its involvement of a variety of nutrients, including folate, vitamins B12 and B6, riboflavin, and amino acids methionine, serine, and glycine. In addition to the importance of these nutrients for DNA synthesis and repair and chromosomal integrity, they directly affect epigenetic processes that determine gene expression and influence cancer risk, including histone modification, levels of non-coding RNAs, and DNA methylation [5, 7-9]. These types of epigenetic modifications may constitute additional oncogenic events required for the development of childhood leukemia [10, 11].

Maternal nutritional factors are clearly important to fetal development, as demonstrated by the ability of folic acid supplementation to prevent neural tube defects [12]. Observational studies of maternal folate intake through diet or supplements and risk of childhood leukemia have yielded mixed findings, with some studies demonstrating a protective effect for folic acid supplementation or food folate intake before or during pregnancy [13-15] and several others finding no association [16-21]. A recent Childhood Leukemia International Consortium study, the largest to date, found that folic acid taken before conception or during pregnancy was associated with a reduced risk of childhood acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) [22]. Only a small number of studies, however, have examined folate intake from food or the role of any other nutrients involved in the one-carbon metabolism cycle and risk of childhood leukemia. In addition, there has been limited consideration of the role of maternal diet on risk of AML [23].

The incidence of ALL differs among racial and ethnic groups, with the highest incidence observed among Hispanics and the lowest in African-Americans [24]. The associations between various genetic and environmental exposures and ALL have also been found to differ by Hispanic ethnicity [25-30], including the effect of genetic variants in the folate pathway [21].

Nevertheless, despite ethnic differences in leukemia risk and distinct dietary patterns among ethnic groups [31], most previous studies have examined the relationship between maternal diet or vitamin supplement use and risk of childhood leukemia in populations with little ethnic diversity and have been unable to explore the influence of maternal ethnicity on these relationships.

The objective of this study is to examine the associations between maternal intake of folate and other one-carbon metabolism nutrients before pregnancy and risk of ALL and AML in children in an ethnically-diverse, population-based case-control study, the California Childhood Leukemia Study (CCLS).

METHODS

Study Population

The CCLS is a population-based case-control study that began in 1995. Phase I of the study (1995-1999) included 17 counties in the San Francisco Bay Area, and Phases II (1999-2002) and III (2002-2008) of the study expanded to include 18 additional counties in the California Central Valley [32]. Incident cases of newly diagnosed childhood leukemia in children 0-14 years old were ascertained from major pediatric clinical centers throughout Northern and Central California, usually within 72 hours after diagnosis. Controls were randomly selected from California birth certificates through the Office of Vital Records at the California Department of Public Health, as described in detail elsewhere [32, 33]. Cases and controls were matched 1:1 or 1:2 on date of birth, gender, Hispanic ethnicity (based on either parent self-reporting as Hispanic), and maternal race (White, Black, or Other). Eligibility was restricted to incident cases and controls who 1) resided in the study area, 2) were under 15 years of age at time of diagnosis for cases and the corresponding date for controls, 3) had at least one parent or guardian who spoke English or Spanish, and 4) had no previous history of any malignancy. This analysis includes ALL and AML case and control participants recruited between 1995 and 2008 whose mothers reported dietary information. Previous CCLS studies examining maternal nutrient intake and childhood leukemia [19, 20] were based only on Phase I and II respondents (less than half of the sample size in this analysis) and did not examine associations with AML or effect modification by maternal ethnicity. Approval for this study was received from the University of California, Berkeley Committee for the Protection of Human Subjects, the California Health and Human Services Agency Committee for the Protection of Human Subjects, and the Institutional Review Boards of the participating hospitals. Prior to the interview, written informed consent was obtained from the responding parent of each participating child, and assent was obtained for children seven years of age and older.

Data Collection

Data were collected by in-person interview in either English or Spanish and were abstracted from birth certificates. Details on dietary data collection have been described elsewhere [19, 20]. In brief, maternal dietary intake was assessed by in-person interview, using a modified version of the Block Food Frequency Questionnaire (FFQ). All biological mothers were asked to report dietary intake and vitamin supplement use in the twelve months before the index pregnancy in order to examine nutritional adequacy at the time of conception and early pregnancy. The FFQ included 76 foods items and questions about regular (at least weekly) use of vitamins or minerals in the year before pregnancy, as well as type of supplement (multiple vitamins or select single

vitamins: vitamins A, C, and E, beta-carotene, calcium, iron, zinc, and selenium) and frequency of use (no use, 1-3 days/week, 4-6 days/week, and every day). Spanish-speaking respondents were administered a Spanish version of the FFQ by bilingual interviewers. The Spanish FFQ included seven additional food items common in the diets of the Latino population (i.e., evaporated or condensed milk, cooked green peppers, avocado or guacamole, chile peppers or chile sauce, sauces such as mole or sofrito, corn tortillas, and flour tortillas). Dietary nutrients from food were calculated using the BlockSys and NutritionQuest computer programs (NutritionQuest, Berkeley, CA, USA) by multiplying the frequency of consumption of each food by its nutrient content and portion size and summing nutrient intake over all foods. Nutrient intake from vitamin supplements was calculated by multiplying the frequency of consumption of each type of supplement (multiple vitamins and single vitamins) by the amount of the nutrient typically found in compositions of each type; all supplemental B vitamin intake was based on use of multiple vitamins. Dietary folate intake was calculated in units of dietary folate equivalents (DFE) [34] and accounted for the different amounts of folic acid available from food before and after national fortification of grain products with folic acid in 1998. Information on vitamin supplement use during pregnancy (i.e. use of any vitamins or minerals and use of specific types of vitamins during pregnancy) was collected only for Phase III respondents. Women were categorized as using vitamin supplements during pregnancy if they reported use of prenatal vitamins, one-a-day Centrum or Thera-type multiple vitamins, Stresstabs/B-complex vitamins, or folic acid supplements during pregnancy.

Statistical Analysis

Analyses were carried out separately for ALL and AML. Mothers of cases and controls with Down's syndrome (N=32) were excluded due to the distinct genetic risk of leukemia among these participants. We also excluded respondents reporting implausible daily energy consumption of <500 or >6000 calories (N=21). Cases and controls were compared by Pearson chi-square tests for categorical variables and Mann-Whitney rank sum tests for continuous variables. Nutrient intake was examined both as total combined intake from food and supplements and separately by source. Principal components analysis (PCA) was used to create two variables summarizing intake of folate, vitamins B12 and B6, riboflavin, and methionine from 1) food and supplements and 2) food only. The first components accounted for ≥80% of the variance. In addition to models examining the principal component variables, the association between each nutrient and case-control status was examined in separate models due to the high correlations among the nutrients ($r > 0.75$ for most pairs of one-carbon metabolism nutrients). Conditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for nutrient intake and case-control status.

Analyses for all mothers included respondents of all races/ethnicities, while stratified analyses were restricted to the three major ethnic groups represented in this study (Hispanic, non-Hispanic white, and Asian). The potential modifying influence of maternal ethnicity (Hispanic, non-Hispanic white, or Asian) on nutrient associations with ALL was assessed through the addition of interaction terms to the statistical models; interaction terms with a p-value less than 0.2 were considered statistically significant to account for the low power of tests of homogeneity. Interaction by maternal ethnicity was not assessed for AML due to a small sample size. Because the Spanish version of the FFQ included seven additional foods, stratified analyses for nutrient intake from food and ALL were restricted to Hispanic mothers who responded in Spanish (N=83

Hispanic English respondents excluded), non-Hispanic white mothers who responded in English (N=35 non-Hispanic white Spanish respondents excluded), and Asian mothers who responded in English (N=1 Asian Spanish respondent excluded). Sensitivity analyses examining stratified results among all members of the race/ethnicity categories (responding in both English and Spanish) were applied to determine the extent to which this restriction influenced the results.

Intake of B vitamins from supplements in the year before pregnancy was treated as a binary variable (any versus none). For Hispanic and non-Hispanic white groups, intake of B vitamins from supplements was also categorized into no, moderate, and high intake based on the distribution of intake in the data (moderate intake: >0 and <600 μg folic acid, >0 and <5 μg vitamin B12, and >0 and <1.5 mg vitamin B6 and riboflavin; high intake: ≥600 μg folic acid, ≥5 μg vitamin B12 and ≥1.5 mg vitamin B6 and riboflavin). Categorical analyses were not carried out for Asian women due to the small sample size. Stratified analyses included both English- and Spanish-speaking respondents of a given race/ethnic group since the questions on vitamin supplement use did not differ by language. For Phase III respondents who reported supplement use during pregnancy, vitamin supplement use was also modeled as vitamin supplement intake during pregnancy only (reference group), intake both before and during pregnancy, and no intake before or during pregnancy for ALL and results were stratified by maternal ethnicity.

Covariates were determined a priori based on known or hypothesized associations with maternal diet and childhood leukemia. All multivariable models adjusted for household income, mother's education, father's education, and maternal age at child's birth; models among all mothers also included mother's ethnicity. In order to examine the influence of the source of nutrients, models examining nutrient intake from food also included intake of B vitamins from supplements (yes/no), and vitamin supplement models included the principal component (PC) for nutrient intake from food. All models examining nutrient intake from food and supplements or food only also adjusted for total energy intake to potentially reduce measurement error [35, 36], although this adjustment did not substantially influence results. Maternal body mass index (BMI) before pregnancy was available for only two-thirds of respondents and was not included as a covariate in final models because it did not improve the accuracy of the PC or vitamin supplement models, as assessed through likelihood ratio tests. Because national folic acid fortification reduced the prevalence of folate deficiency in the US population [37, 38], the effect of folic acid fortification on the relationship between folate and ALL was assessed through the addition of an interaction term with fortification period (child's birth date before 1998 and after 1998) to the folate models for all mothers. All statistical tests were two-sided and considered statistically significant if the 95% CI excluded 1.0. Statistical analyses were carried out using STATA version 12.

RESULTS

For ALL, there were significant differences between cases and controls by income ($p<0.01$), mother's education ($p<0.01$), father's education ($p=0.04$), maternal age category ($p<0.01$), and vitamin supplement use in the year before pregnancy ($p=0.01$) (Table 1). For AML, cases and controls differed significantly by income ($p=0.03$) and maternal age category ($p=0.01$). Thirty-five percent (N=650) of women reported taking any single or multiple vitamin supplements in the year before pregnancy. Only 14.7% of Hispanic mothers reported use of folic acid-containing vitamin supplements in the year before pregnancy in contrast to 40.6% of white mothers and

29.5% of mothers of other ethnicities (31.5% of Asian mothers). Source of nutrients differed by maternal ethnicity: for example, Hispanic mothers had significantly higher folate intake from food than white mothers or mothers of other ethnicities ($p<0.001$), but significantly lower folic acid intake from vitamin supplements than non-Hispanic mothers ($p<0.001$) (Table 2).

Childhood ALL

Higher total intake of one-carbon metabolism nutrients from *food and supplements* before pregnancy as summarized in the PC was associated with reduced risk of ALL among all mothers (OR =0.91, 95% CI 0.84-0.99 for a one-unit change) (Table 3). Higher total intake of each individual nutrient was also associated with reduced risk of ALL, although the OR was less pronounced for folate and the 95% CI included 1.0. Folate fortification period did not influence these associations (data not shown). Test for interaction by maternal ethnicity were statistically significant in the models for the PC and every nutrient, with reduced risks observed for the PC and each nutrient for Hispanic and non-Hispanic white women but not for Asian women.

For intake of one-carbon metabolism nutrients from *food only*, the OR for the PC was less than 1.0 and similar across racial/ethnic groups, although the 95% CI included the null value (Table 3). The ORs for each individual nutrient were all less than 1.0 except for folate intake in white women and methionine intake in Asian mothers. Only the association between methionine and ALL was modified by maternal ethnicity ($p=0.04$). The association between folate from food and ALL was not influenced by fortification period (data not shown).

Children of Hispanic women who reported any B vitamin intake from supplements in the year before pregnancy had substantially reduced risk of ALL (OR=0.51; 95% CI 0.28-0.94) (Table 4). When examined as a categorical variable, high intake of B vitamins from multiple vitamin supplements showed a similar reduced risk of ALL among children of Hispanic women. Conversely, for children of non-Hispanic white women, the ORs for any B vitamin intake from supplements and high B vitamin intake from supplements were less pronounced and the 95% CIs included 1.0. There was an increased risk of ALL among children of Asian mothers who reported vitamin supplement use in the year before pregnancy (OR=2.24, 95% CI 0.91-5.51), though random variation remains a possible explanation.

Only 6% (N=62) of mothers interviewed in Phase III did not take vitamin supplements during pregnancy, and of the women who reported prenatal vitamin use, 93% began vitamin use during the first trimester. Measures of association are not presented for the category of no vitamin supplement use before or during pregnancy due to the small number of respondents. Children of Hispanic women who took supplements both before and during pregnancy had reduced risk of ALL (OR=0.34, 95% CI 0.14-0.79), compared to children of Hispanic women who took supplements during pregnancy only (Table 4). Among white women, the OR for taking supplements before and during pregnancy was less than 1.0 but did not reach statistical significance (Table 4). Results are not presented for Asian women due to the small number of Phase III matched sets.

Childhood AML

When examining combined intake of one-carbon metabolism nutrients among all mothers from food and supplements, there was a reduced risk of AML with higher intake as summarized in the

PC (OR = 0.83, 95% CI 0.66-1.04 for a one-unit change), though the CI included 1.0 (Table 5). When nutrients were examined separately, all associations were negative but all 95% CIs included 1.0. The ORs for nutrient intake from food only were slightly more pronounced, most notably for vitamin B6 (OR=0.47, 95% CI 0.23-0.98). There was no evidence of an association of vitamin supplement use in the year before pregnancy and risk of AML (OR = 0.93, 95% CI 0.44-1.95 with 45 discordant matched sets).

DISCUSSION

Our findings indicate that higher maternal intake of one-carbon metabolism nutrients from food and supplements before pregnancy reduced the risk of ALL and possibly AML. This study also found that one-carbon metabolism nutrients other than folate may be more strongly associated with risk of childhood leukemia. When examined by source of nutrients, ORs for intake of one-carbon metabolism nutrient intake from food and ALL were mostly consistent across categories of maternal race/ethnicity and almost all less than 1.0. In contrast, B vitamin intake from supplements in the year before pregnancy greatly reduced the risk of ALL in children of Hispanic women, but not in children of non-Hispanic white women or Asian women.

Few studies have examined if the associations of childhood leukemia with maternal intake of folate and other one-carbon metabolism nutrients from food corroborate the negative associations observed in some studies examining maternal vitamin supplement use and childhood leukemia [22]. One Australian case-control study found that higher intakes of folate and vitamin B12 from food in the last six months of pregnancy were associated with a decreased risk of ALL, whereas higher dietary intakes of vitamin B6 were paradoxically associated with an increased risk for ALL [15]. The inverse associations of childhood leukemia with greater maternal micronutrient intakes are consistent with findings from other studies, including those from the CCLS, that have found maternal consumption of vegetables and fruits before or during pregnancy decreases risk of leukemia in children [19, 20, 39]. There are several plausible biological mechanisms by which maternal micronutrient intake may influence childhood leukemia risk, supported by a large body of literature examining the importance of one-carbon metabolism nutrients for genetic and epigenetic processes involved in fetal development and the importance of maternal nutritional status in the establishment of the child's immune system [9, 40-43].

The substantial differences in the effect of maternal vitamin supplement use on children's risk of ALL by maternal ethnicity are consistent with the modifying influence of Hispanic ethnicity on associations of other exposures with risk of ALL, including the effect of genetic variants in the folate pathway [21]. Nutrient levels in the body are influenced by dietary intake, genetic polymorphisms, behavioral factors, and nutrient-nutrient interactions [44-46]. These factors are often distributed unequally across ethnic groups. For example, the frequency of some polymorphisms that influence nutrient levels differs by race and ethnicity, with the prevalence of the MTHFR 677C→T polymorphism involved in folate metabolism significantly higher among Mexican-Americans compared to non-Hispanic whites or non-Hispanic blacks [47]. The distribution of nutrient intakes also differs between ethnic groups [48]. In this study, a smaller proportion of Hispanic women had nutrient intakes below the recommended daily allowance than non-Hispanic white women or Asian women, which is contradictory to some national data

[38, 48, 49]. The additional foods included in the Spanish FFQ may partially account for the higher intake of nutrients from food observed in Hispanic mothers, although other studies have found that Hispanics tend to have a healthier diet than non-Hispanic whites in the US [50]. Although it seems that our findings cannot be explained by lower micronutrient intakes among Hispanic women in our population, Hispanics may have higher nutrient requirements due to a higher frequency of particular single nucleotide polymorphisms that influence one-carbon metabolism nutrient levels [51, 52].

There are important differences in socioeconomic status between these ethnic/racial groups, with Hispanic women comprising the majority of women with low household income and low education levels. In this study, Hispanic women had a much lower prevalence of vitamin supplement use than white women, which is consistent with data examining folic acid intake among women of childbearing age in the US [38, 48, 49]. Because most Hispanic women who used vitamin supplements had higher education and income, vitamin supplement use may be a surrogate for other exposures related to socioeconomic status. Finally, the findings for B vitamin intake from supplements and ALL in Hispanic and Asian women are based on a smaller number of matched sets discordant on exposure, and bias away from the null value can occur in conditional logistic regression if there are too few discordant sets or adjustment for too many covariates [53]. Because the heterogeneity by maternal ethnicity observed in the vitamin supplement findings was not found for maternal nutrient intakes from food, this finding may be due to systematic error and must be replicated.

The strengths of this study include a large sample size and extensive dietary and vitamin supplement data collected through the FFQ and interview, which allowed for detailed exposure categorization. Furthermore, the study population is representative of the California population. Despite these strengths, there are potential limitations. There is substantial measurement error in the estimation of food and nutrient intakes by FFQs, and most mothers in this study were asked to recall their usual diet several years in the past. There is evidence that women are able to accurately recall their diets during past pregnancies [54] and that FFQs can be used to capture habitual diet reasonably well up to ten years in the past [55]. However, the large degree of random error associated with dietary assessment methods will usually bias results towards null findings or small effect sizes [35, 56]. Differences in the sensitivity and specificity of maternal recall or reporting may differ by case-control status and result in differential misclassification, biasing measures of association [57, 58]. Recall bias may be less likely in this study because maternal diet is not an established or publicized risk factor for childhood leukemia [58]. Furthermore, a maternal diet reliability sub-study in the CCLS (N=85) found that the reliability of five select FFQ questions did not differ by case-control status (unpublished data). Finally, one-carbon metabolism nutrients are highly correlated with other nutrients that are important for fetal development, such as iron and calcium; some of these nutrients have been previously associated with childhood leukemia [59]. Thus, though it is difficult to attribute a reduction in risk specifically to the B vitamins, our findings are consistent with the importance of the one-carbon metabolism cycle in genetic and epigenetic processes that influence carcinogenesis.

In conclusion, this study found that maternal intake of one-carbon metabolism nutrients from food and supplements was associated with a reduced risk of ALL and possibly AML, and suggests that nutrients other than folate may be important in reducing risk. Our observation that

the association with vitamin supplements differs by maternal ethnicity is not fully explained. Although childhood leukemia is an increasingly curable disease, the illness continues to result in substantial morbidity, and advances in understanding the role of modifiable risk factors such as diet are important in efforts to prevent the disease.

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Table 1: Select characteristics of matched case and control children, by leukemia subtype: the California Childhood Leukemia Study

	ALL		AML	
	Cases N (%)	**Controls** N (%)	**Cases** N (%)	**Controls** N (%)
Total	681	931	103	145
Child sex				
Male	390 (57.3)	538 (57.8)	56 (54.4)	80 (55.2)
Female	291 (42.7)	393 (42.2)	47 (45.6)	65 (44.8)
Child's age at diagnosis/reference date (years)				
< 2	83 (12.2)	106 (11.4)	28 (27.2)	44 (30.3)
2-6	396 (58.2)	543 (58.3)	19 (18.5)	28 (19.3)
6-9	96 (14.1)	132 (14.2)	15 (14.6)	19 (13.1)
≥ 9	106 (15.6)	150 (16.1)	41 (39.8)	54 (37.2)
Child's ethnicity				
Hispanic	312 (45.9)	414 (44.5)	40 (38.8)	56 (38.6)
Non-Hispanic White	256 (37.7)	365 (39.2)	44 (42.7)	62 (42.8)
Non-Hispanic Other	112 (16.5)	152 (16.3)	19 (18.5)	27 (18.6)
Mother's ethnicity				
Hispanic	285 (41.9)	364 (39.1)	35 (34.0)	50 (34.5)
Non-Hispanic White	298 (43.8)	437 (46.9)	55 (53.4)	75 (51.7)
Non-Hispanic Other	98 (14.4)	130 (14.0)	13 (12.6)	20 (13.8)
Household annual income (USD)				
<15,000	105 (15.4)	93 (10.0)	21 (20.4)	12 (8.3)
15,000-29,999	119 (17.5)	116 (12.5)	20 (19.4)	22 (15.2)
30,000-44,999	106 (15.6)	116 (12.5)	13 (12.6)	15 (10.3)
45,000-59,999	104 (15.3)	126 (13.5)	9 (8.7)	20 (13.8)
60,000-74,999	51 (7.5)	103 (11.1)	11 (10.7)	14 (9.7)
75,000+	196 (28.8)	377 (40.5)	29 (28.2)	62 (42.8)
Mother's education				
None or elementary	84 (12.3)	71 (7.6)	12 (11.7)	14 (9.7)
High school or similar	211 (31.0)	251 (30.0)	34 (33.0)	38 (26.2)
Some college or similar	188 (27.6)	293 (31.5)	24 (23.3)	38 (26.2)
Bachelor's degree or higher	198 (29.1)	316 (33.9)	33 (32.0)	55 (37.9)
Father's education				
None or elementary	78 (11.8)	99 (11.1)	13 (13.0)	14 (9.9)
High school or similar	238 (36.1)	271 (30.3)	36 (36.0)	44 (31.0)
Some college or similar	137 (20.8)	231 (25.8)	17 (17.0)	35 (24.7)
Bachelor's degree or higher	207 (31.4)	295 (32.9)	34 (34.0)	49 (34.5)
Maternal age at child's				

birth (years)				
<25	231 (33.9)	237 (25.5)	34 (33.0)	25 (17.2)
25-35	342 (50.2)	516 (55.4)	55 (53.4)	89 (61.4)
>35	108 (15.9)	178 (19.1)	14 (13.6)	31 (21.4)
Vitamin supplement use before pregnancy[a]				
Yes	213 (31.5)	347 (37.5)	34 (33.3)	56 (38.9)
No	463 (68.5)	579 (62.5)	68 (66.7)	88 (61.1)
Dietary nutrient intake before pregnancy	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Folate (DFE)[b]	506.90 (282.0)	508.08 (279.6)	471.70 (253.6)	482.23 (293.1)
Vitamin B12 (μg)	5.07 (3.0)	5.19 (3.3)	5.36 (3.2)	5.39 (3.3)
Vitamin B6 (mg)	2.14 (1.0)	2.13 (1.0)	2.19 (1.1)	2.15 (1.1)
Riboflavin (mg)	2.20 (1.0)	2.21 (1.0)	2.34 (1.3)	2.25 (1.1)
Methionine (g)	1.94 (0.9)	1.95 (1.0)	2.09 (1.0)	2.00 (1.1)

[a] Any use of single or multiple vitamins
[b] Dietary folate equivalent (DFE)

Table 2: Nutrient intake and supplemental B vitamin intake before pregnancy among controls, stratified by maternal race/ethnicity

	Hispanic Mothers[a] N=365	**Non-Hispanic White Mothers**[a] N=499	**Asian Mothers**[a] N=99
Nutrient intake from food and supplements	**Median (25^{th}-75^{th} percentiles)**	**Median (25^{th}-75^{th} percentiles)**	**Median (25^{th}-75^{th} percentiles)**
Folate (DFE)	607.1 (398.1-921.3)	546.4 (341.2-976.1)	561.3 (298.0-1018.7)
Vitamin B12 (µg)	5.8 (3.9-9.1)	6.0 (3.6-9.3)	4.8 (2.9-8.6)
Vitamin B6 (mg)*	2.6 (1.9-3.5)	2.3 (1.6-3.5)	2.2 (1.4-3.2)
Riboflavin (mg)*	2.6 (1.9-3.6)	2.4 (1.6-3.4)	2.1 (1.3-2.9)
Nutrient intake from food			
Folate (DFE)*	552.4 (375.3-780.6)	390.4 (270.2-536.1)	447.3 (280.2-597.8)
Vitamin B12 (µg)*	5.2 (3.6-7.5)	4.0 (2.9-5.9)	3.9 (2.3-5.8)
Vitamin B6 (mg)*	2.3 (1.8-3.1)	1.7 (1.3-2.3)	1.7 (1.1-2.5)
Riboflavin (mg)*	2.4 (1.8-3.2)	1.8 (1.4-2.4)	1.6 (1.2-2.3)
Methionine (g)*	2.0 (1.5-2.7)	1.6 (1.2-2.1)	1.6 (1.1-2.1)
Any B vitamin intake from supplements	N (%)	N (%)	N (%)
Yes	62 (17.0)	207 (41.5)	29 (29.3)
No	303 (83.0)	292 (58.5)	70 (70.7)

* Kruskal-Wallis test $p<0.05$

[a] Because of differences in the Spanish and English FFQ, Hispanic mothers include those who responded to the Spanish FFQ and white and Asian mothers include those who responded to the English FFQ.

Table 3: Association of childhood ALL with intake of one-carbon metabolism nutrients before pregnancy from food and supplements and food only, by maternal race/ethnicity

	All mothers[a] 645 cases, 854 controls	Hispanic mothers[a] 185 cases, 226 controls	White mothers[a] 253 cases, 357 controls	Asian mothers[a] 68 cases, 79 controls
Nutrients from food and supplements	**Odds Ratio (95% CI)**	**Odds Ratio (95% CI)**	**Odds Ratio (95% CI)**	**Odds Ratio (95% CI)**
Principal component[b]	0.91 (0.84-0.99)	0.79 (0.64-0.97)	0.92 (0.81-1.03)	1.08 (0.81-1.44)
Folate (100 DFE/day)	0.97 (0.94-1.01)	0.92 (0.84-1.01)	0.98 (0.93-1.03)	1.02 (0.89-1.17)
Vitamin B12 (1 µg/day)	0.96 (0.93-1.00)	0.94 (0.87-1.02)	0.95 (0.90-1.01)	1.01 (0.89-1.14)
Vitamin B6 (1 mg/day)	0.89 (0.79-1.00)	0.68 (0.50-0.92)	0.90 (0.75-1.08)	1.11 (0.74-1.65)
Riboflavin (1 mg/day)	0.88 (0.77-1.00)	0.69 (0.49-0.97)	0.87 (0.72-1.07)	1.07 (0.66-1.73)
Nutrients from food only				
Principal component[b]	0.93 (0.83-1.05)	0.86 (0.68-1.08)	0.89 (0.72-1.09)	0.75 (0.43-1.33)
Folate (100 DFE/day)	0.99 (0.93-1.06)	0.96 (0.85-1.09)	1.00 (0.90-1.12)	0.87 (0.66-1.13)
Vitamin B12 (1 µg/day)	0.97 (0.92-1.02)	0.98 (0.90-1.07)	0.91 (0.82-1.02)	0.85 (0.68-1.07)
Vitamin B6 (1 mg/day)	0.91 (0.74-1.12)	0.69 (0.45-1.08)	0.88 (0.59-1.29)	0.75 (0.34-1.63)
Riboflavin (1 mg/day)	0.91 (0.74-1.12)	0.78 (0.51-1.20)	0.85 (0.60-1.19)	0.59 (0.24-1.43)
Methionine[c] (1 g/day)	0.90 (0.73-1.10)	0.75 (0.50-1.15)	0.78 (0.54-1.14)	1.39 (0.70-2.75)

Conditional logistic regression models adjusted for father's education, mother's education, household income, maternal age at child's birth, and energy intake. Models for all mothers also adjusted for mother's ethnicity. Models for nutrient intake from food only also adjusted for B vitamin intake from supplements (yes/no).

[a] All mothers includes mothers of all races/ethnicities who responded in English and Spanish. Hispanic mothers include those who responded to the Spanish FFQ and white and Asian mothers include those who responded to the English FFQ.

[b] The principal component represents the combined dietary intake of folate, vitamins B12 and B6, riboflavin and methionine from food and supplements and from food only.

[c] Methionine was measured from food only.

Table 4: Association of childhood ALL with intake of vitamin supplements containing B vitamins before and during pregnancy, by maternal race/ethnicity

Vitamin supplements before pregnancy	**Hispanic mothers**[a] 234 cases, 296 controls		**White mothers**[a] 265 cases, 374 controls		**Asian mothers**[a] 68 cases, 79 controls	
	Discordant sets (%)	**Odds Ratio (95% CI)**	**Discordant sets (%)**	**Odds Ratio (95% CI)**	**Discordant sets (%)**	**Odds Ratio (95% CI)**
Any B vitamins from multiple vitamins	64 (27.4)		162 (61.1)		28 (41.2)	
No		(Ref)		(Ref)		(Ref)
Yes		0.51 (0.28-0.94)		0.91 (0.64-1.31)		2.24 (0.91-5.51)
Level of B vitamin intake from multiple vitamins[b]	68 (29.1)		177 (66.8)			
None		(Ref)		(Ref)		
Moderate intake		1.12 (0.44-2.84)		1.25 (0.75-2.07)		---
High intake		0.36 (0.17-0.74)		0.76 (0.50-1.16)		---
Vitamin supplements during pregnancy	194 cases, 229 controls		154 cases, 261 controls			
Pregnancy only	53 (41.7)	(Ref)	86 (71.1)	(Ref)		---
Before and during pregnancy[c]		0.34 (0.14-0.79)		0.66 (0.39-1.11)		---

[*]Conditional logistic regression models adjusted for adjusted for father's education, mother's education, household income, and maternal age at child's birth and the principal component for nutrient intake from food.

[a] Race/ethnic categories for vitamin supplement use include both English and Spanish respondents because questions did not differ by language.

[b] For folic acid, moderate intake is >0 & <600 µg and high intake is ≥600 µg. For vitamins B12, B6, and riboflavin, moderate intake is >0 & <5 µg B12 and <1.5 mg B6 and riboflavin, and high intake is ≥5 µg B12 and ≥1.5 mg B6 and riboflavin.

[c] Multiple vitamin use before pregnancy and use of prenatal vitamins, one-a-day Centrum or Thera-type multiple vitamins, Stresstabs/B-complex vitamins, or folic acid during pregnancy.

Table 5: Association of childhood AML with intake of one-carbon metabolism nutrients before pregnancy from food and supplements and food only

	All Mothers 98 cases, 128 controls
Nutrients from food and supplements[a]	**Odds Ratio (95% CI)**
Principal component[b]	0.83 (0.66-1.04)
Folate (100 DFE/day)	0.93 (0.85-1.03)
Vitamin B12 (1 µg/day)	0.92 (0.84-1.02)
Vitamin B6 (1 mg/day)	0.72 (0.51-1.04)
Riboflavin (1 mg/day)	0.85 (0.60-1.20)
Nutrients from food only[a]	
Principal component[b]	0.68 (0.46-1.02)
Folate (100 DFE/day)	0.90 (0.76-1.07)
Vitamin B12 (1 µg/day)	0.86 (0.73-1.02)
Vitamin B6 (1 mg/day)	0.47 (0.23-0.98)
Riboflavin (1 mg/day)	0.85 (0.49-1.50)
Methionine (1 g/day)	0.68 (0.35-1.34)

[a] Conditional logistic regression models adjusted for mother's ethnicity, father's education, mother's education, household income, maternal age at child's birth, and energy intake. Models for nutrients from food additionally adjusted for B vitamin intake from supplements (yes/no).

[b] The principal component represents the combined dietary intake of folate, vitamins B12 and B6, riboflavin and methionine from food and supplements and from food only.

Chapter 3:

Systematic error in case-control studies examining maternal vitamin supplement use and childhood leukemia: a review and quantitative bias analysis

INTRODUCTION

Vitamin supplement use is an important contributor to micronutrient status among men and women in the United States [1], and extensive efforts have been made to elucidate the relationship between vitamin supplement use and diverse health outcomes. However, the study of the effect of vitamin supplement use on health outcomes has been fraught with difficulties, with randomized controlled trials and observational studies frequently producing conflicting results [2]. Vitamin supplement users differ from non-users along a wide spectrum of socio-demographic factors and health behaviors: they are more likely to be older, female, non-Hispanic white, have higher income and education, have better dietary patterns, engage in physical activity, and refrain from the use of tobacco products [3, 4]. Thus, results from observational studies examining the effects of vitamin supplement use are likely to be confounded by lifelong social and behavioral factors that are difficult to adequately account for in multivariable models [2]. Although these limitations have been widely acknowledged, there remains much interest in the relationship between vitamin supplement use and health outcomes due to the central role of nutrition in disease risk and groundbreaking findings on the health effects of supplement use, such as the substantial reduction in the prevalence of neural tube defects with folic acid supplementation [5].

There has been great interest in the possible association between maternal prenatal vitamin supplement use and childhood leukemia after the unexpected finding from an Australian case-control study that maternal use of vitamin supplements containing folic acid or iron during pregnancy substantially reduced the risk of acute lymphoblastic leukemia (ALL) in children [6]. Many studies have attempted to replicate this association [7]. A recent pooled analysis found that maternal use of vitamin supplements before or during pregnancy was associated with a reduced risk of childhood ALL and possibly childhood acute myeloid leukemia (AML) [7]. This study also found that the reduced risk of childhood ALL and AML was more pronounced in families with lower education levels, yet the investigators were unable to conclude whether this finding was due to parental education serving as a surrogate for other unmeasured lifestyle and socio-demographic variables, differential maternal recall or genetic background within study populations, or the distribution of education levels by case-control status [7].

Due to the rarity of childhood leukemia, case-control studies are generally the only feasible way to examine the relationship between maternal diet or vitamin supplement use and risk of leukemia in children. However, there is widespread concern about the potential for systematic error to bias case-control findings [8]. A critical facet of case-control studies is that controls are representative of the source population of cases in regards to exposure [8]. However, participating controls often have higher socioeconomic status (SES) than cases or non-participating first-choice controls [9-11]. Because factors of etiologic interest are commonly related to measures of SES such as education, income, occupation, and neighborhood, differential participation by cases and controls according to these factors may result in biased effect estimates [10, 12, 13].

Compounding the potential for selection bias, dietary exposures are generally laden with measurement error, and a common concern in nutritional epidemiologic studies is that the large degree of random error associated with dietary assessment methods will bias results towards null

findings or small effect sizes [14, 15]. Furthermore, differences in the sensitivity and specificity of recall or reporting for certain exposures may differ by case-control status and result in differential misclassification and biased measures of association [16]. In case-control studies of child health outcomes, the most common concern is that parents of cases will have enhanced recall of exposures believed to influence disease risk due to their desire to identify the cause of their child's illness, resulting in higher sensitivity of exposure classification among cases due to improved accuracy or higher specificity among controls due to spurious reporting of exposures by case parents [16-18].

Consequently, there is substantial uncertainty regarding the extent to which systematic error may have influenced the observed measures of association between maternal vitamin supplement use and risk of childhood leukemia. Sensitivity analysis, also referred to as quantitative bias analysis, provides a quantitative estimate of the direction and magnitude of possible biases in observed epidemiologic associations [19-21]. Such analyses are critical to elucidate the extent to which systematic errors such as selection bias, exposure misclassification, and uncontrolled confounding may alter the associations of interest. There were two objectives of this study. The first objective was to review the literature on maternal vitamin supplement use and risk of childhood leukemia and qualitatively examine the potential for selection bias and exposure misclassification to have influenced reported findings. The second objective was to quantitatively assess the degree to which selection bias and exposure misclassification could modify the observed odds ratio (OR) in data from the California Childhood Leukemia Study (CCLS).

METHODS

Systematic Literature Review

Study Inclusion Criteria and Search Strategy

Studies were included in the review if they examined the association between maternal use of folic acid-containing vitamin supplements any time before or during pregnancy and risk of childhood leukemia. This included research that presented (or allowed calculation of) a measure of association (e.g. OR) between childhood leukemia and maternal use of any type of vitamin supplements containing folic acid, including multivitamins, before the birth of the case or control child. Reviews and meta-analyses and any studies examining maternal or infant exposure to vitamins or vitamin supplements that did not contain folic acid (e.g. other single vitamins) were excluded. Prenatal vitamins and multivitamins were assumed to contain folic acid unless otherwise specified. Papers were identified through a systematic search of PubMed/MEDLINE from inception to April 10, 2015 (search strategy is provided in Appendix A). After excluding papers by title and abstract review, each potentially relevant article was reviewed to determine if it met inclusion criteria. In addition, the reference list of the Childhood Leukemia International Consortium (CLIC) study [7] was reviewed for manuscripts that may not have been identified by the initial search strategy.

Data Abstraction and Analysis

From each study, the first author name, year of publication, and name of the study; country; years of case and control recruitment; sample size; outcome (i.e. type of leukemia); exposure (i.e. type of vitamin supplements) and method of assessment; case and control participation; and

primary findings were abstracted. Criteria to qualitatively assess the potential for selection bias and exposure misclassification in the included studies was guided by published recommendations for quality assessment of case-control studies [22-26]. The potential for selection bias was qualitatively assessed by consideration of: 1) ascertainment of cases and selection of controls, and 2) case and control losses (i.e. the number of non-participants or participants selected as first, second or later choice). The potential for exposure misclassification was qualitatively assessed by the following criteria: 1) exposure assessment (e.g. question regarding specific types of vitamin supplements or open-ended questions); 2) assumptions made in the calculation or categorization of exposure; and 3) blinding of interviewers regarding respondents' case or control status.

Quantitative Bias Analysis

The objective of the quantitative bias analysis was to determine the possible influence of selection bias and exposure misclassification on the association between maternal use of folic acid-containing vitamin supplements in the year before pregnancy and childhood ALL in the CCLS [27]. (The potential influence of uncontrolled confounding was not examined because the original analysis adjusted for many covariates, and it is unlikely that there is a single uncontrolled confounder with a large enough association with leukemia to account for the observed associations.) Because the conditional adjusted OR was very similar to the unconditional unadjusted OR (Appendix B), the unadjusted data was used to perform the bias analysis. Because maternal ethnicity was found to modify this association, the quantitative bias analysis was carried out separately for Hispanic and non-Hispanic white mothers. Furthermore, because these two groups of women differ in important socio-demographic characteristics (e.g. household income and education) and the prevalence of vitamin supplement use (19% of Hispanic ALL control mothers versus 42% of non-Hispanic white ALL control mothers), carrying out the bias analysis separately for these two groups is instructive for understanding how systematic error may influence observed associations in different kinds of populations.

Internal and external validation and reliability data were reviewed to determine the range of values for the bias parameters. For selection bias parameters, internal data from the CCLS on participation rates and socio-demographic characteristics of non-participating cases and controls was reviewed. For exposure misclassification parameters, literature on the sensitivity and specificity of self-reported vitamin supplement use and literature examining maternal recall in case-control studies, as well as internal data on the reliability of reported vitamin supplement use was reviewed. A range of values for the selection bias parameters (i.e. selection probabilities of exposed and unexposed cases and controls) and exposure misclassification parameters (i.e. sensitivity and specificity of exposure classification among cases and controls) were examined in order to estimate the possible influence of selection bias and exposure misclassification on the observed OR (formulas provided in Appendix B). This step, referred to as multidimensional bias analysis, generates a range of corrected ORs but does not give an indication of which corrected estimate of association is most likely under an assumed bias model [20]. Probabilistic bias analysis extends multidimensional bias analysis by specifying probability distributions for each of the bias parameters and using Monte Carlo sampling techniques to generate a frequency distribution of ORs corrected for selection bias and exposure misclassification [20]. This approach produces a measure of central tendency and bias interval limits of the corrected estimates based on the assigned probability distribution (e.g. the 2.5^{th} and 97.5^{th} percentiles are

the limits of an interval that contain 95% of the simulated estimates) [19, 20]. For probabilistic bias analysis, uniform or trapezoidal probability distributions were assigned to the bias parameters and sampled from 20,000 times. Finally, multiple bias analysis methods produced estimates corrected for the potential influence of selection bias and misclassification simultaneously. Multidimensional bias analysis was carried out in Excel (using spreadsheets developed by Fox et al. 2007 [28]) and R, and probabilistic bias analysis was carried out in STATA version 12 [29].

Selection Bias Parameters

Because there is no information on the prevalence of vitamin supplement use among nonparticipating cases and controls in the CCLS, selection proportions must be postulated based on the participation rates in cases and controls [20]. Case ascertainment was high in the CCLS: a comparison of case ascertainment with the California Cancer Registry (1997–2003) indicated that the CCLS ascertained 96% of children diagnosed with leukemia in seven Phase I participating hospitals and 93% in the nine Phase II hospitals [30]. Of eligible subjects who were ascertained, 86% of cases consented to participate. Among both participating and non-participating hospitals within the 35 study counties, 76% of all diagnosed cases were ascertained [30].

In contrast, 86% of eligible controls agreed to participate in the CCLS, but only 45% of these were first-choice controls [30]. A recent study on the potential role of selection bias in the association between childhood leukemia and exposure to residential magnetic fields compared a subset of participating to non-participating controls and found that mothers of participating controls in the CCLS had a significantly higher level of education, were older at the child's birth, and were more likely to live in a single-family home or in a neighborhood with a high SES than mothers of non-participating controls [11]. The participation of first-choice controls was lower within levels of lower neighborhood SES: participation of first choice controls was 26% in the low neighborhood SES category and 50% in the high neighborhood SES category. Due to the overall low participation of first-choice controls and the gradient of declining participation with higher SES, it possible that participating controls systematically differed by unmeasured factors (e.g. health behaviors) from those who declined to participate. Thus, due to the association of vitamin supplement use with determinants of control participation (i.e. higher education level, higher household income, and older age) [4], it is plausible that participating controls were more likely to use vitamin supplements than the source population (i.e. all eligible participating and non-participating controls). Estimates of the prevalence of folic-acid containing supplement use among women of child-bearing age from the National Health and Nutrition Examination Survey (1988-2010) have ranged from 12% to 20% for Hispanic or Mexican-American women and from 31% to 37% for non-Hispanic white women [31-33]. The prevalence of vitamin supplement use among mothers of controls in the CCLS are in line with or slightly higher than the upper bounds of these estimates: 19.2% of Hispanic ALL control mothers and 41.6% of non-Hispanic white ALL control mothers reported use of folic-acid containing supplements before pregnancy in the CCLS.

The study on the role of selection bias in the association between childhood leukemia and exposure to residential magnetic fields also found that participating cases had slightly higher SES than non-participating cases [11]. Thus, it is plausible that participating cases were more

likely to use vitamin supplements than non-participating cases. However, due to the overall high participation of cases, it is likely that selection probabilities among exposed and unexposed cases differ to a lesser degree than for the controls.

Based on this data, the following assumptions are made for the specification of selection probabilities:

1) The probability of selecting exposed and unexposed cases is higher than the probabilities of selecting (exposed and unexposed) controls.
2) The probability of selecting exposed cases may be slightly higher than the probability of selecting unexposed cases (based on finding that participating cases have slightly higher SES than non-participating cases).
3) The probability of selecting exposed controls is likely higher than the probability of selecting unexposed controls (based on finding that participating controls have higher SES than non-participating controls).
4) Under the assumption that the prevalence of vitamin supplement use was lower in non-participating cases and controls compared to participating cases and controls, the lowest possible selection probability of unexposed cases is 0.80 and 0.73 among Hispanic and white women, respectively, and the lowest possible selection probability of unexposed controls is 0.56 and 0.48 among Hispanic and white women, respectively (Appendix B).

Exposure Misclassification Parameters

A relatively small number of validation studies have been carried out to assess the sensitivity and specificity of self-reported vitamin supplement use [34]. In validation studies of self-reported vitamin supplement use among adult men and women in various countries (i.e. the United States, Sweden, the Netherlands and Japan), sensitivity has ranged from 66% to 98% and specificity has ranged from 89% to 100%, with the exception of one study that reported a low specificity of 52% and 59% among cases and controls, respectively [16, 35-39] (Appendix B). There is also evidence from validation studies that questions specifying type of supplements improve sensitivity, and that respondents are able to correctly classify their supplements into broad categories of supplement type (e.g. once-a-day versus B complex mixtures) [36, 40].

A study was carried out among a subgroup of CCLS participants who completed a food frequency questionnaire (FFQ) in order to determine the reliability of self-reported dietary exposures and to determine if there was a difference in reliability between cases and controls and by duration of recall (unpublished data). Biological case and control mothers who completed an English FFQ during the Phase I interview (prior to May 31, 2004) were administered a brief telephone questionnaire consisting of five questions from the original FFQ three or more months after the original interview. The repeat questionnaire was administered to 85 mothers (41 case mothers and 44 control mothers). In order to determine if the age of the child at diagnosis (i.e. length of the recall period) influenced reliability, the sample included 48 mothers of children less than four years of age and 37 mothers of children seven years of age or older. For the question on any regular vitamin or mineral supplement use in the year before pregnancy, 73% of responses to the initial question agreed with the follow-up response. There was no evidence of systematic differences by recall period or case-control status: among mothers with children less than four years or at least seven years of age, 71% and 76% of responses agreed, respectively, and among cases and controls, 68% and 77% agreed, respectively. The Australian Study of Causes of Acute

Lymphoblastic Leukemia in Children (Aus-ALL) was also able to compare responses to maternal vitamin supplement questions first reported on a postal self-administered questionnaire and subsequently in an FFQ, and found that 87% of women who reported folate use in the original questionnaire also reported it in the FFQ [41].

Many of the validation studies examining the extent of maternal recall bias in case-control studies of child birth outcomes have found no systematic differences in recall according to case-control status [16, 42-48]. That is, contrary to the common conjecture that mothers of cases will more accurately report exposure history, these studies found that sensitivity of exposure classification was not consistently higher for case mothers; nor were there substantial case-control differences in specificity of exposure classification. Additionally, these studies suggest that the accuracy of recall and the likelihood of case-control differences in accuracy are likely to vary by type of exposure [16, 43, 49]. Socially undesirable behaviors may be underreported by both case and control mothers [42], or even underreported to a greater degree by case mothers [46]. Of relevance to this analysis, two studies examining prenatal vitamin use did not find systematic case-control differences in exposure classification [16, 44].

While these studies do not refute the possibility or presence of recall bias in case-control studies examining maternal exposures, particularly given the limitations of the validation data [44], they do suggest that it is difficult to make assumptions about whether the misclassification of exposure is occurring differentially or non-differentially among cases and controls. However, several of these studies found poor maternal recall of past exposures [46, 48], and the internal and external data on validity and reliability of self-reported past vitamin supplement use suggest that there is likely to be some degree of misclassification in reported vitamin supplement use. For this reason, a wide range of misclassification parameters were explored with the following assumptions:

1) Specificity is higher than sensitivity and likely ranges from 0.9-1.0.
2) Sensitivity is greater than 0.6.
3) Misclassification could be differential or non-differential by case-control status.

RESULTS

Search Yield

The search identified 109 articles, of which 53 were excluded based on title and 23 were excluded by abstract review. One study was unable to be retrieved for review [50]. Twenty-three studies were excluded by full review. Three studies from Aus-ALL presenting overlapping data were excluded [51-53]; the paper presenting the most thorough methodological description and results on the association between maternal vitamin supplement use and childhood ALL is included here [41]. Three previous CCLS studies based on smaller overlapping samples [54-56] were excluded, and the most recent (unpublished) findings from the entire CCLS study population are included here [27]. The CLIC study [7] reported data published elsewhere [41, 54, 56-61], as well as data from four unpublished studies in Brazil, Costa Rica, Egypt and Greece. It is not included in this review because it does not provide details on the methodological aspects of interest (e.g. exposure assessment) for the four unpublished studies. A bias analysis of a previously published association between vitamin supplement use and childhood leukemia

among children with Down syndrome was excluded [34]; the original paper reporting the association is included in this review [62]. Two additional studies [57, 63] were identified through reference lists, resulting in 12 total studies meeting the inclusion criteria (Table 1).

Characteristics of Studies

Studies on the association of childhood leukemia with maternal vitamin supplement use were carried out in the Australia, Canada, France, Germany, New Zealand, and the United States (Table 1). All studies employed a case-control design. Six studies examined both ALL and AML [27, 58, 59, 62, 64, 65] and six studies examined only ALL [6, 41, 57, 60, 61, 63]. One study was restricted to common ALL [6]; one study was restricted to cases and controls with Down syndrome [62]; one study was restricted to infant leukemia cases (i.e. cases diagnosed under one year of age) [65]; and one study included undifferentiated or biphenotypic cases [58]. All studies employed frequency matching or individual matching of cases and controls on age or date of birth; all studies except for three [61, 62, 65] matched on gender; six matched on a measure of geography/region (i.e. location of residence, community, telephone area code or exchange area; the CCLS discontinued matching on geography after Phase I of the study [9]) [6, 41, 59, 61, 63, 65]; and one matched on maternal and child race/ethnicity [27].

Selection and Participation of Cases and Controls

Cases were ascertained from hospitals in four studies [6, 27, 41, 57], from cancer registries in three studies [58, 59, 64], from cancer registries and hospitals in two studies [60, 63], and from Children's Cancer Group/Children's Oncology Group clinical trials and treatment institutions in three studies [61, 62, 65]. Five studies used random-digit dialing (RDD) to identify and select controls [41, 58, 61, 63, 64], and one study first used random-digit dialing and then switched to random selection of controls from state birth registries [65] (Table 2). Two studies randomly selected controls from state birth certificates [27] or the national birth registry [60]. One study selected controls from the files of the local resident registration offices [59]; one selected controls from family allowance files or provincial health insurance agency files [57]; one from a postal survey of people randomly selected from the state electoral roll [6]; and one from rosters of pediatric patients with Down syndrome provided by physicians treating cases [62]. For case participation, five studies had over 90% participation [57, 58, 60, 61, 64], four studies had 80-89% participation [6, 27, 41, 59], and three studies had less than 80% participation [62, 63, 65]. Control participation was generally lower than case participation. Among identified and eligible controls, six studies had between 80-89% participation (although Thompson et al. estimated the true rate of participation to be 75%) [6, 27, 57, 62-64], four studies had between 70-79% participation (although Milne et al. estimated the true rate of participation to be 55%) [41, 58, 59, 61], and two studies had under 70% control participation [60, 65]. Studies often failed to identify and determine eligibility for a substantial portion of controls through these selection approaches [41, 58, 62, 64]. Only two studies [60, 63] did not report socio-demographic differences between cases and controls. Of those that reported case-control differences, all studies except for two [41, 64] reported that participating controls had higher SES, according to measures of income or education, than participating cases.

Exposure Assessment

Four studies examined maternal vitamin supplement use during pregnancy [6, 57, 59, 63], and eight studies examined multiple periods before and during pregnancy [27, 41, 58, 60-62, 64, 65].

Six studies specifically mentioned or asked about folic acid or folic acid-containing supplements [41, 57-60, 64], in contrast to the other studies that asked about use of any vitamin supplements or medications. Most studies included questions about vitamin supplement use in a structured telephone [57, 58, 61, 62, 64, 65] or in-person interview [6, 27, 60, 63], but two studies employed a self-administered postal survey [41, 59]. Each study interview included specific questions about vitamin supplement use except for one study that examined medication use during pregnancy and included an open-ended question about any other drug use not previously mentioned [6]. One study did not report details on assessment of vitamin supplement use and only examined vitamin supplement use as an effect modifier [63]. Two studies asked about the specific brand of the vitamin supplement [64] or included an open text field to specify brand name [59]. Five studies asked about drug dose [6] or about number of times or frequency of consumption before [27] or during pregnancy [41, 57, 61]. Two studies calculated presumed dosage based on standard vitamin supplement formulations [27, 41]. No studies indicated whether or not interviewers were blinded to case-control status.

Association of Childhood Leukemia with Maternal Vitamin Supplement Use

The associations between maternal vitamin supplement use and childhood leukemia in the included studies are summarized elsewhere [7]. Briefly, of the studies examining ALL as the outcome, four studies found a negative association between maternal use of vitamin supplements before [27] or during [6, 27, 58, 61] the index pregnancy and risk of ALL. In addition, the study examining risk of leukemia in children with Down syndrome found that vitamin supplement use in the year before and during pregnancy was associated with a reduced risk of ALL, but noted a possible increased risk of ALL with vitamin supplement use initiated only after knowledge of the pregnancy [62]. The remaining seven studies found no statistically significant associations with vitamin supplement use before [41, 60, 64, 65] or during pregnancy [41, 57, 59, 60, 63-65]. Milne et al. found that ORs for two different exposure assessment methods differed slightly although no associations were statistically significant [41]. One study found a significant negative association between maternal folic acid supplementation and risk of acute non-lymphoblastic leukemia (comprising mostly AML cases but also including a small number of undifferentiated or biphenotypic leukemia cases) [58]. No other studies found statistically significant association of AML with maternal vitamin supplement use before [27, 64, 65] or during [59, 64, 65] pregnancy, including the study carried out among children with Down syndrome only [62].

Quantitative Bias Analysis

Multidimensional Selection Bias Analysis among Hispanic and White Women

Quantitative examination of potential bias in the CCLS measure of association observed among Hispanic women (uncorrected OR = 0.49) found that, under the assumption of lower exposure prevalence among non-participating controls, ORs corrected for selection bias through multidimensional bias analysis ranged from 0.39 to 0.88 (Table 3). Although most selection probability scenarios resulted in corrected ORs closer to the null value, almost all ORs adjusted for selection bias were less than 0.80, even at extreme differences in control selection probabilities (e.g. 1.0 for exposed controls and 0.56 for unexposed controls, assuming the same or similar case selection probabilities).

Among non-Hispanic white women (uncorrected OR = 0.88), ORs corrected for selection bias ranged from 0.70 to 1.83 under the assumption that exposure prevalence was lower in non-participating controls (Table 3). Several corrected ORs were greater than 1.0, and approached or crossed the null value even at relatively modest differences in control selection probabilities (e.g. 0.68 for exposed controls and 0.57 for unexposed controls, assuming the same or similar case selection probabilities).

Multidimensional Exposure Misclassification Analysis among Hispanic and White Women
In multidimensional bias analysis for exposure misclassification, corrected ORs among Hispanic women ranged from 0.02 to 0.78 under scenarios of differential and non-differential misclassification (Figure 1). No corrected ORs were greater than 1.0. Corrections for non-differential misclassification and differential sensitivity and specificity resulted in ORs further away from the null value; only corrections for differential sensitivity (under scenarios of non-differential specificity) produced corrected ORs closer to the null than the uncorrected estimate, although these ORs were still less than 0.8. Under each misclassification scenario, adjustments for reduced specificity resulted in ORs much further away from the null (ORs ranging from 0.02-0.08).

Odds ratios corrected for exposure misclassification among non-Hispanic white women ranged from 0.23 to 2.09 under scenarios of differential and non-differential misclassification (Figure 2). Scenarios of differential misclassification (differential sensitivity and non-differential specificity, and differential sensitivity and specificity) produced a wide range of corrected ORs extending to both sides of the null value. However, scenarios of higher sensitivity in cases compared to controls (at levels of differential or non-differential specificity) resulted in negative associations further from the null value than the uncorrected estimate. Only scenarios of higher sensitivity among controls (at levels of differential or non-differential specificity) resulted in positive associations. Corrections for non-differential misclassification resulted in ORs similar to the uncorrected estimate at all levels of specificity (i.e. 100%, 95%, and 90%).

Probabilistic and Multiple Bias Analysis among Hispanic Women
For the various misclassification scenarios (Table 4), probabilistic bias analysis for exposure misclassification produced results similar to multidimensional bias analysis among Hispanic women, with similar limits to the corrected ORs (Table 5). The interval limits included 1.0 under the differential misclassification scenarios of higher sensitivity and specificity among cases or controls (Models 2 and 3). Under both non-differential and differential misclassification scenarios, the median corrected ORs were all farther away from null value than the uncorrected original estimate. Replacing trapezoidal distributions with uniform distributions produced similar results (data not shown).

Probabilistic bias analysis for selection bias among Hispanic women produced median estimates slightly closer to the null value and bias intervals that included 1.0 with the incorporation of random error. Multiple bias analysis corrections for both exposure misclassification and selection bias among Hispanic women did not substantially change the median estimates but limits for model three (the scenario of sensitivity and specificity higher in controls) included 1.0. The only bias parameters that did not appear to fit the data well were low specificity among Hispanic

cases, with specificity truncated slightly higher than the lower limit of 0.85 (but less than 0.9), which is consistent with the findings from multidimensional bias analysis.

Probabilistic and Multiple Bias Analysis among White Women
Probabilistic bias analysis for exposure misclassification among white women also produced similar results to multidimensional bias analysis, with a wide range of corrected ORs falling on both sides of the null value, ranging from 0.48 to 1.89 (Table 5). All median estimates were relatively close to the uncorrected OR, with scenarios of non-differential misclassification and differential misclassification with higher sensitivity and specificity in cases resulting in corrected ORs slightly farther away from the null value, and differential misclassification with sensitivity and specificity higher in controls resulting in a median estimate slightly closer to 1.0.

Corrections for selection bias only and multiple bias analysis corrections for both selection bias and exposure misclassification produced median estimates greater than 1.0, with wide intervals extending to both sides of the null value.

DISCUSSION

The body of literature examining the association between maternal vitamin supplement use and childhood leukemia has grown rapidly in the last fifteen years. Although most studies have not found an association of childhood leukemia with maternal vitamin supplement use, some studies have reported strong negative associations. The inconsistency in these findings highlights the uncertainty regarding the extent to which both random and systematic error may be influencing these reported results.

Most studies included in this review had high case ascertainment and over 80% case participation. In contrast, many studies had lower participation of eligible first-choice controls and failed to assess eligibility for a large proportion of controls. For example, the estimates of control participation rates from random digit dialing do not typically take into account the loss of eligible subjects when called numbers are never answered [66]. Aus-ALL investigators attempted to calculate a better estimate of control participation by applying the observed proportion of residences with an eligible child to all identified and presumed residences and estimated that the best estimate of control participation was 55% (likely range of 52% to 70%) in contrast to 70% participation from contacted, eligible controls [66]. Additionally, this study found that higher SES controls were more likely to agree to participate and complete components of data collection. Lower participation of controls with low SES has also been reported in studies that use other control selection methods [11, 66]. Almost all studies included in this review reported that participating controls had higher SES than participating cases. While measures of SES are typically adjusted for in multivariable analyses, there remains the concern that participating controls may not be representative of the source population in regards to exposure within strata of the controlled variables, resulting in biased measures of association even after statistical adjustment [67].

The extent to which exposure misclassification will influence findings on vitamin supplement use depends in part on the length of recall and thoroughness of the questions related to vitamin supplement use or micronutrient intake [36]. Previous research has found that open-ended

questionnaires for retrospective data collection can be very insensitive [46], and that error increases when single and multiple vitamins are not asked about separately [40]. Additionally, these errors can occur differentially by socio-demographic characteristics such as race [40]. After the initial finding from Thompson et al. of a strong negative association of prenatal vitamin supplement use and ALL, all studies in this review included specific questions about maternal vitamin supplement use in study questionnaires. However, the error due to recollection of exposures occurring several years in the past was reflected in the reliability data from the CCLS and Aus-ALL in which 13% and 27% of mothers, respectively, reported contradictory responses regarding vitamin supplement use at different times and with different methods of questioning. Length of recall period, however, did not differ between cases and controls, due to the implementation of individual or frequency matching of cases and controls on age or date of birth by all studies included in this review. The extent of exposure misclassification may also depend on how exposure is categorized in analyses. Calculation of levels of micronutrient intake depends on assumptions about vitamin supplement brand, formulation, and dose; because this information is rarely collected, exposure categorization is usually based on standard formulations when there may in fact be a broad range of products in use [36, 40]. The influence of exposure assessment on the observed measure of association was illustrated in Aus-ALL which found that the ORs (and even the direction of effect) differed by method of assessment and categorization of variables, although the inference was the same since no findings were statistically significant [41].

The proportion of the study population that uses supplements will influence positive and negative predictive values and the degree to which differential recall influences the odds ratio [18]. In contrast to widely cited assumptions regarding the expected influence of misclassification (e.g. non-differential misclassification will bias results towards the null) [68], the impact of exposure misclassification on measures of association is often unpredictable. Differential recall by case-control status does not always bias measures of association away from the null [16, 18]. Recall that is only approximately non-differential by case-control status is sufficient to bias the odds ratio away from null value, and certain circumstances (e.g. dependence between misclassification and other errors) can lead misclassification with exact non-differentiality to also result in bias away from the null value [68]. Additionally, recall bias may not substantially alter the observed odds ratio [16, 44, 47], as in the situation in which bias away from the null value due to enhanced recall among cases may be counterbalanced by bias towards the null value due to lack of specificity [18].

The results of the quantitative bias analysis suggest that decreased specificity strongly influenced the OR among Hispanic women, which is consistent with previous research indicating that specificity is a stronger determinant of the observed OR than sensitivity if exposure prevalence is low [18]. In contrast, decreased specificity had a less notable impact on ORs among white women, whereas corrections for differential sensitivity by case-control status resulted in a wide range of ORs in this population with much higher exposure prevalence [18]. The CLIC study found that the percentages of mothers reporting vitamin supplement use before and during pregnancy varied considerably by country (prevalence of vitamin supplement use ranged from 1% to 39% before pregnancy and 9% to 95% during pregnancy) [7]. Thus, considerations of the particular study context are important in postulating the possible influence of exposure

misclassification on study results, and quantitative bias analysis is a useful tool for explicitly assessing the range of possible measures of association in different populations.

A wide range of bias parameters were explored in order to assess the degree of selection bias and exposure misclassification necessary to account for our findings. Because most ORs corrected for selection bias and exposure misclassification among Hispanic women were substantially less than 1.0, these biases do not appear to account for the reduced risk of leukemia in children of Hispanic mothers who used vitamin supplements, given our assumptions about the bias parameters were correct. Conversely, the inference about the association of vitamin supplement use with leukemia among children of non-Hispanic white mothers is much less certain given the large range of corrected ORs under the bias parameter assumptions, with a substantial portion of ORs corrected for selection bias and exposure misclassification both further away from and greater than the null value. However, all corrections for higher sensitivity among cases resulted in negative associations further away from the null value than the uncorrected estimate. If we believe this to be the more likely scenario in case-control studies, these findings suggest that exposure misclassification may have biased results towards the null value among white women in the CCLS.

Some research has found that systematic errors in nutrition research may occur differentially by race/ethnicity [40, 69]. Although there is no evidence that this occurred in the CCLS, the results of the quantitative bias analysis suggest that it is plausible that the observed heterogeneity in the measure of association between vitamin supplement use and ALL by maternal ethnicity could be due to relatively modest differences in the occurrence of these systematic errors. For example, among white mothers, a higher sensitivity of exposure classification in cases compared to controls (0.9 versus 0.7, with the same specificity among cases and controls of 1.0) produced a corrected OR nearly identical to the OR observed among Hispanic women (corrected OR = 0.51). Although there are plausible genetic mechanisms for differences in the effect of vitamin supplement use on risk of leukemia in children of Hispanic women [70-72], studies attempting to replicate these findings should address the possible role of systematic error in producing heterogeneity by maternal ethnicity.

A limitation of this type of analysis is that the results and inference are dependent on the accuracy of the assumptions made in assigning the bias parameters and parameter probability distributions [20]. Additionally, the results of the quantitative bias analysis do not preclude the possibility that findings are due to chance or biases other than those specifically addressed through the bias analysis. Although the possible influence of uncontrolled confounding was not assessed, it is unlikely that there is a single confounder with a large enough association with leukemia to account for the observed association among Hispanic women [67]. However, the broad, systematic socio-demographic and behavioral differences between vitamin supplement users and non-users over the life course suggests that residual confounding could influence findings [2].

Despite a call for broader use of sensitivity analysis, quantitative assessment of bias is still infrequently implemented [19, 73]. However, there appears to be increasing efforts to explicitly acknowledge and examine the possible role of systematic error in case-control findings [11, 26, 74-78]. These efforts will be substantially improved by the collection, whenever possible, of

validation data for exposure assessment and data on characteristics of non-participating cases and controls [19]. Because so many research questions can only feasibly be examined through observational studies, quantitative bias analysis is essential to gauge our confidence in the inferences and conclusions drawn from our findings [19]. Although the limited validation data available for this study precludes firm conclusions about which bias scenario and corrected effect estimate is most accurate, the impact of these bias parameters on possible values of the OR in this population sheds light on the possible mechanisms underlying the different findings reported in the twelve studies examining maternal vitamin supplement use and childhood leukemia, including the potential for both differential and non-differential exposure misclassification to bias results towards null or small effect sizes. Future reviews and meta-analyses should give more consideration to the possible influence of systematic error on results when synthesizing findings from a body of studies carried out among different populations and in different environments.

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Table 1: Characteristics of studies assessing the association of childhood leukemia with maternal vitamin supplement use before or during pregnancy

Author and year of publication (Name of study[a])	Country	Years[b]	Outcome	N	Control sampling	Control matching	Exposure
Singer et al., unpublished (CCLS)	US	1995-2008	ALL and AML	681 ALL cases, 103 AML cases, and 1,076 controls	Randomly selected from birth certificates	Matched 1:1 or 1:2 on sex, date of birth, child Hispanic ethnicity and maternal race	Maternal use of vitamin supplements containing B vitamins before pregnancy and maternal use of any vitamin supplements before and during pregnancy.
Ajrouche et al. 2014 (ESTELLE)	France	2010-2011	ALL and AML	636 ALL cases, 100 AML cases, 1,421 controls	Random-digit dialing	Frequency-matched on age and gender	Folic acid supplements three months before pregnancy and during pregnancy by trimester
Amigou et al. 2012 (ESCALE)	France	2003-2004	ALL and ANLL	434 ALL cases, 51 AML cases, 8 undifferentiated or biphenotypic leukemia cases, and 1,681 controls	Random-digit dialing	Frequency matched with the cases on age and gender	Folic acid or multivitamin supplements in the month preceding conception and in the first, second and last quarter of the pregnancy and child folate pathway single nucleotide polymorphisms.
Milne et al. 2010 (Aus-ALL)	Australia	2003-2007	ALL	416 cases and 1,361 controls	Random digit dialing	Frequency matched on age (within 1 year), sex and State of residence	Folic acid and other vitamin supplements before and during the index pregnancy: any folate or iron; any folate; any iron; folate with iron; folate without iron; and iron without folate.
Linabery et al. 2010 (COG)	US and Canada	1996-2006	Infant leukemia (ALL and AML)	443 infant leukemia cases (263 ALL and 172 AML) and 324 controls	Random-digit dialing from 1996-2002; randomly selected from state birth registries from	Frequency matched on birth year and location of residence	Prenatal vitamin supplements anytime in the year before or during the index pregnancy (in the year before pregnancy; early in but before knowledge of pregnancy; and after knowledge of pregnancy).

					2003-2006		
Dockerty et al. 2007	New Zealand	1990-1993	ALL	97 ALL cases and 303 controls	Randomly selected from birth records.	Matching 1:1 on age and sex	Vitamin and mineral supplements during pregnancy: folic acid (any, with or without iron); iron (any, with or without folic acid); iron without folic acid; multivitamins; and other vitamins or mineral supplements
Schuz et al. 2007	Germany	1992-1997	ALL and AML	650 ALL cases, 105 AML cases, 755 matched controls, 2,057 total (unmatched) controls	Selected from the files of the local resident registration offices	Matched 1:1 on gender, data of birth within 1 year, and community	Maternal medication use during pregnancy, including vitamin, folate, or iron supplements as a category
Ross et al. 2005 (COG)	US	1997-2002	ALL and AML in children with Down syndrome (DS)	97 ALL cases with DS, 61 AML cases with DS, and 173 controls with DS	Randomly selected from rosters of pediatric patients with DS provided by physicians treating cases	Frequency matched on age at leukemia diagnosis	Maternal use of vitamin supplements in the year before pregnancy, during the index pregnancy but before knowledge of pregnancy, and after knowledge of pregnancy
Shaw et al. 2004	Canada	1980-2000	ALL	789 ALL cases and 789 controls	Selected from family allowance files or provincial health insurance agency files	Matched 1:1 on sex and age	Maternal medication use during pregnancy, including two vitamin supplement categories: vitamins that included folic acid (alone or in combination with other vitamins or minerals) or other vitamins/minerals that did not include folic acid
Wen et al. 2002 (CCG)	US	1989-1993	ALL	1,842 ALL cases and 1,986 controls	Random-digit dialing	Matched 1:1, 1:2 and 1:3 on age, race, and telephone area code and exchange	Maternal medication use during the one year before the index pregnancy and maternal use during the pregnancy and nursing of the index child.

Thompson et al. 2001	Australia	1984-1992	Common ALL	83 ALL cases and 166 controls	Postal survey of people randomly selected from the state electoral roll	Matched 1:2 on sex, date of birth (within 6 months), and region of residence	Medication use during pregnancy: iron or folate; iron and folate; folate with or without iron; iron alone.
Sarasua & Savitz 1994	US	1976-1983	ALL	56 ALL cases and 206 controls	Random-digit dialing	Matched on age (within three years), gender, and telephone exchange area	Maternal cured and broiled meat consumption; maternal vitamin supplement use was analyzed as an effect modifier.

[a] Australian Study of Causes of Acute Lymphoblastic Leukemia in Children (Aus-ALL); California Childhood Leukemia Study (CCLS); Children's Oncology Group (COG); Children's Cancer Group (CCG)
[b] Years of case and control ascertainment.

Table 2: Primary methods and findings of studies assessing the association of childhood leukemia with maternal vitamin supplement use before or during pregnancy

Authors	Case and control participation rates	Exposure assessment	Primary findings (Statistical method)
Singer et al.	86% of eligible cases consented to participate. 86% of eligible controls who agreed to participate in the CCLS, but only 45% of these were first-choice controls.	In-person interview with modified Block Food Frequency Questionnaire (FFQ) to assess dietary intake and vitamin supplement use during the year before the index pregnancy. Daily folate intake was calculated as a composite variable of vitamin/supplement use and dietary intake from natural and fortified foods.	Use of any supplements containing B vitamins before pregnancy: Hispanic women, OR = 0.51, 95% CI 0.28-0.94); white women, OR = 0.91, 95% CI 0.64-1.31; Asian women, OR = 2.24, 95% CI 0.91-5.51. Vitamin supplements before and during pregnancy: Hispanic women, OR = 0.34, 95% CI 0.14-0.79; white women, OR = 0.66, 95% CI 0.39-1.11. (Conditional logistic regression)
Ajrouche et al. 2014	93% of eligible cases and 86% of contacted eligible controls consented to participate. Contact with a household was made for only 11% of all selected phone numbers. 83% of all identified residences were screened for eligibility.	Telephone interview using structured questionnaire. Mothers were asked about use of folic acid supplements or other vitamins before or during the index pregnancy, specific period of intake (3 months before pregnancy or trimester of pregnancy), and the proprietary name of the drug for each period.	Folic acid supplementation 3 months before pregnancy, ALL: OR = 0.7 (95% CI 0.5-1.1); ANLL: OR = 0.4 (0.1-1.2). No associations observed by trimester of pregnancy (e.g. first trimester, ALL: OR = 1.1 (95% CI 0.9-1.5), ANLL: OR = 1.0 (95% CI 0.5-1.7)). (Unconditional logistic regression)
Amigou et al. 2012	91% of cases participated. Of 50,217 phone numbers dialed, 22,584 (45%) did not connect to a household, 24,411 (49%) connected to ineligible households, and 862 to respondents who hung up before eligibility could be assessed. 71.2% of 2,361 remaining numbers participated.	Telephone interview using structured questionnaire. Mothers were asked about folic acid or multivitamin supplements in the month preceding conception and in the first, second and last quarter of the pregnancy. "Maternal folic acid supplementation" refers to supplements with a minimum folic acid dosage of 0.4 mg/day. "Multivitamin supplementation" refers to vitamin supplementation containing folic acid at any dosage.	Folic acid supplementation, ALL: OR = 0.4 (95% CI 0.3-0.6); ANLL: OR = 0.3 (95% CI 0.1-0.9). Folic acid or multivitamin supplementation, ALL: OR = 0.7 (95% CI 0.5-0.9); ANLL: OR = 0.6 (95% CI 0.3-1.1). (Unconditional or polychotomous logistic regression)

Milne et al. 2010	80% of cases participated; 70% of controls where an RDD number was answered participated. An estimated 55% of all eligible controls participated. 94% of consenting cases and 92% of recruited controls responded to vitamin intake questions.	Self-administered postal questionnaire on medical history and postal food frequency questionnaire (FFQ) focused on dietary folate intake. Medical history questionnaire asked about ‘‘folate supplement’’ use 1 month before conception, during the first trimester, or during the second/third trimester. The FFQ included questions about dietary intake and an open-ended question about any vitamin, mineral or other dietary supplement use before or during pregnancy.	No association of ALL with any folate use in the month before conception (self-administered questionnaire (SAQ): OR = 0.99 (95% CI 0.75-1.31); FFQ: 0.88 (95% CI 0.66-1.16)) or during the first 3 (SAQ: OR = 1.19 (95% CI 0.91-1.56); FFQ: OR = 0.95 (95% CI 0.72-1.26)) or final 6 months of pregnancy (SAQ: OR = 0.83 (95% CI 0.65-1.06); FFQ: OR = 1.00 (95% CI 0.76-1.29)). (Logistic regression)
Linabery et al. 2010	From 1996-2002, 69% of eligible cases and 59% of random-digit dialing selected controls completed interviews. From 2003-2006, 59% of cases and 27% of birth certificate controls completed interviews.	Telephone interviews of mothers with questions about consumption of vitamin supplements anytime in the year before or during the index pregnancy (in the year before pregnancy; early in but before knowledge of pregnancy; and after knowledge of pregnancy). Questions regarding type of supplements and whether or not supplements were prescribed by healthcare providers were asked for each time period. These questions were also asked about iron supplementation exceeding the dose found in multivitamins.	No associations between vitamin supplement use in the year before and/or during pregnancy, in the periconceptional period, after knowledge of pregnancy, or over all periods (any prenatal vitamin consumption and infant ALL: OR=0.63, 95% CI 0.34-1.18; any prenatal vitamin consumption and infant AML: OR=1.20, 95% CI 0.53-2.75). (Unconditional logistic regression)
Dockerty et al. 2007	93% of eligible ALL cases participated; 69% participation of first-choice controls.	Home interviews using structured questionnaires. Mothers were asked "*Did you take any vitamins or mineral supplements during your pregnancy, in the 3 months before, or while breastfeeding? Include iron or folate and any others*." Vitamin supplement users were asked to specify the name of the supplement and use during the periods of interest. Analyses examined folic acid (any, with or without iron), iron (any, with or without folic acid), iron without folic acid, multivitamins, and other vitamins or mineral supplements.	In both unconditional and conditional analyses, there were no associations between the mother's use of folate (any, with or without iron) before pregnancy, during pregnancy, or while breastfeeding and the risk of ALL (OR=1.01, 95% CI 0.5-2.7 for any folate during pregnancy). There were no associations between other categories of vitamin supplements and ALL. (Unconditional and conditional logistic regression)

Schuz et al. 2007	82.6% of all childhood cancer cases (not specified for ALL); 70.9% of all controls (not specified for ALL matched controls)	Self-administered postal questionnaire assessing medication use, including a medication group for vitamin, folate or iron supplements with open text field for brand name	In conditional logistic regression analyses, there was no association of ALL with vitamin, folate, and/or iron supplements (OR=0.96, 95% CI 0.75-1.22). In frequency-matched unconditional logistic regression, the OR for vitamin, folate and/or iron supplements was 0.84 (95% CI 0.69-1.01) for ALL and 1.13 (95% CI 0.74-1.72) for AML. (Conditional and frequency-matched unconditional logistic regression)
Ross et al. 2005	75% of cases participated. Of 329 controls randomly selected from the rosters, no name or address was available for 114 (35%). 80.5% of 215 controls with names and addresses were interviewed.	Telephone interview using a structured, computer-assisted questionnaire, including questions about maternal use of vitamin supplements in the year before pregnancy, during the index pregnancy but before knowledge of pregnancy, and after knowledge of pregnancy	Any use of vitamins in the periconceptional period (i.e. in the year before pregnancy and during early pregnancy but before knowledge of pregnancy): ALL: OR=0.51, 95% CI 0.30-0.89. AML: OR=0.92, 95% CI 0.48-1.76). Use of vitamins after knowledge of pregnancy: OR=1.61, 95% CI 1.00-2.58. (Unconditional logistic regression)
Shaw et al. 2004	93.2% of cases participated; 86.4% of controls participated	Telephone interview using a structured questionnaire with a section on maternal use of medication during pregnancy, including two vitamin supplement categories: vitamins that included folic acid (alone or in combination with other vitamins or minerals) or other vitamins/minerals that did not include folic acid.	There was no association of ALL with vitamins with folic acid (OR=1.0, 95% CI 0.8-1.2) or with other vitamins (OR=1.0, 95% CI 0.7-1.3). (Conditional logistic regression)
Wen et al. 2002	Of 2081 eligible cases and 2597 eligible controls identified, the mother's interview was completed for 1914 cases (92.0%) and 1987 controls (76.5%).	Telephone interview using a structured questionnaire with questions on maternal and paternal use of a variety of medications during the one year before the index pregnancy and maternal use during the pregnancy and nursing of the index child (medication name, total times used, and duration of use).	Maternal use of vitamins only before pregnancy: OR = 1.2 (95% CI 0.4-3.0); both before and during pregnancy: OR = 0.7 (95% CI 0.5-1.0); only during pregnancy: OR = 0.7 (95% CI 0.5-1.0). (Conditional logistic regression)

Thompson et al. 2001	82% of cases participated. 26% of potential controls with one or more children younger than age 15 years responded to the original contact letter; 82% agreed to participate. The authors estimated overall control participation corrected for nonresponse was 74%.	In-person interview with nurse with questions about the use of medications during pregnancy. Mothers were asked an open-ended question about any other drug use not previously mentioned, type, strength and dose of drugs, when the drugs were used during the pregnancy, and duration of use. Analyses examined iron or folate; iron and folate; folate with or without iron; and iron alone.	Iron or folate supplementation in pregnancy was inversely associated with ALL (OR=0.37, 95% CI 0.21–0.65). Associations of "iron and folate" and "folate with or without iron" were similar. Only one mother took folate without iron. These associations did not substantially vary by time of first use of supplements or duration of use. (Conditional logistic regression)
Sarasua and Savitz 1994	70.8% of eligible cancer (ALL, brain tumor, lymphoma, and soft tissue sarcoma) cases were interviewed. Control response to RDD estimated at 78.6%; of 278 potential controls identified, 79.9% were interviewed.	In-home interview with parent, generally the mother. No details provided on assessment of maternal vitamin supplement use during pregnancy.	Vitamins during pregnancy and ALL, OR = 0.50 (95% CI 0.22-1.13) (calculated from raw data presented in the paper).

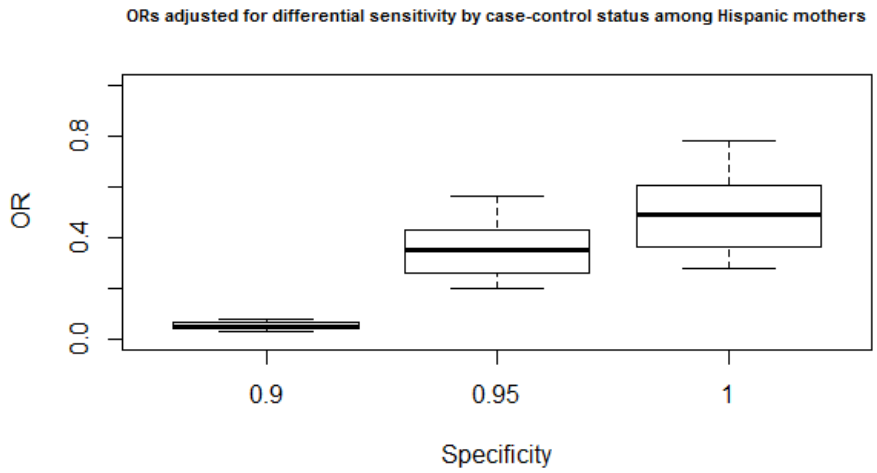
Table 3: Corrected odds ratios for postulated selection probabilities among exposed and unexposed cases and controls among Hispanic and non-Hispanic white mothers

Assumed exposure prevalence among mothers of missing Hispanic controls	**$S(Cont_E)/S(Cont_U)$**	**Corrected OR $S(Case_E)/S(Case_U)$ = (1.0/1.0)**	**Corrected OR $S(Case_E)/S(Case_U)$ = (1.0/0.9)**	**Corrected OR $S(Case_E)/S(Case_U)$ = (1.0/0.8)**
0.192*	0.61/0.61	0.49	0.44	0.39
0.15	0.67/0.6	0.55	0.49	0.44
0.10	0.75/0.58	0.63	0.57	0.51
0.05	0.86/0.57	0.74	0.67	0.59
0.0	1.0/0.56	0.88	0.79	0.70
Assumed exposure prevalence among mothers of missing white controls				
0.416*	0.61/0.61	0.88	0.79	0.70
0.35	0.65/0.58	0.99	0.89	0.79
0.30	0.68/0.57	1.05	0.94	0.84
0.25	0.72/0.55	1.15	1.04	0.92
0.0	1.0/0.48	1.83	1.65	1.47

*Prevalence in participating controls

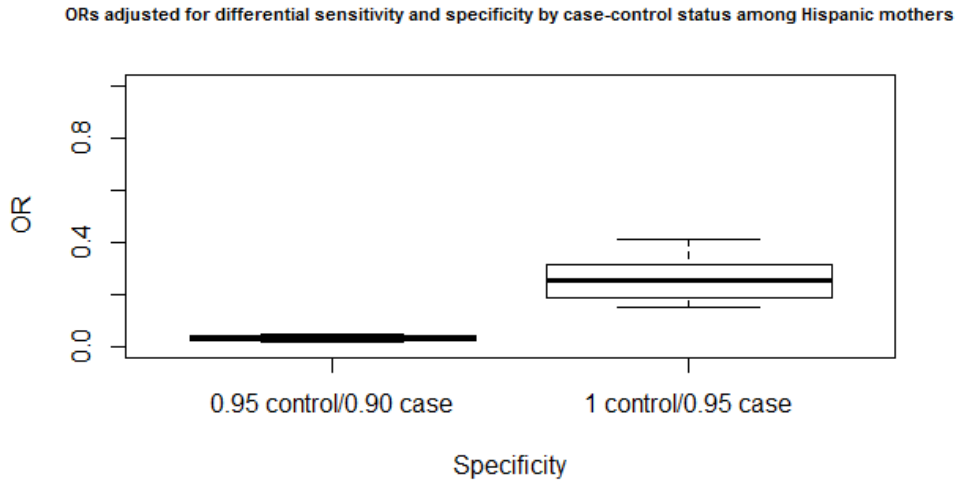
Figure 1: Bias intervals of corrected odds ratios for various exposure misclassification scenarios among Hispanic mothers

a)



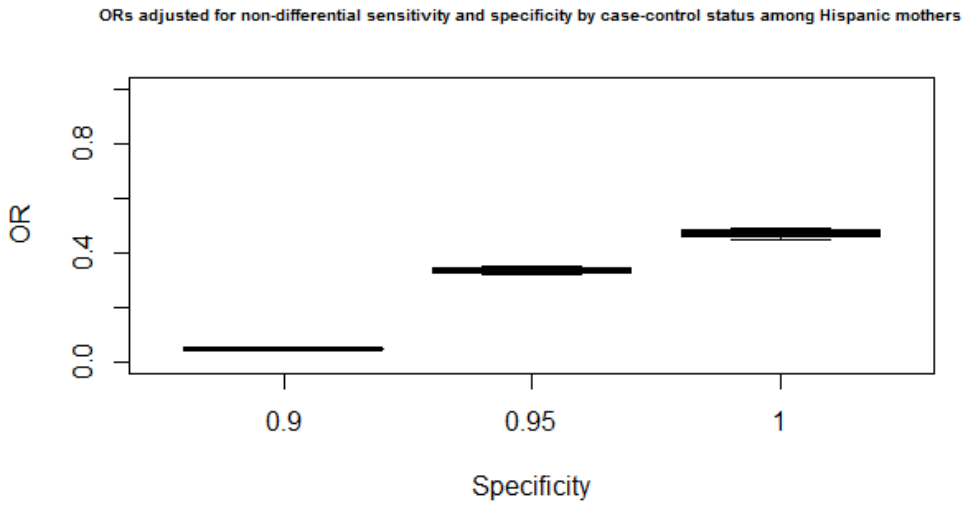
*Sensitivity ranged from 0.6-0.9. Differential sensitivity by case-control status (i.e. scenarios of both higher sensitivity in cases and higher sensitivity in controls) was plotted at different levels of non-differential specificity among cases and controls.

b)



*Sensitivity ranged from 0.6-0.9. Differential sensitivity by case-control status (i.e. scenarios of both higher sensitivity in cases and higher sensitivity in controls) was plotted at different levels of differential specificity among cases and controls (i.e. higher specificity among controls).

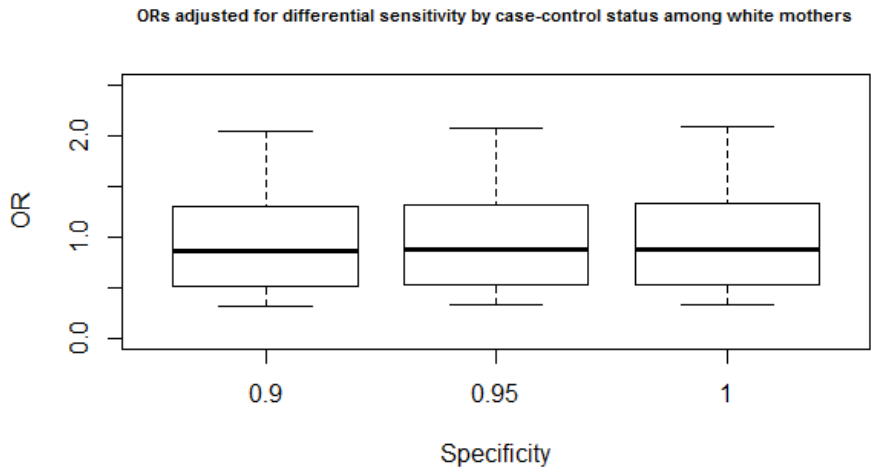
c)



*Sensitivity ranged from 0.6-0.9. Non-differential sensitivity by case-control status was plotted at different levels of non-differential specificity among cases and controls.

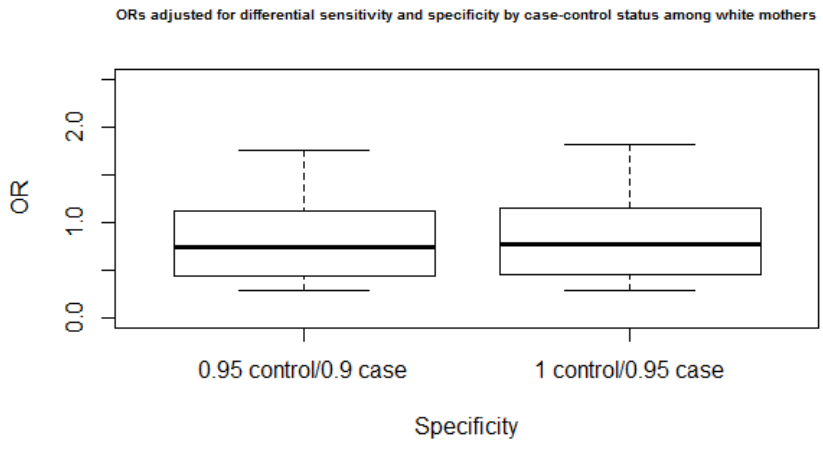
Figure 2: Bias intervals of corrected odds ratios for various exposure misclassification scenarios among white mothers

a)



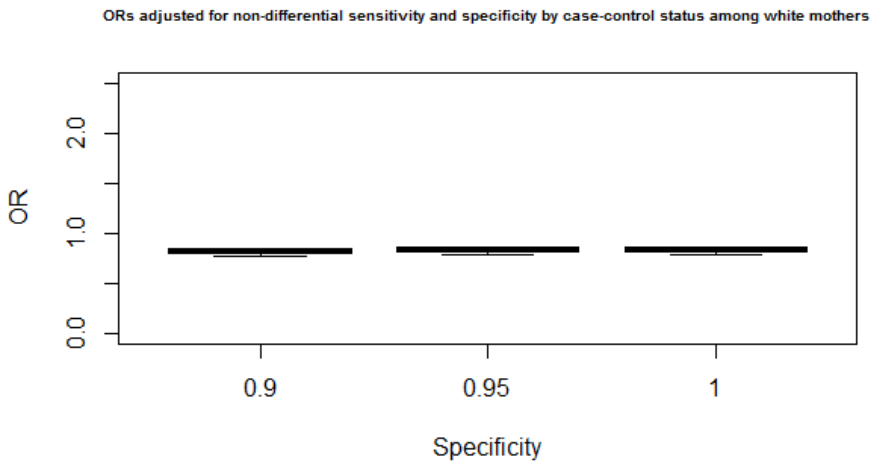
*Sensitivity ranged from 0.6-0.9. Differential sensitivity by case-control status (i.e. scenarios of both higher sensitivity in cases and higher sensitivity in controls) was plotted at different levels of non-differential specificity among cases and controls.

b)



*Sensitivity ranged from 0.6-0.9. Differential sensitivity by case-control status (i.e. scenarios of both higher sensitivity in cases and higher sensitivity in controls) was plotted at different levels of differential specificity among cases and controls (i.e. higher specificity among controls).

c)



*Sensitivity ranged from 0.6-0.9. Non-differential sensitivity by case-control status was plotted at different levels of non-differential specificity among cases and controls.

Table 4: Probabilistic bias analysis parameter probability distributions

Non-Differential Misclassification of Exposure by Case-Control Status				
Model (Scenario)	**Se_{case}**	**Sp_{case}**	**$Se_{control}$**	**$Sp_{control}$**
1	Trapezoidal (0.6, 0.7, 0.85, 0.95)	Trapezoidal (0.85, 0.9, 0.95, 1.0)	Trapezoidal (0.6, 0.7, 0.85, 0.95)	Trapezoidal (0.85, 0.9, 0.95, 1.0)
Differential Misclassification of Exposure by Case-Control Status				
2 (Se and sp higher in cases)	Trapezoidal (0.7, 0.8, 0.9, 0.95)	Trapezoidal (0.85, 0.9, 0.95, 1.0)	Trapezoidal (0.6, 0.7, 0.8, 0.95)	Trapezoidal (0.80, 0.85, 0.90, 1.0)
3 (Se and sp higher in controls)	Trapezoidal (0.6, 0.7, 0.8, 0.95)	Trapezoidal (0.80, 0.85, 0.90, 1.0)	Trapezoidal (0.7, 0.8, 0.9, 0.95)	Trapezoidal (0.85, 0.9, 0.95, 1.0)
Selection Bias among Hispanic Women				
	$S(Case_E)$	**$S(Case_U)$**	**$S(Cont_E)$**	**$S(Cont_U)$**
	Uniform (0.9, 1.0)	Uniform (0.8, 1.0)	Uniform (0.61, 1.0)	Uniform (0.56, 0.61)
Selection Bias among White Women				
	Uniform (0.9, 1.0)	Uniform (0.8, 1.0)	Uniform (0.61, 1.0)	Uniform (0.48, 0.61)

Table 5: Multiple bias analysis results of the association of childhood acute lymphoblastic leukemia with maternal vitamin supplement use corrected for selection bias and exposure misclassification, by maternal race/ethnicity

	Hispanic Women		White Women	
Bias Model	Median	2.5, 97.5 percentile intervals	Median	2.5, 97.5 percentile intervals
Adjusted for exposure misclassification, no random error				
Model 1	0.28	0.03, 0.45	0.84	0.79, 0.86
Model 2	0.36	0.04, 0.93	0.77	0.53, 1.15
Model 3	0.21	0.02, 0.77	0.90	0.62, 1.30
Adjusted for exposure misclassification and random error				
Model 1	0.27	0.03, 0.57	0.84	0.62, 1.14
Model 2	0.35	0.04, 1.05	0.77	0.48, 1.26
Model 3	0.21	0.02, 0.87	0.90	0.55, 1.44
Adjusted for selection bias, no random error	0.64	0.47, 0.84	1.23	0.87, 1.68
Adjusted for selection bias and random error	0.64	0.36, 1.09	1.22	0.78, 1.89
Adjusted for exposure misclassification and selection bias, no random error				
Model 1	0.35	0.04, 0.64	1.16	0.82, 1.59
Model 2	0.45	0.05, 1.26	1.08	0.65, 1.77
Model 3	0.27	0.02, 1.04	1.24	0.75, 2.02
Adjusted for exposure misclassification and selection bias and random error				
Model 1	0.33	0.04, 0.76	1.16	0.73, 1.81
Model 2	0.44	0.05, 1.26	1.07	0.59, 1.94
Model 3	0.27	0.02, 1.14	1.24	0.68, 2.20

Chapter 4:

Maternal diet quality before pregnancy and risk of childhood leukemia

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INTRODUCTION

Maternal nutrition during pregnancy may influence risk of leukemia in children through its role in fetal development, including the synthesis and repair of DNA, development of epigenetic processes, and establishment of the child's immune system. While most research to date has focused on the relationship between maternal folic acid intake and risk of childhood leukemia [1], there is evidence that maternal consumption of specific food groups may influence childhood leukemia risk. Previous research, including findings from our study, the California Childhood Leukemia Study (CCLS), has suggested that higher maternal consumption of fruits and vegetables may be associated with a reduced risk of childhood acute lymphoblastic leukemia (ALL) [2-4] and possibly infant leukemia (i.e. acute leukemia diagnosed under one year of age) [5]. This research also found that other food groups, specifically protein sources such as fish and seafood [3] and beans and beef [2, 4], may reduce risk of ALL. There is also some evidence that maternal consumption of certain foods, such as sugars or syrups, may increase risk of ALL [3, 6]. However, research examining maternal diet and childhood leukemia risk has generally been limited to specific nutrients [1, 7] or specific food components, such as processed meats [8], coffee and alcohol [9, 10], and dietary inhibitors of the nuclear enzyme topoisomerase II [5, 11, 12].

Measures of overall diet quality may better represent nutritional status and the complex biological interaction of multiple nutrients [13]. High quality diets characterized by diet quality indices are often positively correlated with biological markers of micronutrient intake and have been associated with reduced risk of all-cause mortality, cancer risk, and cardiovascular disease [14-18]. Maternal dietary patterns and quality have also been associated with birth outcomes, such as neural tube and congenital heart defects [19, 20]. The objective of this study is to examine the association between maternal diet quality, as assessed by a diet quality index, and risk of childhood ALL and acute myeloid leukemia (AML) in a population-based case-control study in California.

METHODS

Study Population

The CCLS is a population-based case-control study conducted in up to 35 counties in the San Francisco Bay Area and the California Central Valley [21]. Incident cases of newly diagnosed childhood leukemia in children 0-14 years old were ascertained from major pediatric clinical centers from 1995 to 2008 and matched on date of birth, gender, Hispanic ethnicity (based on either parent being Hispanic), and maternal race (White, Black, and Other/Mixed) to controls (ratio 1:1 or 1:2) randomly selected from California birth certificates through the Office of Vital Records at the California Department of Public Health. Control selection procedures and eligibility criteria have been described elsewhere [21, 22]. In brief, participation of ascertained and eligible cases and controls to the main questionnaire was approximately 86% [22], and dietary information in the year before pregnancy was provided by 98% of all respondents (970 cases and 1,187 controls). Approval for this study was received from the University of California, Berkeley Committee for the Protection of Human Subjects, the California Health and Human Services Agency Committee for the Protection of Human Subjects, and the Institutional Review Boards of all participating hospitals. Written informed consent was obtained prior to

interview from the responding parent of each participating child, and assent was obtained from children seven years of age and older.

Data Collection

Data were collected by in-person interview in either English or Spanish and from birth certificates. Details on dietary data collection have been described elsewhere [2, 4]. In brief, a modified version of the Block Food Frequency Questionnaire (FFQ) was administered during an in-person interview with the biological mother to assess her dietary intake and vitamin supplement use in the twelve months before the index pregnancy. This time period was chosen in order to examine nutritional adequacy at the time of conception and early pregnancy. The FFQ contained 76 food items and questions on vitamin supplement use before pregnancy. The FFQ also included five questions about if the mother consumed more, the same, or less fruit, vegetables, tofu or soy, tea and water *during* the pregnancy with the child. Spanish-speaking respondents were administered a Spanish version of the FFQ by bilingual interviewers. The Spanish FFQ included seven additional items common in the diets of the Latino population (i.e. evaporated or condensed milk, cooked green peppers, avocado or guacamole, chile peppers or chile sauce, sauces such as mole or sofrito, corn tortillas, and flour tortillas). Frequency of consumption of food groups was calculated by summing the reported frequency for all foods in a given food group; component foods of food groups are reported elsewhere [4]. The BlockSys and NutritionQuest computer programs (NutritionQuest, Berkeley, CA, USA) were used to calculate dietary nutrients from food by multiplying frequency of consumption of each food by its nutrient content and reported portion size, and then summing over all foods. Dietary folate intake was calculated in units of dietary folate equivalents (DFE) [23] and accounted for the different amounts of folic acid available from food before and after national fortification of grain products with folic acid in 1998. Nutrients obtained from vitamin supplements were estimated by multiplying the frequency of consumption of each type of supplement (multiple vitamins and specific single vitamins) times the amount of the nutrient in typical compositions of each type.

Diet Quality Index

Food frequency data were used to calculate scores for a modified version of the 2010 Healthy Eating Index (HEI-2010). The HEI-2010 is a measure of diet quality that assesses conformance to federal dietary guidance and was updated in 2010 to reflect the 2010 Dietary Guidelines for Americans, the basis for all US government nutrition recommendations and policies [24]. The HEI-2010 is considered an appropriate measure of diet quality for women who are pregnant or lactating [24]. The HEI-2010 comprises 12 nutritional components: nine "adequacy" components (total fruit, whole fruit (excluding fruit juice), total vegetables, greens and beans, whole grains, dairy, total protein foods, seafood and plant proteins, fatty acids) and three "moderation" components (refined grains, sodium, empty calories) [24]. Due to the lack of data for all HEI-2010 components, the index used in these analyses does not include separate components for whole fruit and seafood and plant proteins, although foods in these categories are incorporated into other components. Because we could not distinguish between whole and refined grains in our data, these components are excluded. Additionally, our modified index uses dietary fiber from beans for the greens and beans category, and uses percent calories from sweets and grams of dietary trans fat per day to represent empty calories. We added iron and folate from food as components due to their inclusion in dietary quality indices for pregnancy [25, 26] and previous studies indicating that higher maternal iron and folate intake is associated with reduced risk of

ALL [7, 27-29]. Given that the CCLS data were based on a semi-quantitative food frequency questionnaire, components were scored by quartiles (based on the distribution in controls) instead of at the level of the nutritional standard: for adequacy components, 0 points were assigned to those in the lowest quartile; 1, 2, and 3 points were assigned to those in the second, third, and fourth quartiles, respectively; and vice versa for moderation components (i.e. the lowest quartile received the maximum score of three points) [20]. All components except for the fatty acids ratio and percent calories from sweets were scored on a density basis (i.e. per 1000 kcal) to account for the diverse energy consumption of respondents. All component scores were summed to obtain a total diet quality score ranging from 0 (worst) to 33 (best).

Statistical Analysis

After excluding mothers of cases and controls with Down's syndrome (N=36) due to the distinct genetic risk of leukemia among these children, and excluding respondents reporting daily energy consumption of <500 or >6000 calories (N=20), 681 ALL cases and 931 matched ALL controls and 103 AML cases and 145 matched AML controls were available for analysis. The associations between diet quality score and select covariates were examined through t-tests and ANOVA among controls. Pearson correlation coefficients were calculated to examine the relationship between index components and overall diet quality score among controls. Conditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association of ALL and AML with diet quality score, as well as the association with each of the index components. We also examined the association of ALL with more fruit consumption (yes/no) and more vegetable consumption (yes/no) during pregnancy and whether or not adding these variables to the diet quality score model substantially changed the OR (>10%). Separate analyses were conducted for ALL and AML. Diet quality score was examined as both a continuous variable and in quartiles. Models were adjusted for the following covariates, which were selected a priori based on known or hypothesized associations with maternal diet and childhood leukemia: mother's Hispanic ethnicity, annual household income, father's education, mother's education, maternal age category, and vitamin supplement use in the year before pregnancy. The potential modifying influence of maternal Hispanic ethnicity (Hispanic versus non-Hispanic white or other), maternal vitamin supplement use (yes/no), and child's age at diagnosis (< or ≥ 5 years) on the association between diet quality score and ALL was assessed through the addition of interaction terms to the statistical models; interaction terms with a p-value less than 0.2 were considered a statistically significant indication of lack of additivity. We also stratified results for ALL by vitamin supplement use. Models for AML had an insufficient sample size for stratification or test of interaction. All results were considered statistically significant if the 95% CI excluded 1.0. Statistical analyses were carried out using STATA version 12.

RESULTS

Compared to ALL cases, controls had parents with higher household income and education, and mothers were older at the time of the child's birth and more likely to report vitamin supplement use in the year before pregnancy (Table 1). Controls matched to AML cases had parents with higher household income, and mothers were older at the time of the index child's birth.

Among cases and controls, diet quality score ranged from 2 to 30. The mean (SD) score was 15.7 (5.2) and 16.5 (5.3) among cases and controls, respectively. The 25th, 50th, and 75th percentiles were 12, 16, and 19 among all cases and 13, 16, and 20 among all controls. Among all controls, mean diet quality score was significantly higher in mothers who did not smoke in the three months prior to pregnancy ($p<0.001$), who used vitamin supplements in the year before pregnancy ($p<0.001$), and who were older at the time of the index pregnancy ($p<0.001$). Maternal body mass index (BMI) before pregnancy was available for only two-thirds of respondents, and diet quality score did not significantly differ by BMI. Hispanic women had a higher mean diet quality score than non-Hispanic white women or women of other races/ethnicities ($p<0.001$). Consequently, mean diet quality score among controls was highest in the lowest education group, comprised of 98% Hispanic women, followed by the highest education group (65% non-Hispanic white, 12% Hispanic, and 23% non-Hispanic other race/ethnicity). Correlations of diet quality score with the index components among controls were in the expected directions, ranging from -0.70 to 0.64 (Table 2).

Higher maternal diet quality score was associated with a reduced risk of childhood ALL (OR = 0.88, 95% CI 0.78-0.98 for each five point increase). When examined by quartiles, the reduction in risk was most pronounced for those in the highest quartile of diet quality score (OR = 0.66, 95% CI 0.47-0.93 for highest versus lowest quartile). Maternal Hispanic ethnicity did not modify these associations ($p=0.62$ for interaction term). While interaction by vitamin supplement use was not statistically significant ($p=0.65$), the reduction in ALL risk associated with higher diet quality score was greater among non-users of vitamin supplements (Table 4). There was a stronger negative association of diet quality score with ALL among children younger than five years at diagnosis (OR = 0.79, 95% CI 0.68-0.92 for a five point increase in diet quality score among 382 cases and 503 controls), whereas there was no association among children diagnosed at five years of age or older (OR = 1.02, 95% CI 0.84-1.24 for a five point increase among 256 cases and 340 controls; $p=0.08$ for interaction term).

Higher maternal diet quality score was also associated with a reduced risk of AML, though the 95% CI included one (OR = 0.76, 95% CI 0.52-1.11) (Table 3). There was a similar trend of decreasing AML risk with higher diet quality score when examined by quartiles (OR = 0.42, 95% CI 0.15-1.15 for highest versus lowest quartile).

When components of the diet quality index were examined separately, a reduction in risk of ALL and AML was observed for higher maternal consumption of fruit (Table 5). Other index components were not associated with ALL or AML. Due to the strong association between daily fruit servings and ALL and AML, we calculated diet quality score without fruit consumption as a component and found that its associations with ALL and AML did not substantially change (i.e. OR = 0.89, 95% CI 0.79-1.01 and OR = 0.79, 95 % CI 0.53-1.17, respectively, for a five unit change in score). Although fruit consumption was positively correlated with this revised score ($r = 0.37$), there was a substantial proportion of women with high fruit consumption and lower diet quality scores, suggesting that the association of diet quality score with ALL and AML is not entirely due to the influence of this one component.

Children of women who reported consuming much more or somewhat more vegetables during pregnancy had a reduced risk of ALL (OR = 0.70, 95% CI 0.56-0.89 among 593 cases and 774

controls, with adjustment for household income, parental education, maternal age, vitamin supplement use before pregnancy, and diet quality score), as did children of women who reported consuming much more or somewhat more fruit during pregnancy, although the confidence interval included 1.0 (OR = 0.87, 95% CI 0.69-1.09 among 622 cases and 819 controls, with adjustment for household income, parental education, maternal age, vitamin supplement use before pregnancy, and diet quality score). Adding more fruit or more vegetable consumption during pregnancy to the diet quality score model as covariates did not substantially change the OR for diet quality score (data not shown).

DISCUSSION

This is the first study to examine maternal diet quality in relation to childhood leukemia. Our data suggest a reduction in risk of ALL, and to a lesser extent AML, with higher maternal diet quality. No single food group or nutrient that was part of the diet quality score appeared to be driving the results, suggesting that the quality of the whole diet and the cumulative effects of many dietary components may be important in influencing childhood leukemia risk.

While much attention has been focused on the role of folate in children's health outcomes, there is increasing evidence of the importance of other micronutrients for prenatal development and birth outcomes, such as iron, vitamin D, and iodine [30, 31]. A measure of diet quality may provide a holistic representation of maternal diet, since diet quality index scores are positively associated with a wide range of beneficial nutrients (e.g. antioxidants, carotenoids) and negatively associated with intake of potentially harmful dietary components (e.g. saturated fat) [14]. Previous research has found that HEI score is strongly correlated with biomarkers of several micronutrients important for maternal and child health, including folate, vitamins C and E, and carotenoids [15, 32].

Maternal diet quality may influence leukemia risk in children through the influence of specific nutrients like folic acid on DNA synthesis and repair or epigenetic processes [33, 34]. An alternative or complementary pathway by which maternal nutrition may influence childhood leukemia risk is through its impact on the development of the child's immune system both before and after birth. Immune system development begins early in gestation, with a possible period of heightened vulnerability in immune cell development thought to occur when tissues are being seeded by precursors of immune cells (i.e. 4-7 weeks for myeloid derived cells and 8-18 weeks for lymphoid cells) [35]. There are three hypothesized pathways by which maternal malnutrition may influence the development of the fetal immune system [36]. First, maternal malnutrition may be a stressor that activates the hypothalamic-pituitary-adrenal axis, leading to a high concentration of maternal cortisol that has been shown to influence the developing fetal immune system. Second, low levels of micronutrients may interfere with organogenesis and the normal proliferation of immune cells. For example, animal studies have found that gestational zinc deficiency reduces the size of lymphoid organs and is associated with decreased antibody concentrations in offspring [37], and trials of maternal zinc supplementation during pregnancy in humans have found effects on cytokine production in infants [38]. Finally, poor maternal nutrition can alter the quality and quantity of immune factors transferred prenatally through the placenta or postnatally through the mammary gland [35, 36].

Further understanding of the biological mechanisms by which maternal diet quality may influence childhood leukemia risk is needed. We found that the influence of maternal diet quality on risk of ALL was more pronounced among children diagnosed under five years of age, which strengthens the inference that maternal nutritional status may influence the developmental processes occurring *in utero* that are related to the initiation of leukemia prior to birth [39, 40]. Furthermore, increased vegetable and possibly fruit consumption during pregnancy was associated with a reduced risk of ALL even after controlling for pre-pregnancy diet quality, suggesting that both maternal nutrition around the time of conception and throughout the pregnancy may influence risk of childhood leukemia. This finding is consistent with previous research which found that the associations between maternal vitamin supplement use and childhood leukemia did not differ by period of supplementation (i.e. preconception, during pregnancy, and by trimester) [1].

We used a measure of diet quality defined a priori based on a validated index that measures conformance with federal dietary guidelines [41]. Although we were unable to validate our modified index, the construct validity of this index is supported by its ability to successfully distinguish between groups with known differences in diet quality (e.g. smokers and nonsmokers) [41]. In our index, each component received the same weight, which we believe is appropriate given the limited evidence on the association between maternal consumption of food groups and risk of childhood leukemia. Calculation of our score by quartiles produced a smaller range of component scores and overall score than the traditional HEI-2010, but the estimated 5 and 95 percentiles of total score (7.5 and 25, respectively) indicated that there was a wide range of scores among individuals.

Diet quality is associated with higher food costs [42], and income and education are positively associated with better diet quality among adults [43, 44]. However, the observation in this study that Hispanic women had higher diet quality scores than women of other ethnicities/races despite having lower income and education levels is consistent with findings from other research [45]. A recent NHANES analysis found that Hispanics have better diet quality than whites or blacks, with greater consumption of fruits, vegetables, and legumes [43]. In addition, a growing body of research has suggested that diet quality among Hispanic women in the United States declines with increasing levels of acculturation [46-49]. However, we did not observe substantial heterogeneity in the association between maternal diet quality and ALL by maternal ethnicity.

The strengths of this study include the population-based design and the thorough assessment of maternal dietary intake in the year before pregnancy. Potential limitations include measurement error in the estimation of maternal food and nutrient intakes occurring several years in the past, which may increase the likelihood of null findings or small effect sizes [50, 51]. Although recall bias is possible, we believe it is minimal for a complex exposure such as diet quality, which is based on reported intake of diverse food groups and nutrients calculated from 76 food items. The socio-demographic characteristics and health behaviors of mothers with higher diet quality differ from mothers with low diet quality, and it is possible that we did not adjust for all relevant confounders. However, adjustment for several potential confounders, including measures of socio-economic status, had little influence on the associations between diet quality score and ALL and AML.

A measure of maternal diet quality attempts to better capture intake of the myriad nutrients and bioactive components consumed from foods, in contrast to a limited focus on particular nutrients. Given the importance of multiple nutrients and food components during pregnancy and lactation, this representation of maternal diet may be better suited to capture the complex interplay of diverse nutritional factors on fetal development, birth outcomes, and child health. Our finding of a strong association between maternal diet quality score and risk of childhood leukemia suggest that maternal nutritional status during pregnancy may play a role in the development of leukemia, and that the cumulative effects of many dietary components may be more important than the effect of single nutrients.

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Table 1: Select characteristics of matched case and control children, by leukemia subtype: the California Childhood Leukemia Study

	ALL		AML	
	Cases	**Controls**	**Cases**	**Controls**
	N (%)	N (%)	N (%)	N (%)
Total	681	931	103	145
Child's sex				
Male	390 (57.27)	538 (57.79)	56 (54.37)	80 (55.17)
Female	291 (42.73)	393 (42.21)	47 (45.63)	65 (44.83)
Child's age at diagnosis/reference date (years)				
< 2	83 (12.19)	106 (11.39)	28 (27.18)	44 (30.34)
2-6	396 (58.15)	543 (58.32)	19 (18.45)	28 (19.31)
6-9	96 (14.10)	132 (14.18)	15 (14.56)	19 (13.10)
≥ 9	106 (15.57)	150 (16.11)	41 (39.81)	54 (37.24)
Child's ethnicity				
Hispanic	312 (45.9)	414 (44.5)	40 (38.8)	56 (38.6)
Non-Hispanic White	256 (37.7)	365 (39.2)	44 (42.7)	62 (42.8)
Non-Hispanic Other	112 (16.5)	152 (16.3)	19 (18.5)	27 (18.6)
Mother's race/ethnicity				
Hispanic	285 (41.85)	364 (39.10)	35 (33.98)	50 (34.48)
Non-Hispanic White	298 (43.76)	437 (46.94)	55 (53.40)	75 (51.72)
Non-Hispanic Other	98 (14.39)	130 (13.96)	13 (12.62)	20 (13.79)
Household annual income (USD)				
<15,000	105 (15.42)	93 (9.99)	21 (20.39)	12 (8.28)
15,000-29,999	119 (17.47)	116 (12.46)	20 (19.42)	22 (15.17)
30,000-44,999	106 (15.57)	116 (12.46)	13 (12.62)	15 (10.34)
45,000-59,999	104 (15.27)	126 (13.53)	9 (8.74)	20 (13.79)
60,000-74,999	51 (7.49)	103 (11.06)	11 (10.68)	14 (9.66)
75,000+	196 (28.78)	377 (40.49)	29 (28.16)	62 (42.76)
Mother's education				
None or elementary	84 (12.33)	71 (7.63)	12 (11.65)	14 (9.66)
High school or similar	211 (30.98)	251 (29.96)	34 (33.01)	38 (26.21)
Some college or similar	188 (27.61)	293 (31.47)	24 (23.30)	38 (26.21)
Bachelor's degree or higher	198 (29.07)	316 (33.94)	33 (32.04)	55 (37.93)
Father's education				
None or elementary	78 (11.82)	99 (11.05)	13 (13.00)	14 (9.86)
High school or similar	238 (36.06)	271 (30.25)	36 (36.00)	44 (30.99)
Some college or similar	137 (20.76)	231 (25.78)	17 (17.00)	35 (24.65)

Bachelor's degree or higher	207 (31.36)	295 (32.92)	34 (34.00)	49 (34.51)
Maternal age at child's birth (years)				
<25	231 (33.92)	237 (25.46)	34 (33.01)	25 (17.24)
25-35	342 (50.22)	516 (55.42)	55 (53.40)	89 (61.38)
>35	108 (15.86)	178 (19.12)	14 (13.59)	31 (21.38)
Vitamin supplement use in year before pregnancy				
Yes	213 (31.5)	347 (37.5)	34 (33.3)	56 (38.9)
No	463 (68.5)	579 (62.5)	68 (66.7)	88 (61.1)
Healthy eating index score				
Mean (SD)	15.7 (5.2)	16.5 (5.2)	15.6 (5.0)	16.5 (5.1)

Table 2: Descriptive information about components of the modified Healthy Eating Index (HEI) 2010 among controls

Modified HEI-2010 Components	Criterion for maximum score of 3	Median (25^{th}-75^{th} percentiles)	Correlation with score among controls
Adequacy			
Fruit (daily servings/1,000 kcal)	Highest quartile	0.5 (0.3-0.8)	0.53
Vegetables (daily servings/1,000 kcal)	Highest quartile	1.2 (0.8-1.9)	0.40
Dietary fiber from beans (g/1,000 kcal)	Highest quartile	1.1 (0.6-2.2)	0.39
Dairy (serving/1,000 kcal)	Highest quartile	0.9 (0.5-1.3)	0.19
Total protein foods (g/1,000 kcal)	Highest quartile	39.7 (34.6-45.0)	0.34
Fatty acids ((PUFAS+MUFAS)/SFAs)	Highest quartile	1.8 (1.6-2.1)	0.28
Dietary iron (mg/1,000 kcal)	Highest quartile	7.1 (6.1-8.4)	0.46
Dietary folate (DFE/1,000 kcal)	Highest quartile	226.2 (165.7-289.7)	0.64
Moderation			
Sodium (g/1,000 kcal)	Lowest quartile	1.2 (1.1-1.3)	-0.06
Trans fat (g/1,000 kcal)	Lowest quartile	3.3 (2.4-4.3)	-0.70
Percent calories from sweets	Lowest quartile	7.3 (4.0-13.0)	-0.59

Table 3: Association between the modified Healthy Eating Index (HEI) 2010 and risk of childhood ALL and AML

	ALL 638 cases, 843 controls	**AML** 96 cases, 125 controls
Modified HEI-2010	**Odds Ratio (95% CI)**	**Odds Ratio (95% CI)**
Continuous score[a]	0.88 (0.78-0.98)	0.76 (0.52-1.11)
Quartile 1 (<13)	(Ref)	(Ref)
Quartile 2 (13-15)	0.71 (0.51-1.00)	0.65 (0.25-1.69)
Quartile 3 (16-19)	0.73 (0.54-1.01)	0.60 (0.21-1.68)
Quartile 4 (>20)	0.66 (0.47-0.93)	0.42 (0.15-1.15)

*Models adjusted for mother's Hispanic ethnicity, father's education, mother's education, household income, maternal age at child's birth, and vitamin supplement use before pregnancy.
[a] Odds ratios for a 5 point increase in HEI-2010 score.

Table 4: Association between the modified Healthy Eating Index (HEI) 2010 and risk of childhood ALL among vitamin supplement users and non-users

	Vitamin supplement users 109 cases, 130 controls	**Vitamin supplement non-users** 321 cases, 371 controls
Modified HEI-2010	**Odds Ratio (95% CI)**	**Odds Ratio (95% CI)**
Continuous score[a]	1.00 (0.74-1.36)	0.76 (0.63-0.92)
Quartile 1 (<13)	(Ref)	(Ref)
Quartile 2 (13-15)	0.83 (0.34-2.01)	0.63 (0.37-1.06)
Quartile 3 (16-19)	0.74 (0.30-1.83)	0.52 (0.32-0.86)
Quartile 4 (>20)	1.03 (0.41-2.57)	0.43 (0.25-0.76)

*****Models adjusted for mother's Hispanic ethnicity, father's education, mother's education, household income, and maternal age at child's birth.

[a] Odds ratios for a 5 point increase in HEI-2010 score.

Table 5: Associations between individual components of the modified Healthy Eating Index (HEI) 2010 and risk of childhood ALL and AML

	ALL 638 cases, 843 controls	**AML** 96 cases, 125 controls
Modified HEI-2010 Components	**Odds Ratio (95% CI)**	**Odds Ratio (95% CI)**
Adequacy		
Fruit (1 serving/1,000 kcal)	0.70 (0.52-0.94)	0.23 (0.08-0.70)
Vegetables (1 serving/1,000 kcal)	0.97 (0.86-1.10)	0.84 (0.54-1.30)
Dairy (1 serving/1,000 kcal)	1.01 (0.84-1.22)	0.87 (0.48-1.57)
Dietary fiber from beans (1 g/1,000 kcal)	0.95 (0.88-1.02)	1.03 (0.80-1.34)
Protein (10 g/1,000 kcal)	0.91 (0.79-1.05)	1.00 (0.63-1.59)
Fatty acid ratio	1.07 (0.78-1.45)	1.08 (0.42-2.77)
Dietary iron (1 mg/1,000 kcal)	0.98 (0.93-1.03)	0.87 (0.73-1.03)
Dietary folate (100 DFE/1,000 kcal)	0.97 (0.83-1.12)	0.83 (0.56-1.21)
Moderation		
Sodium (100 mg/1,000 kcal)	1.05 (0.99-1.11)	0.99 (0.85-1.17)
Trans fat (1 g/1,000 kcal)	1.07 (0.99-1.16)	1.11 (0.85-1.44)
Percent calories from sweets (10%)	1.09 (0.94-1.26)	1.40 (0.84-2.34)

*Separate models for each energy-adjusted food group/nutrient as continuous variables adjusted for maternal Hispanic ethnicity, household income, mother's education, father's education, maternal age category, and vitamin supplement use. Fatty acid ratio and percent calories from sweets were not energy-adjusted.

Chapter 5:

Conclusion

SUMMARY OF FINDINGS

This dissertation examined the relationship between maternal diet and vitamin supplement use before and during pregnancy and the risk of leukemia in children in a large, population-based case-control study in California. This dissertation was the first to use principal components analysis to examine the combined influence of one-carbon metabolism nutrients on childhood leukemia risk and to assess if maternal ethnicity modified these associations. This study also examined how characteristics of case-control studies on maternal vitamin supplement use and childhood leukemia could result in the occurrence of systematic error, and quantitatively explored the possible influence of selection bias and exposure misclassification on the association between childhood leukemia and maternal vitamin supplement use observed in the California Childhood Leukemia Study (CCLS). This dissertation was also the first to employ a diet quality index to assess the relationship between overall maternal diet quality before pregnancy and risk of childhood leukemia.

To reflect the biological interaction among nutrients that contribute to one-carbon metabolism [1], principal components analysis was used to create a variable summarizing dietary intake of folate, vitamins B12 and B6, riboflavin, and methionine. Higher maternal intake of one-carbon metabolism nutrients from food and supplements was associated with a reduced risk of acute lymphoblastic leukemia (ALL) and possibly acute myeloid leukemia (AML). When examining combined nutrient intake from food only, there were no systematic differences observed by maternal ethnicity. In contrast, intake of B vitamins from supplements (any versus none) before pregnancy was associated with a reduced risk of ALL in children of Hispanic women, but there was no association among children of non-Hispanic white women and a possible increased risk among children of Asian women. Differences in the prevalence of one-carbon metabolism pathway polymorphisms by race/ethnicity is a plausible mechanism for this observed heterogeneity, which is consistent with the modifying influence of Hispanic ethnicity on associations of ALL with genetic variants in the folate pathway [2]. Differences in the distribution of nutrient intakes by race/ethnicity may also explain this observation [3]. However, because the heterogeneity by maternal ethnicity was not observed when examining nutrient intake from food only, this finding may be due to systematic error.

In order to assess the possible influence of systematic error on findings from studies examining maternal vitamin supplement use and childhood leukemia, case and control participation and exposure assessment was reviewed in twelve studies. The review found that most studies had low control participation and that controls usually had higher socioeconomic status (SES) than participating cases, which increases the likelihood of selection bias in studies of exposures associated with SES. Additionally, half of the included studies examined broad categories of vitamin supplements (e.g. prenatal vitamins) and few asked about brand or frequency of consumption. Because prenatal vitamins frequently contain multiple micronutrients, it is difficult to attribute the observed effects in these studies to a specific vitamin such as folate. The binary categorization of the vitamin supplement variable employed in most studies precluded the determination of the particular levels of micronutrient intake that may be beneficial or harmful and the possible presence of a threshold effect. Time period of supplementation before and during pregnancy also varied across studies. The inconsistency of findings across studies may be due in part to systematic errors occurring as a result of these study characteristics.

A bias analysis was carried out to provide a quantitative estimate of the possible influence of selection bias and exposure misclassification on the association of maternal vitamin supplement use and childhood leukemia in the CCLS. The quantitative bias analysis suggested that, under the assumed bias parameters, selection bias and exposure misclassification are unlikely to account for the association of vitamin supplement use and ALL observed in Hispanic women. The odds ratios (OR) corrected for these systematic errors among non-Hispanic white women varied widely. However, all exposure misclassification corrections for higher sensitivity in cases produced negative associations further away from the null than the uncorrected estimate. If one assumes that this scenario is more likely than higher sensitivity among controls, as is commonly hypothesized for case-control studies [4, 5], these findings suggest that the negative association observed among non-Hispanic white women may have been underestimated. Results of the bias analysis suggest that relatively modest differences in the occurrence of systematic errors between Hispanic and white women could account for the heterogeneity observed in the association between vitamin supplement use and ALL by maternal ethnicity.

This dissertation found that higher maternal diet quality score, calculated through a diet quality index, was associated with a reduced risk of childhood ALL, with a more pronounced reduction in risk among younger children and children of women who did not use vitamin supplements before pregnancy. There was a similar reduced risk of AML with increasing maternal diet quality score, although the confidence interval included 1.0. No single food group or nutrient included in the index appeared to account for the results, suggesting that the quality of the whole diet and the cumulative effects of many dietary components may be important in influencing childhood leukemia risk. Additionally, increased fruit and vegetable consumption during pregnancy was associated with a reduced risk of ALL, suggesting that both maternal nutritional status before pregnancy (and around the time of conception) and throughout pregnancy may influence the developmental processes that could contribute to the risk of developing childhood leukemia.

FUTURE DIRECTIONS

Methodological improvements in the design and analysis of case-control studies of maternal diet and childhood leukemia

1. *Control Selection and Participation*
   Participation rates in epidemiologic studies have been declining over the last several decades, due primarily to increased refusal to participate by persons invited to take part in research and increased difficulty in finding and contacting potential participants [6]. Persons who do agree to participate in research are likely to have higher SES and differ from non-participants in other ways related to exposures of interest, including health status and behaviors [6]. Efforts should continue to be made to improve control participation in case-control studies, through strategies such as providing incentives for participation, using face-to-face recruitment and data collection, and providing participants with different options for data collection (e.g. mail or internet) [6]. Because control recruitment and retention will continue to be a primary challenge in the implementation of case-control studies, an important approach to addressing the limitations of case-control studies will be attempts to collect socio-demographic and exposure data for a sample of non-participating subjects, which can potentially be used to adjust measures of association for nonparticipation.

2. *Exposure Assessment*

The collection of high quality dietary information is a critical facet of all nutritional epidemiologic studies. However, there is no dietary assessment method that is capable of capturing true intake without measurement error [7]. Despite limitations [8], food frequency questionnaires will continue to be the primary dietary assessment method for case-control studies attempting to examine diet as an exposure [9]. The use of more objective measures of exposure, such as serum nutrient concentrations, will have limited applicability in case-control studies attempting to measure nutrient exposures occurring in the past. However, novel applications of biomarkers in case-control studies should be pursued, such as the measurement of nutrient status at birth from stored newborn dried blood spots [10].

The calculation of total nutrient intake from food and supplements, requiring detailed dietary information captured through FFQs, will allow more thorough exploration of the complex ways in which nutrition affects disease risk, including the levels of intake at which effects are observed, possible threshold effects or dose-response relationships, and interactions among nutrients and food components [7]. Of potential importance in childhood leukemia is the potential effect of both micronutrient deficiency and micronutrient excess, both of which have been shown to cause genome damage [11]. The finding in this study that Asian mothers who took vitamin supplements in the year before pregnancy had a possible increased risk of ALL was unexpected, but it is plausible that such an effect could occur if this population had certain micronutrient intakes above the tolerable upper intake levels. There are concerns about the adverse effects of high micronutrient intake during pregnancy: for example, there is evidence that high intake of vitamin A has teratogenic effects [12], that vitamin E supplementation during pregnancy may increase maternal risk of severe eclampsia and gestational hypertension [13], and that excessive iron intake in non-anemic pregnant women can increase risk of gestational hypertension, gestational diabetes mellitus, and small-for-gestational-age birth rate [14]. The possible adverse effects of excessive micronutrient intakes among women before and during pregnancy should be explored further in relation to childhood leukemia risk.

Detailed exposure information will also be critical to assessing if the association of childhood leukemia with maternal diet differs during various time windows before and during pregnancy. Although some studies have attempted to address this question, limited exposure information has not allowed sufficient examination of the influence of maternal nutrient intake during different time periods (e.g. trimesters of pregnancy). Efforts should be made to identify if there are critical windows during which maternal diet is of particular importance for childhood leukemia risk, just as research has identified the early first trimester as the period of importance for risk of neural tube defects [15].

3. *Analytic Approaches*

A particular challenge in the analysis of nutritional epidemiologic studies is that the high correlations between components of the diet makes it challenging, if not impossible, to attribute associations to one particular nutrient or facet of the diet [7]. Various analytic approaches have been implemented in nutritional epidemiologic research to try to address

this issue, including the implementation of principal components or cluster analysis and the use of dietary quality indices [9]. However, these approaches have not been commonly implemented in research examining maternal diet and childhood leukemia. The findings of this dissertation point to the usefulness of these analytic techniques and suggest that they should be more broadly considered in examining the relationship between maternal diet and risk of childhood leukemia.

The Childhood Leukemia International Consortium has allowed the pooling of data from twenty-two case-control studies on childhood leukemia carried out in twelve countries [16]. The large sample sizes attained through this approach will allow the exploration of novel research questions and will reduce the influence of random error on results. However, the statistical power attained through large sample sizes will have little effect on reducing the influence of systematic error on study findings [17]. Consequently, sensitivity analyses and quantitative bias analysis should be more broadly implemented in order to determine the possible influence of systematic error on findings and report a range of possible associations given the bias parameters [18]. The collection of validation and reliability data, whenever possible, will greatly contribute to the usefulness of these analyses. The potential influence of systematic error should also be given greater consideration in the examination of heterogeneity across studies in reviews and meta-analyses [19].

Exploration of mechanisms and pathways by which maternal diet may influence childhood leukemia subtypes

There are several plausible pathways by which maternal diet may influence childhood leukemia risk, but there is little empiric evidence on how diet influences the mechanisms involved in the development of childhood leukemia or how these pathways may differ by leukemia subtype. For example, there is increasing evidence that high intake of folic acid has the potential to promote cancer growth after preneoplastic lesions have developed [20, 21]. Consequently, the association of high maternal folic acid intake during pregnancy might influence the risk of infant leukemia, which is initiated *in utero* [22, 23], differently than leukemia cases that are diagnosed later in childhood. Only one study has examined the association between maternal vitamin supplement use and infant leukemia [24], and the small number of infant leukemia cases in the CCLS precluded examination of this question. Differences in the associations of maternal diet or vitamin supplement use with ALL and AML suggest that maternal diet may influence risk of childhood leukemia subtypes in distinctive ways and via various pathways. Mediation or pathway analyses could shed light on the particular mechanisms involved in the relationship between maternal diet and childhood leukemia, with the following pathways of primary interest:

1. *Epigenetic Mechanisms*

   Recent analyses have attempted to characterize the DNA methylation profiles among children with acute leukemia, with studies identifying global hypomethylation of the genome and region-specific hypermethylation in leukemia cells, consistent with other cancers [25-27]. Research has also found that fetal exposure to folate and folate-related intermediates (e.g. homocysteine) are determinants of DNA methylation at birth [28-30]. Ongoing research at the CCLS is examining the association of maternal diet with DNA

methylation in controls at birth. Future studies should examine the relationship between maternal folate intake or child folate status at birth (e.g. as measured through newborn dried blood spots) and the epigenetic changes associated with childhood leukemia, as has been done in neural tube defects research [31].

2. *Infection*
   Full understanding of how maternal nutrition influences the development of the child's immune system both before and after birth is still needed. Research should examine how maternal nutrition impacts the proliferation of fetal immune cells involved in the development leukemia and how maternal diet is related to the child's early immune system functioning [32, 33]. Alternative analytic approaches could be used to explore the simultaneous involvement of epigenetic and infection-related pathways involved in the association of particular micronutrients with childhood leukemia: for example, one recent study employed structural equations modelling to explore the influence of various one-carbon metabolism and immune system factors on lung carcinogenesis and found that factors representing both methionine-homocysteine metabolism and immune activation had a direct protective effect [34].

3. *Birth Weight*
   Numerous studies have found an increased risk of ALL and AML with increasing birth weight or among children who were large-for-gestational-age [35-40]. A recent meta-analysis of twelve case-control studies from the Childhood Leukemia International Consortium reported a statistically significant increased risk of ALL for children who were large for gestational age relative to appropriate for gestational age (OR =1.24 (95% CI 1.13-1.36) among 7,348 cases and 12,489 controls [41]. Although birth weight is known to be affected by a variety of factors [42-44], increasing evidence indicates that maternal prenatal supplementation with multiple micronutrients [45] and maternal intake of one-carbon metabolism nutrients in particular [46-49] plays a role in fetal growth and birth weight. Maternal serum levels of micronutrients like vitamin B12 have also been associated with DNA methylation patterns in insulin-like growth factor genes in cord blood [50]. The influence of maternal prenatal diet on birthweight or epigenetic changes in fetal growth genes and the interaction of these factors in influencing childhood leukemia risk should be explored.

The findings of this dissertation suggest that maternal nutrition before and during pregnancy influences risk of leukemia in children. However, there much remains much to be known about the complex interplay of maternal diet and other prenatal factors involved in the development of this disease. Methodological improvements in case-control studies examining maternal diet and childhood leukemia will allow future studies to explore important research questions such as the levels of micronutrient intake that have potentially beneficial or harmful effects, interactions between maternal dietary factors and other exposures, and the importance of various time windows in this relationship. The exploration of particular pathways by which maternal diet may influence leukemia subtypes will also shed light on the maternal dietary factors of primary consequence and potential avenues for intervention to prevent future cases of disease.

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Appendices

Appendix A: Full PubMed search strategy

((((("child" OR "childhood" OR "infant" OR "adolescent")) AND ("leukemia" OR "leukaemia" OR "Precursor Cell Lymphoblastic Leukemia-Lymphoma/etiology"[Mesh])) AND ("folic acid" OR "folate" OR "vitamin" OR "diet" OR "nutrition" OR "supplement" OR "Dietary Supplements"[Mesh] OR "Folic Acid"[Mesh])) AND ("maternal" OR "parental" OR "mother" OR "prenatal" OR "pregnancy")

Appendix B: Additional methodological description and formulas for quantitative bias analysis

Because the conditional adjusted odds ratio was very similar to the unconditional unadjusted odds ratio, the unadjusted data was used to perform the bias analysis.

Table 1: Bivariable and multivariable odds ratios computed from conditional and unconditional logistic regression among Hispanic and white mothers in the California Childhood Leukemia Study

	Hispanic Mothers			**White Mothers**		
	Case	Control	Total	Case	Control	Total
Vitamin supplement users	30	70	100	115	182	297
Vitamin supplement non-users	255	294	549	183	255	438
Total	285	364	649	298	437	735
Odds Ratios						
Unconditional bivariable OR	0.49 (0.31-0.78)			0.88 (0.65-1.19)		
Conditional bivariable OR	0.48 (0.29-0.79)			0.87 (0.64-1.19)		
Conditional multivariable OR	0.51 (0.28-0.94)			0.91 (0.64-1.31)		

Adjustment of the odds ratio for possible selection bias

The selection bias factor is given by the exposed versus unexposed selection probabilities comparing cases (Pr selection cases exposed/PR selection cases unexposed) and controls (PR selection controls exposed/PR selection controls unexposed) [1-3]. If the selection probabilities among cases and controls do not differ across exposure status, no bias exists (selection factor = case ratio/control ratio = 1). To adjust the OR for potential selection bias, divide the observed OR by the selection bias factor [1].

Selection probabilities	Exposure = 1	Exposure = 0
Cases	$S_{case,1}$	$S_{case,0}$
Controls	$S_{control,1}$	$S_{control,0}$

Selection bias factor = $[S_{case,1}/S_{case,0}]/[S_{control,1}/S_{control,0}]$
$OR_{adj} = OR / [(S_{case,1}/S_{case,0})/(S_{control,1}/S_{control,0})]$

Of ascertained eligible cases, 86% agreed to participate. In Phase I and Phase II, an average of 95% of cases were ascertained in participating hospitals. Thus, under the assumption that all non-ascertained cases were eligible, we assume that we are missing 64 cases among Hispanics (285/0.86 = 331; 331/0.95 = 349; 349-285 = 64 missing cases) and 67 cases among non-Hispanic whites (298/0.86 = 347; 347/0.95 = 365; 365-298 = 67 missing cases).

Among contacted and eligible controls, 86% agreed to participate. However, only 45% of participating controls were first-choice controls. Thus, we assume that we are missing 233 first-choice controls among Hispanics (364 – (364*0.45) = 200 missing first-choice controls; 200/0.86 = 233 missing first-choice controls) and 279 first-choice controls among whites (437 – (437*0.45) = 240 missing first-choice controls; 240/0.86 = 279 missing first-choice controls).

We can calculate the limits of selection bias by assuming that all non-participating case and control mothers were all either vitamin supplement users of non-users. Participating cases and controls had higher SES that non-participating cases and controls, and our exposure of interest is associated with higher SES. Thus, we created the scenarios in which all non-participating cases and controls are non-users of vitamin supplement use (i.e. prevalence of vitamin supplement use is overestimated in our high-SES case and control population).

Table 2: Selection bias factors and adjusted odds ratio limits under the assumption that non-participating case and control mothers were all either vitamin supplement users or non-users

Hispanic Mothers			
Missing case/control exposure assumption	Selection proportions (E=1 cases, E=0 cases, E=1 controls, E=0 controls)	Selection bias factor	Adjusted odds ratio
User/User	0.32, 1.0, 0.23, 1.0	1.38	0.35
User/Non-user	0.32, 1.0, 1.0, 0.56	0.18	2.75
Non-user/User	1.0, 0.80, 0.23, 1.0	5.42	0.09
Non-user/Non-user	**1.0, 0.80, 1.0, 0.56**	**0.70**	**0.70**
White Mothers			
Missing case/control exposure assumption	Selection proportions (E=1 cases, E=0 cases, E=1 controls, E=0 controls)	Selection bias factor	Adjusted odds ratio
User/User	0.63, 1.0, 0.39, 1.0	1.60	0.55
User/Non-user	0.63, 1.0, 1.0, 0.48	0.30	2.92
Non-user/User	1.0, 0.73, 0.39, 1.0	3.50	0.25
Non-user/Non-user	**1.0, 0.73, 1.0, 0.48**	**0.65**	**1.35**

Adjustment of the odds ratio for possible exposure misclassification

The probability that a subject who was truly exposed was correctly classified as exposed is the classification scheme's sensitivity. The probability that a subject who was truly unexposed was correctly classified as unexposed is the classification scheme's specificity. The sensitivity and specificity of exposure classification among cases and controls is postulated in order to calculate the OR adjusted for potential misclassification [1].

Table 3: Formulas for calculation of data corrected for exposure misclassification, given the observed data and the bias parameters

	Observed		Corrected	
	E_1	E_0	E_1	E_0
Cases	a	b	$[a-D_{+total}(1-SP_{D+})]/[SE_{D+} - (1-SP_{D+})]$	D_{+total} - A
Controls	c	d	$[c-D_{-total}(1-SP_{D-})]/[SE_{D-} - (1-SP_{D-})]$	D_{-total} - C
	a+c	b+d	A+C	B+D

*Reproduced from Lash et al. [1]

A+C is the corrected total number of exposed individuals and B+D is the corrected total number of unexposed individuals

Table 4: Studies reporting or allowing calculation of sensitivity and specificity of self-reported vitamin supplement use

	Population (Country)	Sensitivity	Specificity
Drews et al. 1990[a] [4]	Mothers of sudden infant death syndrome cases and controls (United States)	85% (cases); 82% (controls)	52% (cases); 59% (controls)
Dorant et al. 1994 [5]	Adult men and women aged 55-69 years (The Netherlands)	65.9%	98.5%
Patterson et al. 1998[b] [6]	Adult men and women (United States)	98%	100%
Ishihara et al. 2001 [7]	Adult men and women (Japan)	80.6%	89.2%
Satia-Abouta et al. 2003[b] [8]	Adult men and women aged 50-75 years (United States)	88%	100%
Messerer et al. 2004 [9]	Middle-aged and elderly men (Sweden)	78%	93%

[a] Sensitivity and specificity for prenatal iron use; specificity was >0.8 for almost all other exposures for cases and controls.
[b] Sensitivity and specificity calculated from the data presented in the paper.

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