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Serum folate levels and fatality among diabetic adults: A 15-y follow-up study of a national cohort

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ABSTRACT

Objective: Folate is involved in carbohydrate metabolism, a process that can have clinical implications regarding diabetes management. The aim of this study was to assess the relationship between serum folate and fatality among adults with diabetes.
Methods: A retrospective cohort study was conducted with 532 adults with diabetes who participated in Phase II of NHANES III (National Health and Nutrition Examination Survey III; 1991–1994). This study served as baseline and was linked to the National Death Index database for a 15-y (1991–2006) follow-up study. Estimates of hazard ratios (HRs) for all-cause and cancer-related deaths, cardiovascular disease (CVD), and diabetes for individuals with different serum folate levels were obtained from Cox proportional hazards regression.
Results: The mean age of adults with diabetes and detected serum folate at baseline was 63.2 y (SD 13.8 y). During follow-up, diabetes was listed as a contributor for 138 of 299 deaths. For all-cause deaths, the fatality rate of the upper quartile (74.30/1000 person-years [PY]) was almost twofold higher than the lower quartile (41.75/1000 PY) of serum folate levels. After adjusting for several covariates, including serum vitamin B_{12}, cotinine, homocysteine and CVD history at baseline; the HRs for all-cause fatalities were 1.00 (reference), 1.62 (95% confidence interval [CI], 1.06–2.47) and 1.76 (95% CI, 1.09–2.83) among adults with diabetes in the lower, intermediate, and upper quartiles of serum folate levels, respectively.
Conclusion: Results indicate that high serum folate concentrations are associated with an increased fatality risk among adults with diabetes. Further studies are warranted to determine the mechanism(s) of this phenomenon.

Introduction

Folic acid (or folate), a form of water soluble B vitamin, has been found crucial for proper functioning of the brain. Folate is an essential vitamin that is added to grain products in many countries to prevent major birth defects, with respect to brain and spine development, such as spina bifida and anencephaly [1]. It is also involved in the physiological process of converting carbohydrates into glucose for energy [2]. This metabolic activity can have significant biological and clinical implications in the management of diabetes, which is characterized by chronic hyperglycemia, with disturbances of carbohydrate, fat, and protein metabolism [3]. Because folate cannot be synthesized de novo by

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the body, it must be obtained through food sources or dietary supplements. It is a naturally occurring vitamin in staples such as leafy green vegetables, legumes, egg yolks, liver, and citrus fruit; whereas its synthetic form, folic acid, is found in dietary supplements, fortified foods, and pharmaceutical vitamins [4,5]. It is worth noting that vitamin B_{12} plays a significant role in some of the critical physiological functions of folate. Dietary sources of vitamin B_{12} in the serum or blood are mainly from animal-based foods, including meat, milk, eggs, fish, and shellfish; however, a few plant-based foods (eg, certain types of mushrooms) contain considerable amounts of vitamin B_{12} [6].

At optimum physiological levels of folate and vitamin B_{12} (as coenzyme), methionine synthase converts homocysteine (HCY) into methionine or HCY is trans-sulfated into the glutathione (GSH) biosynthetic pathway. Disruption of this trans-sulfation process, thus depleting the GSH levels, has been associated with oxidative stress [7,8]. The combination of high concentration of HCY in the blood and oxidative stress can influence the pathogenesis of type 2 diabetes [7]. However, the relationship between serum folate levels and diabetes has not been adequately examined in patients with type 2 diabetes [9]. A limited number of scientific investigations examining this serum folate–diabetes relationship have been conducted in experimental studies [7], small-scale clinical trials [9–13], and children's studies [11]. The only observational cohort study with a relatively large sample size was conducted among a population surveyed before folate fortification; however, the investigation did not adjust for HCY and vitamin B_{12} levels, which are key confounders in studies to determine a potential association between diabetes and serum folate [14].

Although these pieces of evidence have been reported, the relationship between serum folate levels and diabetes-related mortality has not been clearly defined. To our knowledge, there have been no studies performed on a nationally representative US population to examine the relationship between serum folate and fatality among individuals with diabetes since NHANES II (Second National Health and Nutrition Examination Survey, 1976–1980). NHANES II was conducted before folic acid fortification; therefore, a data set obtained from NHANES III (1988–1994) was used for this study. Thus, a nationally representative cohort population was selected from the NHANES III survey to determine the relationship between serum folate concentrations and fatality among diabetic adults.

Materials and methods

Study population

A retrospective cohort study was conducted with adults with diabetes who participated in Phase II of NHANES III (1991–1994). Serving as the baseline, this study was linked to the National Death Index database for a 15-y (1991–2006) follow-up study. NHANES III was conducted by the National Center for Health Statistics (NCHS), a subsidiary of the Centers for Disease Control and Prevention (CDC), and consists of a nationwide probability sample of noninstitutionalized civilians. The survey combined multiple components including household interviews, physical examinations, and laboratory tests; and was conducted from October 1988 through October 1994 in two phases. Phase I (October 18, 1988–October 24, 1991) and Phase II (September 20, 1991–October 15, 1994) were conducted in 44 and 45 different locations, respectively. Because serum HCY is a major confounding variable to the current investigation, it was tested on examinees ages ≥12 y in Phase II only. Analyses were restricted to 648 adults (age 19+ y) who had been diagnosed with diabetes before the baseline survey and also had vital status information at the end of the follow-up. An adult with diabetes was defined as an individual who answered "yes" to the question "Have you ever been told by a doctor that you have diabetes or sugar diabetes?" or if he or she reported current use of insulin, oral antidiabetic medications, or both. Women with gestational diabetes and individuals without data on serum folate, family income, educational attainment, smoking, and drinking were excluded. After exclusions, 532 adults with diabetes were retained for analysis. The present study was exempt from ethics review by the Georgia Southern University Institutional Review Board (IRB); however, the NHANES protocol was reviewed and approved by the IRB of the NCHS.

Baseline data collection

Baseline data were collected as part of the NHANES III during in-home interviews and subsequent visits by participants to a mobile examination center. Demographic characteristics and health-related information used in the present study were collected using standardized questionnaires.

Health risk factors and medical history

Current alcohol drinkers were defined as "heavy" if they reported consuming five or more drinks for >10 d within the past 12 mo, and "moderate" if they consumed five drinks or more for ≤10 d within the past 12 mo. Smoking was defined as "heavy" if respondents reported that they smoked ≥40 cigarettes in the past 5 d, and as "moderate" if < 40 but >0 cigarettes were smoked. In the baseline interview, participants were asked "Have you ever been told by a doctor that you had one or more of the following general medical illnesses: asthma, arthritis, cancer, chronic bronchitis, diabetes, hypertension, gout, lupus, stroke, or thyroid disease?" These conditions were selected from the National Health Interview Survey to represent conditions that are both prevalent and associated with substantial morbidity in the US population [15].

Serum folate concentrations and other biomedical measurements

Following a standardized protocol and maintaining controlled and constant environmental conditions, trained technicians collected blood samples from participants and processed them in a mobile examination center. Whole blood, which was not treated with an anticoagulant, was collected into serum separator tubes and held at room temperature for 30 to 60 min before centrifugation. Serum was separated, frozen at −20°C, and transported on dry ice to the CDC central laboratory for priority analyses. Samples were stored at −70°C for 8 mo to 3 y before being analyzed. Serum concentrations of folate and vitamin B_{12} were measured using the Quantaphase II Radioassay Kit (Bio-Rad Laboratories, Hercules, CA, USA) during Phase II of the NHANES III. Briefly, analyses of folate and vitamin B_{12} were carried out at the Inorganic Toxicology and Nutrition Branch of the Division of Laboratory Sciences, National Center for Environmental Health. A 400-μL serum sample each was used for the serum folate and vitamin B_{12} assays [16,17]. A distribution curve was created based on levels of detected serum folate. The upper quartile of the distribution included those individuals with serum folate levels ≥8.8 ng/mL, the lower quartile included those with levels <4 ng/mL, and the intermediate group (50th percentile) included those with levels between 4 and 8.7 ng/mL.

Serum HCY was measured at the US Department of Agriculture–Human Nutrition Research Center on Aging using the high-performance liquid chromatography (HPLC) method as previously reported [18]. Also, serum cotinine levels were measured using HPLC/atmospheric pressure chemical ionization tandem mass-spectrometry and serum vitamin B_{12} was measured using the same protocol used for serum folate measurements. All laboratory measurements were carried out in a blinded fashion with respect to fatality and other clinical data.

Socioeconomic status

Ethnicity was coded as non-Hispanic white, non-Hispanic black, or Mexican-American; the remaining participants were defined as "others" in this study. Educational attainment was defined by the participants' highest completed grade of school regardless of age, and categorized into three levels: high school/equivalent or below, some college, and college graduate or higher. Socioeconomic status (SES) was assessed using the poverty to income ratio (PIR) [19]. PIR was calculated from the previous year's family income and family size, by comparing the midpoint for the category and the family size with the federal poverty line [15].

Fatality follow-up and identification of diabetes-related deaths

Twelve identifiers (including social security number, sex, date of birth, and other demographic characteristics) were used to link NHANES III participants with the National Death Index to ascertain vital status and cause of death [20]. More than 96% of the deceased participants and almost all living participants were successfully followed up and correctly classified. The person-year [PY] contributions from each participant were calculated as the time between the person's baseline examination and the date of death or December 31, 2006 (if still alive). Average follow-up time was 10.46 y with a maximum of 18.08 y. Cause of death was determined using the underlying cause listed on the death certificate. The International Classification of Diseases, Injuries and Cause of Death (ICD–10th Revision), was used to code the cause of deaths into several main groups, including heart disease, diabetes, hypertension, stroke, and unnatural diseases.