Nutrition, Metabolism & Cardiovascular Diseases (2017) **27**, 1015–1020

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

Red blood cell folate concentrations and coronary heart disease prevalence: A cross-sectional study based on 1999–2012 National Health and Nutrition Examination Survey

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Received 1 June 2017; received in revised form 28 June 2017; accepted 17 July 2017
Handling Editor: F. Galletti
Available online 25 July 2017

KEYWORDS
Folate;
Red blood cell;
Coronary heart disease;
National Health and Nutrition Examination Survey;
Cross-sectional

Abstract *Background and aim:* Folate is involved in a number of metabolic pathways. Red blood cell (RBC) folate is a well-established indicator of folate intake. However, studies focused on the association between RBC folate and coronary heart disease (CHD) are limited. The aim of the current study was to investigate the effect of RBC folate concentrations on the presence of CHD in a nationally representative sample of American adults.
Methods and results: In the 1999–2012 National Health and Nutrition Examination Survey (NHANES), 22,499 subjects aged 30–74 years with RBC folate concentrations, CHD status and responses to co-variates questions were included; 822 (3.65%) participants were identified as having CHD. Bio-Rad Quanta Phase II radioassay and microbiological assay were used to measure RBC folate concentrations. Firstly, we treated RBC folate as a categorical variable, based on RBC folate tertiles, and used logistic regression analysis to display the RBC folate and CHD relationship. Secondly, we explored associations using a combination of restricted cubic spline and logistic regression models, stratified by sex. After adjusting for several well-established traditional CHD risk factors, RBC folate was positively related to CHD presence in the total population and the association was more pronounced among males than females. A J-shaped pattern was observed in RBC folate concentrations for females.
Conclusion: Elevated RBC folate concentrations were associated with higher CHD risk. Further investigation is needed to test the association in large-scale follow-up studies.

Introduction

Folate is a water-soluble vitamin and plays a crucial role in multiple metabolic pathways, among which are methylation and synthesis of DNA, methylation of RNA and protein [1–3]. Folate occurs naturally in foods, especially vegetables, cereals, fruits, nuts, seeds and dairy products [4]. Apart from being given as supplements [5], folic acid, the synthetic form of folate, has been added in number of fortified food, like enriched bread, cereals, flours, cornmeals, pastas, rice, and other grain products, by US government [6]. Red blood cell (RBC) and serum folate are widely used biomarkers to reflect folate status [7]. RBC folate is a measure of the folate intake over the past 90–120 days, whereas serum folate is a reflection of the recent folate intake [8].

Coronary heart disease (CHD) remains one of the leading causes of mortality in many countries, including US [9],

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http://dx.doi.org/10.1016/j.numecd.2017.07.007
0939-4753/

Portugal [10], Brazil [11] and China [12]. It has been shown that insufficient folate intake contributed to elevation of plasma homocysteine concentrations [13], a potential risk factor of CHD [14]. Recently, a few studies have focused on the relationship between serum folate status and CHD risk, though they reported inconsistent findings [15–19]. Further, few studies have explored the association between RBC folate concentrations, a biomarker of longer-term folate status, and CHD risk [20–22].

In the current study, we sought to explore the relationship between RBC folate and CHD presence using data from the 1999–2012 National Health and Nutrition Examination Survey (NHANES), a large-scale US nationally representative survey.

Methods

Study population

We obtained data from the public-access files of NHANES, which was organized by National Center for Health Statistics (NCHS) [23]. NHANES used a stratified and multistage design to retrieve a representative sample of the general US population. We restricted our analyses to those who were between age 30 and 74 years in the 1999–2012 NHANES datasets (n = 23,691). Then, 1192 participants were excluded due to missing information on any of RBC folate, CHD status or co-variates. Finally, 22,499 subjects were included in the present study. The NCHS Research Ethics Review Board reviewed and approved NHANES and all participants signed written informed consent. The School of Medicine Low Risk Ethical Review Committee in the University of Queensland approved our study (approval number 2016-SOMILRE-0161).

RBC folate concentrations

Two laboratory methods were used to measure RBC folate concentrations in our study. In 1999–2006, Bio-Rad (BR) Quanta Phase II radioassay was used to measure the RBC folate concentrations. In 2007–2012, RBC folate concentrations were measured using a microbiological assay (MA). To make the results comparable, we used the following steps, as recommended by NHANES, to convert BR results to MA results [8].

Step 1: Convert the 1999–2006 BR RBC folate (RBC folate, nmol/L) to whole blood folate (WBF, nmol/L) using the hematocrit (HCT, %) and the BR serum folate (Serum folate, nmol/L) in the following equation:

$$\text{WBF} = \text{RBC folate} \times \frac{\text{HCT}}{100} + \text{Serum folate} \times \left(1 - \frac{\text{HCT}}{100}\right)$$

Step 2: Apply the following forward linear regression to obtain an adjusted WBF ($\text{WBF}_{\text{adjusted}}$) using WBF from step 1 to match the MA WBF:

$$\text{WBF}_{\text{adjusted}} = 10^{0.2204 + 1.017 \log_{10} \text{WBF}}$$

Step 3: Convert the 1999–2006 BR serum folate (Serum folate, nmol/L) results to equivalent values to match the MA serum folate (Serum $\text{folate}_{\text{adjusted}}$, nmol/L) results using the forward fractional polynomial regression equation specified in the analytic note on serum folate:

$$\text{Serum folate}_{\text{adjusted}} = 10^{0.0188x^3 - 2.7109x^{-0.5} + 3.8276},$$

where $x = \log_{10}\text{Serum folate}$

Step 4: Calculate RBC $\text{folate}_{\text{adjusted}}$ by using $\text{WBF}_{\text{adjusted}}$ (from step 2) and Serum $\text{folate}_{\text{adjusted}}$ (from step 3):

$$\text{RBC folate}_{\text{adjusted}} = \left[\text{WBF}_{\text{adjusted}} - \text{Serum folate}_{\text{adjusted}} \times \left(1 - \frac{\text{HCT}}{100}\right)\right] \times \frac{100}{\text{HCT}}$$

CHD status

The participants were asked whether a doctor or other health professional has told them that they had CHD. The subjects were regarded as positive for CHD presence if they answered yes to this question.

Co-variates

In the multivariate analysis, we adjusted the 10-year Framingham CHD risk to eliminate the influences of traditional CHD risk factors [24]. Thus, information on ages, sex, total cholesterol, high-density lipoprotein-cholesterol, blood pressure, diabetes and smoking were obtained. Age, sex, smoking status, and current use of hypertension medication were collected by interview. Systolic and diastolic blood pressures were measured during participants' visit to the examination center. Participants were considered current smokers if they smoked at least 100 cigarettes in their entire life and were currently smoking. Total cholesterol and high-density lipoprotein cholesterol concentrations were obtained by enzymatic assays on a Hitachi 704 Analyzer (Roche Diagnostics, Indianapolis, Indiana).

Statistical analysis

We used medians and interquartile ranges (IQRs) to describe continuous variables and utilized frequencies and percentages to display categorical variables.