Atherosclerosis 235 (2014) 94–101

Contents lists available at ScienceDirect

Atherosclerosis

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

Plasma homocysteine, dietary B vitamins, betaine, and choline and risk of peripheral artery disease

Monica L. Bertoia [a,b,*], Jennifer K. Pai [c,d], John P. Cooke [e], Michel M. Joosten [a,b,f,g], Murray A. Mittleman [a,c], Eric B. Rimm [b,c,d], Kenneth J. Mukamal [a,b]

[a] Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA, USA
[b] Department of Nutrition, Harvard School of Public Health, Boston, MA, USA
[c] Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA
[d] Channing Division of Network Medicine, Department of Medicine, Brigham & Women's Hospital, Harvard Medical School, Boston, MA, USA
[e] Houston Methodist Research Institute, Houston, TX, USA
[f] Top Institute Food and Nutrition, Wageningen, The Netherlands
[g] Department of Internal Medicine, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

ARTICLE INFO

Article history:
Received 18 December 2013
Received in revised form
6 April 2014
Accepted 9 April 2014
Available online 28 April 2014

Keywords:
Peripheral artery disease
Homocysteine
Folate
Vitamin B12
Vitamin B6
Riboflavin
Betaine
Choline

ABSTRACT

Objective: Few studies have examined the roles of homocysteine and related nutrients in the development of peripheral artery disease (PAD). We examined the associations between plasma homocysteine, dietary B vitamins, betaine, choline, and supplemental folic acid use and incidence of PAD.
Methods: We used two cohort studies of 72,348 women in the Nurses' Health Study (NHS, 1990–2010) and 44,504 men in the Health Professionals Follow-up Study (HPFS, 1986–2010). We measured plasma homocysteine in nested matched case–control studies of clinically recognized PAD within both cohorts, including 143 PAD cases and 424 controls within the NHS (1990–2010) and 143 PAD cases and 428 controls within the HPFS (1994–2008). We examined the association between diet and risk of incident PAD in the cohorts using a food frequency questionnaire and 790 cases of PAD over 3.1 million person-years of follow-up.
Results: Higher homocysteine levels were positively associated with risk of PAD in men (adjusted IRR 2.17; 95% CI, 1.08–4.38 for tertile 3 vs. 1). There was no evidence of an association in women (adjusted IRR 1.14; 95% CI, 0.61–2.12). Similarly, higher folate intake, including supplements, was inversely associated with risk of PAD in men (adjusted HR 0.90; 95% CI, 0.82–0.98 for each 250 μg increase) but not women (HR 1.01, 95% CI, 0.88–1.15). Intakes of the other B vitamins, betaine, and choline were not consistently associated with risk of PAD in men or women.
Conclusion: Homocysteine levels were positively associated and dietary folate intake was inversely associated with risk of PAD in men but not in women.

1. Introduction

Elevated levels of the amino acid homocysteine are positively associated with endothelial dysfunction, oxidation of low-density lipoprotein, and monocyte adhesion [1]. Despite the widely-speculated influence of endothelial dysfunction and oxidative stress in peripheral artery disease (PAD) [2], the relationships between homocysteine, B vitamins and PAD have not been well studied. Furthermore, despite the well-known metabolic pathways that govern homocysteine, no prior studies have examined PAD risk in relation to the combination of plasma homocysteine and its dietary determinants using validated measurements, including dietary intakes of B vitamins, related betaine and choline, and supplements.

B vitamins lower homocysteine levels by promoting homocysteine metabolism. Homocysteine can be removed from circulation by catabolism to cysteine through a pyridoxal phosphate (vitamin B_6) dependent pathway or remethylation to methionine. Betaine or folate (vitamin B_9) can donate the methyl group, the latter of which requires cobalamin (vitamin B_{12}) and riboflavin (vitamin B_2) [3]. Choline plays a peripheral role as betaine can be endogenously synthesized from choline.

* Corresponding author. 655 Huntington Ave, Building 2, room 309, Boston, MA 02115, USA. Tel.: +1 607 592 7583; fax: +1 617 432 2435.
E-mail address: mbertoia@hsph.harvard.edu (M.L. Bertoia).

http://dx.doi.org/10.1016/j.atherosclerosis.2014.04.010
0021-9150/

Abbreviations list

PAD	peripheral artery disease
CVD	cardiovascular disease
MI	myocardial infarction
ABI	ankle-brachial index
NHS	Nurses' Health Study
HPFS	Health Professionals Follow-up Study
FFQ	food frequency questionnaire
IRR	incidence rate ratio
SD	standard deviation
CI	confidence interval
HR	hazard ratio
EDTA	ethylenediaminetetraacetic acid
HDL-C	high-density lipoprotein cholesterol
LDL-C	low-density lipoprotein cholesterol
CRP	c-reactive protein
HbA_{1c}	hemoglobin A_{1c}
BMI	body mass index

The observed association between homocysteine levels and risk of CVD [4] led to a series of randomized controlled trials of B vitamin supplementation. Although these clinical trials consistently lowered homocysteine levels using supplemental folate, vitamin B_6, and vitamin B_{12}, meta-analyses show no impact on risk of cardiovascular events including myocardial infarction (MI) and, and death [5]; however, findings are mixed for stroke [5,6]. Furthermore these studies found no change in underlying atherosclerosis [7]. In contrast, prospective studies and clinical trials of homocysteine and PAD have so far presented inconclusive findings.

One previous prospective cohort study reported a positive association between homocysteine and PAD [8] but two reported no association [9,10]. Of three clinical trials, two found no effect of B vitamin supplementation on atherosclerotic progression [11], arterial stiffening [11], ankle-brachial index (ABI) [12], or carotid and femoral ultrasonography [12] however a third found small improvements in pulse wave velocity and ABI [13]. To address this question more fully, we examined the associations between plasma homocysteine, dietary B vitamins, betaine, choline, and supplemental folic acid use and risk of PAD in two prospective cohort studies including sizable numbers of both men and women. We hypothesized that homocysteine levels would be positively associated and B vitamins, betaine, and choline inversely associated with risk of PAD.

2. Materials and methods

2.1. Study population

2.1.1. Cohort studies

The Nurses' Health Study (NHS) is a prospective cohort of 121,700 female nurses [14]. All women were age 30–55 years at baseline (1976) and continue to be followed. PAD cases in the NHS were confirmed beginning in 1990 until 2010; therefore, our analyses are restricted to 1990–2010. Women were excluded from our analyses if they had confirmed CVD (myocardial infarction, stroke, PAD, or revascularization of the coronary, carotid, or peripheral beds) at baseline. We additionally excluded women who reported implausible dietary energy intake (<600 or >3500 kcal/day) at baseline or during follow-up.

The Health Professionals Follow-up Study (HPFS) is a parallel prospective cohort of 51,529 male health professionals age 40–75 years at baseline (1986) [15]. PAD cases were confirmed in the HPFS through 2010; our analyses include follow-up time between 1986 and 2010. We used the same exclusion criteria for men, with the exception of a higher cutoff for implausible energy intake, <800 or >4200 kcal/day.

Of the 121,700 women participating in the NHS, 42,816 were missing dietary data at baseline in 1990 (after 14 years of follow-up), 594 cases of clinically significant PAD were reported before 1990, 1652 MI, 454 revascularization, 3038 angina, and 485 stroke. After additionally excluding women with missing covariate data on age ($n = 23$), smoking ($n = 207$), and BMI ($n = 83$), 72,348 women remained in our analyses. Of the 51,529 men participating in the HPFS, 1595 were missing dietary data at baseline, 5 died before all baseline data was collected, 2219 reported a history of MI before baseline, 967 revascularization, 732 angina, and 254 stroke. After additionally excluding men with missing data on age ($n = 36$), BMI ($n = 1027$) and physical activity ($n = 190$), 44,504 men remained in our analyses.

2.1.2. Nested case–control studies

In 1990 in NHS and 1994 in HPFS, surviving participants received blood collection kits. Participants collected fasting blood samples (heparin in women and EDTA in men) and shipped them on ice overnight to a central laboratory. Upon arrival, bloods were centrifuged under refrigeration and the blood components were aliquotted and stored in liquid nitrogen at −130 to −196 °C. Among the subcohorts who provided blood specimens and were free of CVD at the time of blood collection, homocysteine was measured in nested 1:3 matched case–control studies within both cohorts, including 143 PAD cases and 424 controls within the NHS (1990–2010) and 143 PAD cases and 428 controls within the HPFS (1994–2008). Cases and controls were matched using risk set sampling on age, smoking, race, month of blood draw, and fasting status. Men and women who provided blood samples were younger on average, but otherwise similar to those who did not provide blood samples [16,17].

2.2. Exposures

2.2.1. Plasma homocysteine

Plasma homocysteine was measured in all case–control samples (men and women) by the same laboratory. The lab used an enzymatic assay to measure homocysteine on the Roche P Modular system (Roche Diagnostics - Indianapolis, IN), with reagents and calibrators from Catch Inc. (Seattle, WA). In this assay, reduced homocysteine with serine was catalyzed by cystathionine b-synthase (CBS) to form L-cystathionine, which in turn was broken down by cystathionine b-lyase (CBL) to form homocysteine, pyruvate and ammonia. The pyruvate was then reduced by lactate dehydrogenase, with NADH forming NAD. The concentration of homocysteine in the sample was directly proportional to the amount of NADH converted to NAD. The change in absorbance was measured spectrophotometrically at 340 nm. Coefficients of variation for split homocysteine samples were 3.8% for women and 7.1% for men.

2.2.2. Dietary intakes of B vitamins, betaine, and choline

Food frequency questionnaires (FFQs) collected every four years from 1990 to 2006 were used to measure the intake of four B vitamins (folate, vitamin B_6, vitamin B_{12}, and riboflavin) and related compounds betaine, and choline in the NHS. The same FFQ was collected every four years from 1986 to 2006 in the HPFS. The semiquantitative FFQ [18] asked participants to report servings of specified portions of foods over the previous year in 9 categories ranging from "never or <1/mo" to "≥6/d." The Harvard University food composition database, derived from the US Department of