



Homocysteine Is Associated With Future Venous Thromboembolism in 2 Prospective Cohorts of Women

Aaron W. Aday¹, Edward K. Duran, Martin Van Denburgh, Eunjung Kim, William G. Christen, JoAnn E. Manson¹, Paul M. Ridker¹, Aruna D. Pradhan

OBJECTIVE: Case-control studies have identified plasma homocysteine as a risk marker for venous thromboembolism (VTE). Prospective data, particularly among women, are sparse. We examined whether plasma homocysteine associates with incident VTE in 2 large prospective cohorts of women.

APPROACH AND RESULTS: In the WHS (Women's Health Study), a prospective cohort study of 27 555 women ≥ 45 years old and free of cardiovascular disease and VTE, we assessed baseline homocysteine concentration along with other thrombotic biomarkers for association with future VTE ($n=743$), pulmonary embolism ($n=363$), and deep vein thrombosis ($n=545$). We used a second cohort of 2672 women ($n=102$ VTE events) in the WAFACS (Women's Antioxidant and Folic Acid Cardiovascular Study) to corroborate our findings. In age-adjusted analyses, elevated homocysteine, hsCRP (high-sensitivity C-reactive protein), fibrinogen, and sICAM-1 (soluble intercellular adhesion molecule-1) were associated with incident VTE (P for extreme quartile comparisons and P -trend < 0.05). In multivariable models adjusting for body mass index and other traditional VTE risk factors, only the association for homocysteine persisted (HR_{Q4} 1.31 [95% CI, 1.06–1.63]). Elevated homocysteine levels were associated with unprovoked pulmonary embolism (HR_{Q4} 2.13 [95% CI, 1.30–3.51]) and deep vein thrombosis (HR_{Q4} 1.59 [95% CI, 1.05–2.40]) but not provoked events. In WAFACS, elevated homocysteine levels were also associated with VTE events (P -trend 0.023).

CONCLUSIONS: Higher plasma homocysteine levels associate with VTE events in 2 cohorts of middle-aged and older women. Among VTE subtypes, homocysteine was associated with unprovoked, but not provoked, events. These data suggest a plausible biological role for homocysteine in the development of VTE.

REGISTRATION: URL: <https://www.clinicaltrials.gov>; Unique identifier: NCT00000479, NCT00000541.

GRAPHIC ABSTRACT: A [graphic abstract](#) is available for this article.

Key Words: biomarker ■ homocysteine ■ plasma ■ pulmonary embolism ■ venous thromboembolism

Homocysteine is an amino acid synthesized during methionine metabolism via both vitamin B₆- and vitamin B₁₂-dependent pathways.¹ In vitro and in vivo experimental data demonstrate that homocysteine contributes to oxidative stress, endothelial dysfunction, inflammation, and thrombosis.^{2–7} Elevations in homocysteine arise through a number of mechanisms, including genetic determinants of methionine metabolism, deficiencies in vitamin cofactors, and environmental exposures

such as transition to high animal protein diets in industrializing nations, growing use of dietary protein supplements, and PPAR (peroxisome proliferator-activated receptor)- α agonist therapy in lipid management.^{8–10}

Epidemiological studies have shown associations between elevations in plasma homocysteine and arterial atherothrombosis,^{11–16} but the epidemiological link between homocysteine and venous thromboembolism (VTE) has been more limited.^{17–23} Few studies have been

Correspondence to: Aaron W. Aday, MD, MSc, Division of Cardiovascular Medicine, Vanderbilt University Medical Center, 2525 West End Avenue Suite 300, Nashville, TN 37203. Email aaron.w.aday@vumc.org

The Data Supplement is available with this article at <https://www.ahajournals.org/doi/suppl/10.1161/ATVBAHA.121.316397>.

For Sources of Funding and Disclosures, see page 2223.

© 2021 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol is available at www.ahajournals.org/journal/atvb

Nonstandard Abbreviations and Acronyms

BMI	body mass index
DVT	deep vein thrombosis
HOPE-2	Heart Outcomes Prevention Evaluation 2
HR	hazard ratio
hsCRP	high-sensitivity C-reactive protein
LITE	Longitudinal Investigation of Thromboembolism Etiology
PE	pulmonary embolism
sICAM-1	soluble intercellular adhesion molecule-1
VITRO	Vitamins and Thrombosis
VTE	venous thromboembolism
WAFACS	Women's Antioxidant and Folic Acid Cardiovascular Study
WHS	Women's Health Study

prospective in nature, and prospective data have shown weaker associations than in retrospective analyses.²² Prior data suggest elevated homocysteine concentration is a risk marker for recurrent, rather than first, VTE,^{24,25} and may have a stronger effect among women than among men.^{17,20} Analyses from 3 randomized placebo-controlled clinical trials of homocysteine-lowering supplements did not show a reduction in VTE.^{26–28} However, low event rates limited the ability to detect modest treatment effects and to evaluate idiopathic (unprovoked) events and VTE subtypes. Furthermore, whether concurrent elevations in other thrombotic risk markers account for previously observed risk associations have not been explored.

In light of these knowledge gaps, we examined the association between plasma homocysteine and total VTE, provoked and unprovoked VTE events, and VTE subtypes in the WHS (Women's Health Study), a large, prospective cohort of >27 000 healthy middle-aged women with measured baseline homocysteine levels and among whom a total of 743 VTE events have accrued. We evaluated additional biomarkers, including hsCRP (high-sensitivity C-reactive protein), fibrinogen, sICAM-1 (soluble intercellular adhesion molecule-1), and lipoprotein(a), based on prior data suggesting a link between each biomarker and VTE. All markers were measured at baseline in the cohort. We also sought to validate our findings in the WAFACS (Women's Antioxidant and Folic Acid Cardiovascular Study), a second independent prospective cohort of 2672 middle-aged women and among whom risk associations with baseline homocysteine have not been previously analyzed.

MATERIALS AND METHODS

Data Availability

The data will not be made available to other researchers for purposes of reproducing the results. However, further details

Highlights

- We found that baseline plasma concentration of homocysteine is associated with future risk of venous thromboembolism in 2 independent cohorts of women.
- In the Women's Health Study, this risk was further increased among women with elevated body mass index.
- Elevated homocysteine was associated with unprovoked, but not provoked, deep vein thrombosis and pulmonary embolism

of the methods used in the analysis are available upon reasonable request.

Study Population

For the primary analysis, we used participants in WHS, a previously completed randomized, placebo-controlled trial of low-dose aspirin and vitamin E for the primary prevention of cardiovascular disease.²⁹ Between 1992 and 1995, the study enrolled 39 876 female health care professionals in the United States ≥ 45 years of age without prior cancer, myocardial infarction, stroke, coronary revascularization, or peripheral artery revascularization. At enrollment, women provided demographic, anthropometric, medical, and lifestyle information. After completion of the trial, individuals were invited to participate in the ongoing longitudinal observational component of the WHS, and additional health outcomes data were collected using annual questionnaires.

Before randomization, 28 345 of the participants provided nonfasting blood samples, which were stored in liquid nitrogen (-150°C to -180°C) until analysis. Of these samples, 27 939 were of sufficient quality to be used for subsequent analyses. Subjects with prerandomization VTE, including both pulmonary embolism (PE) and deep vein thrombosis (DVT), were excluded from the analysis. The final study population ($n=27\,555$) was followed for a median of 20.5 years.

To validate our findings from the primary analysis, we used participants in WAFACS. This was a randomized, placebo-controlled trial of combination therapy with folic acid, vitamin B₉, and vitamin B₁₂ in the prevention of cardiovascular disease.³⁰ The trial population consisted of high risk female health care professionals in the United States ≥ 42 years of age with a history of cardiovascular disease or at least 3 cardiovascular risk factors. Cardiovascular disease was defined as history of myocardial infarction, stroke, coronary or peripheral revascularization, angina pectoris, or transient ischemic attack. Cardiovascular risk factors included hypertension, hypercholesterolemia, diabetes, parental history of myocardial infarction before age 60, body mass index (BMI) ≥ 30 kg/m², and active cigarette use. Women were excluded if they had a history of cancer within the previous 10 years or were currently receiving anticoagulation. A total of 5442 individuals were randomized and followed for 7.3 years. In addition to providing baseline demographic, anthropometric, medical, and lifestyle data, participants completed annual health questionnaires. Before randomization, 2672 women in WAFACS (Women's Antioxidant and Folic Acid Cardiovascular Study) also provided blood samples in

which homocysteine levels were measured. All participants provided written informed consent, and the institutional review board at Brigham and Women's Hospital approved both studies.

Outcome Ascertainment

In WHS, health outcomes were initially ascertained by self-report using health questionnaires at randomization, 6, 12 months, and annually. The primary outcome in our study was incident VTE, including both PE and DVT. VTE events were adjudicated by physician review of medical records in a blinded manner. Documentation required to confirm a PE included an invasive pulmonary angiogram, computed tomographic angiogram, or ventilation-perfusion scan with ≥ 2 mismatched defects. PE resulting in death was confirmed using autopsy reports, medical history, preceding symptoms, and circumstances surrounding death. Confirmation of DVT required either a lower extremity venous duplex ultrasound or an invasive venogram. Unprovoked VTE was defined as an event occurring in the absence of malignancy (diagnosed prior or ≤ 3 months following the VTE event), trauma, hospitalization ≥ 3 days, or surgery within the preceding 3 months. Provoked VTE included those that occurred in patients with documented malignancy or in the setting of trauma or surgery. Only confirmed end points were used in this analysis.

In WAFACS, health outcomes were similarly ascertained by self-report using annual health questionnaires. The primary outcome of interest was again incident VTE, including both PE and DVT. Events were adjudicated by physician review of medical records in a blinded manner, and the documentation required for both PE and DVT was identical to that of WHS.

Laboratory Analysis

A core laboratory certified by the National Heart, Lung, and Blood Institute/Centers for Disease Control and Prevention Lipid Standardization Program performed all laboratory analyses. Low-density lipoprotein cholesterol was measured using a homogeneous direct method with a Hitachi 917 analyzer using reagents from Roche Diagnostics (Indianapolis, Ind). High-density lipoprotein cholesterol was measured using a direct enzymatic colorimetric assay, and triglycerides were measured enzymatically with correction for endogenous glycerol. Apolipoproteins B₁₀₀ and A-1 were measured using immunoturbidimetric assays (DiaSorin, Stillwater, MN). hsCRP was measured by a high-sensitivity immunoturbidimetric assay (Denka Seiken, Niigata, Japan).

In both cohorts, homocysteine was measured with an enzymatic assay (Catch Inc, Seattle, Wash; Roche Diagnostics, Hitachi 917 analyzer). Fibrinogen was calculated with an immunoturbidimetric assay (Kamiya Biomedical Co, Seattle, Wash). Lipoprotein(a) was measured with a latex-enhanced turbidimetric assay (Denka Seiken, Niigata, Japan). sICAM-1 was measured using quantitative sandwich ELISA (R&D Systems, Minneapolis, MN). Hemoglobin A1c was calculated with a turbidimetric assay (Roche Diagnostics, Indianapolis, IN).

Statistical Analysis

Continuous data are summarized as either mean \pm SD or median with interquartile range depending on normality of the

distributions. Categorical data are reported as percentages. Between-group differences were assessed by a *t* test or the Wilcoxon rank-sum test for continuous data and the χ^2 test for categorical data. Biomarkers were divided into quartiles based on the population distribution of each biomarker. Cox proportional-hazards models were used to estimate the hazard ratio (HR) and 95% CI for each biomarker quartile, and results are presented as top quartile compared with bottom (reference) quartile unless otherwise noted. Tests of linear trend across quartiles were performed using the median value from each quartile. Unadjusted event rates were used for each quartile and were reported for the full duration of follow-up.

For the primary analysis within WHS, all Cox regression models were adjusted for age followed by the addition of the following covariates: active smoking, BMI, and postmenopausal hormone therapy (Model 1). Fully adjusted models (Model 2) were adjusted for age (years), active smoking (baseline smoking: yes/no), BMI (kg/m²), postmenopausal hormone therapy (baseline use: yes/no), physical activity level (rarely/never, <1 time weekly, 2–3 times weekly, 4+ times weekly), metabolic syndrome (baseline: yes/no), postmenopausal status (baseline: yes/no/unknown), and glomerular filtration rate. Metabolic syndrome was defined using a modified Adult Treatment Panel III definition that has been previously validated in WHS.^{31,32} All regression results in the text are presented for Model 2 unless otherwise noted. Additionally, all models were adjusted for randomized treatment assignment. Covariates were selected based on a priori knowledge linking them to incident VTE.

Previous studies have shown a positive correlation between BMI and plasma homocysteine concentration^{33,34} as well as a strong association between BMI and risk of VTE.^{35–37} To evaluate the joint effects of plasma homocysteine concentration as well as BMI, individuals were classified into 4 groups based on the values of each measure relative to the population median.

In our confirmatory study conducted in the WAFACS, Cox regression analyses were adjusted for age, active smoking, elevated BMI (>26.70 kg/m²), height, physical activity level, diabetes, postmenopausal status, and history of hypertension. Because more than half of WAFACS participants did not have baseline lipid measures, this regression analysis additionally adjusted for diabetes (as a surrogate for hyperglycemia) and history of hypertension to account for potential residual confounding from these components of metabolic syndrome. Models were additionally adjusted for baseline history of venous thromboembolism (91.1% of subjects reported no prior VTE).

All statistical analyses were performed using SAS statistical software version 9.4 (SAS Institute, Cary, NC). All 95% CIs are 2-tailed, and the *P* cutoff for all analyses was 0.05.

RESULTS

As shown in Table 1, women in WHS with incident VTE were older with a larger mean BMI. They also had a greater prevalence of metabolic syndrome and hypertension and were more likely to be postmenopausal at baseline. Baseline diabetes was rare in the overall study population, and there were no significant differences in active smoking, self-reported physical activity, or hormonal therapy between the 2 groups. Median plasma measures of total cholesterol, low-density lipoprotein

Table 1. Baseline Characteristics of the WHS Population

	Women remaining free of VTE (n=26 812)*	Women developing VTE (n=743)†	P value
Age, y, mean (SD)	54.6 (7.1)	56.9 (7.5)	<0.0001
BMI, kg/m ² , mean (SD)	25.9 (4.9)	27.4 (5.5)	<0.0001
Height, in, mean (SD)	64.6 (2.5)	65.0 (2.6)	<0.0001
Non-Hispanic White, %	25 298 (95.2)	713 (96.7)	0.045
Current smoking, %	3103 (11.6)	80 (10.8)	0.52
Alcohol abstinence, %	11 829 (44.1)	332 (44.7)	0.76
Diabetes, %	644 (2.4)	18 (2.4)	0.90
Metabolic syndrome, %	6423 (24.2)	232 (31.5)	<0.0001
Hypertension, %	6668 (24.9)	214 (28.8)	0.016
Treatment for hypercholesterolemia, %	851 (3.2)	21 (2.8)	0.67
Exercise ≥1 time/wk, %	11 596 (43.3)	302 (40.7)	0.16
Current HT use, %	11 395 (42.6)	329 (44.3)	0.35
Post-menopausal, %	14 465 (54.1)	482 (65.1)	<0.0001
Hemoglobin A _{1c} , %	5.0 (4.8–5.2)	5.0 (4.9–5.2)	0.07
GFR, mL/min per 1.73 m ²	91.8 (79.6–105.5)	89.5 (77.2–103.7)	0.005
Standard chemical lipids, mg/dL			
Total cholesterol	208 (184–235)	214 (189–240)	0.002
HDL-cholesterol	52 (43–62)	51 (42–62)	0.08
LDL-cholesterol	121 (100–144)	125 (103–147)	0.006
Triglycerides	118 (83–174)	132 (92–187)	<0.0001
Apolipoproteins, mg/dL			
Apolipoprotein A-1	149 (132–168)	148 (132–170)	0.94
Apolipoprotein B ₁₀₀	100 (84–121)	107 (87–125)	<0.0001
hsCRP, mg/L	2.0 (0.8–4.3)	2.6 (1.2–5.2)	<0.0001
Fibrinogen, mg/dL	350.1 (307.0–401.9)	362.1 (319.3–415.9)	<0.0001
sICAM-1, ng/mL	342.2 (300.6–394.0)	351.4 (311.0–404.3)	0.0003
Homocysteine, μmol/L	10.4 (8.7–12.9)	10.9 (9.1–13.7)	<0.0001
Lipoprotein(a), mg/dL	10.6 (4.4–32.8)	10.5 (4.7–35.4)	0.82

Values are median (25th–75th percentile) unless otherwise indicated. *P* Value for continuous variables were obtained from the *t* test (age, BMI, height) or the Wilcoxon rank-sum test. *P* Value for categorical variables were obtained using χ^2 test. BMI indicates body mass index; GFR, glomerular filtration rate; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; HT, hormonal therapy; LDL, low-density lipoprotein; sICAM-1, soluble intercellular adhesion molecule-1; and VTE, venous thromboembolism.

*Number missing: 22 for BMI; 17 for height; 226 for race; 24 for current smoking; 6 for alcohol abstinence; 15 for diabetes; 216 for metabolic syndrome; 7 for hypertension; 18 for treatment for hypercholesterolemia; 9 for exercise; 49 for current HT use; 48 for postmenopausal status; 432 for HbA_{1c}; 377 for GFR; 378 for total cholesterol; 377 for HDL-cholesterol, LDL-cholesterol, and triglycerides; 505 for apolipoprotein A-1; 509 for apolipoprotein B₁₀₀; 377 for hsCRP; 504 for fibrinogen; 512 for sICAM-1; 509 for homocysteine; 514 for Lp(a).

†Number missing: 1 for BMI; 1 for height; 6 for race; 6 for metabolic syndrome; 1 for treatment for hypercholesterolemia; 1 for current HT use; 2 for postmenopausal status; 12 for HbA_{1c}; 12 for GFR; 12 for total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides; 14 for apolipoproteins A-1 and B₁₀₀; 12 for hsCRP; 14 for fibrinogen; 16 for sICAM-1; 15 for homocysteine; 14 for Lp(a).

cholesterol, triglycerides, apolipoprotein B₁₀₀, hsCRP, fibrinogen, sICAM-1, and homocysteine were all greater among women with incident VTE, although absolute differences were small.

Table 2 shows the results of Cox regression analyses for each biomarker and incident VTE based on quartiles of the population distribution for each biomarker. In age-adjusted analyses, hsCRP had the strongest positive risk association (HR_{Q4}, 1.84 [95% CI, 1.47–2.30]; *P*-trend <0.001) followed by fibrinogen, homocysteine,

and sICAM-1 (all *P*-trend <0.05). In multivariable-adjusted models, the associations for hsCRP, fibrinogen, and sICAM-1 no longer reached statistical significance. However, there remained a strong association between plasma homocysteine concentration and incident VTE in multivariable-adjusted models after adjustment for BMI and other risk factors (HR_{Q4}, 1.31 [95% CI, 1.06–1.63]; *P*-trend 0.006). Incidence rates for VTE increased across homocysteine quartiles: 1.18, 1.34, 1.53, and 1.80 per 1000 person-years, respectively. Each 5 μmol/L

Table 2. Associations of Biomarkers With Incident VTE in the WHS

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P value for linear trend
hsCRP					
Range, mg/L	≤0.80	0.81–2.00	2.01–4.34	≥4.35	
Cases, n	117	184	202	228	
Age-adjusted HR (95% CI)	1.00	1.50 (1.19–1.89)	1.61 (1.28–2.02)	1.84 (1.47–2.30)	<0.001
Model 1 h (95% CI)	1.00	1.32 (1.05–1.68)	1.28 (1.00–1.63)	1.27 (0.98–1.64)	0.45
Model 2 h (95% CI)	1.00	1.34 (1.06–1.70)	1.29 (1.01–1.65)	1.30 (1.00–1.68)	0.37
Fibrinogen					
Range, mg/dL	≤307.3	307.4–350.6	350.7–402.5	≥402.6	
Cases, n	130	177	199	223	
Age-adjusted HR (95% CI)	1.00	1.31 (1.05–1.64)	1.43 (1.14–1.78)	1.58 (1.27–1.96)	<0.0001
Model 1 h (95% CI)	1.00	1.22 (0.97–1.53)	1.24 (0.99–1.56)	1.21 (0.96–1.53)	0.18
Model 2 h (95% CI)	1.00	1.21 (0.96–1.52)	1.22 (0.97–1.53)	1.20 (0.95–1.51)	0.22
siCAM-1					
Range, ng/dL	≤300.8	300.8–342.5	342.6–394.2	≥394.3	
Cases, n	149	168	198	212	
Age-adjusted HR (95% CI)	1.00	1.06 (0.85–1.33)	1.22 (0.99–1.52)	1.34 (1.09–1.66)	0.002
Model 1 h (95% CI)	1.00	1.01 (0.81–1.25)	1.08 (0.87–1.34)	1.10 (0.88–1.37)	0.34
Model 2 h (95% CI)	1.00	1.01 (0.81–1.26)	1.08 (0.87–1.35)	1.11 (0.88–1.39)	0.30
Homocysteine					
Range, μmol/L	≤8.6	8.7–10.5	10.5–12.9	≥12.9	
Cases, n	151	169	190	218	
Age-adjusted HR (95% CI)	1.00	1.08 (0.87–1.35)	1.20 (0.97–1.49)	1.39 (1.13–1.71)	<0.001
Model 1 h (95% CI)	1.00	1.05 (0.85–1.31)	1.14 (0.92–1.42)	1.33 (1.07–1.64)	0.004
Model 2 h (95% CI)	1.00	1.04 (0.83–1.29)	1.14 (0.91–1.41)	1.31 (1.06–1.63)	0.006
Lipoprotein(a)					
Range, mg/dL	≤4.4	4.5–10.6	10.7–32.9	≥33.0	
Cases, n	174	196	165	194	
Age-adjusted HR (95% CI)	1.00	1.13 (0.92–1.39)	0.93 (0.75–1.15)	1.11 (0.90–1.36)	0.47
Model 1 h (95% CI)	1.00	1.15 (0.94–1.41)	0.93 (0.75–1.16)	1.12 (0.91–1.38)	0.43
Model 2 h (95% CI)	1.00	1.15 (0.94–1.42)	0.94 (0.76–1.17)	1.13 (0.92–1.39)	0.40

BMI indicates body mass index; HR, hazard ratio; hsCRP, high-sensitivity C-reactive protein; siCAM-1, soluble intercellular adhesion molecule-1; VTE, venous thromboembolism; and WHS, Women's Health Study.

Model 1 adjusted for age, smoking, BMI, hormonal therapy, and randomization status.

Model 2 adjusted for age, smoking, BMI, hormonal therapy, randomization status, activity level, metabolic syndrome, postmenopausal status, and GFR.

increase in homocysteine concentration was also associated with an increased risk of VTE (HR, 1.09 [95% CI, 1.03–1.16]; $P=0.005$ [data not shown]). There was no significant association between plasma concentrations of lipoprotein(a) and incident VTE in age-adjusted or multivariable-adjusted regression models.

As homocysteine was the only biomarker positively associated with incident VTE in both age-adjusted and multivariable-adjusted regression models, we then assessed the association between homocysteine and different sub-categories of VTE (Table 3). Homocysteine concentration was associated with total PE (HR_{Q4}, 1.44 [95% CI, 1.07–1.94]; P -trend 0.005) and unprovoked PE (HR_{Q4}, 2.28 [95% CI, 1.41–3.69]; P -trend

<0.001) cases in age-adjusted models. In multivariable-adjusted models, however, homocysteine was only associated with incident unprovoked PE (HR_{Q4}, 2.13 [95% CI, 1.30–3.51]; P -trend 0.002). There was no significant association between plasma homocysteine concentration and provoked PE in age-adjusted or multivariable-adjusted models.

We found similar associations for DVT subtypes (Table 3). Homocysteine was positively associated with total DVT (HR_{Q4}, 1.30 [95% CI, 1.02–1.65]; P -trend 0.009) and unprovoked DVT (HR_{Q4}, 1.56 [95% CI, 1.05–2.32]; P -trend 0.01) in age-adjusted models. These associations were slightly attenuated for total DVT (HR_{Q4}, 1.24 [95% CI, 0.96–1.59]; P -trend 0.03) but persisted

Table 3. Risk Associations Between Homocysteine and Incident Provoked and Unprovoked Pulmonary Embolism and Deep Vein Thrombosis in the WHS

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P value for linear trend
Total PE					
Homocysteine					
Range, $\mu\text{mol/L}$	≤ 8.6	8.7–10.5	10.5–12.9	≥ 12.9	
Cases, n	74	78	95	110	
Event rate (per 1000 person-years)	0.58	0.62	0.76	0.90	
Age-adjusted HR (95% CI)	1.00	1.02 (0.74–1.40)	1.23 (0.91–1.67)	1.44 (1.07–1.94)	0.005
Model 1 h (95% CI)	1.00	0.97 (0.71–1.34)	1.14 (0.84–1.55)	1.32 (0.98–1.78)	0.03
Model 2 h (95% CI)	1.00	0.96 (0.70–1.33)	1.17 (0.86–1.59)	1.36 (1.00–1.85)	0.02
Unprovoked PE					
Homocysteine					
Range, $\mu\text{mol/L}$	≤ 8.6	8.7–10.4	10.5–12.8	≥ 12.9	
Cases, n	24	35	42	55	
Event rate (per 1000 person-years)	0.19	0.28	0.34	0.45	
Age-adjusted HR (95% CI)	1.00	1.43 (0.85–2.41)	1.71 (1.03–2.83)	2.28 (1.41–3.69)	<0.001
Model 1 h (95% CI)	1.00	1.38 (0.82–2.32)	1.57 (0.95–2.61)	2.08 (1.28–3.38)	0.002
Model 2 h (95% CI)	1.00	1.37 (0.81–2.31)	1.60 (0.96–2.66)	2.13 (1.30–3.51)	0.002
Provoked PE					
Homocysteine					
Range, $\mu\text{mol/L}$	≤ 8.6	8.7–10.4	10.5–12.8	≥ 12.9	
Cases, n	48	43	52	54	
Event rate (per 1000 person-years)	0.38	0.34	0.42	0.44	
Age-adjusted HR (95% CI)	1.00	0.85 (0.57–1.29)	1.02 (0.69–1.51)	1.07 (0.72–1.58)	0.52
Model 1 h (95% CI)	1.00	0.81 (0.54–1.23)	0.96 (0.65–1.43)	0.98 (0.66–1.46)	0.79
Model 2 h (95% CI)	1.00	0.80 (0.53–1.22)	0.98 (0.66–1.46)	1.02 (0.68–1.53)	0.62
Total DVT					
Homocysteine					
Range, $\mu\text{mol/L}$	≤ 8.6	8.7–10.4	10.5–12.8	≥ 12.9	
Cases, n	117	116	139	159	
Event rate (per 1000 person-years)	0.92	0.92	1.12	1.31	
Age-adjusted HR (95% CI)	1.00	0.95 (0.74–1.23)	1.13 (0.88–1.44)	1.30 (1.02–1.65)	0.009
Model 1 h (95% CI)	1.00	0.93 (0.72–1.21)	1.09 (0.85–1.39)	1.27 (1.00–1.62)	0.02
Model 2 h (95% CI)	1.00	0.92 (0.71–1.19)	1.07 (0.83–1.37)	1.24 (0.96–1.59)	0.03
Unprovoked DVT					
Homocysteine					
Range, $\mu\text{mol/L}$	≤ 8.6	8.7–10.4	10.5–12.8	≥ 12.9	
Cases, n	40	45	58	64	
Event rate (per 1000 person-years)	0.31	0.36	0.47	0.53	
Age-adjusted HR (95% CI)	1.00	1.10 (0.72–1.68)	1.41 (0.94–2.11)	1.56 (1.05–2.32)	0.01
Model 1 h (95% CI)	1.00	1.10 (0.72–1.68)	1.39 (0.93–2.09)	1.60 (1.07–2.40)	0.01
Model 2 h (95% CI)	1.00	1.10 (0.71–1.68)	1.39 (0.92–2.09)	1.59 (1.05–2.40)	0.01
Provoked DVT					
Homocysteine					
Range, $\mu\text{mol/L}$	≤ 8.6	8.7–10.4	10.5–12.8	≥ 12.9	
Cases, n	74	68	79	90	
Event rate (per 1000 person-years)	0.58	0.54	0.64	0.74	
Age-adjusted HR (95% CI)	1.00	0.88 (0.63–1.22)	1.00 (0.73–1.37)	1.14 (0.84–1.55)	0.22
Model 1 h (95% CI)	1.00	0.85 (0.61–1.18)	0.95 (0.69–1.31)	1.09 (0.80–1.49)	0.34
Model 2 h (95% CI)	1.00	0.82 (0.59–1.14)	0.93 (0.67–1.28)	1.05 (0.76–1.45)	0.46

Event rates were calculated using the Kaplan-Meier estimator and are reported for the full duration of follow-up. Model 1 adjusted for age, smoking, BMI, hormonal therapy, and randomization status. Model 2 adjusted for age, smoking, BMI, hormonal therapy, randomization status, activity level, metabolic syndrome, postmenopausal status, and GFR. BMI indicates body mass index; DVT, deep vein thrombosis; HR, hazard ratio; PE, pulmonary embolism; and WHS, Women's Health Study.

for unprovoked DVT ($HR_{0.4}$, 1.59 [95% CI, 1.05–2.40]; P -trend 0.01) after adjusting for additional VTE risk factors. As with provoked PE, there was no significant association between homocysteine and provoked DVT.

Women were classified as having high/low BMI and high/low homocysteine concentration based on the population median of each measure to evaluate the joint role of these risk factors on incident VTE, PE, and DVT (Figure 1; Table I in the [Data Supplement](#)). Even among women with a low BMI, an elevated plasma homocysteine concentration identified women at heightened risk for VTE, PE, and DVT, although the association was strongest for PE (HR , 1.61 [95% CI, 1.10–2.34]). Women with elevated BMI were at heightened risk for all VTE events regardless of plasma homocysteine concentration, although elevations in homocysteine further identified women in the highest risk group (HR , 2.25 [95% CI, 1.78–2.84]; HR , 2.99 [95% CI, 2.10–4.27]; and HR , 2.20 [95% CI, 1.68–2.89] for incident VTE, PE, and DVT respectively). Across all categories, both BMI and homocysteine concentration were associated with a greater risk of PE than VTE or DVT.

Finally, we examined the risk association between plasma homocysteine concentration and incident VTE in WAFACS (Figure 2). In WAFACS, women in the highest quartile of homocysteine had an increased risk of VTE ($HR_{0.4}$, 2.15 [95% CI, 1.17–3.96]; P -trend 0.02). There was no statistically significant increased risk for women in the second or third quartiles.

DISCUSSION

In 2 prospective cohorts of women, we found that plasma homocysteine concentration was independently

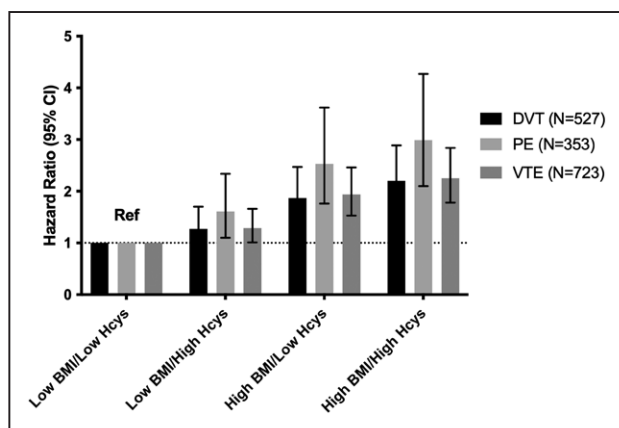


Figure 1. Risk of incident venous thromboembolism (VTE), pulmonary embolism (PE), and deep vein thrombosis (DVT) by baseline body mass index (BMI) and homocysteine concentration.

Participants are divided into low/high categories based on the study population median for body mass index (24.9 kg/m^2) and plasma homocysteine (Hcys; $10.5 \text{ }\mu\text{mol/L}$); Adjusted for age, smoking, hormonal therapy, activity level, metabolic syndrome, post-menopausal status, glomerular filtration rate, and randomized treatment.

associated with a heightened risk for incident VTE. In the primary prevention WHS, these findings for homocysteine differed in comparison to other biomarkers of atherothrombosis, including hsCRP, fibrinogen, sICAM-1, and lipoprotein(a), which were not associated with incident VTE in multivariable-adjusted models. Homocysteine concentration was associated with unprovoked, but not provoked, cases of incident PE and DVT. Our data also support a joint association of BMI with homocysteine, as women with elevations in both BMI and plasma homocysteine concentration were at highest risk.

Our data must be placed in the context of previous, at times conflicting, analyses of homocysteine and VTE. The first published case-control analysis found no association between homocysteine and VTE.³⁸ However, subsequent retrospective analyses have shown more consistent associations. A meta-analysis of case-control studies found that every $5 \text{ }\mu\text{mol/L}$ increase in plasma homocysteine associates with a 60% increased risk of VTE (odds ratio [OR], 1.60 [95% CI, 1.10–2.34]).²² Furthermore, in a case-control analysis of 269 patients with first DVT in the Leiden Thrombophilia Study, a homocysteine concentration above the 95th percentile ($>18.5 \text{ }\mu\text{mol/L}$) was associated with a 2.5-fold increased odds of VTE (95% CI, 1.2–5.2).¹⁷ This risk association persisted even with a lower cutoff of the 90th percentile value ($>16.6 \text{ }\mu\text{mol/L}$). Notably, in subgroup analyses, the risk association did not materially increase until reaching a threshold $>18 \text{ }\mu\text{mol/L}$, with a dramatically increased risk among those with a concentration $>22 \text{ }\mu\text{mol/L}$. This is in contrast to our present analysis, which showed not only a dose-response relationship between homocysteine and incident VTE but also a heightened risk association at lower concentrations (lower limit for top quartile $\geq 12.9 \text{ }\mu\text{mol/L}$).

Retrospective case-control analyses do not account for the potential impact of acute VTE events as well as associated medical interventions on homocysteine levels. In a prospective nested case-control analysis of initially healthy men in the Physicians' Health Study, investigators measured plasma homocysteine concentration in 145 individuals who subsequently developed incident VTE as well as 646 controls.²¹ Over 10 years of follow-up, men with homocysteine levels >95 th percentile ($17.25 \text{ }\mu\text{mol/L}$) were at increased risk of idiopathic (unprovoked), but not total, VTE (relative risk [RR], 3.4 [95% CI, 1.6–7.3]; $P=0.002$ versus RR, 1.6 [95% CI, 0.8–3.3]; $P=0.2$, respectively). In the present analysis from WHS, we similarly found the strongest risk associations for unprovoked DVT and PE compared with either total or provoked events. Notably, our analysis showed a risk association at even more modest homocysteine levels (top quartile $\geq 12.9 \text{ }\mu\text{mol/L}$; HR , 1.31 [95% CI, 1.06–1.63]), potentially identifying important sex differences for homocysteine risk.

Until the present analysis, the largest prospective study examining homocysteine and VTE was a nested

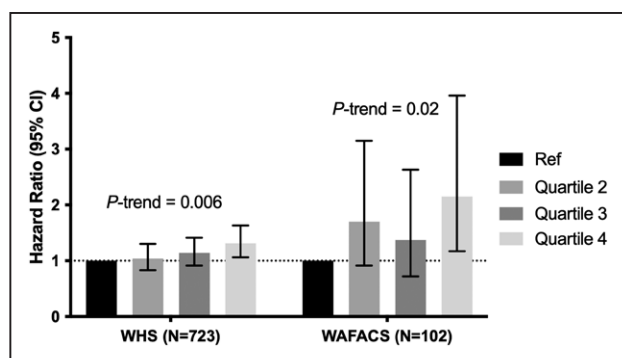


Figure 2. Association between homocysteine and incident venous thromboembolism in WHS (Women's Health Study) and WAFACS (Women's Antioxidant and Folic Acid Cardiovascular Study).

Hazard ratios and 95% CIs for homocysteine in both WHS and WAFACS. Models for WHS adjusted for age, smoking, body mass index (BMI), hormonal therapy, activity level, metabolic syndrome, postmenopausal status, glomerular filtration rate, and randomized treatment. Models for WAFACS adjusted for age, smoking, BMI, height, activity level, diabetes, postmenopausal status, history of hypertension, randomized treatment, and baseline venous thromboembolism.

case-control analysis of 303 VTE cases and 635 matched controls in the LITE (Longitudinal Investigation of Thromboembolism Etiology) study.²⁰ Homocysteine levels in the top quintile ($>15.9 \mu\text{mol/L}$) were not associated with a statistically significant increased risk of a composite of incident and recurrent VTE compared with those in the lowest quintile (OR, 1.55 [95% CI, 0.93–2.58]). However, when restricted to individuals age 45 to 64, a range closer to that of WHS, the risk association reached statistical significance (OR, 2.05 [95% CI, 1.10–3.83]). This may have been because of a stronger link between homocysteine and competing arterial thromboembolic events among older participants.²⁰

Despite these epidemiological findings, the null results from randomized clinical trials of homocysteine lowering therapy have posed the greatest challenge to the “homocysteine hypothesis” in VTE (Table 4). The VITRO (Vitamins and Thrombosis) study enrolled 701 individuals with a history of VTE.²⁶ Three hundred forty-one participants had homocysteine levels ≥ 75 th percentile (≥ 8.5 – $10.6 \mu\text{mol/L}$ depending on sex and the local population homocysteine distribution), and 360 had homocysteine levels < 75 th percentile. Participants were randomized to either a combination of folic acid, vitamin B₆, and vitamin B₁₂ or placebo. After a planned follow-up of 2.5 years, vitamin supplementation led to 46% and 33% reductions in homocysteine in the hyperhomocysteinemic and normohomocysteinemic groups, respectively. However, there was no reduction in recurrent VTE events, although with only 43 and 50 events in the intervention and placebo arms, respectively, this study may have been underpowered. VITRO was also limited to individuals with a prior history of VTE, and it is possible that medical interventions following the initial events could have both altered baseline homocysteine

levels as well as individual response to vitamin therapy. It is also possible that the intervention occurred too late in the disease process to yield any benefit.

The HOPE-2 trial (Heart Outcomes Prevention Evaluation 2) enrolled 5522 men and women age 55 or older with either a history of atherosclerotic cardiovascular disease or diabetes plus an additional risk factor for cardiovascular disease.²⁷ Participants were randomized to a combination of folic acid, vitamin B₆, and vitamin B₁₂ or placebo for a mean duration of 5 years. Although vitamin supplementation reduced plasma homocysteine levels compared with placebo, it did not reduce the rates of DVT, PE, or unprovoked VTE.²⁷ In subgroup analyses, there were also no differences based on sex, age, baseline oral anticoagulant use, or baseline homocysteine level. In the WAFACS, the combination of folic acid, vitamin B₆, and vitamin B₁₂ did not lower VTE compared with placebo in the total trial population, though once again the event rate was low.³⁹ There was, however, a significant interaction by obesity such that among 2690 obese women (BMI $\geq 30 \text{ kg/m}^2$), the treatment-associated risk reduction was 43% (95% CI, 9%–67%]; $P=0.019$). Our data from WHS complement this finding by demonstrating the highest incidence rates in overweight women with high levels of homocysteine.

Despite the large sample size and prospective nature of our study, there are several potential limitations that warrant discussion. Our analysis was restricted to women, and WHS participants were largely White and healthy at baseline. Therefore, our results may not be generalizable to a more diverse population. Participants in WAFACS, the secondary prevention cohort used to validate our findings, had a higher prevalence of cardiovascular disease and cardiovascular risk factors at baseline, thus mitigating some of this limitation. Variability in preanalysis sample processing conditions, such as time between blood draws and freezing, number of freeze-thaw cycles, and the presence or absence of hemolysis, may limit our ability to compare results across different studies, although prior data suggest homocysteine concentration is minimally impacted by multiple freeze-thaw cycles.⁴⁰ We also did not utilize fasting homocysteine concentration in our analysis. However, data suggest the impact of fasting is likely minimal, and fasting homocysteine levels may not accurately reflect the typical physiological state of individuals.⁴¹ Measures of renal function were unavailable in WAFACS, and renal function is known to strongly correlate with homocysteine concentration.⁴² However, adjusting for renal function in WHS did not significantly alter any of our findings. Finally, during the course of WHS and WAFACS, the Food and Drug Administration implemented mandatory folic acid supplementation, which began in 1996 and was largely completed by mid-1997.⁴³ Although this may have influenced homocysteine concentration subsequent to participants' blood draws, this would have biased our results toward the null. The results of VITRO and HOPE-2 further suggested that folic acid supplementation had no influence on VTE risk.

Table 4. Summary of Prior Placebo-Controlled Clinical Trials Evaluating Folic Acid and B Vitamins for VTE Prevention

Study population	VITRO hyperhomocysteinemic ²⁶	VITRO normohomocysteinemic ²⁶	HOPE-2 ²⁸	WAFACS ³⁹
	Secondary VTE prevention	Secondary VTE prevention	High CV risk	High CV risk
No. of subjects	360	341	5522	2672
No. of women	152	187	1559	2672
Geometric mean homocysteine, $\mu\text{mol/L}$	15.9*	9.0	11.5	12.2
Folic acid dose	5 mg/d	5 mg/d	2.5 mg/d	2.5 mg/d
Vitamin B ₆ (pyridoxine) dose	50 mg/d	50 mg/d	50 mg/d	50 mg/d
Vitamin B ₁₂ (cyanocobalamin) dose	0.4 mg/d	0.4 mg/d	1.0 mg/d	1.0 mg/d
Duration of follow-up	2.5 y	2.5 y	5 y	7.3 y
No. of total VTE events	50	43	88	132
No. of unprovoked VTE events	Not classified	Not classified	42	Not classified
Treatment effect, $\mu\text{mol/L}^\dagger$	-8.5	-2.5	-2.2	-2.4
Treatment effect (hazard ratio [95% CI])	1.14 (0.65-1.98)	0.58 (0.32-1.08)	1.01 (0.66-1.53)	0.82 (0.59-1.16)
Key secondary analyses	Per 5 $\mu\text{mol/L}$ higher baseline homocysteine: 1.13 (1.05-1.20)		Unprovoked VTE: 1.21 (0.66-2.23)	Unprovoked VTE: 0.68 (0.44-1.04) BMI \geq 30 kg/m ² : 0.57 (0.36-0.91)

CV indicates cardiovascular; HOPE, Heart Outcomes Prevention Evaluation; VITRO, Vitamins and Thrombosis; VTE, venous thromboembolism; and WAFACS, Women's Antioxidant and Folic Acid Cardiovascular Study.

*Geometric mean in placebo group.

[†]Not placebo adjusted as these were not available in all trials.

In summary, in what is to our knowledge the largest prospective analysis of homocysteine and VTE, higher plasma homocysteine concentrations were associated with a heightened future risk of VTE. Specifically, homocysteine was associated with unprovoked, rather than provoked, PE and DVT. The risk association between homocysteine and VTE was also seen in a second validation cohort. Although prior clinical trials of homocysteine reduction have not been effective in reducing VTE risk, our data suggest homocysteine remains an important risk marker. Rather than focusing on homocysteine reduction, future studies may use circulating biomarkers like homocysteine and other markers of procoagulability,⁴⁴ as well as additional markers like polygenic risk scores, to help clinicians implement primary and secondary prevention strategies to reduce the risk of VTE.

supported the biomarker analyses in the Women's Health Study (P.M. Ridker). A.D. Pradhan received support from the National Heart, Lung, and Blood Institute of the National Institutes of Health under Award Number R01HL111156. The Women's Health Study was funded by grants CA047988, HL043851, HL080467, HL099355, and UM1 CA182913. WAFACS (Women's Antioxidant and Folic Acid Cardiovascular Study) was funded by grant HL47959.

Disclosures

A.W. Aday has served as a consultant to OptumCare. P.M. Ridker is listed as a coinventor on patents held by the Brigham and Women's Hospital that relate to the use of inflammatory biomarkers in cardiovascular disease, which have been licensed to AstraZeneca and Siemens, has received investigator research support from Kowa Research Institute, Novartis, Pfizer, and Astra-Zeneca, has served as a consultant to Janssen, Novartis, and Sanofi-Regeneron, and serves as co-Principal Investigator of the PROMINENT trial (Pemafibrate to Reduce Cardiovascular Outcomes by Reducing Triglycerides in Patients With Diabetes; NCT03071692). A.D. Pradhan: receives investigator-initiated research support from Kowa Research Institute and serves as co-Principal Investigator of the PROMINENT trial (NCT03071692). The other authors report no conflicts.

REFERENCES

- Welch GN, Loscalzo J. Homocysteine and atherothrombosis. *N Engl J Med*. 1998;338:1042-1050. doi: 10.1056/NEJM199804093381507
- Harker LA, Slichter SJ, Scott CR, Ross R. Homocystinemia. Vascular injury and arterial thrombosis. *N Engl J Med*. 1974;291:537-543. doi: 10.1056/NEJM197409122911101
- Harker LA, Ross R, Slichter SJ, Scott CR. Homocystine-induced arteriosclerosis. The role of endothelial cell injury and platelet response in its genesis. *J Clin Invest*. 1976;58:731-741. doi: 10.1172/JCI108520
- Welch GN, Upchurch G Jr, Loscalzo J. Hyperhomocyst(e)inemia and atherothrombosis. *Ann N Y Acad Sci*. 1997;811:48-58; discussion 58. doi: 10.1111/j.1749-6632.1997.tb51988.x
- Weiss N, Keller C, Hoffmann U, Loscalzo J. Endothelial dysfunction and atherothrombosis in mild hyperhomocysteinemia. *Vasc Med*. 2002;7:227-239. doi: 10.1191/1358863x02vm428ra
- de Groot PG, Willems C, Boers GH, Gonsalves MD, van Aken WG, van Mourik JA. Endothelial cell dysfunction in homocystinuria. *Eur J Clin Invest*. 1983;13:405-410. doi: 10.1111/j.1365-2362.1983.tb00121.x
- Eberhardt RT, Forgione MA, Cap A, Leopold JA, Rudd MA, Trolliet M, Heydrick S, Stark R, Klings ES, Moldovan NI, et al. Endothelial dysfunction in a murine model of mild hyperhomocyst(e)inemia. *J Clin Invest*. 2000;106:483-491. doi: 10.1172/JCI8342

ARTICLE INFORMATION

Received August 4, 2020; accepted May 4, 2021.

Affiliations

Division of Preventive Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA (A.W.A., E.K.D., M.V.D., E.K., W.G.C., J.E.M., P.M.R., A.D.P.). Now with Vanderbilt Translational and Clinical Cardiovascular Research Center, Division of Cardiovascular Medicine, Vanderbilt University Medical Center, Nashville, TN (A.W.A.). Now with Cardiovascular Division, University of Minnesota, Minneapolis (E.K.D.). Division of Cardiovascular Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA (P.M.R.). Division of Cardiovascular Medicine, VA Boston Medical Center, Boston, MA (A.D.P.).

Acknowledgments

We wish to thank statistical programmer M. Vinayaga Moorthy for his efforts.

Sources of Funding

This work was supported by the National Institutes of Health under Award Number T32 HL007575 (A.W. Aday and E.K. Duran), K12 HL133117 (A.W. Aday), K23 HL151871 (A.W. Aday), and by the Donald W Reynolds Foundation which

8. Pradhan AD, Paynter NP, Everett BM, Glynn RJ, Amarenco P, Elam M, Ginsberg H, Hiatt WR, Ishibashi S, Koenig W, et al. Rationale and design of the pemafibrate to reduce cardiovascular outcomes by reducing triglycerides in patients with diabetes (PROMINENT) study. *Am Heart J*. 2018;206:80–93. doi: 10.1016/j.ahj.2018.09.011
9. Herrmann M, Whiting MJ, Veillard AS, Ehnholm C, Sullivan DR, Keech AC; FIELD study investigators. Plasma homocysteine and the risk of venous thromboembolism: insights from the FIELD study. *Clin Chem Lab Med*. 2012;50:2213–2219. doi: 10.1515/cclm-2012-0078
10. Kumar A, Palfrey HA, Pathak R, Kadowitz PJ, Gettys TW, Murthy SN. The metabolism and significance of homocysteine in nutrition and health. *Nutr Metab (Lond)*. 2017;14:78. doi: 10.1186/s12986-017-0233-z
11. Arnesen E, Refsum H, Bønaa KH, Ueland PM, Førde OH, Nordrehaug JE. Serum total homocysteine and coronary heart disease. *Int J Epidemiol*. 1995;24:704–709. doi: 10.1093/ije/24.4.704
12. Ridker PM, Manson JE, Buring JE, Shih J, Matias M, Hennekens CH. Homocysteine and risk of cardiovascular disease among postmenopausal women. *JAMA*. 1999;281:1817–1821. doi: 10.1001/jama.281.19.1817
13. Bots ML, Launer LJ, Lindemans J, Hoes AW, Hofman A, Witteman JC, Koudstaal PJ, Grobbee DE. Homocysteine and short-term risk of myocardial infarction and stroke in the elderly: the Rotterdam Study. *Arch Intern Med*. 1999;159:38–44. doi: 10.1001/archinte.159.1.38
14. Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *JAMA*. 2002;288:2015–2022. doi: 10.1001/jama.288.16.2015
15. Casas JP, Bautista LE, Smeeth L, Sharma P, Hingorani AD. Homocysteine and stroke: evidence on a causal link from mendelian randomisation. *Lancet*. 2005;365:224–232. doi: 10.1016/S0140-6736(05)17742-3
16. Shai I, Stampfer MJ, Ma J, Manson JE, Hankinson SE, Cannuscio C, Selhub J, Curhan G, Rimm EB. Homocysteine as a risk factor for coronary heart diseases and its association with inflammatory biomarkers, lipids and dietary factors. *Atherosclerosis*. 2004;177:375–381. doi: 10.1016/j.atherosclerosis.2004.07.020
17. den Heijer M, Koster T, Blom HJ, Bos GM, Briet E, Reitsma PH, Vandenbroucke JP, Rosendaal FR. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *N Engl J Med*. 1996;334:759–762. doi: 10.1056/NEJM199603213341203
18. Ray JG. Meta-analysis of hyperhomocysteinemia as a risk factor for venous thromboembolic disease. *Arch Intern Med*. 1998;158:2101–2106. doi: 10.1001/archinte.158.19.2101
19. Langman LJ, Ray JG, Evrosvki J, Yeo E, Cole DE. Hyperhomocyst(e) inemia and the increased risk of venous thromboembolism: more evidence from a case-control study. *Arch Intern Med*. 2000;160:961–964. doi: 10.1001/archinte.160.7.961
20. Tsai AW, Cushman M, Tsai MY, Heckbert SR, Rosamond WD, Aleksic N, Yanez ND, Psaty BM, Folsom AR. Serum homocysteine, thermolabile variant of methylene tetrahydrofolate reductase (MTHFR), and venous thromboembolism: longitudinal Investigation of Thromboembolism Etiology (LITE). *Am J Hematol*. 2003;72:192–200. doi: 10.1002/ajh.10287
21. Ridker PM, Hennekens CH, Selhub J, Miletich JP, Malinow MR, Stampfer MJ. Interrelation of hyperhomocyst(e)inemia, factor V Leiden, and risk of future venous thromboembolism. *Circulation*. 1997;95:1777–1782. doi: 10.1161/01.cir.95.7.1777
22. Den Heijer M, Lewington S, Clarke R. Homocysteine, MTHFR and risk of venous thrombosis: a meta-analysis of published epidemiological studies. *J Thromb Haemost*. 2005;3:292–299. doi: 10.1111/j.1538-7836.2005.01141.x
23. Mäkelburg AB, Lijfering WM, Middeldorp S, Hamulyák K, Veeger NJ, Prins MH, Büller HR, van der Meer J. Low absolute risk of venous and arterial thrombosis in hyperhomocysteinemia - a prospective family cohort study in asymptomatic subjects. *Thromb Haemost*. 2009;101:209–212.
24. den Heijer M, Blom HJ, Gerrits WB, Rosendaal FR, Haak HL, Wijermans PW, Bos GM. Is hyperhomocysteinemia a risk factor for recurrent venous thrombosis? *Lancet*. 1995;345:882–885. doi: 10.1016/s0140-6736(95)90008-x
25. Eichinger S, Stümpflen A, Hirschl M, Bialonczyk C, Herkner K, Stain M, Schneider B, Pabinger I, Lechner K, Kyrle PA. Hyperhomocysteinemia is a risk factor of recurrent venous thromboembolism. *Thromb Haemost*. 1998;80:566–569.
26. den Heijer M, Willems HP, Blom HJ, Gerrits WB, Cattaneo M, Eichinger S, Rosendaal FR, Bos GM. Homocysteine lowering by B vitamins and the secondary prevention of deep vein thrombosis and pulmonary embolism: a randomized, placebo-controlled, double-blind trial. *Blood*. 2007;109:139–144. doi: 10.1182/blood-2006-04-014654
27. Ray JG, Kearon C, Yi Q, Sheridan P, Lonn E; Heart Outcomes Prevention Evaluation 2 (HOPE-2) Investigators. Homocysteine-lowering therapy and risk for venous thromboembolism: a randomized trial. *Ann Intern Med*. 2007;146:761–767. doi: 10.7326/0003-4819-146-11-200706050-00157
28. Lonn E, Yusuf S, Arnold MJ, Sheridan P, Pogue J, Micks M, McQueen MJ, Probstfield J, Fodor G, Held C, et al; Heart Outcomes Prevention Evaluation (HOPE) 2 Investigators. Homocysteine lowering with folic acid and B vitamins in vascular disease. *N Engl J Med*. 2006;354:1567–1577. doi: 10.1056/NEJMoa060900
29. Ridker PM, Cook NR, Lee IM, Gordon D, Gaziano JM, Manson JE, Hennekens CH, Buring JE. A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women. *N Engl J Med*. 2005;352:1293–1304. doi: 10.1056/NEJMoa050613
30. Albert CM, Cook NR, Gaziano JM, Zaharris E, MacFadyen J, Danielson E, Buring JE, Manson JE. Effect of folic acid and B vitamins on risk of cardiovascular events and total mortality among women at high risk for cardiovascular disease: a randomized trial. *JAMA*. 2008;299:2027–2036. doi: 10.1001/jama.299.17.2027
31. Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. *Circulation*. 2003;107:391–397. doi: 10.1161/01.cir.0000055014.62083.05
32. Conen D, Rexrode KM, Creager MA, Ridker PM, Pradhan AD. Metabolic syndrome, inflammation, and risk of symptomatic peripheral artery disease in women: a prospective study. *Circulation*. 2009;120:1041–1047. doi: 10.1161/CIRCULATIONAHA.109.863092
33. Sanlier N, Yabancı N. Relationship between body mass index, lipids and homocysteine levels in university students. *J Pak Med Assoc*. 2007;57:491–495.
34. Karatela RA, Sainani GS. Plasma homocysteine in obese, overweight and normal weight hypertensives and normotensives. *Indian Heart J*. 2009;61:156–159.
35. Klarin D, Emdin CA, Natarajan P, Conrad MF, Kathiresan S; INVENT Consortium. Genetic analysis of venous thromboembolism in UK Biobank identifies the ZFPM2 locus and implicates obesity as a causal risk factor. *Circ Cardiovasc Genet*. 2017;10:e001643. doi: 10.1161/CIRCGENETICS.116.001643
36. Eichinger S, Hron G, Bialonczyk C, Hirschl M, Minar E, Wagner O, Heinze G, Kyrle PA. Overweight, obesity, and the risk of recurrent venous thromboembolism. *Arch Intern Med*. 2008;168:1678–1683. doi: 10.1001/archinte.168.15.1678
37. Klovaite J, Benn M, Nordestgaard BG. Obesity as a causal risk factor for deep venous thrombosis: a Mendelian randomization study. *J Intern Med*. 2015;277:573–584. doi: 10.1111/joim.12299
38. Brattström L, Tengborn L, Lagerstedt C, Israelsson B, Hultberg B. Plasma homocysteine in venous thromboembolism. *Haemostasis*. 1991;21:51–57. doi: 10.1159/000216202
39. Glynn RJ, Manson JE. Abstract 5062: Effect of folic acid and B vitamins on the occurrence of venous thromboembolism: a randomized trial in women at high cardiovascular risk. *Circulation*. 2008;118:S_1137–S_1137.
40. Eggebrecht L, Prochaska JH, Schulz A, Arnold N, Jünger C, Göbel S, Laubert-Reh D, Binder H, Beutel ME, Pfeiffer N, et al. Intake of vitamin K antagonists and worsening of cardiac and vascular disease: results from the population-based gutenber health study. *J Am Heart Assoc*. 2018;7:e008650. doi: 10.1161/JAHA.118.008650
41. Fokkema MR, Gilissen MF, Van Doormaal JJ, Volmer M, Kema IP, Muskiet FA. Fasting vs nonfasting plasma homocysteine concentrations for diagnosis of hyperhomocysteinemia. *Clin Chem*. 2003;49:818–821. doi: 10.1373/49.5.818
42. van Guldener C. Why is homocysteine elevated in renal failure and what can be expected from homocysteine-lowering? *Nephrol Dial Transplant*. 2006;21:1161–1166. doi: 10.1093/ndt/gfl044
43. Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *N Engl J Med*. 1999;340:1449–1454. doi: 10.1056/NEJM199905133401901
44. Kyrle PA, Stümpflen A, Hirschl M, Bialonczyk C, Herkner K, Speiser W, Weltermann A, Kaider A, Pabinger I, Lechner K, et al. Levels of prothrombin fragment F1+2 in patients with hyperhomocysteinemia and a history of venous thromboembolism. *Thromb Haemost*. 1997;78:1327–1331.