The PAr index, an indicator reflecting altered vitamin B-6 homeostasis, is associated with long-term risk of stroke in the general population: the Hordaland Health Study (HUSK)

Hui Zuo,[1] *Grethe S Tell,*[1,3] *Per M Ueland,*[2,4] *Ottar Nygård,*[2,5] *Stein E Vollset,*[1,6] *Øivind Midttun,*[7] *Klaus Meyer,*[7] *and Arve Ulvik*[7]

Departments of [1]Global Public Health and Primary Care and [2]Clinical Science, University of Bergen, Bergen, Norway; [3]Domain for Health Data and Digitalization, Norwegian Institute of Public Health, Bergen, Norway; [4]Laboratory of Clinical Biochemistry; [5]Department of Heart Disease, Haukeland University Hospital, Bergen, Norway; [6]The Norwegian Institute of Public Health, Bergen, Norway; and [7]Bevital A/S, Bergen, Norway

ABSTRACT

Background: Vitamin B-6 homeostasis is altered during inflammation and immune activation. It is unknown whether altered vitamin B-6 homeostasis is associated with the risk of stroke.

Objective: We investigated the relation between the ratio plasma 4-pyridoxic acid: (pyridoxal + pyridoxal-5′-phosphate) (PAr) as an indicator of altered vitamin B-6 homeostasis and the risk of stroke in the general population.

Design: We conducted a prospective analysis of the community-based Hordaland Health Study (HUSK) in 6891 adults (born during 1925–1927 and 1950–1951) without known stroke at baseline (1998–1999). Participants were followed via linkage to the CVDNOR (Cardiovascular Disease in Norway) project and the Cause of Death Registry. HRs and 95% CIs were calculated using Cox proportional hazards analyses.

Results: A total of 390 participants (193 men and 197 women) developed stroke over a median follow-up period of 11 y. Study participants with elevated PAr experienced a higher risk of incident stroke in an essentially linear dose-response fashion. The HR (95% CI) for the highest compared with the lowest quartile of PAr was 1.97 (1.42, 2.73; *P*-trend <0.001) for total stroke and 2.09 (1.42, 3.09; *P*-trend <0.001) for ischemic stroke after adjustment for age, sex, body mass index (BMI), smoking, education, physical activity, estimated glomerular filtration rate, hypertension, diabetes, total cholesterol, and statin use. PAr had greater predictive strength than did C-reactive protein, current smoking, diabetes, hypertension, estimated glomerular filtration rate, and physical activity. The associations were similar in subgroups stratified by age group, sex, BMI, current smoking, hypertension, diabetes, and statin use at baseline.

Conclusions: Higher plasma PAr was independently associated with increased risk of incident stroke in all participants and across all subgroups stratified by conventional risk predictors. Our novel findings point to and expand the range of inflammation and immune activation processes that may be relevant for the pathogenesis and prevention of stroke. This trial was registered at clinicaltrials.gov as NCT03013725. *Am J Clin Nutr* 2018;107:105–112.

Keywords: vitamin B-6, biomarker, stroke, inflammation, cohort, risk

INTRODUCTION

Stroke is the second most common cause of death worldwide and a major cause of serious long-term disability (1). About 80% of all strokes are ischemic (2). In the past few decades, significant advances have been made in the identification and treatment of conventional risk factors, including high blood pressure, diabetes, smoking, and physical inactivity (3). However, only 60–80% of ischemic strokes may be attributed to these factors (4), a finding that has motivated a search for novel risk factors.

Chronic inflammation has been suggested to be involved in the development of stroke (5). For example, the commonly used inflammatory marker C-reactive protein (CRP) has been linked to ischemic stroke, although results from individual studies are inconsistent (6, 7). Neopterin, a marker of cellular immunity activation, has been related to prognostic outcome and mortality after stroke (8, 9).

Vitamin B-6 has attracted attention because of its widespread involvement in the body metabolism and function (10). The major circulating B-6 vitamers include the active form pyridoxal-5′-phosphate (PLP), the transport form pyridoxal, and the catabolite

The authors reported no funding received for this study.

Supplemental Table 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

Address correspondence to HZ (e-mail: hui.zuo@uib.no).

Abbreviations used: CHD, coronary heart disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HUSK, Hordaland Health Study; ICD, International Classification of Diseases; PA, 4-pyridoxic acid; PAr, 4-pyridoxic acid/(pyridoxal + pyridoxal-5′-phosphate); PLP, pyridoxal-5′-phosphate.

Received July 11, 2017. Accepted for publication October 19, 2017.

First published online January 26, 2018; doi: https://doi.org/10.1093/ajcn/nqx012.

4-pyridoxic acid (PA) (11, 12). There are consistent reports on the inverse associations of low plasma PLP with markers of inflammation and clinical conditions linked to low-grade inflammation, including cardiovascular disease and stroke (12). Recently, we proposed the PA:(PLP + pyridoxal) ratio (PAr), as a possible marker of altered vitamin B-6 homeostasis during cellular immune activation (11, 13). Key properties of PLP-catabolizing enzymes indicate a plausible association of high PAr with aldehyde and oxidative stress (13). Furthermore, the acute inflammatory response (as indicated by, for example, CRP) was found to be associated with a redistribution of PLP from the plasma to other tissues (14), thereby linking elevated PAr to other inflammatory modalities as well.

Recent observations demonstrate that PAr predicts the risk of inflammatory-related conditions including cancer (15), especially lung (15) and colorectal cancer (16), as well as long-term mortality in patients with coronary artery disease (17). However, no published studies, to our knowledge, have explored a possible association of PAr with stroke.

We therefore tested the hypothesis that altered vitamin B-6 homeostasis during inflammation and immune activation, captured by plasma PAr, predicts long-term risk of cerebral stroke in a prospective cohort of community-dwelling adults.

METHODS

Study population

The Hordaland Health Study (HUSK) is a community-based study with baseline measurements and survey conducted during 1997–1999 as collaboration between the University of Bergen, the National Institute of Public Health, and the Municipal Health Service in Hordaland, Western Norway (http://husk.b.uib.no). The Regional Committee for Medical and Health Research Ethics in Western Norway (REK-Vest) approved the study protocol. Written informed consent was obtained from all participants. Details of the study design and methodology have been described elsewhere (18, 19). The source population in the current study was 17,361 participants born during 1925–1927 and 1950–1951 and living in the city of Bergen and its surrounding areas who participated in the Hordaland Homocysteine Study in 1992–1993 (19). Of these, 9187 participants were invited to the HUSK study, and a total of 7074 participants met for physical examinations and completed self-administered questionnaires (19), yielding a participation rate of 77%. The present study cohort comprised 7050 men and women who donated blood samples. Of these, 67 were excluded due to hospitalization with stroke before enrollment, and 92 with missing data on plasma measurements of PLP, pyridoxal or PA. This left 6891 participants (3036 men and 3855 women) in the final study cohort, as described in **Figure 1**.

Biochemical analyses

Non-fasting plasma specimens were collected at baseline and stored at –80°C until analysis. Plasma PLP, pyridoxal, PA, neopterin, and cotinine were measured using liquid chromatography/tandem mass spectrometry (20). In addition, serum total cholesterol was measured using standard methods and creatinine measured colorimetrically using the alkaline picrate method (18). Plasma high-sensitivity CRP was measured by an immuno-matrix-assisted laser desorption/ionization mass spectrometry assay (21). All biochemical analyses were performed at Bevital A/S, Bergen (www.bevital.no), except for serum total cholesterol and creatinine which were analyzed at Ullevål University Hospital, Oslo. The coefficients of variation for PLP, pyridoxal, PA, and neopterin were 2.8–3.7% (within-day) and 5.7–7.1% (between-day) (20).

Follow-up and outcome ascertainment

The cohort participants were followed from baseline to the date of stroke diagnosis, death, emigration, or 31 December 2009 (the end of follow-up), whichever came first. Follow-up was 100% complete with a total of 70,662 person-years. Stroke cases were ascertained via record linkage to national hospital discharge diagnoses data obtained through the Cardiovascular Disease in Norway (CVDNOR) project, 1994–2009 (www.cvdnor.no) (22–24). The primary outcome was hospitalization or death attributed to stroke [total stroke: International Classification of Diseases (ICD)-9 codes 430–434, 436 and ICD-10 codes I60–I61, I63–I64 except I63.6; ischemic stroke: ICD-9 codes 433, 434 and ICD-10 codes I63 except I63.6]. Information on death was collected from the Cause of Death Registry at Statistics Norway and coded according to ICD-10. If >1 stroke event occurred in a participant during the follow-up period, only the first event was considered. An 11-digit personal identifier, unique to each Norwegian resident, was used to link baseline variables with study endpoints.

Assessments of covariates

Information on education (highest level of completed education), physical activity, statin use, and a history of coronary heart disease (CHD, defined as myocardial infarction or angina pectoris) at baseline was collected via self-administered questionnaires. Height and weight were measured and BMI was calculated as weight in kilograms divided by height in meters squared. Smoking status was based on self-reported smoking status and corrected by plasma cotinine (i.e., self-reported nonsmokers with plasma cotinine concentrations ≥85 nmol/L were reclassified as current smokers) (25). Participants were considered to have hypertension at baseline if they reported use of antihypertensive medication or had a blood pressure ≥140/90 mm Hg (26). Diabetes was defined based on self-reported diabetes, use of hypoglycemic medication, or blood glucose concentration at baseline (27). Calculation of estimated glomerular filtration rate (eGFR) was based on serum creatinine concentrations using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (28). Vitamin B-6 supplementation was assessed by a validated self-administered food frequency questionnaire (29). Fasting was defined as no caloric intake for ≥8 h.

Statistical analysis

Since PAr levels were markedly higher in the older age group (median: 0.46 for 70–74 y compared with 0.34 for 46–49 y), age-specific quartiles were defined for the 2 age groups separately. Baseline characteristics of study participants are described for all participants combined and according to quartiles of PAr, and variables are expressed as percentages or medians (interquartile ranges). Trends across quartiles were tested by logistic and quantile (median) regression for categorical and continuous variables,

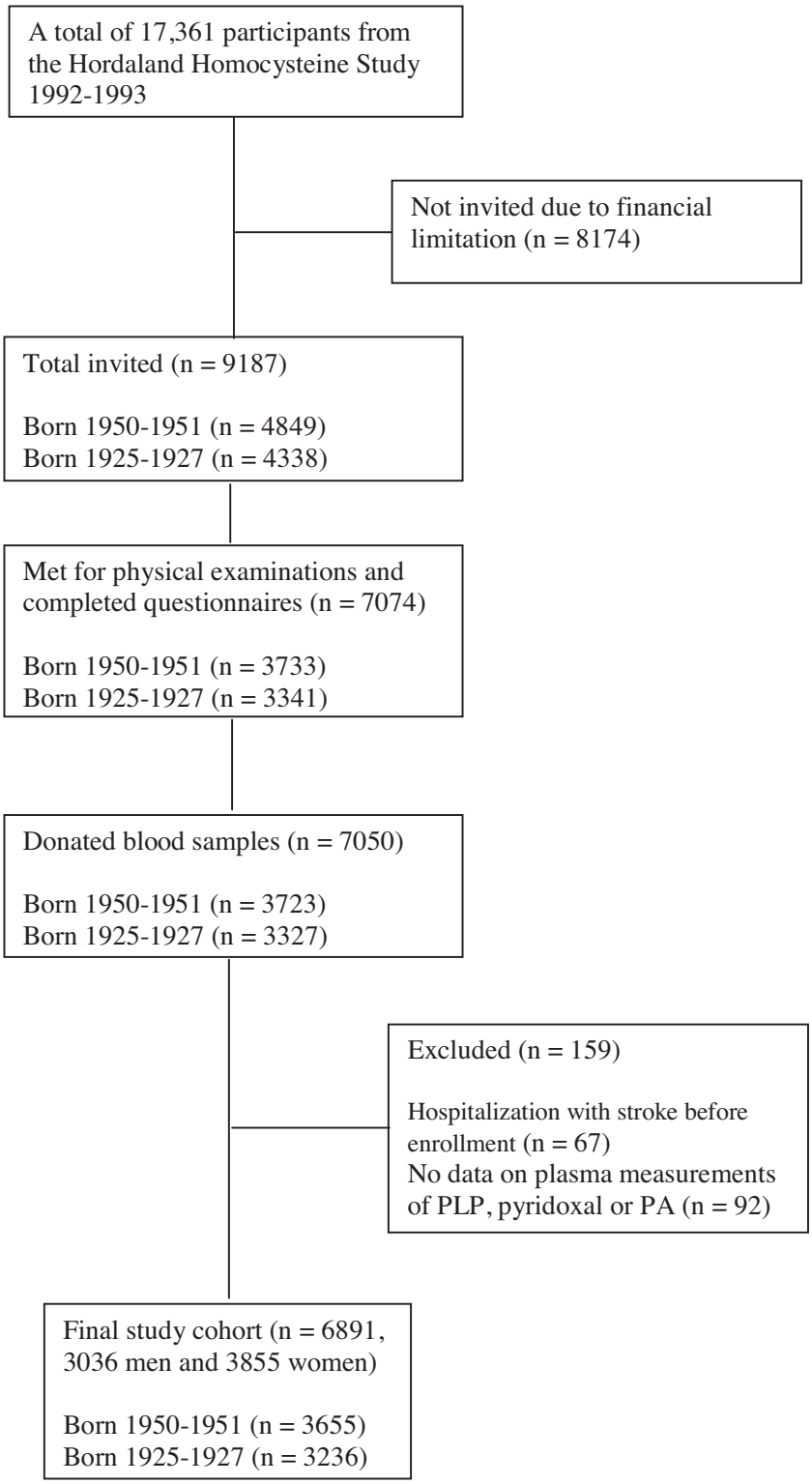

FIGURE 1 Flow chart of study participants. PA, 4-pyridoxic acid; PAr, 4-pyridoxic acid/(pyridoxal + pyridoxal-5′-phosphate).

respectively. Natural log transformation was applied to plasma biomarkers to normalize distributions.

We used Cox proportional hazards regression to evaluate PAr and long-term stroke risk. HRs and corresponding 95% CIs are reported per 1-SD increment. To estimate the independent association of PAr with risk of stroke (total and ischemic stroke, respectively), 3 multivariate models were constructed: model 1 was the basic model adjusted for age (46–49 compared with 70–74 y) and sex; model 2 was adjusted as model 1 plus lifestyle risk factors—BMI (continuous), current smoking (yes or no), education (≤10, 11–13, or ≥14 y) and physical activity (none/light or moderate/vigorous); model 3 was further adjusted for

TABLE 1
Baseline characteristics of all participants and by PAr quartiles in the HUSK[1]

Characteristic	All	PAr quartile				*P*-trend[2]	*P*-trend[3]
		Q1	Q2	Q3	Q4		
Participants, *n*	6891	1722	1723	1723	1723		
Men, %	44.1	46.4	46.3	43.6	39.9	<0.001	<0.001
BMI, kg/m^2	25.4 (23.1, 27.9)	25.5 (23.3, 27.8)	25.6 (23.3, 28.1)	25.2 (23.0, 27.9)	25.1 (22.8, 27.9)	0.002	0.02
Current smoking, %	28.7	23.6	27.0	29.4	34.6	<0.001	<0.001
Physical activity, %							
None/light	43.6	43.3	41.9	43.9	45.3	0.14	0.35
Moderate/vigorous	56.4	56.7	58.1	56.1	54.7		
Education, y							
≤10	31.1	30.1	31.1	33.3	29.6		
11–13	40.8	41.1	40.5	39.5	42.2	0.67	0.57
≥14	28.1	28.8	28.4	27.2	28.2		
eGFR, mL · min^{-1} · 1.73 m^{-2}	79.9 (69.0, 89.8)	82.3 (71.8, 92.4)	80.6 (70.3, 90.3)	79.3 (68.6, 88.9)	77.0 (66.0, 87.7)	<0.001	<0.001
Hypertension, %	42.3	40.9	41.7	43.2	43.5	0.08	0.04
Diabetes, %	2.7	2.0	2.4	3.3	2.8	0.06	0.07
Total cholesterol, mmol/L	5.9 (5.2, 6.7)	6.0 (5.3, 6.8)	6.0 (5.3, 6.7)	6.0 (5.2, 6.7)	5.7 (5.1, 6.5)	<0.001	<0.001
Statin use, %	7.2	7.0	7.7	6.9	7.0	0.72	0.65
History of CHD, %	8.3	6.5	8.1	8.5	10.0	<0.001	<0.001
Plasma biomarkers							
PAr	0.39 (0.29, 0.52)	0.24 (0.21, 0.29)	0.33 (0.30, 0.40)	0.43 (0.38, 0.51)	0.66 (0.53, 0.82)		
CRP, mg/L	1.6 (0.7, 3.6)	1.2 (0.6, 2.4)	1.4 (0.6, 3.1)	1.7 (0.7, 3.9)	2.1 (0.9, 5.4)	<0.001	<0.001
Neopterin, nmol/L	7.6 (6.4, 9.3)	7.0 (6.0, 8.3)	7.4 (6.2, 9.0)	7.8 (6.6, 9.4)	8.4 (7.0, 10.7)	<0.001	<0.001

[1]Values are presented as medians (IQRs) or percentages. CRP, C-reactive protein; CHD, coronary heart disease; eGFR, estimated glomerular filtration rate; HUSK, Hordaland Health Study; PAr, 4-pyridoxic acid/(pyridoxal + pyridoxal-5′-phosphate); Q, quartile.
[2]Derived from logistic regression for categorical variables and quantile (median) regression for continuous variables, unadjusted.
[3]Adjusted for sex.

potential mediators—eGFR (continuous), hypertension (yes or no), diabetes (yes or no), total cholesterol (continuous), and statin use (yes or no). In addition, we also assessed the associations after additional adjustment for CRP (continuous), neopterin (continuous), vitamin B-6 supplementation (yes or no), and fasting status (yes or no) in the models. The proportionality assumption was tested using Schoenfeld residuals. To test for potential confounding or mediation by plasma CRP or neopterin, we also examined the associations by additional adjustment for these 2 biomarkers. Potential nonlinear associations of PAr levels with stroke risk were modeled by generalized additive models using penalized splines with multivariable adjustment as indicated in model 3 (30). To corroborate the findings, the risk associations were also evaluated across age-specific quartiles of PAr, and *P*-trend was calculated by modeling the quartile number as a continuous variable in the regression models.

Potential effect modification by age group, sex, BMI (below or above median), current smoking, hypertension, diabetes, and statin use was assessed in stratified analyses and significance of interactions was assessed based on first-degree multiplicative models for each stratification variable separately. Sensitivity analyses were performed to determine the robustness of findings in the primary analysis. We restricted the risk-association analyses to participants who had no self-reported history of CHD or stroke at baseline. In addition, the analyses were repeated after excluding the first 2 y of follow-up.

We performed forward and backward stepwise selection of predictors, keeping age and sex as adjustments at all times using the R-package *crrstep* (31). The impact on the model fit from the addition or removal of predictors was evaluated with the use of the modified Bayesian information criterion for competing risk as recommended by the developers of the package (31). To assess improvement in model discrimination and reclassification of the study participants, we calculated the integrated discrimination index and the continuous net reclassification index (>0) in logistic regression models containing the same covariates as the multivariate Cox model, with and without PAr (32, 33).

Statistical analyses were conducted with the SAS statistical software (version 9.4; SAS Institute, Inc.) and R (version 3.4.0, www.r-project.org). All tests were 2 sided and a P value <0.05 was considered statistically significant.

RESULTS

Among the 6891 participants, a total of 390 (193 men and 197 women) developed stroke over a median follow-up period of 10.9 y (range: 0.1–11.7 y), of which 279 (72%) were ischemic. Baseline characteristics of the study participants as a whole and trends across quartiles of PAr are shown in **Table 1**. Participants in higher PAr quartiles were more likely to be women, current smokers ($P < 0.001$), and to have hypertension, diabetes, and a history of CHD at baseline. PAr levels were inversely associated with BMI, eGFR, and total cholesterol concentrations. As expected, PAr was positively associated with the plasma biomarkers CRP ($P < 0.001$) and neopterin ($P < 0.001$) at baseline. In addition, use of vitamin B-6 supplements was a weak predictor of PAr, whereas it was an important predictor of PLP, pyridoxal, and PA. Fasting status contributed no additional explained variation in either PAr or any individual vitamin B-6 forms (**Supplemental Table 1**).

TABLE 2
HRs (95% CI) for risk of total and ischemic stroke by PAr in the HUSK[1]

	PAr quartile					Continuous	
	Q1	Q2	Q3	Q4	*P*-trend	Per 1-SD increment of log-transformed PAr	*P*
Total stroke							
Cases/*n*	72/1722	89/1723	106/1723	123/1723		390/6891	
Model 1[2]	1	1.25 (0.91, 1.70)	1.50 (1.11, 2.02)	1.94 (1.45, 2.59)	<0.001	1.28 (1.16, 1.42)	<0.001
Model 2[3]	1	1.31 (0.94, 1.84)	1.53 (1.11, 2.12)	1.88 (1.37, 2.59)	<0.001	1.27 (1.14, 1.42)	<0.001
Model 3[4]	1	1.33 (0.95, 1.86)	1.56 (1.12, 2.16)	1.97 (1.42, 2.73)	<0.001	1.29 (1.15, 1.45)	<0.001
Ischemic stroke							
Cases/*n*	48/1722	62/1723	80/1723	89/1723		279/6891	
Model 1[2]	1	1.30 (0.89, 1.89)	1.69 (1.18, 2.42)	2.07 (1.46, 2.94)	<0.001	1.31 (1.16, 1.48)	<0.001
Model 2[3]	1	1.31 (0.88, 1.97)	1.66 (1.13, 2.44)	1.95 (1.33, 2.86)	<0.001	1.28 (1.12, 1.46)	<0.001
Model 3[4]	1	1.33 (0.89, 1.99)	1.71 (1.16, 2.52)	2.09 (1.42, 3.09)	<0.001	1.31 (1.15, 1.50)	<0.001

[1]Cox proportional hazards regression models were used to calculate the HRs and 95% CIs. eGFR, estimated glomerular filtration rate; HUSK, Hordaland Health Study; PAr, 4-pyridoxic acid /(pyridoxal + pyridoxal-5′-phosphate).
[2]Model 1 was adjusted for age (46-49 y vs. 70–74 y) and sex.
[3]Model 2 was adjusted as for model 1 plus BMI (continuous), current smoking (yes or no), education (≤10, 11–13, or ≥14 y) and physical activity (none/light or moderate/vigorous).
[4]Model 3 was adjusted as for model 2 plus eGFR (continuous), hypertension (yes or no), diabetes (yes or no), total cholesterol (continuous), and statin use (yes or no).

As shown in **Table 2**, higher levels of PAr were significantly associated with an increased risk of total stroke and ischemic stroke in models adjusted for age and sex, in models with further adjustments for BMI, current smoking, education, and physical activity, and in fully adjusted models with additional adjustments for eGFR, hypertension, diabetes, total cholesterol, and statin use. The multivariable-adjusted HR for total stroke was 1.29 (95% CI: 1.15, 1.45) per 1-SD increment of log-transformed PAr, and 1.97 (95% CI: 1.42, 2.73, *P*-trend <0.001) for the highest compared with the lowest PAr quartile. Additional adjustments for CRP (HR/SD: 1.26, 95% CI: 1.11, 1.42), neopterin (HR/SD: 1.27, 95% CI: 1.12, 1.43), vitamin B-6 supplementation (HR/SD: 1.29, 95% CI: 1.15, 1.45) and fasting status (HR/SD: 1.29, 95% CI: 1.15, 1.45) did not materially affect the risk associations. Vitamin B-6 supplementation was not associated with the risk of total stroke (multivariable-adjusted HR/SD: 1.11, 95% CI: 0.74, 1.68). Similar risk estimates were found for ischemic stroke (Table 2). Generalized additive model plots showed that the association between PAr and risk of total and ischemic stroke was essentially linear across the distribution of PAr values (**Figure 2**). When the components of the PAr index, i.e., PA, pyridoxal, and PLP, were evaluated separately, we found only a weak and positive association for PA, and nonsignificant associations for PLP or pyridoxal (data not shown).

The associations between PAr and the risk of total stroke were similar in subgroups stratified by age, sex, BMI, current smoking, hypertension, diabetes, and statin use at baseline (all *P* for interaction >0.30, **Table 3**).

In sensitivity analyses, restricting the study population to the 6322 participants without self-reported history of CHD yielded slightly attenuated associations for total stroke (HR/SD: 1.26, 95% CI: 1.11, 1.42) and ischemic stroke (HR/SD: 1.28, 95% CI: 1.11, 1.49). The risk estimates for total stroke remained unchanged after excluding 113 participants with self-reported history of stroke (HR/SD: 1.29, 95% CI: 1.15, 1.45). In addition, repeating the analyses among the whole study cohort after excluding the first 2 y of follow-up yielded similar results (HR/SD: 1.29, 95% CI: 1.14, 1.46).

We compared PAr to all potential risk predictors included in model 3 as well as CRP and neopterin by analyzing how much they improved the model fit after being added to a Cox regression model that included age and sex (**Table 4**). PAr was the strongest predictor followed by CRP, current smoking, diabetes, hypertension, eGFR, and physical activity among all the 12 potential risk predictors. Forward stepwise selection identified PAr, diabetes, and current smoking, in that order, as the best model for predicting total stroke, whereas inflammatory markers CRP and neopterin, among others, were not selected (Table 4). In reclassification analysis, the addition of PAr to the fully adjusted model (model 3 in Table 2) resulted in significant net reclassification improvement. The category-free net reclassification index for the addition of PAr in predicting total stroke was 0.184 (95% CI: 0.075, 0.294, $P = 0.001$). Similarly, the corresponding integrated discrimination index was 0.004 (95% CI: 0.001, 0.006, $P = 0.003$).

DISCUSSION

Principal findings

In this prospective community-based study, we observed that high plasma PAr, an indicator of altered vitamin B-6 homeostasis, was associated with an increased risk of total and ischemic stroke. The multivariable-adjusted risk estimates for stroke were almost twice as high for participants in the highest compared with the lowest quartile of PAr. The association between PAr and stroke risk appeared to be independent of potential confounding factors, CRP, and neopterin, and was similar across subgroups stratified by age, sex, BMI, current smoking, hypertension, diabetes, or statin use at baseline. Notably, PAr was the strongest predictor of stroke risk among a panel of 12 potential risk factors when evaluated by Cox regression and stepwise selection criteria.

PAr and stroke risk

We found that PAr was positively associated with stroke risk, and the risk increased continuously through the entire

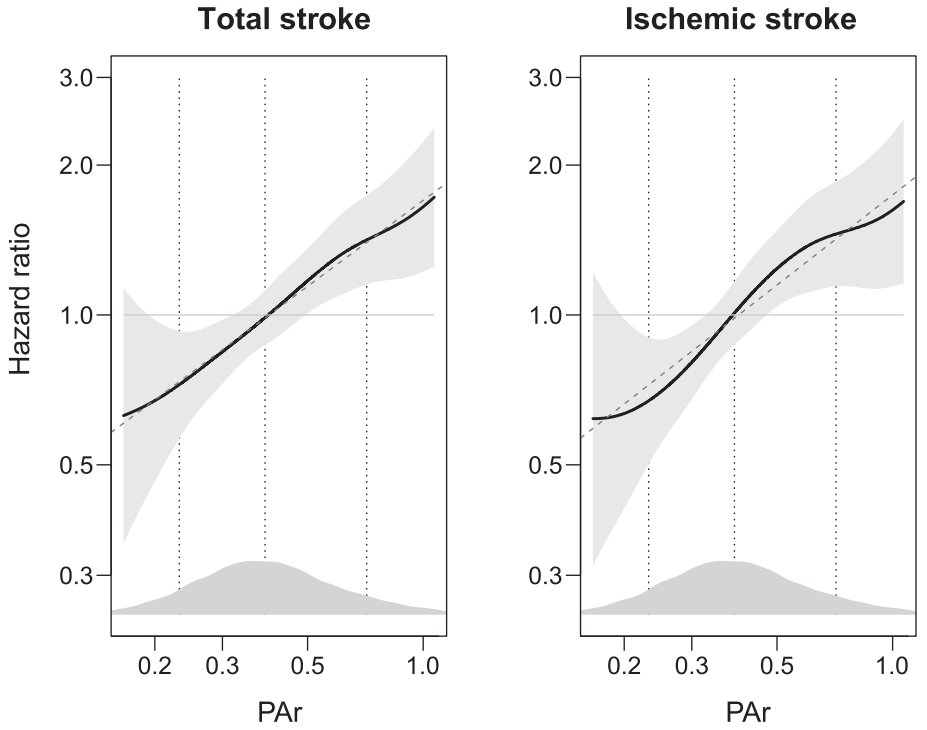

FIGURE 2 The associations of PAr with total and ischemic stroke by generalized additive models ($n = 6891$). The models were adjusted for age (46–49 vs. 70–74 y), sex, BMI (continuous), current smoking (yes or no), education (≤10, 11–13, or ≥14 y), physical activity (none/light or moderate/vigorous), eGFR (continuous), hypertension (yes or no), diabetes (yes or no), total cholesterol (continuous), and statin use (yes or no). The solid lines show HRs and the shaded areas 95% CIs. The dashed lines show HRs by linear regression on logarithmic scale. Density plots indicate the distributions of log-transformed PAr, and dotted lines denote the 10th, 50th, and 90th percentiles. eGFR, estimated glomerular filtration rate; PAr, 4-pyridoxic acid/(pyridoxal + pyridoxal-5′-phosphate).

distribution of PAr levels. Participants in the highest age-specific quartile of PAr had an approximately double risk of total or ischemic stroke compared with those in the first quartile. The associations between PAr and stroke were essentially unaltered in 3 models with gradual adjustments for age, sex, and other potential confounding factors, and did not differ significantly between the various subgroups. This indicates that the association is unlikely to be mediated through traditional risk factors for stroke, such as high BMI, smoking, hypertension, and diabetes (3). In addition, vitamin B-6 supplementation was not associated with stroke risk. Fasting status appeared to have negligible effect on PAr and its risk associations.

The weak or nonsignificant risk associations for the components of PAr (PLP, pyridoxal, and PA) suggest a minor role of vitamin B-6 status (PLP). Apparently, inflammatory processes are more important. Assuming a simultaneous influence of inflammation on PA (increasing) and PLP+ pyridoxal (decreasing), the PAr index is ideally configured to capture both processes.

Notably, PAr was found to be a stronger predictor of stroke risk than the other inflammatory markers CRP and neopterin as well as other potential risk predictors such as current smoking, diabetes, hypertension, and eGFR, which closely resembles the reported findings on PAr and all-cause mortality (17).

Causation and sensitivity analyses

Reverse causality (i.e., preclinical conditions before a diagnosis of stroke could lead to higher levels of PAr) needs attention in observational studies. However, this was probably not the case in our study as indicated by essentially unaltered risk estimates after exclusion of the first 2 y of follow-up. Moreover, only minimal changes in risk estimates were observed when restricting the study population to those without self-reported history of CHD or stroke at baseline. Taken together, these findings all attest to the robustness and independence of PAr as a marker of stroke risk.

Possible mechanisms

Systemic inflammation is strongly linked to the occurrence of stroke, exacerbates ischemic brain damage, and shapes stroke outcome (4, 5, 34). Although not yet fully elucidated, diverse inflammatory processes may trigger a stroke event through a variety of mechanisms, including thrombosis through the coagulation system (5), microvascular injury, and disturbed vascular homeostasis (34), and by promoting atherosclerosis (5). In addition, immune activation due to systemic inflammatory conditions or infection has a detrimental role in stroke pathophysiology (34, 35).

Inflammation links closely with oxidative and aldehydes stress, i.e., increased production of reactive oxygen species and reactive aldehydes (36). In response, aldehyde-scavenging enzymes, including enzymes that catalyze the irreversible catabolic conversion of PLP (via pyridoxal) to PA, are upregulated, leading to an increase in PAr (13). In addition, other independent inflammatory processes lead to an increased uptake or retention of PLP in tissues (12). As PLP appears in the denominator of PAr, these processes are also captured by the PAr index.

TABLE 3
PAr and risk of total stroke by strata in the HUSK[1]

	Cases/*n*	HR (95% CI)	*P*-interaction
Age			0.54
46–49 y	37/3655	1.46 (1.01, 2.09)	
70–74 y	353/3236	1.28 (1.13, 1.44)	
Sex			0.69
Men	193/3036	1.30 (1.11, 1.54)	
Women	197/3855	1.28 (1.09, 1.51)	
BMI (kg/m^2)			0.35
<25	157/3114	1.35 (1.14, 1.62)	
≥25	233/3766	1.26 (1.08, 1.46)	
Current smoking			0.78
No	296/4917	1.29 (1.12, 1.47)	
Yes	94/1974	1.29 (1.03, 1.61)	
Hypertension			0.61
No	121/3974	1.29 (1.05, 1.60)	
Yes	269/2915	1.29 (1.12, 1.48)	
Diabetes			0.58
No	358/6708	1.28 (1.14, 1.45)	
Yes	32/183	1.35 (0.92, 1.98)	
Statin use			0.52
No	347/6398	1.32 (1.16, 1.49)	
Yes	43/493	1.18 (0.82, 1.69)	

[1]HRs (95% CIs) are reported per 1-SD increment of log-transformed PAr. Multivariate adjusted model: adjusted for age (46–49 vs. 70–74 y), sex, BMI (continuous), current smoking (yes or no), education (≤10, 11–13, or ≥14 y), physical activity (none/light or moderate/vigorous), eGFR (continuous), hypertension (yes or no), diabetes (yes or no), total cholesterol (continuous), and statin use (yes or no). eGFR, estimated glomerular filtration rate; HUSK, Hordaland Health Study; PAr, 4-pyridoxic acid/(pyridoxal + pyridoxal-5′-phosphate).

TABLE 4
Comparison of predictive strength for selected variables using ΔAIC in the HUSK ($n = 6891$) [1]

	ΔAIC	Order of strength[2]	Order of selection[3]
PAr	–16.7	1	1
Diabetes	–8.1	4	2
Current smoking	–8.2	3	3
Hypertension	–5.1	5	NA
eGFR	–0.1	6	NA
CRP	–8.7	2	NA
Physical activity	1.2	7	NA

[1]ΔAIC calculated as reduction in the AIC after the inclusion of the variable in a Cox regression model that was adjusted for age and sex. The AIC for the starting model was 5111.7. Potential risk predictors include: PAr (log transformed), CRP (log transformed), neopterin (log transformed), age (46–49 y vs. 70–74 y), sex, BMI (continuous), current smoking (yes or no), education (≤10, 11–13, or ≥14 y), physical activity (none/light or moderate/vigorous), eGFR (continuous), hypertension (yes or no), diabetes (yes or no), total cholesterol (continuous), and statin use (yes or no). AIC, Akaike's information criterion; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HUSK, Hordaland Health Study; NA, not applicable; PAr, 4-pyridoxic acid/(pyridoxal + pyridoxal-5′-phosphate).

[2]Order of predictive strength according to the ΔAIC.

[3]Order of selection in a Cox regression model with the use of forward stepwise selection. Age and sex as adjustments at all times.

In our study, the association between PAr and stroke risk was independent of CRP, a commonly used systemic marker of inflammation. This clearly suggests that inflammatory processes forming the basis for the observed association of PAr with stroke is not fully captured by CRP.

In addition, conventional risk factors, including aging, high BMI, smoking, hypertension, and diabetes (4), are often accompanied by an inflammatory response, characterized by altered glial activity and production of pro- and anti-inflammatory cytokines (34). However, the association of PAr with stroke risk was similar across subgroups stratified by age, BMI, smoking, hypertension, and diabetes, indicating that PAr and these factors are essentially mutually independent risk predictors and suggesting that PAr is an indicator of aspects of the inflammatory response beyond those elicited by the conventional risk factors.

Strengths and limitations

To the best of our knowledge, this is the first study to investigate altered vitamin B-6 homeostasis and stroke risk, allowing a more comprehensive evaluation and understanding of vitamin B-6 in relation to stroke. Other important strengths include the prospective design, long and complete follow-up, and adjustment for several possible confounders. Nevertheless, the true risk associations may have been underestimated due to regression dilution bias (37) because plasma biomarkers were measured at baseline only. The comparatively high intraclass correlation coefficient of PAr (0.75) (13), however, suggests that regression dilution bias probably plays a minor role in the evaluation of PAr with the risk of stroke.

In conclusion, we observed that plasma levels of PAr, a multifactorial indicator of cellular immunity activation, oxidative stress, and related inflammatory responses, have a continuous and independent association with the risk of total and ischemic stroke in community-dwelling men and women. As a risk predictor of stroke, PAr was superior to the established inflammatory markers CRP and neopterin, but also to a complete panel of conventional risk factors of stroke including smoking and diabetes. Our findings highlight inflammation and immunity in the pathogenesis of stroke and point at limitations of established inflammatory markers. Finally, our results suggest additional reaction mechanisms and pathways, some of them apparently captured by the PAr index, which merit greater attention in further studies for the ultimate translation into effective preventive measures for stroke in the future.

The authors' responsibilities were as follows—HZ: drafted the manuscript and had primary responsibility for the final content of the manuscript; HZ and AU: analyzed the data; HZ, AU, GST, and SEV: designed the research; GST, SEV, ØM, and KM: acquired the data; GST, PMU, ON, SEV, ØM, and AU: critically revised the manuscript for important intellectual content; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to this manuscript.

REFERENCES

1. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, de Ferranti SD, Floyd J, Fornage M, Gillespie C, et al. Heart disease and stroke statistics—2017 update: a report from the American Heart Association. Circulation 2017;135:e146–e603.
2. Donnan GA, Fisher M, Macleod M, Davis SM. Stroke. Lancet 2008;371(9624):1612–23.
3. Feigin VL, Roth GA, Naghavi M, Parmar PG, Krishnamurthi R, Chugh S, Mensah GA, Norrving B, Shiue I, Ng MY, et al. Global burden of stroke and risk factors in 188 countries, during 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet Neurol 2016;15:913–24.

4. Bang OY, Ovbiagele B, Kim JS. Nontraditional risk factors for ischemic stroke: an update. Stroke 2015;46:3571–8.
5. Macrez R, Ali C, Toutirais O, Le Mauff B Defer G, Dirnagl U, Vivien D. Stroke and the immune system: from pathophysiology to new therapeutic strategies. Lancet Neurol 2011;10:471–80.
6. Emerging Risk Factors C, Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, Danesh J. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet 2010;375(9709): 132–40.
7. Xu T, Ke K. C-reactive protein and ischemic stroke risk in general population: A dose-response meta-analysis of prospective studies. Int J Cardiol 2015;190:264–7.
8. Zeng X, Zhang G, Yang B, Zhang B, Zhang L, Ni Y, Liu C, Luo Y. Neopterin as a predictor of functional outcome and mortality in Chinese patients with acute ischemic stroke. Mol Neurobiol 2016;53:3939–47.
9. Lin HS, Tsai TH, Liu CF, Lu CH, Chang WN, Chen SF, Huang CW, Huang CR, Tsai NW, Huang CC, et al. Serum level and prognostic value of neopterin in patients after ischemic stroke. Clin Biochem 2012;45:1596–601.
10. Eliot AC, Kirsch JF. Pyridoxal phosphate enzymes: mechanistic, structural, and evolutionary considerations. Annu Rev Biochem 2004;73:383–415.
11. Ueland PM, Ulvik A, Rios-Avila L, Midttun O, Gregory JF. Direct and functional biomarkers of vitamin B6 status. Annu Rev Nutr 2015;35:33–70.
12. Ueland PM, McCann A, Midttun O, Ulvik A. Inflammation, vitamin B6 and related pathways. Mol Aspects Med 2017;53:10–27.
13. Ulvik A, Midttun O, Pedersen ER, Eussen SJ, Nygard O, Ueland PM. Evidence for increased catabolism of vitamin B-6 during systemic inflammation. Am J Clin Nutr 2014;100:250–5.
14. Chiang EP, Smith DE, Selhub J, Dallal G, Wang YC, Roubenoff R. Inflammation causes tissue-specific depletion of vitamin B6. Arthritis Res Ther 2005;7:R1254–62.
15. Zuo H, Ueland PM, Eussen SJ, Tell GS, Vollset SE, Nygard O, Midttun O, Meyer K, Ulvik A. Markers of vitamin B6 status and metabolism as predictors of incident cancer: the Hordaland Health Study. Int J Cancer 2015;136:2932–9.
16. Gylling B, Myte R, Schneede J, Hallmans G, Haggstrom J, Johansson I, Ulvik A, Ueland PM, Van Guelpen B Palmqvist R. Vitamin B-6 and colorectal cancer risk: a prospective population-based study using 3 distinct plasma markers of vitamin B-6 status. Am J Clin Nutr 2017;105:897–904.
17. Ulvik A, Pedersen ER, Svingen GF, McCann A, Midttun O, Nygard O, Ueland PM. Vitamin B-6 catabolism and long-term mortality risk in patients with coronary artery disease. Am J Clin Nutr 2016;103:1417–25.
18. Vikse BE, Vollset SE, Tell GS, Refsum H, Iversen BM. Distribution and determinants of serum creatinine in the general population: the Hordaland Health Study. Scand J Clin Lab Invest 2004;64:709–22.
19. Refsum H, Nurk E, Smith AD, Ueland PM, Gjesdal CG, Bjelland I, Tverdal A, Tell GS, Nygard O, Vollset SE. The Hordaland Homocysteine Study: a community-based study of homocysteine, its determinants, and associations with disease. J Nutr 2006;136(6 Suppl):S1731–40.
20. Midttun O, Hustad S, Ueland PM. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 2009;23:1371–9.
21. Meyer K, Ueland PM. Targeted quantification of C-reactive protein and cystatin c and its variants by immuno-MALDI-MS. Anal Chem 2014;86:5807–14.
22. Igland J, Tell GS, Ebbing M, Nygård O, Vollset SE, Dimoski T. Cardiovascular Disease in Norway 1994-2009 Description of data and data quality. 2013. Internet: https://cvdnor.b.uib.no/ accessed Date Accessed).
23. Sulo G, Igland J, Vollset SE, Nygård O, Øyen N, Tell GS. Cardiovascular disease and diabetes mellitus in Norway during 1994–2009 CVDNOR—a nationwide research project. Nor Epidemiol 2013;23:101–7.
24. Sulo G, Igland J, Nygard O, Vollset SE, Ebbing M, Tell GS. Favourable trends in incidence of AMI in Norway during 2001–2009 do not include younger adults: a CVDNOR project. Eur J Prev Cardiol 2014;21:1358–64.
25. Jarvis MJ, Tunstall-Pedoe H, Feyerabend C, Vesey C, Saloojee Y. Comparison of tests used to distinguish smokers from nonsmokers. Am J Pub Health 1987;77:1435–8.
26. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Despres JP, Fullerton HJ, et al. Heart disease and stroke statistics—2016 update: a report from the American Heart Association. Circulation 2016;133:e38–360.
27. American Diabetes Association. 2. Classification and diagnosis of diabetes. Diabetes Care 2016;39 Suppl 1:S13–22.
28. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, et al. A new equation to estimate glomerular filtration rate. Ann Int Med 2009;150:604–12.
29. Andersen LF, Solvoll K, Johansson LR, Salminen I, Aro A, Drevon CA. Evaluation of a food frequency questionnaire with weighed records, fatty acids, and alpha-tocopherol in adipose tissue and serum. Am J Epidemiol 1999;150(1):75–87.
30. Wood SN. Generalized additive models: an introduction with R: Boca Raton: Chapman & Hall/CRC; 2006.
31. Kuk D, Varadhan R. Model selection in competing risks regression. Statistics in medicine 2013;32(18):3077–88.
32. Pencina MJ, D'Agostino RB Sr., D'Agostino RB Jr., Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med 2008;27:157–72; discussion 207–12.
33. Pencina MJ, D'Agostino RB Sr., Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. Stat Med 2011;30:11–21.
34. Murray KN, Buggey HF, Denes A, Allan SM. Systemic immune activation shapes stroke outcome. Mol Cell Neurosci 2013;53:14–25.
35. Fu Y, Liu Q, Anrather J, Shi FD. Immune interventions in stroke. Nat Rev Neurol 2015;11:524–35.
36. Grimsrud PA, Xie H, Griffin TJ, Bernlohr DA. Oxidative stress and covalent modification of protein with bioactive aldehydes. J Biol Chem 2008;283:21837–41.
37. Hutcheon JA, Chiolero A, Hanley JA. Random measurement error and regression dilution bias. BMJ 2010;340.