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Plasma Vitamin B_6 and Risk of Myocardial Infarction in Women

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Abstract

Background—Vitamin B_6 is widely involved in amino acid metabolism and is a modulator of several reactions important to cardiovascular health. We prospectively evaluated relationships between fasting plasma levels of vitamin B_6, as pyridoxal phosphate (PLP), to subsequent myocardial infarction risk in women. We also evaluated the predictors of fasting plasma concentration of pyridoxal phosphate.

Methods—Participants were adult nurses who completed questionnaires, and updated exposures every 2 years since 1976. Subjects for this analysis were selected by a nested case control design. Blood samples were collected between 1989 and 1990. We restricted our analysis to those women who had provided fasting blood samples (≥10 hours since last meal). During follow-up through June 1998, 144 were diagnosed with myocardial infarction (fatal and non-fatal). Cases were matched 1:2 by age, cigarette smoking status, and month and fasting status at the time of blood collection. Conditional logistic regression was used to adjust for potential confounders, including anthropometric factors, dietary intake, and selected biomarkers. Linear regression was used to determine which variables predict fasting total PLP concentration among control women.

Results—Median age at blood collection was 63. Among controls, lower estimated creatinine clearance, plasma total homocysteine and body mass index were statistically significant predictors of higher plasma PLP, as were higher dietary vitamin B_6, and folate intake (all $P < 0.05$). Plasma levels of pyridoxal phosphate were inversely associated with risk of MI, the multivariable adjusted rate ratio (RR) between extreme quarters was 0.22 (95% CI 0.09,0.55; Ptrend=0.05). The effect of plasma PLP varied by age. Among women who were aged less than 60 at blood sampling, the RR (95%CI) comparing top vs. bottom quarter was 0.03 (0.002,0.48), whereas among older women the corresponding RR (95%CI) was 0.43 (0.15,1.25).

Conclusions—Fasting plasma concentration of pyridoxal phosphate was inversely associated with MI risk. Plasma PLP is positively correlated with dietary vitamin B6, and is inversely correlated with renal function and body mass index. Future studies are needed to better understand both dietary and

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non-dietary determinants of plasma and tissue vitamin B6 status, and how these can be optimized to prevent MI and other diseases.

Keywords

vitamins; nutrition; women; myocardial infarction; risk factors

Introduction

Coronary Artery Disease (CAD) remains the leading cause of mortality among postmenopausal women in the USA [1,2]. Several epidemiological studies suggest that vitamin B_6 may be important in the prevention of CAD[3,4]. Few studies have adequately assessed the role of plasma levels of vitamin B_6 in relation to MI risk. Vitamin B_6 is widely involved in one-carbon transfer[5,6] and is a cofactor for more than 100 human enzymes[7], that are essential in amino acid metabolism (including the homocysteine-cystathionine-cysteine conversion), carbohydrate and lipid metabolism, and neurotransmitter production. In circulation, it mainly exists as pyridoxal 5′ phosphate (PLP). In addition to its role in macronutrient metabolism, vitamin B_6 is important in steroid receptor interactions[8], immune response[9], and mineral transport across cell membranes[10]. One published study found that plasma concentrations of PLP are inversely associated with C-reactive protein concentration, independent of plasma total homocysteine. Another epidemiological study has demonstrated that dietary vitamin B_6 is inversely related to risk of myocardial infarction (MI)[3], and others have suggested an inverse relationship between plasma PLP and CAD risk[2,11,12].

Apart from dietary intake plasma concentration of PLP likely depends on variability in absorption by the gastrointestinal tract, its uptake in the liver, its activation and de-activation by various metabolic processes, and its excretion by the kidneys. These factors have so far not been systematically studied in epidemiological studies. Plasma concentration of PLP is sensitive to feeding status[13] and changes after glucose ingestion[14].

In this paper, using prospectively collected samples, selected using a nested case control design within the Nurses' Health Study cohort, we seek to answer two primary questions: 1) What are the major determinants of the fasting plasma concentration of PLP in predominantly postmenopausal women?; and 2) What is the relationship between plasma levels of PLP, in the fasting state, on subsequent risk of myocardial infarction in predominantly postmenopausal women? An additional aim of this research is to determine the extent to which this relationship is independent of dietary pyridoxine intake. Further, we explored whether the association varied by fasting homocysteine concentration, renal function, body mass index (BMI), or age at blood sampling.

Methods

Study Population

The Nurses' Health Study cohort was established in 1976 when 121,700 female registered nurses, 30–55 years of age, completed and returned a mailed questionnaire in order to study the relationship between diet and lifestyle and subsequent disease. The cohort continues to be followed every 2 years by questionnaire to update exposure status and to identify cases of newly diagnosed disease. Data have been collected on many coronary artery disease risk factors, including height, weight, cigarette smoking, alcohol use, physical activity, age at menopause, post-menopausal hormone use, diagnosis of hypertension and diabetes mellitus, history of aspirin use, and parental family history of myocardial infarction. Body mass index was calculated by dividing the most recent weight prior to blood collection by the square of height reported in 1976.

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Dietary information was collected using food frequency questionnaires that had been completed by the participants in 1980, 1984, and 1986. These questionnaires assessed the average consumption of a specific amount of each food during the past year, and it allowed nine frequency responses, ranging from "never" to "six or more times per day." Nutrient intake per day was calculated by multiplying the frequency response by the nutrient content of the specified portion sizes. Total alcohol intake per day was calculated as the sum of the alcohol content contributed from beer, wine, and liquor, assuming 12.8 g of ethanol for 360 mL (12 oz) of beer, 11.0 g for 120 mL (4 oz) of wine, and 14.0 g for 45 mL (1.5 oz) of liquor. Duration, brand, and type of multivitamin supplement use were updated in the biennial questionnaires or the food frequency questionnaires, and a comprehensive database on the vitamin content of the multivitamin preparations was developed. The validity and reliability of the food frequency questionnaires used in the Nurses' Health Study have been described previously[15–17].

From 1989 through 1990, blood samples were collected from 32,826 cohort members (27% of the original cohort) who were then 43–69 years of age. Details regarding the blood collection methods have been published previously[18]. Briefly, each woman arranged to have her blood drawn and then shipped, via overnight courier with an ice pack, to our laboratory, where it was processed and separated into plasma, red blood cells, and white blood cell components. Within 24–36 hours of being drawn, 97% of the samples were received in our laboratory. Since collection, samples have been archived at −130 °C or colder in continuously monitored liquid nitrogen freezers. As of 1998, follow-up of women who provided blood samples was 99.8% complete.

We included as cases, women who provided a blood sample after fasting 10 hours or more, reported no myocardial infarction before blood collection, and who were diagnosed with myocardial infarction after blood collection but before June 1, 1998. Overall, 144 cases of myocardial infarction (21 fatal) with adequate plasma were identified. For all cases of myocardial infarction, we requested hospital records (we could not obtain records for three cases, but all cases were retained for analysis). Myocardial infarction was classified as confirmed if symptoms met the criteria of the World Health Organization (typical symptoms and either diagnostic electrocardiographic changes or elevated cardiac enzymes).

The median (10^{th} to 90^{th} percentiles) time from blood collection to diagnosis was 48 months (16, 91). Two control subjects were selected at random, matched to the case subject by age (±2 years), cigarette smoking status (current, past, and never smoker), month of blood collection, and fasting status at the time of blood collection (≥10 hours since a meal). 81% percent of control matches were exact; the most relaxed match was within ±3 years of age, and within ±6 months of blood collection. The matched sets were analyzed together. The study complies with the Declaration of Helsinki, and was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital. All subjects gave their informed consent in order to participate.

Since exposure and covariate data were collected prospectively, they are independent of case control status.

Laboratory Analyses

Plasma levels of pyridoxal phosphate (PLP) were determined by an enzymatic procedure using radioactive tyrosine and the apo-enzyme tyrosine decarboxylase.[19,20] During the assay process, we interspersed replicate plasma samples, which were labeled to preclude their identification by the laboratory, to assess laboratory precision. The intra-assay coefficients of variation for plasma pyridoxal phosphate was 8.9%.

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Creatinine was measured using a modified Jaffe method. The intra-assay coefficient of variation was 12.8%. We used a modified version of the Cockcroft-Gault formula to estimate creatinine clearance[21–23]. This formula is based on fat-free body mass and has the advantage of attenuating the overestimation of creatinine clearance (CrCl) in obese individuals with the Cockcroft-Gault equation while providing similar results in average-weight women[22]. The formula for women is $(146 - \text{age}) \times[(0.287 \times \text{weight}) + (9.74 \times \text{height}^2)]/(60 \times \text{creatinine})$, where age is measured in years, weight is measured in kilograms, height is measured in meters, and creatinine is measured in mg/dl; the units for CrCl are ml/min. This formula has been validated compared with measured CrCl[22].

Plasma homocysteine concentrations were measured with high performance liquid chromatography using fluorescence detection at the Jean Mayer United States Department of Agriculture Human Nutrition Research Center on Aging at Tufts University[19,24]. The intra-assay coefficients of variation for plasma total homocysteine, was 7.5%. Total cholesterol was measured enzymatically[25] with an intra-assay coefficient of variation of 1.7%. High-density lipoprotein cholesterol (HDL) was measured using Hitachi 911 analyzer[26], with an intra-assay coefficient of variation of 2.5%. C-reactive protein (CRP) was determined with a high sensitivity immunoturbidimetric assay on a Hitachi 911 analyzer, with an intra-assay coefficient of variation of 1.4%.

All matched case–control blood samples were handled identically and together, handled in the same batch, and assayed in the same analytical run. All assays were conducted by the laboratory personnel without knowledge of the case–control status of the samples.

Data Analysis

Medians and percentiles were calculated for continuous variables in case women and in controls as two separate groups.

Among the samples provided by controls, we conducted linear regression analyses to evaluate the relationship of fasting PLP concentration to quarters of the continuous variables and indicator functions of the categorical variables while controlling for an indicator function of blood analysis batch. The continuous variables were age, plasma total homocysteine, CrCl, plasma CRP, HDL-to-total cholesterol, physical activity, body mass index, and dietary intake of energy-adjusted total protein, total caloric intake, vitamin B_6, folate, vitamin B_{12}, vitamin B_1, vitamin B_2, vitamin C, magnesium, and alcohol. The categorical variables were menopausal status (pre-menopausal, post-menopausal, unknown menopausal status), current post-menopausal hormone use at blood collection, a personal history of being diagnosed with diabetes mellitus, a personal history of being diagnosed with hypertension, parental history of myocardial infarction prior to age 60, and cigarette smoking status (never smoker, past smoker, current smoker <15 cigarettes per day, 15–24 cigarettes per day, and greater than 24 cigarettes per day). A modified backwards stepwise procedure was used to determine which variables should remain in the model as judged by statistical significance (p value<0.05) of the F-test[27] (p value<0.05). The initial model included all of the non-biomarker variables listed above and creatinine clearance; and variables were removed individually in order of largest to smallest p-value on the F-test until the only variables remaining were statistical significant predictors. All three indicators for each continuous variable were considered one variable. The previously removed variables were then individually returned to determine statistical significance in the modified model, at which point, all previously removed variables would be returned and backwards elimination repeated. This procedure was repeated iteratively until no added or removed variable resulted in statistically significant changes to the model, the “final model”. The relationship of biomarkers to plasma PLP were determined in the multivariable model by individually adding the respective variable to this “final model”.

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Conditional logistic regression was used to estimate odds ratios between quarters of plasma PLP concentration and risk of MI. These odds ratios were taken as direct estimates[28] of rate ratios (RRs) and 95% confidence intervals (CIs)[29]. We additionally controlled for potential confounders that were not part of the matching scheme. To control more appropriately for confounding and other co-variation by continuous variables, natural cubic splines (28, 29) with four degrees of freedom (three when four was not feasible) were used to smooth the relationships with the log-odds of myocardial infarction. Physical activity in metabolic equivalents (MET) – hours per week in 1988 was entered into the regression models as indicator functions of the quarters since natural cubic splines did not determine a good fit for this variable. Indicator functions of the categorical covariates were used. We estimated the following models: i) a model that controls for matching factors only; ii) a model that controls for conventional non-biomarker risk factors for MI (considered the final model); iii) a model that additionally controls for creatinine clearance, the ratio of plasma total to HDL-cholesterol, and plasma high-sensitivity C-reactive protein, iv) a model that additionally adjusts for plasma concentrations of total homocysteine; and v) A model that controls for predictors (including dietary pyridoxine and folate) of plasma PLP. There were very few missing values in the covariates physical activity, CRP, creatinine, and HDL to total cholesterol ratio (all less than 4%), and thus medians were used for imputation. Tests for interaction were done by including product terms between indicator functions of halves of the respective variable and indicator functions of quarters of PLP.

We conducted tests for trend by modeling the hormone level as a linear continuous covariate and calculating a Wald statistic[29]. All P values were based on two-sided tests. The software used for statistical analysis were SAS release 9.1[30] and S-plus version 8[31].

Results

Table 1 shows the distribution of risk factors for myocardial infarction at the time of blood sampling among case women and matched controls. As expected, average body mass index, total cholesterol, CRP, histories of diabetes mellitus, hypertension, and having had a parent with early myocardial infarction were all higher in case women. Similarly, the levels of physical activity, alcohol consumption and plasma HDL cholesterol were lower in those who later developed myocardial infarction, compared with control women.

Plasma concentrations of PLP were inversely correlated with estimated creatinine clearance and body mass index, and directly correlated with dietary intake of vitamin B_6 (table 2,3). Our model that included dietary B_6, folate, body mass index, estimated creatinine clearance explained 28.6% of the variation in plasma PLP. After adjustment for estimated creatinine clearance, body mass index, dietary intake of vitamin B_6, and folate, plasma PLP was also inversely correlated with plasma homocysteine (table 3). Plasma concentration of PLP was not predicted by age, menopausal status, use of postmenopausal hormones, protein intake, total caloric intake, physical activity, alcohol consumption, plasma total to HDL cholesterol ratio, or by history of hypertension, diabetes mellitus, cigarette smoking, or by parental history of MI before age 60 (table 3).

Fasting concentrations of PLP were significantly inversely associated with subsequent risk of MI [RR (95%CI) for top vs. bottom quarter 0.22 (0.09,0.55), p value for linear trend 0.047] (Table 4, Figure 1). The relationship between log rate of MI and plasma PLP is more linear than table 4 suggests. Compared to plasma PLP <20 pmol/mL, concentrations of 20–39,40–59,60–79,80–99,100–119,≥120 pmol/mL were associated with MI with estimated rate ratios of 0.4, 0.5, 0.3, 0.3, 0.1, and 0.1 respectively. The shape and magnitude of the relationships changed little with addition of plasma homocysteine or dietary determinants of plasma PLP (dietary pyridoxine and folate).

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The relationship between pyridoxal phosphate and risk of MI appeared to vary by age (χ^2_{3df}=11.2, p=0.011). The effect of plasma PLP was stronger among women who were aged less than 60 at blood sampling [RR (95%CI) for top vs. bottom quarter 0.03 (0.002,0.48)], compared to older women [RR (95%CI) for top vs. bottom quarter 0.43 (0.15,1.25)]. There was no statistically significant effect modification by halves of body mass index, estimated creatinine clearance, or by plasma total homocysteine.

Discussion

The questions addressed by the present study were whether fasting concentrations of plasma pyridoxal phosphate predict later risk of MI among predominantly post-menopausal women, and to determine the major epidemiological predictors of the plasma concentration. The main finding of the study is that a low plasma concentration of pyridoxal phosphate is associated with increased rate of MI among predominantly post-menopausal women. This was demonstrated by the statistically and clinically significant lower risk of MI associated with higher PLP concentration (table 4, figure 1). Although there was a steep decline in MI risk for PLP concentrations above 20 pmol/mL relative to lower, risk continued to decline with higher levels. The effect was more pronounced in younger relative to older women. We also found that estimated creatinine clearance, dietary vitamin B_6 intake, dietary folate intake, and body mass index were statistically significant predictors of fasting plasma levels of PLP. Fasting concentration of PLP was also negatively correlated with total homocysteine.

The finding that plasma concentrations of PLP is inversely related to risk of MI is consistent with the knowledge of the widespread role of vitamin B_6 in protein metabolism, including homocysteine metabolism, and its likely effects on mineral transport across cell membranes[10]. This is also in agreement with the results of other epidemiological studies that have also shown that dietary vitamin B_6 and plasma PLP are inversely related to risk of MI[3] and to the risk of coronary artery disease[2,12]. This is also accordantwith the role of pyridoxal phosphate as a cofactor in the conversion of homocysteine to cysteine in the transulfuration pathway. Although it was not statistically significantly related to plasma levels of CRP in this study, PLP was inversely correlated with CRP in one previous study[32].

The finding that the plasma concentration of PLP is positively correlated with dietary intake of vitamin B_6 is consistent with a number of studies that demonstrated that dietary supplementation of vitamin B_6 results in an increase in plasma concentration[33–36]. The positive association between dietary folate and plasma PLP concentration may reflect a vitamin B_6 - sparing effect of increased folate, by its reduction of the flux of homocysteine via the transulfuration pathway. The negative correlation between estimated creatinine clearance and plasma levels of PLP is consistent with the fact that vitamin B_6 is almost completely excreted via the kidneys[37]. It is unclear whether the negative correlation between BMI and plasma PLP is due to a causal effect of BMI on vitamin B_6. Although animal studies suggest that high protein diets increase requirements for vitamin B_6, we did not find a relationship between protein intake and plasma PLP. Chronic alcohol use results in increased alkaline phosphatase[38], and thus may affect concentration of plasma PLP[39], but we did not find significant relationships in this dataset. Older individuals have been found to have lower PLP concentrations relative to the young in another study[40], but this was not the case in this study. This may be related to the fact all the women in this study were aged above 40 and thus there was not enough variation in age. The fact that our linear regression model explained only 28.6% of the variation in plasma PLP reinforces the fact that there are important determinants not accounted for by the measured variables. This also helps to explain our finding that the relationship between plasma PLP and MI risk was little affected by including the measured determinants of PLP concentration such as dietary pyridoxine in the regression model (table 4).

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To our knowledge, this is the first prospective study that has looked specifically at the relationship of fasting levels of PLP with MI in predominantly post-menopausal women. Major strengths of this study are its prospective design, and its careful control of confounding factors. In addition, we were able to reduce measurement error in PLP as well as other plasma covariates by focusing on those women who had provided blood after fasting for 10 hours or more. However, given the observational nature of the study, we cannot be sure that all unknown confounders have been adequately controlled. Further, inference from our linear regression models are limited in that the estimation was performed in a non-random selection of women, since the controls were matched to the MI cases.

In summary, our investigation revealed that lower fasting concentration of pyridoxal phosphate is significantly associated with increased risk of myocardial infarction in predominantly post-menopausal women, a relationship that may be causal. We also confirmed that plasma PLP is positively correlated with dietary vitamin B_6, and found that it is inversely correlated with a measure of renal function and with body mass index. Future studies are needed to better understand both dietary and non-dietary determinants of plasma and tissue vitamin B_6 status, and how these can be optimized to prevent disease.

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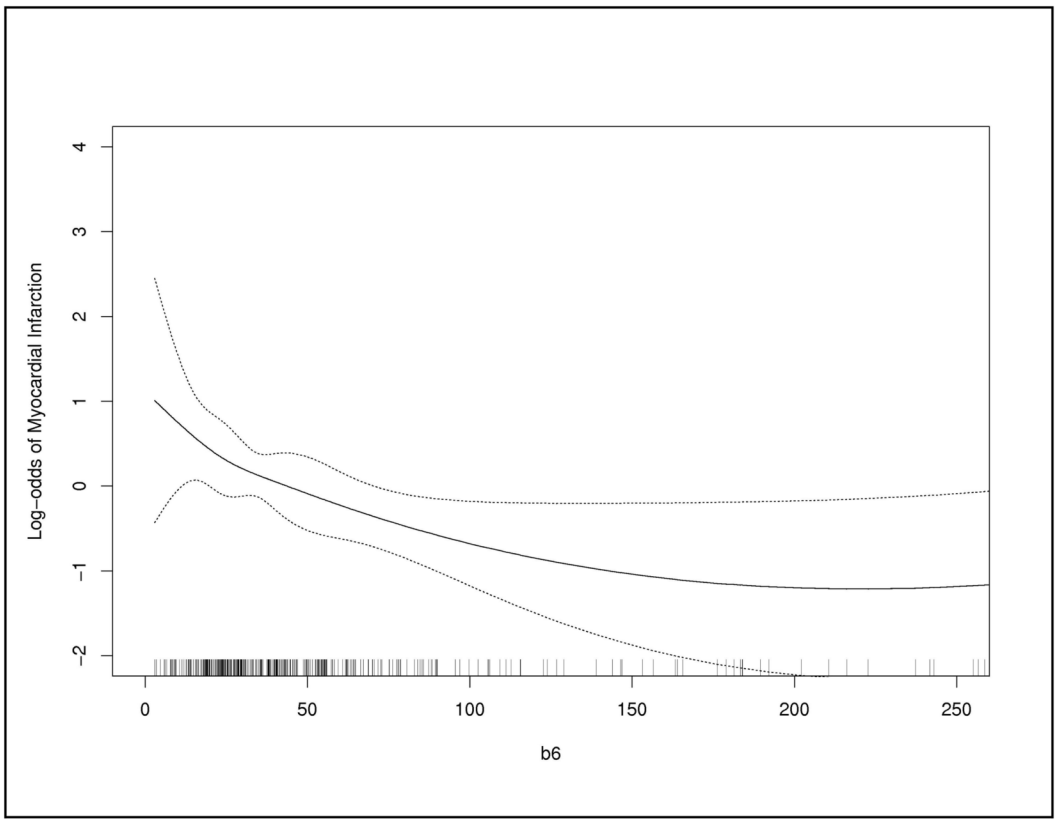


Figure 1. Smoothed relationship between fasting plasma levels of Vitamin B_6 as Pyridoxal 5′ Phosphate and the log odds of myocardial infarction* in predominantly post-menopausal women in the Nurses' Health Study

* Conditional logistic regression model controlling for matching factors (age, cigarette smoking status, and month of blood collection) in addition to conventional (non-biomarker) cardiovascular risk factors [history of diabetes mellitus, history of hypertension, parental history of MI before age 60, menopausal status (postmenopausal vs. pre-menopausal vs. uncertain), current post-menopausal hormone use (use of female hormones in the three months prior to blood draw), physical activity in MET-hours, body mass index and alcohol intake in grams per day].

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Table 1

Distribution of covariates in MI case and matched control participants*

Covariate	Cases	Controls
	Median (10th to 90th percentiles)	Median (10th to 90th percentiles)
Plasma pyridoxal phosphate (pmol/ml)	30.9 (14.3,83.0)	40.5 (19.2, 153.2)
Plasma homocysteine (nmol/ml)	11.0 (7.2,16.0)	9.9 (6.9, 14.8)
Age at blood draw (years)	62.9 (52.6, 68.1)	63.2 (52.0, 67.9)
Current smokers φ	45 (31.3)	78 (30.2)
Body Mass Index (kg/m^2)	25.9 (20.5, 35.4)	24.3 (20.4, 31.3)
Waist to Hip Ratio	0.81 (0.73, 0.89)	0.78 (0.71, 0.88)
Average physical activity (MET-hours per week)	7.8 (0.2, 37.2)	9.0 (0.9, 34.2)
Mean alcohol consumption (grams/day)	0.9 (0, 15.0)	2.7 (0, 20.9)
Parental history of Myocardial Infarction φ	56 (38.9)	58 (22.5)
History of Hypertension φ	86 (59.7)	73 (28.3)
History of Diabetes Mellitus φ	31 (21.5)	15 (5.8)
History of Hypercholesterolemia	17 (11.8)	27 (10.5)
Use of Aspirin in 1988		
None	44 (30.6)	81 (31.4)
1–14 days per month	55 (38.2)	119 (46.1)
>14 days per month	40 (27.6)	51 (19.8)
Post-menopausal φ	128 (89.0)	224 (86.8)
Use of female hormones in the three months prior to blood collection φ	45 (31.0)	94 (36.0)
Use of estrogen in the three months prior to blood collection φ	42 (29.2)	88 (34.1)
Plasma total cholesterol (mg/dL)	240 (180, 292)	229 (181, 279)
Plasma HDL cholesterol (mg/dL)	50.7 (35.5, 71.1)	57.4 (41.7, 85.3)
Ratio of plasma total to HDL cholesterol	4.45 (3.30, 6.93)	3.96 (2.62, 5.66)
Plasma C reactive protein (mg/L)	3.6 (0.6, 15.6)	2.5 (0.5, 9.6)
Plasma Creatinine (mg/dl)	0.73 (0.57,1.03)	0.72 (0.58, 0.88)
Estimated Creatinine Clearance (ml/min)	88.4 (59.8, 121.4)	87.9 (67.4,116.3)
Multi-vitamin tablets φ	56 (40.9)	110 (44.9)
Total Dietary B_6 (mg/day)	2.33 (1.37, 6.87)	2.38 (1.34, 15.1)
Non-supplement B_6 (mg/day)	1.70 (1.32, 2.23)	1.72 (1.28, 2.22)
Non-supplement Folate (mcg/day)	253 (184.3, 363)	271 (195, 375))
Non-supplement B_{12} (mcg/day)	6.23 (3.80,12.1)	6.21 (3.7, 11.8)
Non-supplement Vitamin C (mg/day)	136.0 (72.7, 217.0)	133.5 (84.5, 205.8)
Non-supplement Thiamine (B1) (mg/day)	1.09 (0.87, 1.41)	1.08 (0.87, 1.36)
Non-supplement Riboflavin (B2) (mg/day)	1.65 (1.26, 2.21)	1.61 (1.24, 2.09)
Dietary Magnesium (mg/day)	287 (223, 392)	305 (229, 380)
Dietary Protein (grams/day)	75.0 (61.7, 92.2)	73.7 (60.9, 87.2)

Covariate	Cases	Controls
	Median (10^{th} to 90^{th} percentiles)	**Median (10^{th} to 90^{th} percentiles)**
Total Caloric Intake (kcal/day)	1534 (1004, 2304)	1491 (988, 2182)

$^{\varphi}$Number (percentage) shown.

*There were 144 cases and 258 controls. Median age (range; 10^{th} and 90^{th} percentiles) of diagnosis was 67.0(55.4,73.7). Median time from blood sample to diagnosis was 4.1 years (1.25, 7.6). Waist-hip ratio was available for 101 cases and 178 controls. Information was available for the other covariates in over 95% of study participants.

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Table 2

Spearman's correlation coefficients (P value) between continuous covariates and batch adjusted residuals of $\log_e$(Pyridoxal Phosphate) among control study participants who provided blood after fasting for 10 hours or more.

Covariate	Spearman's correlation coefficient (P value)
Age (years)	0.08 (0.20)
Plasma Total Homocysteine (nmol/ml)	−0.28 (<0.001)
Estimated Creatinine Clearance (ml/min)	−0.21 (<0.001)
BMI (kg/m^2)	−0.11 (0.08)
Average physical activity (MET-hours per week)	0.20 (0.001)
Mean alcohol consumption (grams/day)	−0.01 (0.82)
C-reactive Protein (mg/L)	−0.13 (0.04)
Plasma total:HDL cholesterol ratio	−0.11 (0.07)
Total Dietary Vitamin B_6 (mg/day)	0.45 (<0.001)
Supplemental Vitamin B_6 (mg/day)	0.25 (<0.001)
Non-supplemental Vitamin B_{12} (mcg/day)	0.25 (<0.001)
Non-supplemental Folate (mcg/day)	0.41 (<0.001)
Non-supplemental Vitamin C (mg/day)	0.35 (<0.001)
Non-supplemental Thiamine (B1) (mg/day)	0.22 (<0.001)
Non-supplemental Riboflavin (B2) (mg/day)	0.10 (0.12)
Dietary Magnesium (mg/day)	0.25 (<0.001)
Total Protein Intake (grams/day)	0.09 (0.13)
Total Caloric Intake (kcal/day)	−0.08 (0.21)

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Table 3

Predictors of Log_e(Plasma Pyridoxal Phosphate) among control participants

	Simpler Model[§]			Multivariable Model[†]		
Covariate and unit of measure	**Regression Coefficient (Standard Error)**	**P value**	**P value for Linear Trend**	**Regression Coefficient (Standard Error)**	**P value**	**P value for Linear Trend**
Age in years						
<58	0	0.77	0.18	0	0.37	0.30
58–62	0.10 (0.151)			−0.10 (0.136)		
63–65	0.05 (0.147)			−0.19 (0.139)		
≥66	0.14 (0.144)			−0.24 (0.144)		
Plasma Homocysteine (nmol/ml)						
<8.1	0	<0.001	<0.001	0	0.019	0.001
8-1–9.8	−0.11 (0.141)			−0.12 (0.130)		
9.9–12.1	−0.24 (0.146)			−0.22 (0.133)		
≥12.2	−0.64 (0.143)			−0.42 (0.137)		
Estimated Creatinine Clearance (ml/min)						
<77	0	0.023	<0.001	0	0.09	0.005
77–87	−0.01 (0.145)			0.07 (0.132)		
88–102	−0.20 (0.150)			−0.17 (0.143)		
≥103	−0.39 (0.147)			−0.21 (0.148)		
Body Mass Index (kg/m^2)						
<22.3	0	0.030	0.054	0	0.007	0.010
22.3–24.2	−0.25 (0.145)			−0.18 (0.129)		
24.3–27.8	−0.12 (0.144)			−0.10 (0.128)		
≥27.9	−0.425 (0.146)			−0.45 (0.133)		
Physical Activity (MET-hrs/week)						
<3.6	0	0.007	0.13	0	0.80	0.89
3.6–8	0.25 (0.147)			0.06 (0.136)		
9–19	0.48 (0.145)			0.12 (0.139)		
≥20	0.38 (0.145)			0.02 (0.141)		
Parental History of MI						

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Covariate and unit of measure	Simpler Model§			Multivariable Model†		
	Regression Coefficient (Standard Error)	P value	P value for Linear Trend	Regression Coefficient (Standard Error)	P value	P value for Linear Trend
Not present	0	0.18		0	0.41	
Present	−0.17 (0.125)			−0.09 (0.111)		
History of Diabetes Mellitus						
Not present	0	0.94		0	0.90	
Present	0.02 (0.223)			−0.02 (0.201)		
History of Hypertension						
Not present	0	0.65		0	0.46	
Present	0.05 (0.116)			0.08 (0.103)		
History of Hypercholesterolemia						
Not present	0	0.89		0	0.77	
Present	−0.02 (0.171)			−0.05 (0.159)		
Use of Aspirin in 1988						
None	0	0.96		0	0.91	
1–14 days per month	0.02 (0.119)			0.03 (0.103)		
>14 days per month	0.04 (0.150)			−0.03 (0.132)		
Cigarette Smoking						
Never-smoker	0	0.37		0	0.61	
Ex-smoker	0.03 (0.124)			0.01 (0.110)		
Current smoking (<15 cigs/day)	−0.27 (0.203)			−0.13 (0.183)		
Current smoking (15–24 cigs/day)	−0.12 (0.174)			−0.06 (0.159)		
Current smoking (>24 cigs/day)	−0.24 (0.186)			−0.23 (0.170)		
Alcohol Consumption (grams/day)						
<0.4	0	0.69	0.72	0	0.91	0.46
0.4–2.6	0.13 (0.150)			0.00 (0.134)		
2.7–10.8	0.01 (0.149)			−0.05 (0.134)		
≥10.9	−0.04 (0.152)			−0.08 (0.136)		
Menopausal Status						
Pre-menopausal	0	0.67		0	0.31	
Uncertain	−0.18 (0.323)			−0.39 (0.286)		

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Covariate and unit of measure	Simpler Model[§]			Multivariable Model[†]		
	Regression Coefficient (Standard Error)	P value	P value for Linear Trend	Regression Coefficient (Standard Error)	P value	P value for Linear Trend
Post-menopausal	0.06 (0.171)			−0.20 (0.159)		
Use of current estrogen						
Not present	0	0.70		0	0.35	
Present	−0.04 (0.110)			−0.09 (0.097)		
C-Reactive Protein (mg/L)						
<0.11	0	0.23	0.085	0	0.16	0.14
0.11–0.24	0.11 (0.150)			0.06 (0.132)		
0.25–0.57	−0.10 (0.149)			−0.19 (0.136)		
≥0.58	−0.19 (0.154)			−0.19 (0.142)		
Plasma total: HDL cholesterol ratio						
<3	0	0.32	0.13	0	0.79	0.95
3–3.9	−0.13 (0.148)			−0.11 (0.131)		
4–4.8	−0.07 (0.159)			0.00 (0.144)		
≥4.9	−0.28 (0.158)			−0.07 (0.147)		
Total dietary Vitamin B_6 (mg/day)						
<1.65	0	<0.001	<0.001	0	<0.001	<0.001
1.65–2.37	0.29 (0.134)			0.23 (0.136)		
2.38–3.92	0.50 (0.131)			0.40 (0.137)		
≥3.93	1.05 (0.135)			0.95 (0.142)		
Non-supplement Vitamin B_{12} (mcg/day)						
<4.8	0	0.07	0.82	0	0.10	0.48
4.8–6.1	0.36 (0.149)			0.22 (0.133)		
6.2–8.6	0.03 (0.145)			−0.07 (0.130)		
≥8.7	0.14 (0.148)			−0.07 (0.133)		
Non-supplement Folate (mcg/day)						
<229	0	<0.001	<0.001	0	0.02	0.15
229–269	0.47 (0.146)			0.34 (0.138)		
270–319	0.31 (0.141)			0.03 (0.135)		
≥320	0.60 (0.145)			0.26 (0.140)		

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Covariate and unit of measure	Simpler Model[§]			Multivariable Model[†]		
	Regression Coefficient (Standard Error)	P value	P value for Linear Trend	Regression Coefficient (Standard Error)	P value	P value for Linear Trend
Non-supplement Vitamin B_1 (mg/day)						
<0.98	0	0.054	0.019	0	0.46	0.66
0.98–1.07	0.13 (0.149)			0.00 (0.138)		
1.08–1.22	0.25 (0.145)			0.19 (0.153)		
≥1.23	0.40 (0.149)			0.19 (0.189)		
Non-supplement Vitamin B_2 (mg/day)						
<1.42	0	0.17	0.16	0	0.41	0.77
1.42–1.60	0.30 (0.149)			0.18 (0.134)		
1.61–1.87	0.22 (0.145)			−0.03 (0.140)		
≥1.88	0.27 (0.148)			0.02 (0.146)		
Non-supplement Vitamin C (mg/day)						
<105	0	0.22	0.07	0	0.98	0.97
105–133	0.25 (0.150)			0.00 (0.147)		
134–166	0.27 (0.146)			0.02 (0.163)		
≥167	0.26 (0.150)			−0.04 (0.178)		
Dietary Magnesium Intake (mg/day)						
<262	0	<0.001	<0.001	0	0.39	0.05
262–305	0.21 (0.140)			0.18 (0.134)		
306–338	0.31 (0.147)			0.17 (0.148)		
≥339	0.64 (0.142)			0.26 (0.159)		
Total Protein Intake (grams/day)						
<66.7	0	0.61	0.16	0	0.76	0.29
66.7–73.6	0.07 (0.150)			0.09 (0.133)		
73.7–80.5	0.12 (0.146)			0.12 (0.133)		
≥80.6	0.20 (0.151)			0.14 (0.141)		
Total Caloric Intake (kcal/day)						
<1214	0	0.25	0.61	0	0.59	0.82
1214–1490	0.01 (0.143)			0.09 (0.127)		
1491–1842	0.18 (0.143)			0.16 (0.126)		

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	Simpler Model[§]			Multivariable Model[†]		
Covariate and unit of measure	**Regression Coefficient (Standard Error)**	**P value**	**P value for Linear Trend**	**Regression Coefficient (Standard Error)**	**P value**	**P value for Linear Trend**
≥1843	0.13 (0.143)			0.01 (0.130)		

[§] Linear Regression with Plasma Pyridoxal Phosphate as outcome, controlling for batch

[†] Linear Regression with Plasma Pyridoxal Phosphate as outcome, controlling for batch, estimated creatinine clearance, mean dietary vitamin B_6, mean dietary non-supplement folate intake, and body mass index

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Table 4

Odds ratio (OR) of myocardial infarction and 95% confidence interval (CI) by quarter of fasting plasma Pyridoxal Phosphate, in the Nurses' Health Study.

	Quarters				P value for linear trend
	1	2	3	4	
Pyridoxal Phosphate, (pmol/mL)	<27.9	27.9–40.4	40.5–69.9	≥70	
No. of cases/No. of controls	63/65	23/64	38/65	21/66	
Matching factor adjusted OR (95% CI)	1.0 (referent)	0.34 (0.18,0.64)	0.50 (0.28,0.90)	0.24 (0.12,0.48)	0.031
Adjusted OR† (95%CI)	1.0 (referent)	0.34 (0.16,0.75)	0.66 (0.31,1.41)	0.22 (0.09,0.55)	0.047
Adjusted OR# (95%CI)	1.0 (referent)	0.31 (0.13,0.77)	0.53 (0.22,1.30)	0.18 (0.06,0.51)	0.047
Adjusted OR‡ (95%CI)	1.0 (referent)	0.32 (0.12,0.82)	0.57 (0.23,1.43)	0.19 (0.06,0.56)	0.081
Adjusted OR§ (95%CI)	1.0 (referent)	0.32 (0.14,0.76)	0.53 (0.22,1.28)	0.21 (0.07,0.60)	0.046

†Conditional logistic regression model controlling for matching factors (age, cigarette smoking status, and month of blood collection) in addition to conventional (non-biomarker) cardiovascular risk factors [history of diabetes mellitus, history of hypertension, parental history of MI before age 60, menopausal status (postmenopausal vs. pre-menopausal vs. uncertain), current post-menopausal hormone use (use of female hormones in the three months prior to blood draw), physical activity in MET-hours, body mass index and alcohol intake in grams per day].

#Conditional logistic regression model controlling for † in addition to creatinine clearance, plasma CRP concentration and plasma total to HDL cholesterol ratio

‡Conditional logistic regression model controlling for # in addition to plasma concentrations of homocysteine

§Conditional logistic regression model controlling for † in addition to predictors of plasma pyridoxal phosphate (dietary pyridoxine, folate, and creatinine clearance)