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Long- and Short-term Effects of Tobacco Smoking on Circulating Concentrations of B Vitamins

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BACKGROUND: Smoking is associated with decreased concentrations of several antioxidant vitamins. We sought to determine the relation between circulating concentrations of selected B vitamins and smoking status, with particular attention to longitudinal associations.

METHODS: We used baseline data from 2 B-vitamin intervention trials that included 6837 patients with ischemic heart disease. Smoking habits were ascertained by interview. Vitamins and metabolites, including the nicotine metabolite cotinine, were measured in plasma and serum by microbiological assays or gas/liquid chromatography–tandem mass spectrometry.

RESULTS: The highest circulating concentrations of folate and pyridoxal 5′phosphate (PLP) and lowest concentrations of total plasma homocysteine, a functional marker of folate status, were observed for self-reported never smokers, followed by self-reported ex-smokers and current smokers ($P_{trend} < 0.001$). Cobalamin and its functional marker methylmalonic acid were not associated with smoking status. Based on their low cotinine concentrations, we were able to identify a group of smokers that had abstained from smoking for 3 days or more. Compared with smokers with high plasma cotinine, smokers with low cotinine had significantly higher circulating concentrations of folate, PLP, and riboflavin (all $P < 0.005$), and this trend continued for ex-smokers, with increasing time since smoking cessation.

CONCLUSIONS: Smoking lowered circulating concentrations of folate, PLP, and riboflavin, but concentrations increased significantly after a few days of smoking cessation. We propose that short-term effects may be related to acute smoking-induced oxidative stress, whereas the longer-lasting effects among ex-smokers may reflect changes in diet and/or restoration of vitamin concentrations in tissue during the first few months to years after smoking cessation.

Tobacco smoking is associated with well-known health risks to respiratory organs, but it also affects organs not directly exposed to the toxic substances contained in smoke, indicating that smoking has widespread effects *(1)*. Among systemic effects, there is evidence of increased oxidative stress, inflammation, and lower circulating concentrations of antioxidant vitamins *(1)*.

In a number of studies smokers reported different intake of food items compared to ex- and never smokers *(2–4)*. Specifically, smokers had lower intake of several micronutrients, including carotenes, vitamins C and B6, and folate *(5, 6)*. After data were controlled for diet, however, smokers were still found to have lower circulating concentrations of several vitamins *(6, 7)*. Further adjustments for socioeconomic variables diminished only a few associations, suggesting direct effects of smoking on plasma vitamin concentrations *(6, 7)*. Accordingly, smokers required higher intake to achieve adequate circulating vitamin C and B6 *(8)*. Direct effects of smoking may include oxidative stress that leads to increased turnover or breakdown of vitamins *(1)*.

Although both dietary differences and direct effects of smoking have been found, their relative contributions to the low concentration of circulating vitamins are still poorly characterized. In particular, the mechanisms and time dependence of these effects remain to be established.

In this study we combined data from 2 clinical trials aimed at secondary prevention of cardiovascular events in patients with established ischemic heart disease. Plasma cotinine, the predominant metabolite of nicotine, was used to evaluate self-reported smoking status and smok-

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ing behavior during hospitalization and after the first 1–2 months of follow-up. This information, together with data on duration of smoking abstinence among ex-smokers, enabled us to investigate longitudinal effects of smoking on circulating B vitamins, and also on plasma total homocysteine (tHcy)[9] and methylmalonic acid (MMA) in their capacity as functional indicators of folate and B12 status, respectively.

Patients and Methods

PATIENTS

The patients in this study participated in 1 of 2 randomized, double-blinded, placebo-controlled clinical trials conducted in Norway from 1998 to 2006, the Norwegian Vitamin Trial (NORVIT) or the Western Norway B-Vitamin Intervention Trial (WENBIT). Details and results of the 2 trials are described elsewhere *(9, 10)*. In brief, the primary objective of both trials was to assess whether homocysteine-lowering treatment with folic acid and vitamin B_{12} reduced the risk of cardiovascular events and mortality in patients after they had suffered an acute myocardial infarction (AMI) (NORVIT) or undergone coronary angiography for suspected coronary artery disease (CAD) or aortic valve stenosis (WENBIT). NORVIT participants (n = 3749) were recruited from 35 hospitals throughout the country, and WENBIT participants (n = 3090) from the 2 university hospitals in Western Norway. Only patients with information on smoking habits and a valid plasma cotinine measurement at baseline (n = 6775, 99.1%) were included for the current analysis. The study was approved by the Regional Committee for Medical and Health Research Ethics, the Data Inspectorate, and the Norwegian Directorate of Health, and is registered with ClinicalTrials.gov, NCT00671346.

DATA COLLECTION AND LABORATORY ANALYSES

In both trials clinical information and blood samples were obtained at baseline, at a follow-up visit 1–2 months after randomization, and at a final study visit. At baseline, patients were interviewed by nurses or physicians using trial-specific questionnaires. Smoking status was assessed by asking participants if they were current or former smokers, and for former smokers, how long ago they had quit smoking. Vitamin supplementation was assessed by asking patients about regular use of over-the-counter vitamin supplements. Analyses of serum/plasma B vitamins and metabolites were performed at the laboratory of Bevital AS by use of published methods. Serum folate and cobalamin were analyzed by microbiological assays *(11, 12)*, and the remaining vitamins and metabolites, pyridoxal 5′phosphate (PLP), riboflavin, tHcy, MMA, and cotinine by liquid chromatography–tandem mass spectrometry or GC-MS based assays *(13)*.

For the vitamins and metabolites, only baseline data were used in this report. However, for the evaluation of self-reported smoking status, we also used cotinine measurements from the first follow-up visit 1–2 months after baseline.

STATISTICAL ANALYSES

We used analysis of covariance (ANCOVA) to evaluate differences in circulating vitamin and metabolite concentrations between patients in different smoking categories. Because of skewed (right-tailed) distributions, all outcome variables were log-transformed before analysis. We performed variance component analyses to establish whether the inclusion hospital and/or trial (NORVIT/WENBIT) should be treated as random effects in a mixed model regression analysis. The variance contribution from the inclusion-hospital variable was 1.6% to 2.7% for all outcomes, whereas that from trial was 6% to 11%, leaving the remaining variance (about 90%) between study patients. We therefore used a fixed-effects model with adjustment for trial only. Interaction between explanatory variables was evaluated by inclusion of a product term in the model. All analyses were done by use of the statistical software packages SPSS version 16.0 (SPSS) and R version 2.8.1 for Macintosh *(14)*. For the variance component analysis and adjusted geometrical mean values for smoking categories, we used the R-packages lme (linear mixed effects) and effects, respectively *(14)*. A 2-sided P value of <0.05 was considered statistically significant.

Results

CHARACTERISTICS OF THE STUDY POPULATION

The overall median age was 62.5 years (5th to 95th percentile 44.2–79.4 years), and 76.4% of participants were male. Among NORVIT participants, self-reported current smokers were the largest group followed by ex-smokers and never smokers (46.4%, 32.6%, and 21.0%, respectively). Current smokers were markedly younger than ex-smokers and never-smokers (median age 57.0, 67.8, and 70.2 years, $P < 0.0001$). Among WENBIT participants, by contrast, ex-smokers were most frequent (51.4%), and age differences between smoking categories were modest. Table 1 shows selected characteristics by trial and self-reported smoking categories.

[9] Nonstandard abbreviations: tHcy, total homocysteine; MMA, methylmalonic acid; NORVIT, Norwegian Vitamin Trial; WENBIT, Western Norway B-Vitamin Intervention Trial; AMI, acute myocardial infarction; CAD, coronary artery disease; PLP, pyridoxal 5′phosphate; ANCOVA, analysis of covariance.

Table 1. Characteristics of study participants by trial and smoking categories.[a]

	Trial[b]	Total	Never smokers	Ex-smokers	Smokers with low cotinine[c]	Smokers with high cotinine[c]
Trial	N	3695 (100)	1204 (100)	777 (100)	861 (100)	853 (100)
	W	3080 (100)	734 (100)	1587 (100)	98 (100)	661 (100)
Women[e]	N[d]	969 (26.2)	433 (36.0)	114 (14.7)	266 (30.9)	156 (18.3)
	W[d]	631 (20.5)	266 (36.3)	211 (13.3)	19 (19.4)	135 (20.4)
Age, years[e]	N[d]	63.3 (43.6, 80.6)	70.2 (48.1, 81.8)	67.8 (48.2, 81.3)	57.0 (40.3, 77.0)	57.1 (41.6, 76.2)
	W[d]	61.8 (44.7, 77.3)	61.3 (44.9, 77.0)	62.2 (45.3, 76.9)	61.0 (44.2, 77.1)	62.5 (44.3, 78.0)
BMI, kg/m^2[e]	N[d]	26.0 (20.8, 33.0)	26.6 (21.4, 33.0)	26.3 (21.6, 33.2)	25.6 (20.2, 33.1)	25.5 (20.4, 32.7)
	W[d]	26.5 (21.5, 33.5)	26.3 (21.5, 33.5)	26.5 (21.3, 34.1)	26.5 (21.3, 34.4)	26.6 (21.8, 33.0)
Serum total cholesterol , mmol/L[e]	N	5.7 (3.9, 7.9)	5.8 (3.9, 7.9)	5.7 (3.9, 7.7)	5.8 (4.0, 7.9)	5.7 (4.0, 8.1)
	W[d]	4.9 (3.5, 7.1)	5.0 (3.5, 7.2)	4.9 (3.4, 7.1)	4.9 (3.6, 7.2)	4.9 (3.5, 7.2)
Serum creatinine, mmol/L	N[d]	87 (62, 131)	90 (63, 138)	91 (68, 141)	83 (59, 116)	85 (61, 122)
	W[d]	90 (70, 117)	90 (69, 118)	89 (69, 118)	90 (71, 115)	90 (70, 115)
Daily or regular use of any vitamin supplement[e]	N[d]	1053 (28.5)	372 (30.9)	251 (32.3)	241 (28.0)	189 (22.2)
	W[d]	506 (16.4)	146 (19.9)	250 (15.8)	6 (6.1)	104 (15.7)
Plasma cotinine, nmol/L	N[d]	4.8 (0.0, 733)	0.0 (0.0, 56.6)	0.24 (0.0, 170)	20.9 (0.0, 70.4)	263 (91.9, 1241)
	W[d]	1.7 (0.0, 1693)	0.2 (0.0, 6.9)	0.8 (0.0, 874)	6.0 (0.0, 54.8)	1080 (210, 2119)
Cardiovascular disease history						
Myocardial infarction[e]	N[d]	614 (16.8)	218 (18.3)	191 (25.0)	82 (9.6)	123 (14.7)
	W[d]	1273 (41.3)	241 (32.8)	737 (46.4)	23 (23.5)	272 (41.1)
Myocardial revascularization[e]	N[d]	305 (8.3)	109 (9.1)	91 (11.7)	40 (4.6)	65 (7.6)
	W[d]	918 (29.8)	185 (25.2)	554 (34.9)	12 (12.2)	167 (25.3)
Stroke, TIA, or carotid artery stenosis[e]	N[d]	156 (4.3)	69 (5.8)	44 (5.7)	22 (2.6)	21 (2.5)
	W[d]	192 (6.2)	49 (6.7)	111 (7.0)	3 (3.1)	29 (4.4)
Treatment for hypertension[e]	N[d]	1059 (29.0)	422 (35.4)	275 (36.0)	184 (21.5)	178 (21.0)
	W[d]	1414 (45.9)	362 (49.3)	749 (47.2)	39 (39.8)	264 (39.9)
Lipid-lowering treatment[e]	N[d]	2785 (81.3)	842 (76.3)	610 (83.6)	689 (86.3)	644 (81.2)
	W	2723 (88.4)	624 (85.0)	1412 (89.0)	91 (92.9)	596 (90.2)
Diabetes mellitus[e]	N[d]	364 (9.9)	159 (13.3)	84 (10.9)	59 (6.9)	62 (7.4)
	W	356 (11.6)	85 (11.6)	195 (12.3)	8 (8.2)	68 (10.3)
Diagnosis at inclusion						
Acute myocardial infarction	N[d]	3695 (100)	1204 (100)	777 (100)	861 (100)	853 (100)
	W[d]	321 (10.4)	55 (7.5)	131 (8.3)	49 (50.0)	86 (13.0)
Unstable angina pectoris	W[d]	137 (4.4)	36 (4.9)	71 (4.5)	17 (17.3)	13 (2.0)
Stable angina pectoris	W[d]	2578 (83.7)	622 (84.7)	1369 (86.3)	32 (32.7)	555 (84.0)
Aortic valve stenosis	W	44 (1.4)	21 (2.9)	16 (1.0)	0 (0.0)	7 (1.1)

[a] Numbers are n (%) or median (5th, 95th percentiles). Percentages are of smoking categories or of total, within trial.
[b] N, NORVIT; W, WENBIT; TIA, transient ischemic attack.
[c] Smokers with baseline plasma cotinine concentration below or above 80 nmol/L.
[d] $P < 0.0001$ for difference between smoking categories by χ^2 test or 1-way ANOVA (for age: Kruskal-Wallis test).
[e] $P < 0.0001$ for difference between trials by χ^2-test or *t*-test.

SMOKING CESSATION DURING HOSPITALIZATION FOR AMI

All participants in NORVIT and 321 (10.4%) of participants in WENBIT were included during hospitalization for AMI. Study enrollment and blood collection for vitamin/metabolite analyses occurred either on the day of hospital admission or within the following 1–10 days. During this time the patients had limited opportunities to smoke because of their clinical condition,

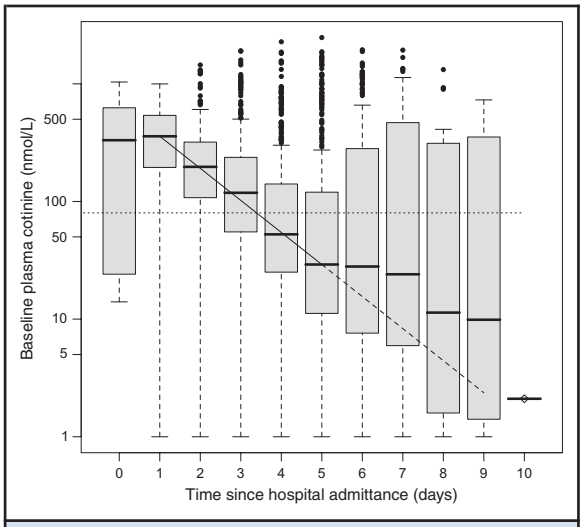

Fig. 1. Boxplot of plasma cotinine concentration among self-reported current smokers by time since hospital admittance for AMI.

The straight line from the median cotinine concentration at day 1 to day 5 suggests similarity to first-order kinetics usually observed for the removal of cotinine from the body [Ahijevych et al. *(16)*]. Only data from NORVIT participants were used for this analysis.

medical procedures, and restrictions on smoking in the hospital area. A semilogarithmic plot of plasma cotinine for self-reported current smokers in the NORVIT trial against time since hospital admittance showed a decrease in median cotinine that was linear between days 1 and 5 (Fig. 1).

SELF-REPORTED SMOKING VS PLASMA COTININE AT BASELINE AND FOLLOW-UP

Plasma cotinine at baseline plotted against measurements at follow-up 1 or 2 month(s) after trial inclusion for each self-reported smoking category are shown in Fig. 2. Results are shown separately for NORVIT and WENBIT participants (WENBIT, 1 month; NORVIT, 2 months). A plasma cotinine cutoff value of 80 nmol/L, a widely used cutoff value used to distinguish smokers from nonsmokers *(15)*, divides each diagram into 4 quadrants, and the number of study patients in each quadrant is given.

In NORVIT 95.6% and in WENBIT 98.9% of self-reported never smokers had low plasma cotinine (<80 nmol/L) at baseline. The corresponding percentages for ex-smokers were 92.1% and 86.9%, and for current smokers 50.2% and 12.9%, respectively. At follow-up, the percentages with low cotinine were similar to baseline for the never- and ex-smoker groups, whereas for self-reported current smokers, the percentages were 40.7% and 20.6%, respectively. Notably, for NORVIT smokers, 43.9% of those with low cotinine at baseline had high cotinine at follow-up, whereas 25.3% of those with high cotinine at baseline had low cotinine at follow-up.

BASELINE CIRCULATING VITAMIN CONCENTRATIONS BY SMOKING CATEGORIES

Table 2 shows adjusted geometric mean plasma/serum concentrations of folate, PLP, riboflavin, and cobalamin, and of tHcy and MMA as functional markers of folate and cobalamin status, respectively, by smoking categories. Folate and PLP concentrations progressively decreased, and tHcy increased in the order never smokers, ex-smokers, current smokers with cotinine <80 nmol/L, and current smokers with cotinine ≥80 nmol/L (all $P_{trend} < 0.0001$). For cobalamin no such trend was evident ($P = 0.14$). Accordingly, MMA concentrations were also not associated with smoking ($P = 0.09$).

Associations for men and women were similar, but slightly stronger for women with respect to PLP ($P_{interaction} = 0.003$), and for men with respect to tHcy ($P_{interaction} = 0.02$). Results were similar by age for all the vitamins. However, the association between tHcy and smoking was weaker among older participants ($P_{interaction} = 0.0002$). There were slightly stronger associations between smoking and circulating concentrations of folate, PLP, and riboflavin for patients who took vitamin supplements compared to those who reported not taking supplements ($P_{interaction} = 0.01$, 0.001, and 0.02, respectively). We compared results among the NORVIT and WENBIT participants after the exclusion of 321 cases of AMI from WENBIT, thereby essentially comparing patients with recent myocardial infarction (NORVIT) to patients with stable angina pectoris (WENBIT). No significant difference between trial/patient groups was found except for riboflavin, for which weaker associations was found among NORVIT than among WENBIT participants ($P_{interaction} = 0.002$).

CIRCULATING VITAMIN CONCENTRATIONS FOR EX-SMOKERS CATEGORIZED BY TIME SINCE SMOKING CESSATION

Time since smoking cessation was available for 2078 of 2364 ex-smokers (87.9%). We divided ex-smokers into 3 subcategories (1–3): Those who quit smoking more than 5 years before study enrollment (median 21 years, n = 1353), those who quit smoking between 1 and 5 years before enrollment (median 2.8 years, n = 244), and those who quit smoking within 1 year before study enrollment (median 0.23 years, n = 481). In this context, self-reported current smokers with cotinine <80 nmol/L could be considered a 4th ex-smoker category with smoking cessation lasting for 3–4 days or more.

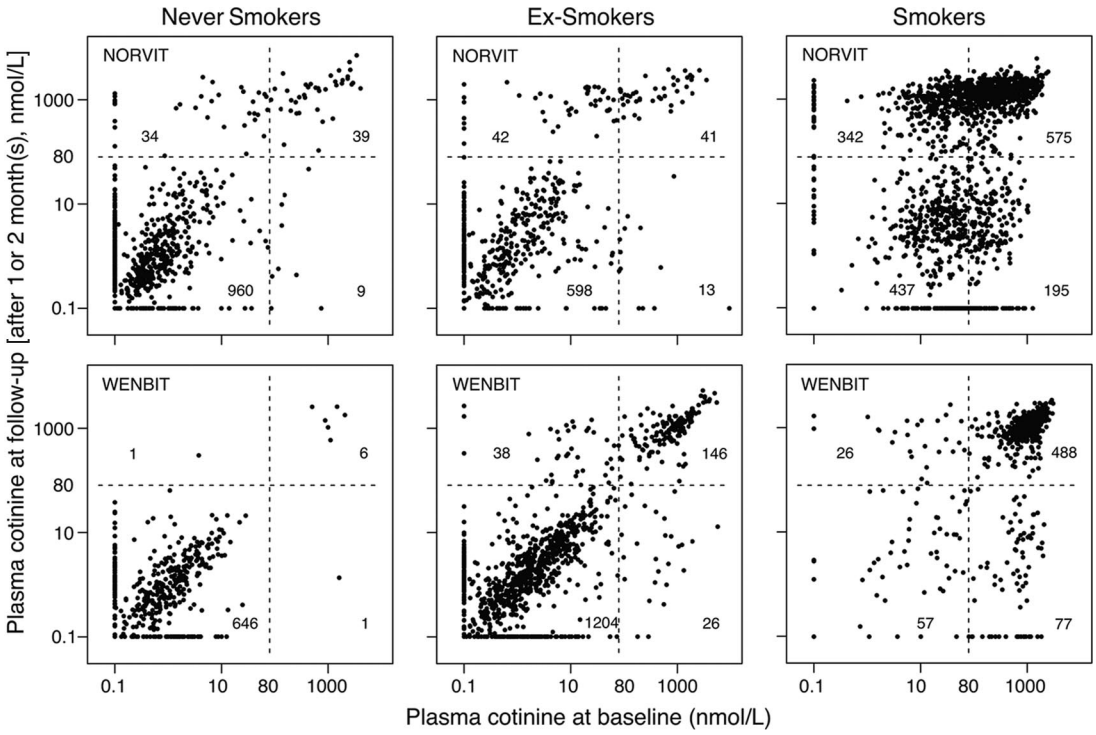

Fig. 2. Plasma cotinine concentrations measured at baseline and at follow-up by self-reported smoking categories.
The upper and lower panels show results for NORVIT and WENBIT participants, respectively. Patients who had no observable cotinine (i.e., below the detection limit) were assigned the value 0.1 nmol/L before plotting. A widely used cutoff value (80 nmol/L) to separate smokers from non-smokers [Jarvis et al. *(15)*] is depicted as dotted lines on both axes, and the number of patients in each resulting quadrant is shown.

Fig. 3 shows that with never smokers as the reference category, there were progressively stronger associations across the ex-smoker subcategories for folate, PLP, and tHcy. A similar, but less pronounced, trend was found for riboflavin.

Discussion

We investigated the association between smoking status and circulating B-vitamin/metabolite concentrations by using baseline data from 6775 men and women with ischemic heart disease included in 2 clinical trials. Compared with never smokers, current smokers had significantly lower circulating concentrations of folate, PLP, and riboflavin and higher concentrations of tHcy. Serum cobalamin and MMA were not associated with smoking. Within the current smoker group, folate, PLP, and riboflavin concentrations were significantly higher for study participants who had abstained from smoking for 3–4 days or more, and this trend continued for ex-smokers with increasing time since quitting smoking. The findings were essentially the same for the 2 trial populations, between sexes, according to age, and for vitamin supplement users vs nonusers.

COMPARISON OF RESULTS FOR NORVIT AND WENBIT PARTICIPANTS

Compared to WENBIT (in which most participants had stable CAD), participants in NORVIT (AMI at baseline) had lower circulating concentrations of folate, PLP, and riboflavin, and higher concentrations of tHcy. Possible explanations for this difference include a transient decrease in food intake, and/or depressed vitamin concentrations associated with increased inflammatory activity after AMI. Additional explanations may include that the majority of participants in WENBIT had been encouraged to quit smoking and to improve their lifestyle, including diet, as a result of previously diagnosed CAD. Ongoing medical treatment at the time of baseline blood sampling also differed according to indications for inclusion in the respective trials. Nevertheless, the associations between smoking

Table 2. Vitamin concentrations in plasma or serum by smoking categories.[a]

	Never smokers	Ex-smokers	Smokers with low cotinine[b]	Smokers with high cotinine[b]	P_{trend}
All					
Folate, nmol/L	10.0 (9.7–10.3)	9.7 (9.4–9.9)	8.8 (8.4–9.1)	7.9 (7.7–8.2)	<0.0001
PLP, nmol/L	37.9 (37.0–38.9)	35.3 (34.5–36.1)	31.1 (30.0–32.3)	28.8 (28.1–29.6)	<0.0001
Riboflavin, nmol/L	12.3 (11.9–12.6)	12.7 (12.3–13.0)	11.5 (11.0–12.0)	10.6 (10.3–11.0)	<0.0001
Cobalamin, pmol/L	338 (329–346)	341 (333–349)	327 (316–340)	330 (320–339)	0.11
tHcy, μmol/L	10.4 (10.3–10.6)	10.6 (10.5–10.8)	11.2 (11.0–11.5)	11.5 (11.4–11.7)	<0.0001
MMA, μmol/L	0.17 (0.17–0.18)	0.17 (0.17–0.17)	0.17 (0.17–0.18)	0.18 (0.17–0.18)	0.09
NORVIT					
Folate, nmol/L	9.1 (8.8–9.4)	8.9 (8.5–9.3)	7.7 (7.4–8.1)	7.1 (6.8–7.4)	<0.0001
PLP, nmol/L	33.1 (32.0–34.2)	31.1 (29.8–32.3)	27.4 (26.4–28.5)	25.5 (24.5–26.5)	<0.0001
Riboflavin, nmol/L	11.9 (11.4–12.3)	12.5 (12.0–13.1)	10.8 (10.4–11.3)	10.6 (10.2–11.1)	<0.0001
Cobalamin, pmol/L	337 (325–349)	346 (331–362)	322 (308–336)	338 (324–353)	0.64
tHcy, μmol/L	11.2 (11.0–11.4)	11.1 (10.9–11.4)	12.0 (11.8–12.3)	12.5 (12.2–12.7)	<0.0001
MMA, μmol/L	0.18 (0.18–0.18)	0.17 (0.17–0.18)	0.18 (0.18–0.19)	0.19 (0.18–0.19)	0.06
WENBIT					
Folate, nmol/L	11.3 (10.8–11.7)	10.9 (10.6–11.1)	10.6 (9.6–11.7)	9.1 (8.7–9.5)	<0.0001
PLP, nmol/L	44.8 (43.2–46.5)	41.3 (40.3–42.4)	34.0 (30.8–37.6)	33.4 (32.1–34.8)	<0.0001
Riboflavin, nmol/L	12.7 (12.1–13.4)	13.1 (12.6–13.5)	13.8 (12.1–15.8)	10.6 (10.0–11.2)	<0.0001
Cobalamin, pmol/L	341 (330–352)	339 (331–346)	360 (331–393)	320 (309–331)	0.009
tHcy, μmol/L	9.5 (9.3–9.7)	9.9 (9.7–10.0)	10.4 (9.7–11.0)	10.6 (10.3–10.8)	<0.0001
MMA, μmol/L	0.16 (0.16–0.17)	0.16 (0.16–0.17)	0.17 (0.16–0.18)	0.16 (0.16–0.17)	0.66

[a] Cells contain adjusted geometrical means with 95% CIs by ANCOVA, with adjustment for trial, sex, age, vitamin supplement user status, and time since hospital admission for AMI.
[b] Smokers with plasma cotinine concentration <80 nmol/L or ≥80 nmol/L.

status and circulating vitamin concentration were similar in the 2 trial populations, indicating that differences in treatment and/or medical condition (including vitamin status itself) did not modify the results.

SHORT-TERM EFFECTS OF SMOKING

We found that for self-reported current smokers in the NORVIT trial log-transformed plasma cotinine decreased linearly as a function of time since hospital admittance. The estimated half-life of the median cotinine concentration between day 1 and 5 was 1.1 days, which is similar to reported half-lives of 0.7–0.9 days for plasma cotinine *(16)*. This finding suggests that smokers with cotinine below a cutoff of 80 nmol/L had been unable to or were restricted from smoking for at least 3–4 days. We found significant differences between this group and smokers with cotinine ≥80 nmol/L, suggesting direct and relatively short-term effects of smoking on circulating levels of the vitamins folate, PLP, and riboflavin.

COMPARISON WITH FINDINGS FROM OTHER STUDIES

Lower circulating folate, PLP, and riboflavin, and higher plasma tHcy among smokers compared with nonsmokers have been reported previously *(6, 17–20)*. Many studies have also demonstrated lower intakes of vitamins among smokers; however, lower intakes were not found to be sufficient to explain all of the differences between never and daily smokers *(6, 7)*. Short-term effects of smoking that normalized within a few days or weeks after smoking cessation have been demonstrated for a number of hematologic parameters *(21)*, as well as for plasma antioxidant status *(22)*, indicating acute effects of smoking not attributable to diet or socioeconomic differences. Moreover, compared with nonsmokers, smokers required higher intakes of vitamin C *(8)* and B6 *(18)* to achieve plasma concentrations that were considered nondeficient.

POSSIBLE MECHANISMS

Tobacco smoke contains a large number of reactive oxygen species that may induce oxidative stress in tis-

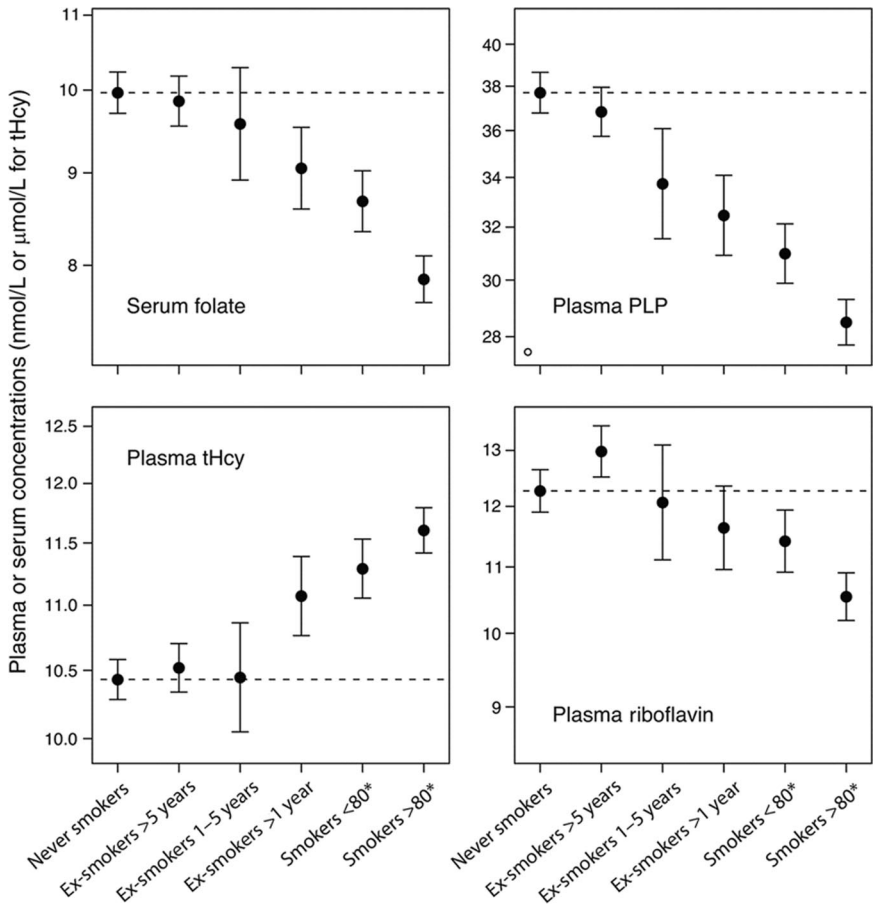

Fig. 3. Serum/plasma B-vitamin concentrations by smoking categories and duration of ex-smoker status.
The ANCOVA model included adjustment for trial, sex, age, vitamin supplement user status, and time since hospital admittance for AMI. The *y* axis is scaled to span 0.7 SD for each outcome variable. *Smokers with plasma cotinine above or below 80 nmol/L.

sues, as indicated by observations of differences in antioxidant concentrations and related enzyme activities between nonsmokers and smokers *(1)*. PLP is involved in the cellular antioxidant defense as cofactor for the 2 enzymes that convert homocysteine to cysteine, the rate-limiting substrate for glutathione synthesis *(23)*, whereas vitamin B2 serves as cofactor for glutathione reductase in addition to a number of other enzymes involved in cellular redox status *(24)*. Thus, for smokers, increased activity of antioxidant defense enzymes could increase the turnover and uptake of PLP and riboflavin into tissues, resulting in decreased circulating vitamin levels.

There is evidence for direct antioxidant effects of folate, B6 species, and riboflavin *(25–27)*. We found that patients with high circulating vitamin concentrations, e.g., vitamin supplement users, and participants in WENBIT (compared to NORVIT) exhibited the same (and, in some cases, larger) relative differences in vitamin concentrations between smoking categories as patients with lower concentrations. This finding is suggestive of a process of vitamin removal or breakdown that is proportional to the concentration of the vitamin, including, possibly, the direct damage by oxidation.

IMPROVED DIET AFTER SMOKING CESSATION?

For folate and PLP there was a continuous trend of increasing serum/plasma concentrations, approaching the level of never smokers, with time since smoking cessation. In addition, the trend for tHcy mirrored the trend for folate. One possible explanation for these findings is a gradual improvement in diet accompanying changes in lifestyle associated with quitting smoking. There was a strong correlation between folate and PLP concentrations (Spearman's $r = 0.40$, $P < 0.0001$), possibly reflecting that the 2 vitamins share important dietary sources *(28)*. This

could underlie the similarity in profiles for folate and PLP with respect to increases in concentration. The trend toward increasing vitamin concentrations with time since smoking cessation was less clear for riboflavin, and no trend was seen for cobalamin (result not shown). Accordingly, these 2 vitamins correlated only weakly with folate and PLP (bivariate correlation coefficients ranging from 0.14 to 0.22).

Previous studies have shown that the diet among ex-smokers is similar to, or intermediate between, that of never smokers and current smokers *(2, 29)*. Notably, vitamin status may depend on time since quitting smoking. Bolton-Smith et al. found a gradual improvement in diet among ex-smokers by duration of ex-smoker status *(30)*. Moreover, smoking is associated with smell and taste impairments that are reversible upon smoking cessation *(31)*. This could lead to changes in taste preferences and ultimately changes in dietary habits over time.

REPLENISHMENT OF VITAMIN STORES AFTER SMOKING CESSATION?

Another explanation for the trend toward increasing vitamin concentrations after quitting smoking may be restoration of vitamin content in tissues. Smoking is associated with reduced red blood cell folate, which may also reflect liver storage *(17, 32)*. Replenishment of vitamin stores could delay the increase in serum folate after smoking cessation. Muscle is the major depot of PLP *(33)*, and plasma PLP concentration is correlated to muscle mass *(34)*. Smoking is associated with impaired muscle protein synthesis and turnover *(35)* and reduced muscle mass *(36)*. Thus, a slow recovery of muscle mass after smoking cessation could be associated with increases in plasma PLP over a time-span of several years, as was observed in this study. A third possibility is decreased vitamin status related to local or systemic oxidative stress associated with persistent low-level inflammation in ex-smokers. A recent study showed that it took more than 20 years before some inflammation markers reverted to levels of never smokers *(37)*.

LIMITATIONS

The main limitation of this study was the lack of food intake data, both at the time of blood sampling and longitudinally. The availability of these data would have aided the interpretation of the results. Additional limitations included no information on use of nicotine replacement therapy or smokeless tobacco in the study population. Notably, the percentages of never and ex-smokers with high cotinine were similar between baseline and follow-up 1–2 months later, whereas the percentage of smokers with low cotinine increased (accounting for the transient smoking cessation for AMI patients). This finding may indicate that true smokers changed their use of tobacco after the diagnosis at trial entry, whereas participants using other forms of nicotine delivery did not. A recent study showed that nicotine by itself had little or no effect on antioxidant vitamins *(38)*. When analyzed as a separate group, never and ex-smokers with high cotinine had only marginally lower vitamin concentrations than never and ex-smokers with low cotinine (results not shown). We therefore concluded that the self-reported smoking status assignments were largely correct. However, we expect that a low level of misclassification of smoker group assignments would result in a slight underestimation of associations.

We had no information about alcohol or coffee intake in the 2 trial populations. Alcohol intake correlates with smoking, and is associated with lower plasma B-vitamin concentrations in some, but not all, studies *(39, 40)*. In a recent study we showed that coffee drinking is associated with reduced concentrations of several B vitamins *(41)*. However, although adjustment for smoking attenuated the associations between coffee and vitamins somewhat, the reverse was not found.

In summary, our results indicate that smoking has direct and short-term effects on circulating levels of the B vitamins folate, PLP, and riboflavin. For folate, PLP, and tHcy we also observed longer-lasting effects. We propose that the short-term effects of smoking may be related to acute oxidative stress, whereas the longer-lasting effects may be partly attributed to modifications in diet associated with smoking that are reversible upon smoking cessation, and partly related to replenishment of vitamin stores during the first few months to years after smoking cessation. In addition, a persistent but slowly decreasing low-level inflammation may prevail among ex-smokers. Our study demonstrates that tobacco smoking affects circulating B-vitamin status, probably by both direct and indirect routes and depending on time since last exposure. It is therefore important to take these effects into account when assessing the independent contribution of smoking and B-vitamin status to human health and disease.

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