

Coffee Consumption and Circulating B-Vitamins in Healthy Middle-Aged Men and Women

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BACKGROUND: Coffee consumption has been associated with several risk factors for coronary heart disease, including increased cholesterol, increased blood pressure, and increased plasma total homocysteine (tHcy). tHcy is determined by several B-vitamins. However, reports about the association between coffee intake and B-vitamin status are few.

METHODS: We measured plasma B-vitamins and tHcy in a cohort of 10 601 healthy, middle-aged Norwegian men and women. Information about lifestyle factors, including coffee consumption, smoking, alcohol use, height, and weight, was obtained by interview.

RESULTS: Coffee consumption was dose-dependently associated with reduced plasma B-vitamin concentrations. Compared with coffee abstainers, individuals drinking ≥ 4 cups/day had 11.7% ($P < 0.001$), 14.1% ($P < 0.001$), and 5.5% ($P = 0.01$) lower plasma concentrations of folate, pyridoxal phosphate, and riboflavin, respectively, and the mean tHcy concentration was 6.8% ($P < 0.001$) higher. Quantile regression analysis showed essentially no difference in B-vitamin concentrations between coffee consumption categories at low vitamin concentrations but a progressive increase in the difference at higher concentrations. This pattern of differences (effect profile) was found independently of smoking status, alcohol intake, and sex. The decrease in folate explained approximately half of the increase in tHcy.

CONCLUSIONS: Coffee consumption was associated with reduced circulating B-vitamin concentrations. The observed effect profiles indicated that coffee consumption preferentially affected the upper, but not the lower, part of the B-vitamin concentration distributions. We hypothesize that coffee consumption may

increase the loss of surplus B-vitamins by excretion in urine.

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Coffee is one of the most widely consumed beverages in the world. It contains more than 1000 compounds, not all of which have been identified, and several have nutritional value or are bioactive (1). The potential health consequences of coffee intake have been extensively investigated. A number of case-control studies have found increased risk of coronary heart disease or myocardial infarction associated with coffee consumption (1, 2); however, these findings have not been confirmed in prospective studies (2). In contrast, recent studies have reported health benefits associated with coffee consumption, including decreased risk of diabetes, Parkinson's disease, and liver disease (1).

Although an association between cardiovascular disease (CVD)³ and coffee consumption remains unproved, coffee has been associated with several CVD risk factors, including increased cholesterol, increased blood pressure, and increased plasma total homocysteine (tHcy). Increased cholesterol was found to be largely attributable to the diterpenes kahweol and cafestol found in boiled coffee, but not in filtered coffee (3). Whereas increased blood pressure is measured as an acute effect from coffee (4), near complete tolerance is established during chronic consumption, and prospective studies have not found convincing evidence of increased risk of hypertension (1). The most consistently reported association has been with moderately increased tHcy, a risk factor for multiple health conditions, including CVD, cognitive impairment, and osteoporosis (5).

One of the main determinants of tHcy is folate status, measured as dietary intake or blood concentration. Plasma concentrations of 3 other B-vitamins,

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³ Nonstandard abbreviations: CVD, cardiovascular disease; tHcy, total homocysteine; PLP, pyridoxal phosphate; BMI, body mass index.

cobalamin (vitamin B₁₂), riboflavin (B₂), and pyridoxal phosphate (B₆), also involved in homocysteine metabolism, are additional determinants of tHcy (6).

Whereas human intervention studies showed that coffee consumption caused an increase in plasma tHcy concentrations, no effects—or only moderate, non-significant effects—on plasma folate, vitamin B₆, and cobalamin were found (7–9). Thus, the mechanism behind the tHcy increase is uncertain, although caffeine, as well as chlorogenic acid, a polyphenol found in large quantities in coffee, were shown to increase tHcy (8, 10). Moreover, the addition of 200 µg/day of folic acid to coffee intervention groups prevented the increase in tHcy (11).

Human observational studies examining the association between coffee consumption and plasma B-vitamin concentrations are scarce. A recent Greek study including 486 participants found a borderline significant decrease in serum folate concentrations associated with coffee drinking (12).

The objective of this study was to examine the association between coffee consumption and plasma folate, pyridoxal phosphate (PLP), riboflavin, and cobalamin in a large cohort of >10 000 middle-aged Norwegian men and women. In addition, we wanted to determine if the increase in tHcy associated with coffee consumption could be explained completely, or partially, through an effect on these vitamins.

Participants and Methods

PARTICIPANTS

We analyzed 10 601 blood samples from individuals enrolled in the Norwegian Colorectal Cancer Prevention (NORCCAP) study. NORCCAP was initiated and organized by the Norwegian Cancer Society and the Norwegian Department of Health. It includes middle-aged (50–64 years old) apparently healthy individuals of both sexes resident in the city of Oslo or county of Telemark in eastern Norway drawn at random from the population registry. Recruitment took place from 1999 to 2001 at 2 hospitals (screening centers), one in each catchment area. Several lifestyle variables were recorded, and blood samples were collected (13). The study was approved by the Regional Ethics Committee and the Data Inspectorate. Written informed consent was obtained from all participants.

BLOOD COLLECTION AND BIOCHEMICAL ANALYSIS

At inclusion, blood samples were collected into EDTA Vacutainer Tubes and tubes without additive. Serum was allowed to clot for 1 h at room temperature, whereas EDTA samples were immediately put on ice. Samples were centrifuged at 1100g for 10 min, and serum and plasma were then separated and frozen at

–80 °C until analysis. We measured folate (14) and vitamin B₁₂ (15) in serum and PLP, riboflavin (16), and tHcy (17) in plasma. We genotyped the *MTHFR* 677C->T polymorphism (18) in packed blood cells according to published methods.

STATISTICAL METHODS

The distributions of folate, PLP, riboflavin, and cobalamin were skewed (right tailed); therefore, analyses were performed on the log-transformed variables. Results are either shown as percentage differences or converted to linear scale.

We analyzed the association between coffee and vitamin concentrations by ANCOVA and quantile regression (19). With both methods, we report results using a model including coffee, smoking, beer, wine, liquor, body mass index (BMI), and *MTHFR* 677C->T genotype as 3-level factors, and age, sex, and study centre (hospital) as covariates. We evaluated interaction between coffee and other explanatory variables by including a product term in the model.

With ANCOVA, only the difference in means between factor levels (e.g., drinking ≥4 cups of coffee vs no coffee) is evaluated; no information is obtained about differential effects at high and low concentrations of the dependent variable. In contrast, quantile regression determines the difference between factor levels at specific quantile (percentile) cutoffs of the distribution of the outcome variable. By choosing a set of cutoffs, a profile of the effect of the factor on the outcome (effect profile) is obtained (19). We obtained effect profiles of coffee on vitamin concentrations applying cutoffs (τ) at the 0.05, 0.10 . . . 0.95 quantiles. The results were plotted graphically displaying the difference between factor levels (e.g., ≥4 vs 0 cups of coffee), vs the vitamin concentration, at each quantile cutoff. (For an illustration of the method, see the Data Supplement that accompanies the online version of this article at <http://www.clinchem.org/content/vol54/issue9>).

We used the open source statistical program environment R (20, 21) with package “quantreg” to obtain quantile regression estimates for the explanatory variables in our statistical model at the chosen set of τ 's. For other statistical analyses, we used SPSS version 13 for Macintosh.

Results

COFFEE CONSUMPTION ACCORDING TO SMOKING, ALCOHOL, BMI, AND AGE

The average (range) age of our study group was 56 (50–64) years. Among 10 555 participants who reported their coffee drinking habits, 9140 (86.6%) drank 1 or more cups a day. Among these, 8726 (94.4%) drank filtered coffee, and 798 (8.6%) drank boiled coffee

Table 1. Association between coffee consumption and lifestyle characteristics in the study population.^a

	Men					Women				
	Total	Coffee, cups/day			<i>P</i> , χ^2 test	Total	Coffee, cups/day			<i>P</i> , χ^2 test
		0	1–3	≥4			0	1–3	≥4	
n	5189	673	1687	2829		5348	741	2183	2424	
Smoking	31.0	14.1	19.9	41.7	<0.001	30.7	15.7	19.9	45.3	<0.001
Beer ^b	50.9	41.1	52.6	51.5	<0.001	19.2	12.4	19.0	21.0	<0.001
Wine ^b	52.1	49.6	58.2	48.8	<0.001	56.5	53.5	61.2	52.5	<0.001
Liquor ^b	34.9	28.0	32.8	37.3	<0.001	14.6	12.7	13.6	16.0	0.13
BMI 3rd tertile ^c	33.3	33.2	30.9	34.7	0.001	33.3	33.2	32.5	34.0	0.17
Mean age, years	56.4	56.0	56.5	56.3	0.03 ^d	56.3	55.9	56.7	56.3	<0.001 ^d

^a Data are %, unless noted otherwise.
^b Percentage of study participants who reported drinking 1 or more glass/14 days.
^c Percentage of study participants in the upper (sex-specific) tertile of BMI.
^d Kruskal-Wallis test.

(518 exclusively). Coffee drinking was strongly associated with smoking, and there were also differences in the frequency of beer, wine, and liquor drinking be-

tween coffee consumption categories (Table 1). Men drank more coffee, beer, and liquor and less wine than women (all $P < 0.001$ by χ^2 test).

Table 2. Coffee consumption and plasma vitamin levels.^a

Vitamin	Coffee, cups/day	All			Men			Women		
		Plasma concentration ^b	Difference		Plasma concentration ^b	Difference		Plasma concentration ^b	Difference	
			%	% of SD		%	% of SD		%	% of SD
Folate, nmol/L	0	16.2	Ref	Ref	14.9	Ref	Ref	17.4	Ref	Ref
	1–3	15.2	–5.7	–10.1	14.3	–3.9	–7.6	16.2	–6.9	–11.7
	≥4	14.2	–11.7	–20.2	13.3	–10.1	–19.2	15.1	–13.7	–22.3
<i>P</i> for trend (ANCOVA)		<0.001			<0.001			<0.001		
PLP, nmol/L	0	55.8	Ref	Ref	54.9	Ref	Ref	56.5	Ref	Ref
	1–3	52.0	–6.4	–9.6	52.9	–2.9	–5.5	51.2	–9.4	–12.5
	≥4	47.8	–14.1	–20.4	46.9	–14.4	–23.7	48.8	–13.9	–18.7
<i>P</i> for trend (ANCOVA)		<0.001			<0.001			<0.001		
Riboflavin, nmol/L	0	11.3	Ref	Ref	10.6	Ref	Ref	12.1	Ref	Ref
	1–3	11.0	–2.2	–2.7	10.8	2.1	2.7	11.3	–6.7	–7.3
	≥4	10.7	–5.5	–6.6	10.1	–4.9	–5.7	11.3	–6.8	–7.4
<i>P</i> for trend (ANCOVA)		0.01			0.02			0.16		
Cobalamin, pmol/L	0	310	Ref	Ref	307	Ref	Ref	312	Ref	Ref
	1–3	306	–0.8	–2.2	305	–0.6	–2.0	309	–0.9	–2.7
	≥4	307	–0.5	–1.4	303	–1.0	–3.3	311	–0.2	–0.4
<i>P</i> for trend (ANCOVA)		0.75			0.44			0.83		

^a Differences between consumption categories are expressed as percentage of the reference category and as percentage of the SD of the dependent variable.
^b Estimated marginal means.

Table 3. Coffee consumption and plasma tHcy concentrations.^a

	All				Men			Women		
	Coffee, cups/day	Plasma concentration ^b	Difference		Plasma concentration ^b	Difference		Plasma concentration ^b	Difference	
			%	% of SD		%	% of SD		%	% of SD
tHcy, $\mu\text{mol/L}$	0	10.1	Ref	Ref	10.7	Ref	Ref	9.5	Ref	Ref
	1–3	10.4	3.5	12.0	11.0	2.5	9.3	9.9	4.5	15.5
	≥ 4	10.8	6.8	23.3	11.2	5.1	18.6	10.3	8.8	29.5
<i>P</i> for trend (ANCOVA)		<0.001			<0.001			<0.001		
tHcy after adjustment for folate, $\mu\text{mol/L}$	0	10.3	Ref	Ref	10.9	Ref	Ref	9.8	Ref	Ref
	1–3	10.5	1.8	6.4	11.0	1.3	4.8	10.0	2.4	8.4
	≥ 4	10.7	3.9	13.4	11.3	2.7	10.0	10.3	5.1	17.6
<i>P</i> for trend (ANCOVA)		<0.001			0.01			<0.001		

^a Differences between consumption categories are expressed as percentage of the reference category and as percentage of the standard deviation (SD) of the dependent variable.
^b Estimated marginal means.

DIFFERENCES IN B-VITAMIN AND tHcy CONCENTRATIONS ACCORDING TO COFFEE CONSUMPTION

Table 2 shows the plasma concentration of folate, PLP, riboflavin, and cobalamin according to coffee intake analyzed by ANCOVA. Coffee drinking was associated with lower plasma concentration of folate, PLP, and riboflavin, but no change was observed for cobalamin. The difference between coffee consumption levels was similar for folate and PLP, but somewhat smaller for riboflavin. Table 3 shows that coffee consumption was

associated with increased tHcy concentration, but that adjustment for folate reduced the difference between coffee consumption categories. Further adjustment for PLP and riboflavin had negligible effect (results not shown).

DIFFERENCES IN B-VITAMIN CONCENTRATIONS ACCORDING TO COFFEE CONSUMPTION BY QUANTILE REGRESSION

Fig. 1 shows the difference between coffee consumption categories by quantile regression with quantile cut points at the 5th, 10th . . . 95th percentiles of the

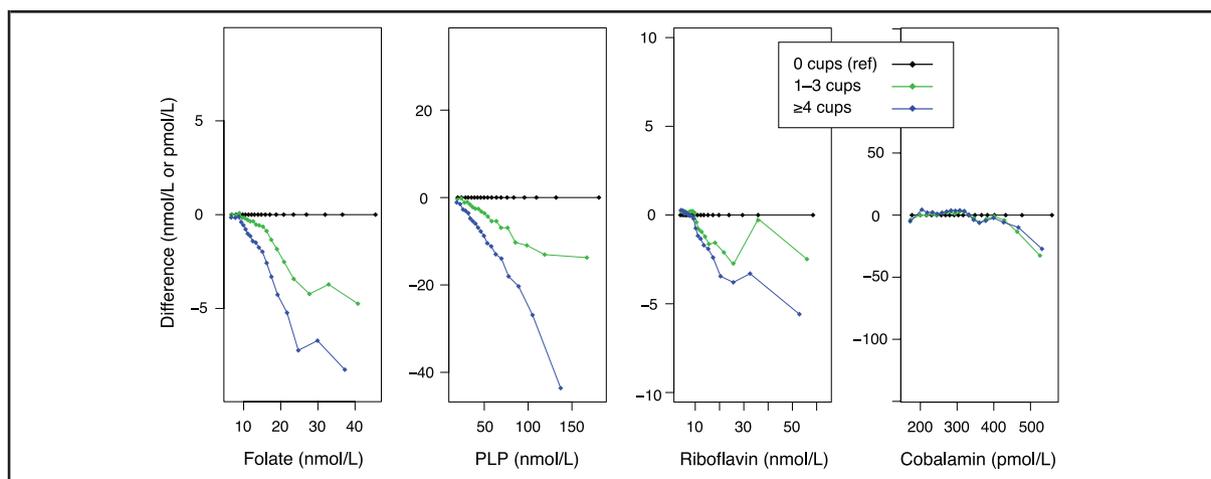
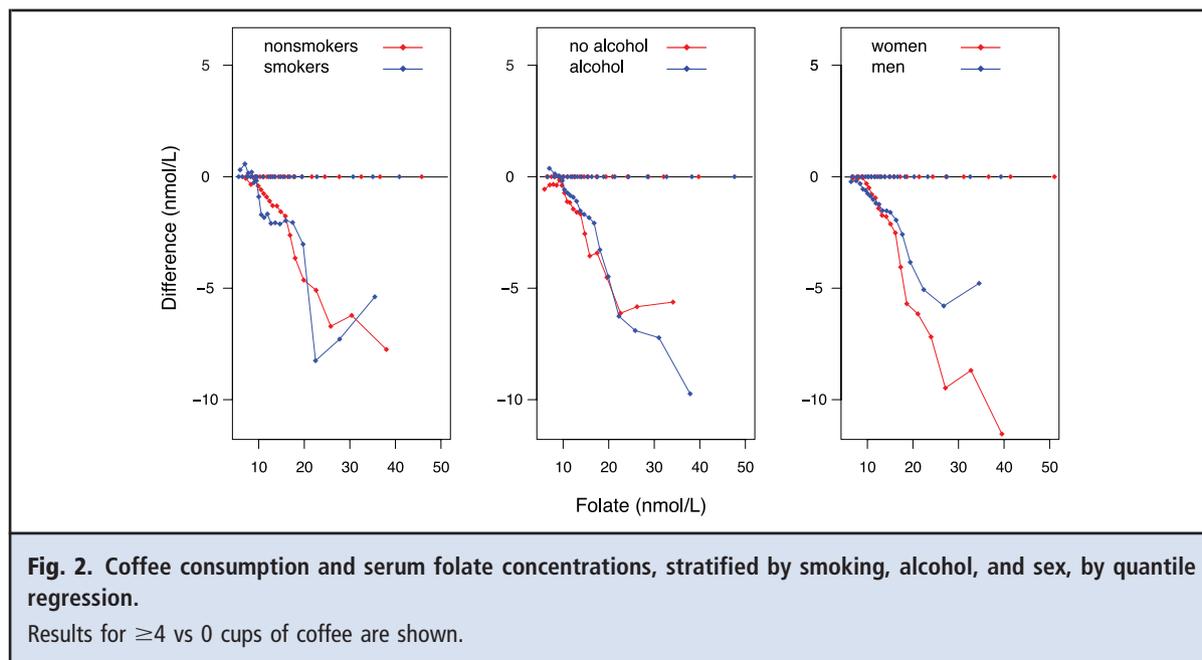


Fig. 1. Coffee consumption and folate, PLP, riboflavin, and cobalamin serum/plasma concentrations by quantile regression.

The vitamin concentration is shown on the x axis, and the difference between 1–3 vs 0 cups and ≥ 4 vs 0 cups of coffee is shown on the y axis. Quantile cut points (19 cut points from the 0.05–0.95 quantile) are denoted by dots.



outcome variable. Similar effect profiles were found for folate, PLP, and riboflavin. At the lower quantiles, there was no difference between coffee consumption categories; at progressively higher quantiles, however, there was an increase in the difference that was approximately proportional to the vitamin concentration for those who drank no coffee (reference).

STRATIFICATION BY SMOKING, ALCOHOL CONSUMPTION, AND SEX

Fig. 2 shows the difference in folate concentrations between the highest (≥ 4 cups/day) level of coffee consumption and no consumption, stratified by smoking status, alcohol consumption, and sex. Smokers had lower folate concentrations, and the entire effect profile for smokers was therefore left-shifted, but otherwise it overlapped the profile for nonsmokers. We also observed this general pattern of overlapping effect profiles when the analyses were stratified by alcohol consumption and sex (Fig. 2), and we obtained similar results for PLP (results not shown). We found no interaction between coffee consumption and other explanatory variables (including those indicated in Fig. 2) (all P interactions > 0.1 by ANCOVA).

CORRESPONDENCE BETWEEN tHcy AND FOLATE RESULTS

Total plasma homocysteine was inversely correlated to folate (Spearman's $\rho = -0.44$, $P < 0.001$). Fig. 3 compares the effect profiles of coffee consumption on tHcy to that on folate. The largest differences between coffee consumption categories were observed at low tHcy concentrations, corresponding with the observation of

largest differences at high folate concentrations. At the opposite ends of the tHcy and folate distributions, however, there was some effect of coffee on tHcy, but essentially no effect of coffee on folate.

Discussion

We investigated the association between coffee consumption and plasma folate, PLP, riboflavin, and cobalamin concentrations in a large population of healthy men and women. A dose-dependent decrease in vitamin concentrations with increasing coffee consumption was found for all vitamins except cobalamin. Using quantile regression, we showed that coffee consumption had no effect at the lower end of the vitamin distributions, but a progressively larger effect at the higher quantiles.

STUDY DESIGN AND POTENTIAL CONFOUNDING

This is, to our knowledge, the largest study to date that addresses the relation between coffee consumption and B-vitamin status. Because of the cross-sectional design, however, true cause-and-effect relationships cannot be proven.

A decrease in vitamin concentrations as a result of coffee consumption could be explained alternatively by an association of coffee consumption with diet, physical exercise, socioeconomic status, or other confounding variables that were not measured or ascertained in the cohort. In several studies using multivariate analysis, coffee consumption was associated with a Western-type diet characterized by high energy content and low

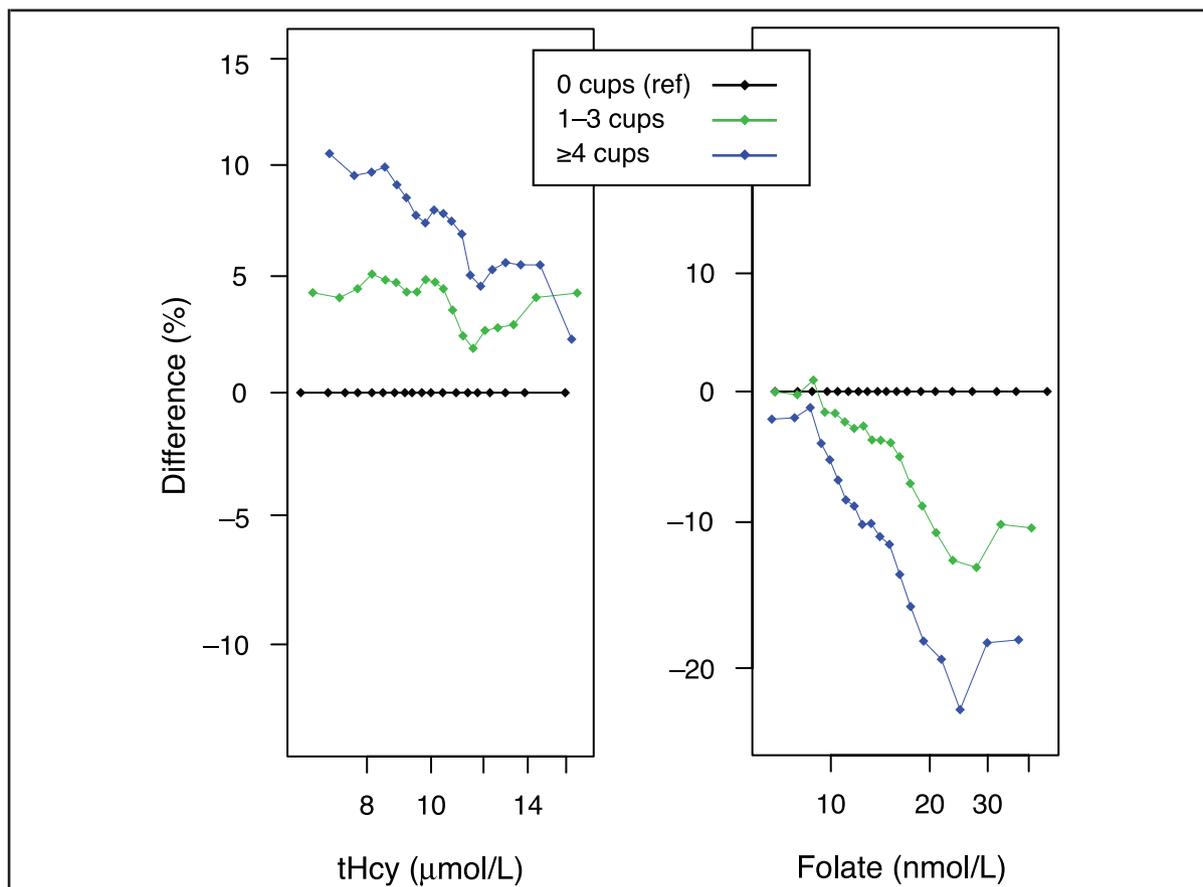


Fig. 3. Comparison of effect profiles of coffee consumption on plasma tHcy and serum folate

The y axis shows differences expressed as percentage deviation from the reference (0 cups) and is scaled to show 0.5 SD of the outcome variable in each direction.

nutrient value (22–24). In a recent Norwegian study on individuals in the relevant age groups, however, coffee was not associated with any particular dietary pattern (25). Furthermore, adjustment for BMI and alcohol use may have corrected, at least partially, for such effects. It is commonly found that smokers have a different diet and lifestyle than nonsmokers (26); however, we found overlapping effect profiles according to smoking. Similarly, alcohol consumption and sex did not modify the associations between coffee consumption and vitamin concentrations.

A potential weakness of this study was the lack of information about vitamin supplement use. In a similar Norwegian cohort, it was found that coffee consumption was associated with less frequent use of vitamin supplements (27). Supplement use could, hypothetically, explain both the dosage pattern and effect profile observed for coffee on the vitamins folate, PLP, and riboflavin, but only if there was an inverse relation between supplement use and the amount of coffee con-

sumed, and more frequent use among individuals with otherwise healthier lifestyles (better vitamin status). Adjustment for supplement use did not remove or appreciably diminish the effect of coffee consumption on tHcy concentrations in the previous study (27).

POSSIBLE MECHANISMS

The difference in vitamin concentrations between coffee consumption categories was proportional to the plasma vitamin concentration. Moreover, the effect profiles were similar and overlapping for groups characterized by differences in vitamin status, e.g., smokers vs nonsmokers. This suggests that coffee consumption may influence one or more physiological processes that regulate the vitamin concentration in blood. Coffee is the main dietary source of caffeine, a stimulant that has effects on many organs, including diuretic effects on the kidneys (28, 29). Experimental studies have shown that a fraction of daily water-soluble vitamin intake is excreted unmetabolized in the urine, and that blood

concentrations as well as the fractional excretion increase with intake (30, 31). The effect profiles of coffee on folate, PLP, and riboflavin could be explained if coffee consumption, through renal mechanisms, increases vitamin excretion at high blood vitamin concentrations. Reports describing increased excretion and lower blood concentrations of micronutrients (including folate, PLP and riboflavin) and higher tHcy in patients given diuretics (32–36) support this hypothesis. Caffeine has been shown not to modify the glomerular filtration rate (37), but it affects the proximal tubules where most of the vitamins are reabsorbed (37–39).

For renal processes to be a significant factor in determining plasma vitamin concentrations, some fraction of the vitamin must be filterable at the glomeruli. Cobalamin is normally 100% bound to haptocorrin and transcobalamin, limiting the fraction that is filtered under normal circumstances (39). At high cobalamin concentrations, however, a non-protein-bound, completely filtered fraction may exist (39). Accordingly, our data suggested a small difference between coffee drinkers and coffee abstainers at the upper end of the cobalamin distribution.

THE INCREASE IN tHcy

The effect of coffee on tHcy was of the same magnitude as on folate (in terms of % SD); after adjusting for folate in the statistical model, approximately half of the effect on tHcy remained. A recent intervention study found that caffeine was responsible for approximately half of

the increase in tHcy after coffee intake (8). Our data suggest that this may be explained by decreases in plasma folate, and, if our hypothesis is correct, the decreases are caused by effects of caffeine on renal excretion. Another study by the same group (10) suggested that the additional rise in homocysteine for coffee drinkers could be due to the chlorogenic acid content of coffee, perhaps through its metabolism by methylation, which leads to increased homocysteine production (40). Both of these mechanisms are compatible with the observation that folate supplementation prevented the increase in tHcy following coffee consumption (11).

We have shown that coffee consumption is associated with reduced plasma concentration of folate, PLP and riboflavin, but that these effects are asymmetric, with coffee intake mainly lowering high vitamin concentrations while having a negligible effect on low concentrations. Our hypothesis that coffee consumption may increase the loss of surplus B-vitamins through excretion in urine could be tested in future experiments.

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