Performance of plasma trigonelline as a marker of coffee consumption in an epidemiologic setting

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ABSTRACT

Background: Coffee is a widely consumed beverage, and studies suggest that drinking coffee has beneficial health effects. The phytohormone trigonelline is present in large amounts in coffee beans, and circulating concentrations of trigonelline have been shown to be positively related to dietary intake of coffee and to increase significantly after the consumption of a bolus dose of coffee.

Objective: We cross-sectionally investigated the utility of plasma trigonelline as a marker of coffee consumption in an epidemiologic setting. We secondarily investigated if coffee intake is related to plasma concentrations of vitamin B-3 (niacin) forms.

Design: In a Norwegian cohort of 3503 participants, we combined questionnaire data on the number of cups of coffee consumed per day with plasma trigonelline to evaluate trigonelline as a marker of coffee intake. The suitability of plasma trigonelline to discriminate those not consuming from those consuming coffee was investigated by receiver operating characteristic (ROC) analysis. Plasma collected at 2 time points 1 y apart was used to determine the within-person reproducibility of trigonelline.

Results: We found that plasma trigonelline concentrations increased strongly with increasing amounts of coffee consumed. ROC analysis showed that trigonelline had an area under the curve of 0.92 (95% CI: 0.90, 0.94) for distinguishing coffee abstainers from coffee drinkers. Plasma trigonelline had a good within-person reproducibility (0.66; 95% CI: 0.64, 0.68) for samples collected 1 y apart. The amount of coffee consumed was not associated with plasma concentrations of the niacin vitamers nicotinamide and N^1-methylnicotinamide.

Conclusion: Plasma trigonelline performs well as a marker of coffee intake. Data used in this study were derived from the clinical trial registered at www.clinicaltrials.gov as NCT00354081. *Am J Clin Nutr* 2018;107:941–947.

Keywords: coffee, trigonelline, vitamin B-3, niacin, smoking, cotinine, plasma

INTRODUCTION

Coffee is the second most-consumed beverage worldwide, and it is also the second-largest trade commodity (1). Epidemiologic studies have found mostly positive health effects of coffee consumption (1–8). Confounding factors that may contribute to the slight heterogeneity of results include differences in coffee cup size, coffee types consumed, biological and genetic variations, and associated lifestyle factors such as smoking (6).

In epidemiologic studies, the amount of coffee consumed is usually obtained from diet records, diet recalls, or questionnaire data and quantified as number of coffee cups consumed per day (2). Uncertainties in such data may be avoided by the use of biomarkers, which are objective measurements and thus considered to be better than dietary assessment data in reflecting intake (9). A suitable biomarker should be valid, reproducible, and sensitive to variation in intake (10, 11). Weaknesses of dietary biomarkers include variation among individuals in absorption, tissue turnover, and renal excretion (11). The use of a continuous measure of coffee consumption may facilitate investigations of nonlinear relations and mechanisms involved.

The alkaloid trigonelline is present in particularly high amounts in coffee beans, where it amounts to ∼1% of the bean dry weight (12). Trigonelline is present in only small amounts in other plants and food items (13–15). It has been reported that serum (16–19) and urine (18, 20) trigonelline concentrations were positively associated with coffee consumption (16, 17) and that plasma and urine concentrations of trigonelline increased strongly in individuals after the consumption of a bolus dose of

Supported by the Western Norway Regional Health Authority (grant 911489); the Norwegian Cancer Society (grant 421798); the Foundation to Promote Research into Functional Vitamin B12 Deficiency, Bergen Norway; and Alpharma, Inc., Copenhagen, Denmark.

Supplemental Tables 1–4 and Supplemental Figure 1 are 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/.

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Abbreviations used: eGFR, estimated glomerular filtration rate; ICC, intraclass correlation coefficient; LOD, limit of detection; mNAM, N^1-methylnicotinamide; NAM, nicotinamide; ROC, receiver operating characteristic; WECAC, Western Norway Coronary Angiography Cohort; WENBIT, Western Norway B-Vitamin Intervention Trial.

Received December 29, 2017. Accepted for publication March 7, 2018.

First published online May 15, 2018; doi: https://doi.org/10.1093/ajcn/nqy059.

coffee (21). However, to the best of our knowledge, no literature exists that thoroughly investigates the performance of this alkaloid as a quantitative marker of coffee consumption in an epidemiologic setting.

The roasting of coffee beans converts trigonelline to nicotinic acid [vitamin B-3 (niacin)], and the extent to which this occurs depends on the temperature and duration of roasting (22, 23). It has therefore been suggested that coffee may be a source of niacin (22, 23), but after consumption of a bolus dose of coffee only a small increase in plasma nicotinamide (NAM), and no change in nicotinic acid or N^1-methylnicotinamide (mNAM), was observed (21).

The positive association of smoking with coffee consumption (24–27) necessitates adjustment for smoking behavior when investigating the health effects of coffee. The biochemical assay (28) used in this work enables simultaneous measurement of circulating concentrations of trigonelline and cotinine [an established marker of recent nicotine exposure (29)]. The combined analysis of these 2 lifestyle markers will be a useful tool when investigating the health effects of coffee consumption and smoking.

We investigated the relation of coffee consumption with plasma concentrations of trigonelline and the niacin vitamers NAM and mNAM in a large cohort of Norwegian adults. The performance of plasma trigonelline as a marker of coffee consumption was investigated by calculating receiver operating characteristics (ROCs) with the use of questionnaire data as a classifier. By analyzing plasma samples collected 1 y apart we also calculated the within-person reproducibility of plasma trigonelline.

METHODS

Study participants

This work included 4609 participants from the Western Norway Coronary Angiography Cohort (WECAC), which consisted of patients undergoing elective coronary angiography due to suspected stable angina pectoris between 2000 and 2004. Details about the WECAC study have been published previously (30). Approximately two-thirds of the WECAC patients participated in the Western Norway B-Vitamin Intervention Trial (WENBIT) (31). Participants with missing or incomplete data on coffee consumption (not reporting type of coffee while reporting to drink >1 cup of coffee/d) were excluded, which left 3503 participants for the final analyses at baseline (**Supplemental Figure 1**).

Written informed consent was obtained from all participants. The WENBIT study protocol was in accordance with the principles of the Declaration of Helsinki, and the trial was approved by the Regional Committee for Medical and Health Research Ethics, the Norwegian Medicines Agency, and the Data Inspectorate.

Assessment of coffee consumption and smoking habits

Coffee consumption was assessed by questionnaire as number of cups of coffee consumed per day in categories (0, ~1, 2–4, 5–8, or >8), and which coffee type they most often consumed (filtered, boiled, powder, decaffeinated, several types, or unknown). Smoking habits were given as never, former, or current smoker.

Blood samples and biochemical analyses

EDTA plasma was drawn at baseline and after 1 y. Plasma samples were stored at −80°C until analysis, and trigonelline (28), NAM (28), mNAM (28), cotinine (28), and creatinine (32) were analysed by isotope dilution liquid chromatography–tandem mass spectrometry by the Bevital Laboratory (www.bevital.no). Ion pairs for trigonelline (138.0/78.0), $^2H_3$-trigonelline (141.0/78.0), NAM (123.0/80.1), $^2H_4$-nicotinamide (127.0/80.1), mNAM (137.3/79.0), and $^2H_4$-N^1-methylnicotinamide (141.3/83.0) were added to an existing assay (28). The limits of detection (LODs) were 50 nmol/L, 20 nmol/L, 5 nmol/L, 1 nmol/L, and 0.25 μmol/L; and within- and between-day CVs were <5%, <12%, <9%, <6%, and <6% for trigonelline, NAM, mNAM, cotinine, and creatinine, respectively. The estimated glomerular filtration rate (eGFR)/1.73 m^2 was calculated by the Modification of Diet in Renal Disease equation (33).

Statistical methods

Trigonelline and cotinine values below the LOD (50 and 1 nmol/L, respectively) were set to the respective LODs; no other biomarkers were below their LOD in any samples. Variables are reported as medians (5th, 95th percentiles). The distribution of coffee consumption is given as numbers [*n* (%) of total population] within each coffee-cup category, and coffee type and smoking as numbers [*n* (%)] within each category of coffee cups consumed per day. The average number of coffee cups consumed per day was set to the mean for each category, except for the highest (>8 cups/d), where it was set to 9.

We calculated linear regression models with ln-transformed concentrations of trigonelline, NAM, and mNAM as outcomes and study center (Bergen = 0, Stavanger = 1), fasting status (nonfasting = 0, fasting = 1), age, sex (male = 0, female = 1), smoking category, coffee-cup consumption category, and ln(eGFR) as predictors. Thus, after exponentiation, the regression coefficients describe the ratio of outcome variables for those with a factor level = 1 divided by those with a factor level = 0. For eGFR, the regression coefficient is given as the estimated ratio of the response for a doubling in eGFR. Few participants reported usually drinking coffee types other than filtered coffee; thus, only the participants reporting no coffee consumption or reporting most often drinking filtered coffee were included in the models. Spearman correlations between biomarkers adjusted for study center, fasting status, age, and sex, and plasma cotinine (marker of recent nicotine exposure) and eGFR (where appropriate) were calculated separately for abstainers and those consuming coffee.

The suitability and optimal cutoff value of plasma trigonelline to correctly discriminate consumers from nonconsumers of coffee were investigated by ROCs with the use of questionnaire data as the classifier. To allow for comparison with an established marker of recent nicotine exposure (34), a ROC analysis was also performed for plasma cotinine compared with smoking habits (current compared with not current) based on data from the same questionnaire.

The within-person reproducibility for trigonelline and cotinine was determined as intraclass correlation coefficients (ICCs) (95% CIs) from samples collected at baseline and after 1 y of

TABLE 1
Demographic characteristics and plasma biomarker concentrations at baseline according to coffee consumption[1]

		Number of cups consumed per day					
	Total	0	1	2–4	5–8	>8	P^2
Participants, *n* (%)	3503 (100)	204 (5.8)	299 (8.5)	1886 (53.8)	918 (26.2)	196 (5.6)	<0.001
Male sex, %	72.7	66.2	62.5	70.4	79.3	86.7	<0.001
Age, y	62 (44, 78)[3]	57 (39, 76)	66 (45.9, 80)	64 (46, 78)	59 (43, 74)	56 (40, 70)	<0.001
Smoking, *n* (%)							<0.001
Never smokers	931 (26.6)	98 (48.0)	126 (42.1)	569 (30.2)	133 (14.5)	5 (2.6)	
Former smokers	1738 (49.7)	77 (37.7)	139 (46.5)	999 (53.0)	456 (49.8)	67 (34.2)	
Current smokers	831 (23.7)	29 (14.2)	34 (11.4)	318 (16.9)	326 (35.6)	124 (63.3)	
Coffee type, *n* (%)							<0.001
None	204 (4.7)	204 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Filtered	2888 (67.0)	0 (0.0)	244 (81.6)	1639 (86.9)	831 (90.5)	172 (87.8)	
Boiled	72 (1.7)	0 (0.0)	7 (2.3)	40 (2.1)	21 (2.3)	3 (1.5)	
Powder	121 (2.8)	0 (0.0)	22 (7.4)	82 (4.3)	14 (1.5)	2 (1.0)	
Decaffeinated	53 (1.2)	0 (0.0)	10 (3.3)	27 (1.4)	10 (1.1)	5 (2.6)	
Several types	124 (2.9)	0 (0.0)	10 (3.3)	71 (3.8)	31 (3.4)	12 (6.1)	
Unknown	850 (19.7)	0 (0.0)	6 (2.0)	27 (1.4)	11 (1.2)	2 (1.0)	
Trigonelline, μmol/L	3.55 (0.25, 13.1)	0.29 (0.05, 2.81)	1.63 (0.16, 7.44)	3.56 (0.54, 12.1)	4.71 (0.83, 14.9)	5.8 (1.24, 21.4)	<0.001
NAM, nmol/L	342 (156, 754)	373 (164, 836)	322 (144, 714)	337 (159, 741)	349 (151, 775)	359 (182, 759)	<0.01
mNAM, nmol/L	85.4 (34.3, 217)	82.7 (29.6, 236)	86.2 (35.2, 236)	85.8 (33.9, 218)	83.6 (36.4, 205)	93.2 (36.7, 211)	0.271
Cotinine, nmol/L	1.57 (1, 1770)	1 (1, 1420)	1 (1, 1100)	1 (1, 1520)	10.6 (1, 1990)	893 (1, 2190)	<0.001
Creatinine, μmol/L	74 (53.5, 105)	72.8 (51.8, 101)	74.1 (53, 111)	75 (53.9, 107)	73.2 (53.6, 101)	71 (53.9, 94.8)	<0.001
eGFR, mL · min^{-1} · 1.73 m^{-2}	92.1 (60, 130)	95.2 (62, 133)	87.7 (53.3, 124)	89.7 (57.5, 126)	96.5 (64.9, 134)	103 (70.9, 136)	<0.001

[1]eGFR, estimated glomerular filtration rate; mNAM, N^1-methylnicotinamide; NAM, nicotinamide.
[2]*P* values were determined by Kruskal-Wallis test (across categories of coffee cups) or chi-square test (across categories of smoking and coffee types).
[3]Median; 5th, 95th percentile in parentheses (all such values).

follow-up by using baseline questionnaire data on amount of coffee consumed and smoking habits (not current compared with current smoking) and ln-transformed values and a random-effects mixed model with participant identification as the random variable. An ICC <0.40 is considered to represent poor reproducibility, 0.40–0.75 represents fair to good reproducibility, and >0.75 represents excellent reproducibility (35). We also calculated the Spearman correlations for trigonelline and cotinine for the 2 time points. For these analyses, complete data sets were available for 2630 participants for trigonelline and for 2591 participants for cotinine.

All of the statistical analyses were performed with the use of R version 3.2.3 for Windows (www.r-project-org). Adjusted Spearman correlations were computed by using the ppcor package, ROC curves by the pROC package, and ICCs by using the ICC package. *P* values <0.05 were considered significant.

RESULTS

Cohort characteristics

At baseline, the median (5th, 95th percentile) age was 62 y (44, 78 y), 72.7% were men, 26.6% of the participants were never smokers, 49.7% were former smokers, and 23.7% were current smokers (**Table 1**).

Coffee consumption

One or more cups of coffee were consumed per day by 94.2% of the participants, with ∼1 cup/d by 8.5% of participants, 2–4 cups by 53.8%, 5–8 cups by 26.2%, and >8 cups/d by 5.6% of participants (Table 1). The median (5th, 95th percentile) age decreased with increasing coffee consumption ($P < 0.001$). Men consumed more coffee than women ($P < 0.001$), and 86.7% of those who reported drinking >8 cups/d were men.

Filtered coffee was by far the most commonly consumed coffee type and was consumed by 88.7% of the participants who provided information on coffee type (**Supplemental Table 1**). The type of coffee consumed varied by age ($P = 0.026$) but not by sex ($P = 0.77$). The average number of cups of coffee per day varied by coffee type ($P < 0.001$; **Supplemental Table 2**), and for all types most individuals (51.9–68.3%) consumed 2–4 cups/d.

The mean (95% CI) number of cups per day consumed was lowest among the never smokers, followed by former smokers and current smokers ($P < 0.001$; **Supplemental Table 3**). Among those reporting no coffee consumption, 48% were never smokers, and this proportion gradually decreased to 2.6% of those drinking >8 cups/d (Table 1). Conversely, only 14.2% of those drinking no coffee were current smokers, increasing to 63.3% among those consuming >8 cups/d.

Biomarker concentrations according to coffee consumption

The median (5th, 95th percentile) plasma concentration of trigonelline was 0.29 μmol/L (0.05, 2.81 μmol/L) in those reporting no coffee consumption and increased according to cups consumed per day, as follows: ∼1 cup [1.63 μmol/L (0.16, 7.44 μmol) trigonelline/L], 2–4 cups [3.56 μmol/L (0.54, 12.1 μmol) trigonelline/L], 5–8 cups [4.71 μmol/L (0.83, 14.9

TABLE 2
Plasma concentrations of trigonelline, NAM, and mNAM at baseline according to coffee consumption, smoking, demographic characteristics, and renal function by linear regression[1]

	Trigonelline, μmol/L		NAM, nmol/L		mNAM, nmol/L	
	β	*P*	*β*	*P*	*β*	*P*
Intercept	14.46 (5.83, 35.85)	<0.001	497 (318, 777)	<0.001	631 (374, 1063)	<0.001
Ratio (compared with reference)						
Men vs. women	0.95 (0.87, 1.03)	0.23	1.03 (0.99, 1.07)	0.17	0.90 (0.86, 0.94)	<0.001
Age (per 10 y)	1.09 (1.05, 1.14)	<0.001	0.92 (0.91, 0.94)	<0.001	0.96 (0.94, 0.98)	<0.001
Fasting vs. nonfasting	0.80 (0.73, 0.88)	<0.001	1.12 (1.07, 1.17)	<0.001	1.23 (1.16, 1.29)	<0.001
Study center (Stavanger vs. Bergen)	0.96 (0.84, 1.09)	0.50	1.09 (1.02, 1.16)	0.01	0.76 (0.71, 0.82)	<0.001
Former smoker vs. never smoker	1.24 (1.13, 1.35)	<0.001	1.05 (1.00, 1.10)	0.03	1.07 (1.01, 1.12)	0.01
Current smoker vs. never smoker	1.48 (1.33, 1.65)	<0.001	1.02 (0.97, 1.07)	0.47	1.01 (0.95, 1.07)	0.86
1 cup of coffee/d vs. no coffee	3.67 (3.05, 4.41)	<0.001	0.97 (0.89, 1.07)	0.56	1.05 (0.94, 1.16)	0.39
2–4 cups of coffee/d vs. no coffee	8.37 (7.24, 9.67)	<0.001	0.98 (0.92, 1.06)	0.66	1.01 (0.93, 1.10)	0.79
5–8 cups of coffee/d vs. no coffee	11.33 (9.72, 13.21)	<0.001	0.96 (0.89, 1.03)	0.28	1.05 (0.96, 1.15)	0.26
>8 cups of coffee/d vs. no coffee	15.08 (12.28, 18.52)	<0.001	0.97 (0.87, 1.07)	0.51	1.14 (1.01, 1.28)	0.03
eGFR (ratio for doubling in eGFR)	0.52 (0.46, 0.59)	<0.001	1.00 (0.94, 1.06)	0.93	0.80 (0.74, 0.86)	<0.001

[1]Values are *β*s (95% CIs) given as ratios compared with the reference level for all predictors, except for eGFR, where the ratio for a doubling in eGFR is given. eGFR, estimated glomerular filtration rate; mNAM, N^1-methylnicotinamide; NAM, nicotinamide.

μmol) trigonelline/L], and >8 cups [5.80 μmol/L (1.24, 21.4 μmol) trigonelline/L] ($P < 0.001$) (Table 1). Plasma trigonelline [median (5th, 95th percentile) varied according to coffee type consumed ($P < 0.001$; Supplemental Table 1)].

In the regression model (**Table 2**), the number of coffee cups consumed per day was a strong predictor of plasma trigonelline concentrations. In the same model, plasma trigonelline was 20% lower in fasting compared with nonfasting participants and increased by 9% per 10 y of age (both $P < 0.01$). Former smokers showed 24% higher and current smokers 48% higher trigonelline concentrations than never smokers ($P < 0.01$), and a doubling in eGFR was associated with 48% lower trigonelline.

Plasma NAM varied slightly according to category of coffee cups per day ($P = 0.01$; Table 1) and was highest in those reporting no coffee consumption. mNAM showed no association with coffee consumption ($P = 0.27$). Neither of the niacin vitamers varied according to type of coffee consumed (Supplemental Table 1).

Among the coffee drinkers, plasma trigonelline was not related to NAM or mNAM by Spearman correlation but was moderately positively correlated with cotinine ($r = 0.25$, $P < 0.001$) and moderately inversely correlated with eGFR ($r = -0.18$, $P < 0.001$) (**Supplemental Table 4**). Among those reporting no consumption of coffee, trigonelline was not correlated with NAM, mNAM, cotinine, or eGFR (Supplemental Table 4).

Cutoff concentration for trigonelline by ROCs

From ROCs, the optimal cutoff for distinguishing consumers from nonconsumers of coffee was a trigonelline concentration of 1.01 μmol/L, with a sensitivity of 0.891, a specificity of 0.887, and an AUC of 0.92 (95% CI: 0.90, 0.94) (**Figure 1**). From ROC analysis of current compared with not-current smokers, the optimal cutoff was found at a cotinine concentration of 28.8 nmol/L, giving a sensitivity of 0.937, specificity of 0.898, and an AUC of 0.95 (95% CI: 0.94, 0.95).

Within-person reproducibility

For blood samples collected 1 y apart, the within-person reproducibility in terms of ICCs was 0.66 (95% CI: 0.64, 0.68) for trigonelline and 0.84 (95% CI: 0.83, 0.85) for cotinine (**Table 3**).

DISCUSSION

Principal findings

With the use of data from a large cohort of 3503 Norwegians we showed that plasma concentrations of trigonelline increased strongly according to self-reported amount of coffee consumed. By using ROCs, we obtained a trigonelline cutoff concentration of 1.01 μmol/L for separating nonconsumers from consumers of coffee, with specificity, sensitivity, and AUCs only slightly lower than those obtained for cotinine, an established marker of recent nicotine exposure (29). The within-person reproducibility (calculated as the ICC) of trigonelline was in the upper range of the fair to good category. We found no relation of the amount of coffee consumed with circulating concentrations of the niacin vitamers NAM and mNAM.

Coffee consumption

We observed that the majority of participants consumed an intermediate amount of coffee (2–4 cups) per day. This consumption amount is similar to that reported for another population from the same geographical region (southwestern) in Norway (24) but less than for a population from northern Norway (25). The present study confirms the results from previous studies on Norwegian populations, showing a higher prevalence of smoking (24–27) as well as being male (25) among participants with a high coffee consumption and that most coffee drinkers consume filtered coffee (24). We observed an inverse relation between age and amount of coffee consumed, which was not observed in a population from northern Norway (25).

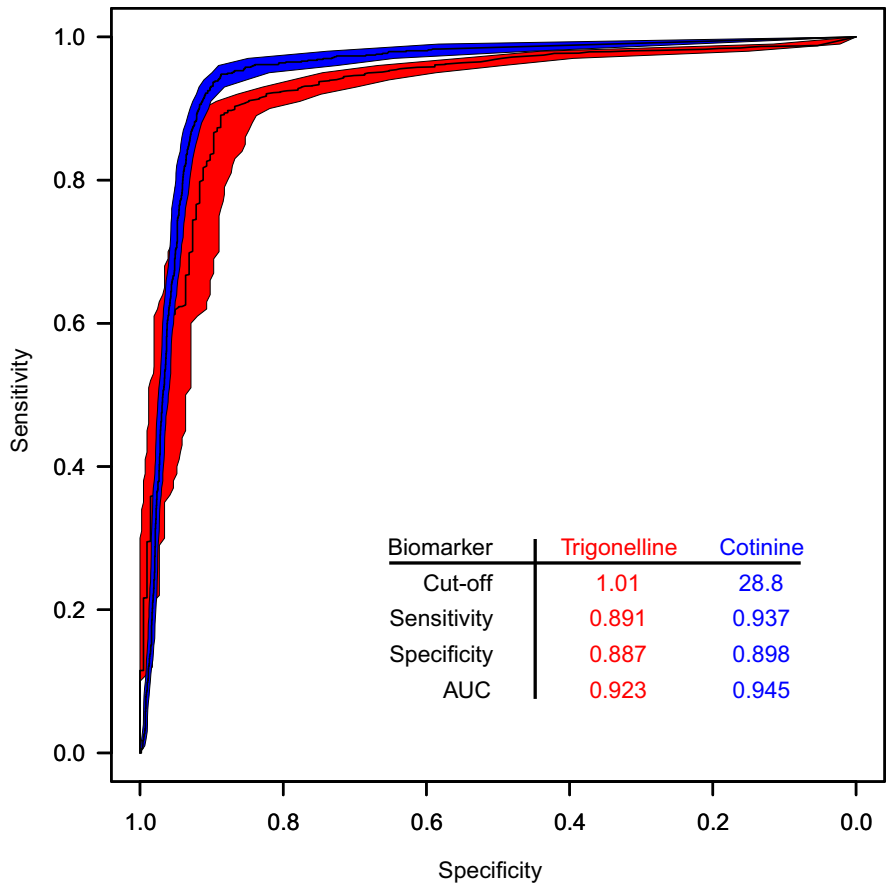

FIGURE 1 ROC analysis in 3503 patients from the WECAC for plasma trigonelline as a marker of coffee consumption (red) and plasma cotinine as marker of current smoking (blue). The curves are shown with 95% CIs. Cutoffs are given in μmol/L for trigonelline and nmol/L for cotinine. ROC, receiver operating characteristic; WECAC, Western Norway Coronary Angiography Cohort.

Plasma concentrations of trigonelline

The increase in plasma trigonelline with increasing number of cups of coffee consumed per day is in line with the positive association of trigonelline with the intake of coffee (16), as well as the strong increase in trigonelline observed after the consumption of a bolus dose of coffee (21). Results from ROC analysis showed that plasma trigonelline performed well as a marker of coffee consumption, approaching the performance of plasma cotinine as a marker of current smoking, as shown in the present and previous studies (29, 36). The ICC for plasma trigonelline over 1 y in this study (0.66) was similar to that found in another study (16) and for questionnaire data on coffee consumption over an 11-y time span (0.60) (4), but was lower than the ICCs reported for questionnaire data over a period of several months (0.77–0.85) (37). Combined, these results suggest that the amount of coffee consumed by individuals is relatively stable over time.

Possible sources of error that may influence the quality of plasma trigonelline as a marker of coffee consumption include the presence of small amounts of trigonelline in other food items, including fruit (14, 15), breakfast cereals (15, 22), and parboiled rice (22). Coffee-containing food items, such as chocolate cakes, might also contribute to plasma trigonelline concentrations. Accordingly, we and others (21) observed low concentrations of trigonelline in plasma from participants who reported no intake

TABLE 3
Concentrations and within-person reproducibility of trigonelline and cotinine in plasma samples collected at 2 time points 1 y apart

		Geometric mean (95% CI)				CV[2] (%)		
	Participants, *n*	Baseline	1 y	*P*[1]	Spearman *ρ*	Within-person	Between-person	ICC[3] (95% CI)
Trigonelline	2630	2.65 (2.56, 2.74)	4.11 (3.93, 4.30)	<0.001	0.63	71	98	0.66 (0.64, 0.68)
Cotinine	2591	10.58 (9.70, 11.53)	9.35 (8.33, 10.48)	0.19	0.79	120	273	0.84 (0.83, 0.85)

[1]Derived by using a paired *t* test comparing geometric mean at the first compared with the second blood collection.
[2]Within- and between-person CVs were estimated by taking the square root of the within- and between-person variance components from a random-effects mixed model on the log-transformed scale.
[3]ICC, intraclass correlation coefficient calculated by using log-transformed biomarker concentrations.

of coffee. Errors in self-reported consumption of coffee may also contribute to inconsistencies of questionnaire data with plasma trigonelline.

The observed differences in plasma trigonelline among coffee consumers may also be partly explained by varying trigonelline content of coffee beans of different types (12) and origins (38). It has also been suggested that the method used to prepare coffee may influence how much trigonelline is obtained from drinking coffee (14), because the conversion of trigonelline to nicotinic acid increases with increasing temperature and duration of roasting of coffee beans (22, 38). Individual differences in renal excretion of trigonelline have also been reported (21).

The within-subject reproducibility of trigonelline was lower than that found for cotinine, with ICCs for trigonelline in the upper range of the category of fair to good (0.40–0.75) and for cotinine in the excellent (≥0.75) category (35). Larger variability in trigonelline than in cotinine over time may reflect the shorter half-life of trigonelline, ~5 h (21), compared with 12–20 h for cotinine (34). This relatively short half-life of trigonelline may also explain the presence of low trigonelline concentrations in some participants who reported drinking coffee. Furthermore, fasting participants had lower plasma trigonelline concentrations than nonfasting participants, which may reflect longer time interval between coffee intake and blood draw in fasting individuals or the effect of food intake on trigonelline bioavailability and kinetics.

Many exposure biomarkers (39), including trigonelline (18, 20, 21), are excreted in the urine, and coffee consumption has also been found to be positively related to eGFR (40). The present work confirmed the published relations of circulating trigonelline and eGFR. We therefore included eGFR as an adjustment when modelling the relation of circulating trigonelline concentrations with self-reported coffee consumption. A number of morbidities are related to renal function, which should therefore be taken into account when investigating coffee consumption in relation to disease risk.

Plasma concentrations of niacin forms

The intake of nicotinic acid has been shown to significantly increase circulating concentrations of NAM (41). Thus, the lack of association of circulating niacin vitamers with coffee consumption in this study, and the small increase observed after the consumption of a bolus dose of coffee (21) suggest that coffee is, at most, a minor contributor to circulating concentrations of niacin.

Strengths and weaknesses

Errors in self-reported consumption of coffee may attenuate the association between reported coffee intake and plasma trigonelline, and could also partly explain the presence of trigonelline in plasma from participants who reported not drinking any coffee. Coffee is present in some food products, such as chocolate cakes, and trigonelline has been found in small amounts in some food items (14, 15, 22). We did not have information on intakes of several food items (14, 15, 22) containing small amounts of trigonelline. The time- and temperature-dependent conversion of trigonelline to nicotinic acid during the roasting of coffee beans (22, 23) may cause variable relations of coffee consumption with circulating trigonelline concentrations, and also attenuate the association. The roasting procedure also influences the content of several biologically active compounds (42), so the method of preparation is also an aspect to consider when investigating the health effects of coffee consumption.

Strengths of this work include a large, well-characterized cohort with information on both amount and type of coffee consumed. Plasma samples drawn 1 y apart enabled the calculation of intra- compared with interindividual variation. The availability of information on smoking and plasma cotinine concentrations enabled comparison of trigonelline with cotinine as markers of exposure in the same cohort.

Conclusions

Circulating concentrations of trigonelline were strongly related to the amount of coffee consumed, and its discriminating ability approaches the performance of cotinine as a marker of recent nicotine exposure. Thus, plasma trigonelline may serve as a useful marker of coffee consumption in epidemiologic studies. The use of a biomarker avoids the problems associated with inaccurate and variable categories in questionnaire data, and as a continuous variable it may also address the issue of variations in coffee-cup size. Continuous variables may provide more detailed information about exposure-outcome relations and also enhance the possibility of investigating nonlinear relations. The combined measurement of trigonelline and cotinine (28) allows for investigations of interaction effects of coffee consumption with smoking, which both are important lifestyle factors with known health effects.

The authors' responsbilities were as follows—PMU and ØM: led the laboratory analysis; ØM and AU: conducted the statistical analysis; ØM: drafted the first version of the manuscript. ON and PMU: initiated and are coordinators of WECAC and WENBIT; ØM: had primary responsibility for final content; and all authors: were involved with data collection and interpretation and critical revisions of the manuscript and read and approved the final manuscript. PMU is member of the steering board of the nonprofit Foundation to Promote Research into Functional Vitamin B12 Deficiency; none of the other authors reported any disclosures.

REFERENCES

1. Butt MS, Sultan MT. Coffee and its consumption: benefits and risks. Crit Rev Food Sci Nutr 2011;51(4):363–73.
2. Ding M, Bhupathiraju SN, Satija A, van Dam RM, Hu FB. Long-term coffee consumption and risk of cardiovascular disease: a systematic review and a dose-response meta-analysis of prospective cohort studies. Circulation 2014;129(6):643–59.
3. Gunter MJ, Murphy N, Cross AJ, Dossus L, Dartois L, Fagherazzi G, Kaaks R, Kuhn T, Boeing H, Aleksandrova K, et al. Coffee drinking and mortality in 10 European countries: a multinational cohort study. Ann Intern Med 2017;167(4):236–47.
4. Park SY, Freedman ND, Haiman CA, Le Marchand L, Wilkens LR, Setiawan VW. Association of coffee consumption with total and cause-specific mortality among nonwhite populations. Ann Intern Med 2017;167(4):228–35.
5. Ding M, Satija A, Bhupathiraju SN, Hu Y, Sun Q, Han J, Lopez-Garcia E, Willett W, van Dam RM, Hu FB. Association of coffee consumption with total and cause-specific mortality in 3 large prospective cohorts. Circulation 2015;132(24):2305–15.
6. Higdon JV, Frei B. Coffee and health: a review of recent human research. Crit Rev Food Sci Nutr 2006;46(2):101–23.
7. Ludwig IA, Clifford MN, Lean ME, Ashihara H, Crozier A. Coffee: biochemistry and potential impact on health. Food Funct 2014;5(8):1695–717.
8. Grosso G, Godos J, Galvano F, Giovannucci EL. Coffee, caffeine, and health outcomes: an umbrella review. Annu Rev Nutr 2017;37:131–56.

9. Kaaks R, Riboli E, Sinha R. Biochemical markers of dietary intake. In:Toniolo P, Boffetta P, Shuker DEG, Rothman N, Hulka B, Pearce N, editors. Application of biomarkers in cancer epidemiology. Lyon (France): International Agency for Research on Cancer; 1997. p. 103–26.
10. Hedrick VE, Dietrich AM, Estabrooks PA, Savla J, Serrano E, Davy BM. Dietary biomarkers: advances, limitations and future directions. Nutr J 2012;11:109.
11. Potischman N. Biologic and methodologic issues for nutritional biomarkers. J Nutr 2003;133(Suppl 3):875S–80S.
12. Stennert A, Maier HG. Trigonelline in coffee. II. Content of green, roasted and instant coffee. Z Lebensm Unters Forsch 1994;199(3):198–200.
13. Tice R. Trigonelline. In: E Zeiger, editor. Review of toxicological literature. NC, Durham: National Institute of Environmental Health Sciences; 1997. p. 27.
14. de Zwart FJ, Slow S, Payne RJ, Lever M, George PM, Gerrard JA, Chambers ST. Glycine betaine and glycine betaine analogues in common foods. Food Chem 2003;83:197–204.
15. Slow S, Donaggio M, Cressey PJ, Lever M, George PM, Chambers ST. The betaine content of New Zealand foods and estimated intake in the New Zealand diet. J Food Comp Anal 2005;18:473–85.
16. Guertin KA, Moore SC, Sampson JN, Huang WY, Xiao Q, Stolzenberg-Solomon RZ, Sinha R, Cross AJ. Metabolomics in nutritional epidemiology: identifying metabolites associated with diet and quantifying their potential to uncover diet-disease relations in populations. Am J Clin Nutr 2014;100(1):208–17.
17. Guertin KA, Loftfield E, Boca SM, Sampson JN, Moore SC, Xiao Q, Huang WY, Xiong X, Freedman ND, Cross AJ, et al. Serum biomarkers of habitual coffee consumption may provide insight into the mechanism underlying the association between coffee consumption and colorectal cancer. Am J Clin Nutr 2015;101(5):1000–11.
18. Playdon MC, Sampson JN, Cross AJ, Sinha R, Guertin KA, Moy KA, Rothman N, Irwin ML, Mayne ST, Stolzenberg-Solomon R, et al. Comparing metabolite profiles of habitual diet in serum and urine. Am J Clin Nutr 2016;104(3):776–89.
19. Playdon MC, Ziegler RG, Sampson JN, Stolzenberg-Solomon R, Thompson HJ, Irwin ML, Mayne ST, Hoover RN, Moore SC. Nutritional metabolomics and breast cancer risk in a prospective study. Am J Clin Nutr 2017;106(2):637–49.
20. Rothwell JA, Fillatre Y, Martin JF, Lyan B, Pujos-Guillot E, Fezeu L, Hercberg S, Comte B, Galan P, Touvier M, et al. New biomarkers of coffee consumption identified by the non-targeted metabolomic profiling of cohort study subjects. PLoS One 2014;9(4):e93474.
21. Lang R, Wahl A, Skurk T, Yagar EF, Schmiech L, Eggers R, Hauner H, Hofmann T. Development of a hydrophilic liquid interaction chromatography-high-performance liquid chromatography-tandem mass spectrometry based stable isotope dilution analysis and pharmacokinetic studies on bioactive pyridines in human plasma and urine after coffee consumption. Anal Chem 2010;82(4):1486–97.
22. Lang R, Yagar EF, Eggers R, Hofmann T. Quantitative investigation of trigonelline, nicotinic acid, and nicotinamide in foods, urine, and plasma by means of LC-MS/MS and stable isotope dilution analysis. J Agric Food Chem 2008;56(23):11114–21.
23. Teply LJ, Prier RF. Nutrients in coffee, nutritional evaluation of coffee including niacin bioassay. J Agric Food Chem 1957;5(5):375–7.
24. Nygard O, Refsum H, Ueland PM, Stensvold I, Nordrehaug JE, Kvale G, Vollset SE. Coffee consumption and plasma total homocysteine: the Hordaland Homocysteine Study. Am J Clin Nutr 1997;65(1):136–43.
25. Enga KF, Braekkan SK, Hansen-Krone IJ, Wilsgaard T, Hansen JB. Coffee consumption and the risk of venous thromboembolism: the Tromso study. J Thromb Haemost 2011;9(7):1334–9.
26. Lukic M, Licaj I, Lund E, Skeie G, Weiderpass E, Braaten T. Coffee consumption and the risk of cancer in the Norwegian Women and Cancer (NOWAC) Study. Eur J Epidemiol 2016;31(9):905–16.
27. Ulvik A, Vollset SE, Hoff G, Ueland PM. Coffee consumption and circulating B-vitamins in healthy middle-aged men and women. Clin Chem 2008;54(9):1489–96.
28. 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 Sp 2009;23:1371–9.
29. Seccareccia F, Zuccaro P, Pacifici R, Meli P, Pannozzo F, Freeman KM, Santaquilani A, Giampaoli S; Research Group of the MATISS Project. Serum cotinine as a marker of environmental tobacco smoke exposure in epidemiological studies: the experience of the MATISS project. Eur J Epidemiol 2003;18(6):487–92.
30. 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(6):1417–25.
31. Ebbing M, Bleie O, Ueland PM, Nordrehaug JE, Nilsen DW, Vollset SE, Refsum H, Pedersen EK, Nygard O. Mortality and cardiovascular events in patients treated with homocysteine-lowering B vitamins after coronary angiography: a randomized controlled trial. JAMA 2008;300(7):795–804.
32. Midttun O, Kvalheim G, Ueland PM. High-throughput, low-volume, multianalyte quantification of plasma metabolites related to one-carbon metabolism using HPLC-MS/MS. Anal Bioanal Chem 2013;405(6):2009–17.
33. Lameire N, Adam A, Becker CR, Davidson C, McCullough PA, Stacul F, Tumlin J; Contrast-Induced Nephropathy Consensus Working Panel. Baseline renal function screening. Am J Cardiol 2006;98(6A):21K–6K.
34. Kim S. Overview of cotinine cutoff values for smoking status classification. Int J Environ Res Public Health 2016;13(12):1236.
35. Rosner B. One-way ANOVA—the random-effects model. In: Fundamentals of biostatistics. Belmont (CA): Duxbury; 2006. p. 613–8.
36. Benowitz NL, Bernert JT, Caraballo RS, Holiday DB, Wang J. Optimal serum cotinine levels for distinguishing cigarette smokers and nonsmokers within different racial/ethnic groups in the United States between 1999 and 2004. Am J Epidemiol 2009;169(2):236–48.
37. Saaksjarvi K, Knekt P, Mannisto S, Heliovaara M. Self-administered questionnaire is a reliable measure of coffee consumption. J Epidemiol 2010;20(5):363–9.
38. Minamisawa M, Yoshida S, Takai N. Determination of biologically active substances in roasted coffees using a diode-array HPLC system. Anal Sci 2004;20(2):325–8.
39. Savitz DA, Wellenius GA. Exposure biomarkers indicate more than just exposure. Am J Epidemiol 2017;187(4):803–5.
40. Herber-Gast GC, van Essen H, Verschuren WM, Stehouwer CD, Gansevoort RT, Bakker SJ, Spijkerman AM. Coffee and tea consumption in relation to estimated glomerular filtration rate: results from the population-based longitudinal Doetinchem Cohort Study. Am J Clin Nutr 2016;103(5):1370–7.
41. Menon RM, Gonzalez MA, Adams MH, Tolbert DS, Leu JH, Cefali EA. Effect of the rate of niacin administration on the plasma and urine pharmacokinetics of niacin and its metabolites. J Clin Pharmacol 2007;47(6):681–8.
42. Esquivel P, Jiménez VM. Functional properties of coffee and coffee by-products. Food Res Int 2012;46:488–95.