Evaluation of various biomarkers as potential mediators of the association between coffee consumption and incident type 2 diabetes in the EPIC-Potsdam Study[1–3]

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ABSTRACT

Background: The inverse association between coffee consumption and the risk of type 2 diabetes (T2D) is well established; however, little is known about potential mediators of this association.

Objective: We aimed to investigate the association between coffee consumption and diabetes-related biomarkers and their potential role as mediators of the association between coffee consumption and T2D.

Design: We analyzed a case-cohort study (subcohort: n = 1610; verified incident T2D cases: n = 417) nested within the European Prospective Investigation into Cancer and Nutrition–Potsdam study involving 27,548 middle-aged participants. Habitual coffee consumption was assessed with a validated, semiquantitative food-frequency questionnaire. We evaluated the association between coffee consumption and several T2D-related biomarkers, such as liver markers (reflected by γ-glutamyltransferase, fetuin-A, and sex hormone–binding globulin), markers of dyslipidemia (high-density lipoprotein cholesterol and triglycerides), inflammation [C-reactive protein (CRP)], an adipokine (adiponectin), and metabolites, stratified by sex.

Results: Coffee consumption was inversely associated with diacyl-phosphatidylcholine C32:1 in both sexes and with phenylalanine in men, as well as positively associated with acyl-alkyl-phosphatidylcholines C34:3, C40:6, and C42:5 in women. Furthermore, coffee consumption was inversely associated with fetuin-A (P-trend = 0.06) and CRP in women and γ-glutamyltransferase and triglycerides in men. Coffee consumption tended to be inversely associated with T2D risk in both sexes, reaching significance only in men [HR (95% CI): women: ≥4 compared with >0 to <2 cups coffee/d: 0.78 (0.46, 1.33); men: ≥5 compared with >0 to <2 cups coffee/d: 0.40 (0.19, 0.81)]. The association between coffee consumption and T2D risk in men was slightly reduced after adjustment for phenylalanine or lipid markers.

Conclusions: Coffee consumption was inversely associated with a diacyl-phosphatidylcholine and liver markers in both sexes and positively associated with certain acyl-alkyl-phosphatidylcholines in women. Furthermore, coffee consumption showed an inverse trend with CRP in women and with triglycerides and phenylalanine in men. However, these markers explained only to a small extent the inverse association between long-term coffee consumption and T2D risk. *Am J Clin Nutr* doi: 10.3945/ajcn.113.080317.

INTRODUCTION

Brewed coffee is among the most widely consumed beverages in the world (1). During recent years, a number of epidemiologic studies have found an inverse relationship between consumption of coffee and the risk of developing type 2 diabetes (T2D)[4] (2).

The association does not depend on race, sex, obesity, or region of the study population (2). Explanations for the protective effect of coffee consumption on the risk of T2D have been related to varying coffee components such as magnesium, lignans, and chlorogenic acid (2). However, little is known about the underlying biologic mechanisms of this association.

A meta-analysis of intervention studies suggests that consumption of unfiltered, but not filtered, coffee increases serum concentrations of triglycerides (3). Evidence from clinical trials suggests furthermore that coffee intake increases adiponectin (4–6), decreases fetuin-A concentrations (5), and has no effect on C-reactive protein (CRP) (5–7), the sex hormone–binding globulin (SHBG)

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[4] Abbreviations used: CRP, C-reactive protein; EPIC, European Prospective Investigation into Cancer and Nutrition; FFQ, food-frequency questionnaire; GGT, γ-glutamyltransferase; SHBG, sex hormone–binding globulin; T2D, type 2 diabetes.

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(8), or HDL cholesterol (3). However, these intervention studies are limited by their short durations and high coffee dosages.

In prospective studies, adjustment for total cholesterol and triglycerides (9) or SHBG (10) led to an attenuation of the association between coffee intake and T2D risk, suggesting that this association might be partly mediated by dyslipidemia or SHBG. To our knowledge, a comprehensive investigation of the association between coffee consumption and diabetes-related biomarkers reflecting different biologic pathways under real-life conditions is lacking. Moreover, a potential mediating role of various biomarkers on the inverse association between coffee consumption and T2D risk has not been investigated. Identification of potential mediators can help to develop hypotheses regarding which metabolic pathways may play a role in the association between coffee consumption and T2D risk.

Therefore, the aim of this study was to evaluate the association between coffee consumption and various diabetes-related biomarkers, reflecting different metabolic pathways, including biomarkers indicating liver function, dyslipidemia, inflammation, adipokines, and several T2D-related metabolites (amino acids, phosphatidylcholines, and sphingomyelins), and their potential mediating role in linking coffee consumption to T2D risk.

MATERIALS AND METHODS

Study population

The European Prospective Investigation into Cancer and Nutrition (EPIC)–Potsdam study is part of the multicenter prospective cohort study EPIC, which was intended to investigate the association between nutrition and risk of cancer and other chronic diseases (11). EPIC-Potsdam included 16,644 women and 10,904 men recruited from the general population of the city of Potsdam, Germany, and surrounding municipalities from 1994 to 1998. The age criterion was mainly 35–64 y in women and 40–64 y in men. The baseline assessment included the collection of blood samples, anthropometric measurements, a self-administered validated food-frequency questionnaire (FFQ), and questions on sociodemographic and lifestyle characteristics. Information on education, smoking, and physical activity was assessed at baseline with a self-administered questionnaire and a personal computer-guided interview (11). Anthropometric measurement procedures followed a standardized protocol (11). Informed consent was obtained from all participants, and approval was given by the Ethics Committee of the Medical Association of the State of Brandenburg (Germany). Within the EPIC-Potsdam study, a case-cohort study was designed. For biochemical measurements, a random sample of 2500 participants was drawn from all participants in EPIC-Potsdam who provided a blood sample (n = 26,444) (12). For the present analysis, participants with treated hypertension (n = 367) were excluded because they consumed less coffee than those without hypertension in our study, suggesting that these participants changed their coffee consumption as a consequence of their diagnosis. Furthermore, it was considered likely that people in poor health as well as particularly health-conscious people might refrain from coffee drinking. To minimize bias, we therefore excluded non–coffee drinkers (n = 74) in this analysis. After exclusion of prevalent or nonverified cases of T2D (n = 120), participants with an uncompleted follow-up questionnaire (n = 58), those taking liver or lipid-lowering medication (n = 72), participants with insufficient filled blood monovettes (n = 126), and individuals with missing or implausible values for biomarkers (n = 73), 1610 participants remained for our analysis. During a mean follow-up time of 7 y, 801 incident cases of T2D were identified in the full cohort. Of these, 417 (254 men and 163 women) remained for analyses after identical exclusion criteria were applied. The age range included participants 20.2–67 y with a mean age of 49.7 y. Because the subcohort was representative of the full cohort at baseline, it included 35 individuals (18 men and 17 women) who developed T2D during follow-up (so-called internal cases in case-cohort studies).

Assessment of coffee consumption

The EPIC-Potsdam participants were questioned about their habitual coffee consumption in the self-administered semiquantitative FFQ at baseline. The questions were as follows: "How frequently did you consume coffee with caffeine during the previous 12 months?" and "How frequently did you consume coffee without caffeine during the previous 12 months?" Ten frequency categories were provided, ranging from "never" to "5 times/day or more." Furthermore, the usual portion size was assessed, with categories ranging from 0.5 to 3 cups (1 cup was defined as 150 mL).

From the frequency and portion size, the usual consumption of caffeinated and decaffeinated coffee was estimated. Because findings from prospective studies of regular and decaffeinated coffee suggest that any protective effects of coffee are unlikely to be solely effects of caffeine but rather likely to involve a broader range of chemical constituents, such as magnesium, lignans, and chlorogenic acids (2), the overall habitual coffee consumption was calculated as the sum of decaffeinated and caffeinated coffee intake and considered the exposure variable. Data from the German part of the EPIC project suggest that the FFQ allows us to estimate coffee consumption reliably (Spearman correlation coefficient, r = 0.71) and in comparison with repeated 24-h recalls with a high relative validity (r = 0.70) (13).

Measurement of biomarkers

For the current analysis, we considered those biomarkers that have been shown to be mainly associated with T2D risk in the EPIC-Potsdam study (12, 14–18): liver markers [such as γ-glutamyltransferase (GGT) and fetuin-A), biomarkers of dyslipidemia (such as HDL cholesterol and triglycerides), biomarkers of inflammation [such as C-reactive protein (CRP)], an adipokine (adiponectin), and several metabolites (amino acids; diacyl-, acyl-alkyl-, and lyso-phosphatidylcholines; and sphingomyelins). Furthermore, SHBG was investigated, given the strong evidence for an association with T2D risk in a Mendelian randomization analysis (19).

During the baseline examination, 30 mL of peripheral venous blood was obtained from each participant, mostly in the nonfasting status. Plasma, serum, red blood cells, and buffy coat were separated by centrifugation (1000 $\times$ g, 10 min, 4°C) and stored at −80°C or lower.

Measurement of plasma HDL cholesterol, triglycerides, GGT, CRP, and fetuin-A followed standard procedures, which are described in detail elsewhere (12). Plasma total adiponectin

concentrations were determined by an enzyme-linked immunosorbent assay (Linco Research). The content of SHBG was determined by an IMMULITE 2000 (Siemens Healthcare Diagnostics Products Ltd). SHBG was analyzed via a solid 2-phase chemiluminescence immunometric assay (detection limit SHBG >180 nmol/L). SHBG values that exceeded the detection limit (n = 17) were substituted with 181 nmol/L. All assays were performed according to the manufacturer's description. Plasma concentrations were multiplied by 1.16 for women and 1.17 for men to obtain concentrations for these citrate plasma samples comparable to those obtainable from EDTA plasma. These factors were based on the hypothesis that, on average, the hematocrit was 0.44 in men and 0.40 in women—similar to the mean hematocrit observed in the German National Health Interview and Examination Survey in 1998 (Robert Koch Institute, unpublished data)—and that, after centrifugation of the blood with anticoagulant, all citrate was in the plasma (20).

Metabolomic measurements were performed in the Genome Analysis Center at the Helmholtz Center Munich. Concentrations of serum metabolites were determined with the Absolute*IDQ* p150 kit (BIOCRATES Life Sciences AG) by using flow injection analysis tandem mass spectrometry. Sample preparation was done according to the manufacturer's protocol (BIOCRATES user's manual UM-P150) and has been described previously (21–23). The metabolomic method simultaneously quantified 163 metabolites. The reliability of 163 serum metabolite concentrations was measured over a 4-mo period and showed an overall good reliability, with a median intraclass correlation coefficient of 0.57 (23). To ensure valid measurements, metabolites below the limit of detection (n = 30) and those with very high analytic variance (n = 6) were excluded, leaving 127 metabolites (17, 23).

Definition of cases of T2D

Every 2–3 y, follow-up questionnaires were sent to participants to identify incident cases of T2D, with response rates of ~95% (24, 25). For this analysis, we considered the data until the end of the fourth follow-up period (August 2005). Systematic information sources for incident cases were self-reports of a T2D diagnosis, T2D-relevant medication, and dietary treatment because of T2D during follow-up. Furthermore, we obtained additional information from death certificates or from random sources, such as a tumor center, physician, or clinic, which provided assessments from other diagnoses. Although self-reports of T2D were generally reliable (26), by including other sources of information, we even improved the completeness of case ascertainment. Once an individual was identified as a potential case, disease status was further verified by sending a standard inquiry form to the treating physician. Only physician-verified cases with a diagnosis of T2D (*International Classification of Diseases, 10th Revision*, code E11) and a diagnosis date after the baseline examination were considered as incident cases in the present analysis.

Statistical methods

The study population was stratified by sex because significant cross-product terms of coffee consumption and sex were detected in the multivariable-adjusted models analyzing associations between coffee consumption and biomarkers (phenylalanine and glycine). Furthermore, in previous studies, sex-dependent associations of coffee consumption and biomarkers were detected (27, 28). Male study participants were divided into 5 coffee consumption categories (>0 to <2, 2 to <3, 3 to <4, 4 to <5, and ≥5 cups/d). Female study participants were divided into 4 coffee consumption categories (>0 to <2, 2 to <3, 3 to <4, and ≥4 cups/d) because of the low number of cases of T2D in women consuming ≥5 cups in our study (n = 18).

A cross-sectional analysis restricted to the subcohort (n = 1610) was performed to investigate associations between coffee consumption and biomarkers by using multiple linear regression. Analyses of SHBG were restricted to men and postmenopausal women, because an association with T2D has been suggested only in these subgroups (19). From the panel of 127 metabolites, we evaluated 13 of the 14 metabolites that have been independently associated with T2D risk in EPIC-Potsdam (17)—namely, phenylalanine; diacyl-phosphatidylcholines C32:1, C36:1, C38:3, and C40:5 and serum glycine; sphingomyelin C16:1; acyl-alkyl-phosphatidylcholines C34:3, C40:6, C42:5, C44:4, and C44:5; and lyso-phosphatidylcholine C18:2. Because the focus of this analysis was on upstream mediators to elucidate different pathways, hexose was excluded in the present analysis. Regarding the associations between coffee consumption and biomarkers, we conducted 3 sensitivity analyses. First, we restricted the analysis to participants consuming black coffee only. Second, we excluded all consumers of decaffeinated coffee. Furthermore, we included cross-product terms of coffee consumption and fasting status in the multivariable-adjusted models by analyzing associations between coffee consumption and biomarkers. For cross-product terms that were statistically significant, we investigated associations between coffee consumption and respective biomarkers (triglycerides, phenylalanine, diacylphosphatidylcholines C36:1 and C38:3, and lyso-phosphatidylcholine C18:2) in an analysis restricted to fasted participants.

In addition, the associations between coffee consumption and 113 metabolites not independently associated with T2D risk (17) were investigated in an exploratory approach. Correlations between these metabolites and coffee have been investigated in a recent analysis of EPIC-Potsdam but with a focus on metabolite networks (29). Taking into account the exploratory nature of this analysis, the P value from the multiple linear regression was adjusted for multiple testing by the Bonferroni-Holm method (30) (n = 113), and a corrected $P < 0.05$ (2-tailed testing) was considered significant.

Unless otherwise stated, metabolite concentrations were z score standardized [mean 0 (SD 1)] and Box-Cox transformed to ensure comparability of the results. We used the $\log_e$ transformation of HDL cholesterol, triglycerides, SHBG, adiponectin, and CRP and the exp ($\log_e$) transformation of GGT to normalize the right-skewed distributions. Two different models were calculated, including an age-adjusted model and a model also adjusted for the following variables to account for potential confounding: alcohol intake (g/d), smoking status (never, past, current <20 units/d, or current ≥20 units/d), education status (in or no training, vocational training, technical school, or technical college or university degree), leisure-time sport activities (no sports, ≤4 h/wk, or >4 h/wk), biking (no biking, <2.5 h/wk, 2.5–4.9 h/wk, or ≥5 h/wk), hormone use in women

(none, oral contraceptive, or hormone replacement therapy), caffeinated tea intake (mL/d), total energy intake (kcal/d), whole-grain bread intake (g/d), red meat intake (g/d), processed meat intake (g/d), BMI (kg/m^2), and waist circumference (cm).

Biomarkers associated with coffee consumption in this cross-sectional analysis were subsequently evaluated as potential mediators in a longitudinal analysis. We used Cox proportional hazards regression analysis stratified by age and adapted for the case-cohort design by the weighting method described by Prentice and Self (31) to estimate multivariable-adjusted HRs and 95% CIs, with age as the primary time-dependent variable (entry and exit time of each participant was defined as the age at recruitment and age at first event of T2D diagnosis, death, or return of the last follow-up questionnaire). Participants with a coffee consumption of >0 to <2 cups/d were used as a reference group. Cox regression models were calculated with and without adjustment for potential mediators to study to which extent the adjustment attenuated the association between coffee consumption and T2D risk. Differences in β-coefficients obtained from the multivariable-adjusted model (reference) and the multivariable-adjusted model also adjusted for potential mediators were calculated and tested for statistical significance [according to Hoffmann et al (32)]. The proportional hazards assumption of the Cox model was tested by plotting Schoenfeld residuals (33).

We performed the statistical analyses with SAS software, release 9.2 (SAS Institute). All statistical tests were 2-tailed except the comparison of the β-coefficients, which was 1-tailed. We considered $P < 0.05$ as statistically significant.

RESULTS

Study participants in the subcohort had a mean ± SD coffee consumption of 3.14 ± 2.10 cups/d. In general, men consumed more coffee (3.38 ± 2.39 cups/d) than did women (3.01 ± 1.91 cups/d).

Baseline characteristics of the participants according to coffee consumption are displayed in **Table 1**. Heavier coffee consumers of both sexes tended to be less educated, were more likely to be heavy smokers, consumed less caffeinated tea and whole-grain bread, and had a higher intake of red meat and processed meat. In both sexes, heavy coffee consumers displayed higher concentrations of plasma triglycerides. Women with high coffee consumption also had higher concentrations of CRP and lower concentrations of SHBG and fetuin-A compared with women with low coffee consumption.

Associations between coffee consumptions and T2D-related biomarkers

Multivariable-adjusted means and 95% CIs of biomarkers by coffee categories stratified by sex are presented in **Table 2**. Age-adjusted means are displayed in Supplemental Table 1 (under "Supplemental data" in the online issue).

Regarding the multivariable-adjusted model, coffee consumption was significantly inversely associated with serum diacyl-phosphatidylcholine C32:1 in both sexes; significantly inversely associated with plasma GGT, phenylalanine, and triglycerides in men; and significantly inversely associated with plasma CRP and nonsignificantly inversely associated with fetuin-A (P-trend = 0.06) in women. Furthermore, coffee consumption showed a nonsignificant inverse trend with serum diacyl-phosphatidylcholines C36:1 (P-trend = 0.05) and C40:5 (P-trend = 0.09) in men.

Coffee consumption was significantly positively associated with serum acyl-alkyl-phosphatidylcholines C34:3, C40:6, and C42:5 in women. Coffee consumption showed a nonsignificant positive trend with plasma adiponectin (P-trend = 0.08) and serum glycine (P-trend = 0.06) in women.

Sensitivity analyses

Restricting participants to those who were fasted at baseline or those who consumed caffeinated coffee yielded similar results (data not shown). Regarding the restriction to fasted participants, we considered only those biomarkers that had a significant interaction term with the cross-product of coffee consumption and fasting status (lyso-phosphatidylcholine C18:2, diacyl-phosphatidylcholines C36:1 and C38:3, phenylalanine, and triglycerides). Restricting participants to those who consumed black coffee only, effects were qualitatively comparable except that coffee consumption showed a positive trend with glycine in both sexes (data not shown).

Exploratory analyses

When evaluating metabolites not associated with T2D risk in an exploratory approach, coffee consumption was positively associated with most serum sphingomyelins, particularly in men, and most acyl-alkyl-phosphatidylcholines in both sexes. Coffee consumption was not clearly associated with acylcarnitines or lyso-phosphatidylcholines in one direction but, particularly in men, inversely associated with most amino acids and diacyl-phosphatidylcholines. However, after adjustment for multiple testing, none of the associations with any of these 113 metabolites remained significant (*see* Supplemental Table 2 under "Supplemental data" in the online issue).

Mediator analyses

Coffee consumption showed an inverse trend with T2D risk in both sexes, reaching significance only in men (**Table 3**). The multivariable-adjusted HR of T2D was 0.78 (95% CI: 0.46, 1.33; $P = 0.36$) for women who drank ≥4 compared with >0 to <2 cups coffee/d and 0.40 (95% CI: 0.19, 0.81; $P = 0.01$) for men consuming ≥5 compared with >0 to <2 cups coffee/d. We next evaluated single biomarkers and their combination as potential mediators by adjustment for these markers in Cox models.

Among men, a considerable part of the association between coffee consumption and T2D risk was not explained after adjustment for biomarkers. Significant attenuations of the association between coffee consumption and T2D risk in men were found after adjustment for CRP, GGT, phenylalanine, and HDL cholesterol or after simultaneous adjustment for HDL cholesterol and triglycerides. However, these attenuations were only modest in magnitude.

The association between coffee consumption and T2D among women was not significant in the first place. Furthermore, β-coefficients were not statistically significantly different after additional adjustment for biomarkers in any of the models.

TABLE 1
Characteristics of a subsample from the EPIC-Potsdam cohort by coffee categories in women and men[1]

	Women (n = 1032)				Men (n = 578)				
	>0 to <2 cups/d	≥2 to <3 cups/d	≥3 to <4 cups/d	≥4 cups/d	>0 to <2 cups/d	≥2 to <3 cups/d	≥3 to <4 cups/d	≥4 to <5 cups/d	≥5 cups/d
n (subcohort)	184	300	208	340	116	132	109	110	111
Age (y)	45.1 (16.6)[2]	45.6 (14.7)	45.3 (15.2)	48.7 (17.0)	50.9 (14.9)	48.4 (14.3)	48.0 (12.0)	53.3 (13.3)	49.0 (14.2)
BMI (kg/m^2)	23.4 (5.85)	24.1 (4.46)	24.7 (4.74)	24.6 (5.62)	25.3 (4.15)	26.1 (3.67)	25.6 (3.72)	26.1 (4.94)	26.5 (4.98)
Waist circumference (cm)	75.5 (15.5)	76.0 (13.5)	78.0 (15.0)	77.8 (15.0)	91.4 (11.5)	90.4 (10.8)	89.5 (10.5)	92.0 (13.0)	94.0 (13.5)
Leisure-time sport activity (h/wk)	0.00 (2.00)	0.00 (1.50)	0.00 (1.50)	0.00 (1.75)	0.00 (2.00)	0.00 (1.00)	0.00 (3.00)	0.00 (2.00)	0.00 (1.00)
Biking (h/wk)	1.00 (3.00)	0.50 (2.50)	0.50 (2.50)	0.50 (2.50)	1.00 (2.50)	0.50 (2.00)	1.00 (2.50)	1.00 (2.00)	0.50 (2.00)
Alcohol intake (g/d)	3.90 (7.90)	5.14 (8.22)	5.42 (8.83)	6.09 (10.8)	15.6 (21.0)	20.4 (24.6)	19.1 (28.9)	15.0 (23.0)	17.0 (27.4)
Energy intake (kcal/d)	1780 (616)	1823 (704)	1832 (724)	1884 (759)	2343 (872)	2313 (865)	2436 (841)	2435 (917)	2686 (1057)
Caffeinated tea intake (mL/d)	302 (472)	162 (277)	74.8 (208)	109 (266)	300 (368)	152 (279)	59.6 (232)	96.2 (242)	49.3 (135)
Whole-grain bread intake (g/d)	41.9 (69.8)	32.3 (56.3)	24.9 (51.0)	28.9 (60.7)	22.6 (48.8)	18.5 (54.4)	20.2 (46.2)	15.3 (54.0)	17.7 (55.9)
Red meat intake (g/d)	26.3 (24.5)	27.5 (22.7)	33.7 (27.7)	34.3 (25.3)	46.2 (35.1)	40.5 (32.2)	47.7 (34.9)	51.6 (38.8)	57.3 (36.6)
Processed meat intake (g/d)	39.6 (33.7)	44.2 (34.7)	44.7 (36.5)	44.4 (34.7)	58.9 (55.4)	62.9 (65.7)	66.2 (61.6)	75.5 (63.6)	73.8 (72.2)
Smoker ≥20 cigarettes/d (%)	2.72	1.33	4.81	5.59	6.90	5.30	9.17	12.7	25.2
Technical college/university (%)	35.9	35.0	34.1	23.5	53.5	56.8	58.7	58.2	47.8
Participants who provided a blood sample in the fasted state (%)	9.78	10.0	15.4	13.2	17.2	22.7	11.0	18.2	9.91
Hormone replacement therapy (%)	17.9	20.7	21.6	25.3	—	—	—	—	—
Contraceptive use (%)	16.9	20.7	17.3	15.0	—	—	—	—	—
Biomarkers									
CRP (mg/L)	0.70 (2.26)	0.81 (1.97)	0.70 (2.44)	0.81 (1.68)	0.59 (0.88)	0.59 (1.29)	0.35 (1.05)	0.59 (1.17)	0.59 (1.64)
Adiponectin (μg/mL)	9.14 (4.11)	9.11 (5.47)	8.97 (5.19)	9.72 (5.36)	6.15 (3.07)	5.63 (3.50)	6.02 (3.06)	6.16 (3.96)	6.10 (4.00)
GGT (U/L)	12.8 (11.0)	12.8 (11.6)	12.8 (11.6)	12.8 (10.4)	27.5 (29.8)	26.9 (30.4)	23.4 (28.1)	25.7 (26.9)	26.9 (26.9)
Fetuin-A (μg/mL)[3]	276 ± 62.5	273 ± 66.1	273 ± 65.8	263 ± 58.0	263 ± 52.3	258 ± 57.8	263 ± 52.4	261 ± 57.9	266 ± 54.7
SHBG (nmol/L)[4]	65.7 (48.5)	63.8 (30.9)	48.4 (22.5)	56.6 (36.1)	35.1 (22.0)	35.3 (19.1)	39.0 (22.4)	34.8 (17.1)	36.9 (18.4)
Phenylalanine (μmol/L)	54.6 (14.3)	54.4 (14.0)	54.0 (12.9)	53.8 (12.1)	54.8 (15.0)	57.4 (15.5)	54.7 (12.9)	54.6 (11.7)	53.2 (13.7)
Glycine (μmol/L)	255 (108)	241 (94.5)	242 (112)	256 (101)	238 (65.5)	243 (66.5)	236 (60.0)	236 (63.0)	224 (65.0)
PC aa C32:1 (μmol/L)	15.0 (10.4)	15.0 (10.3)	13.8 (9.20)	14.8 (10.0)	14.0 (8.85)	14.3 (11.0)	14.1 (8.47)	13.2 (8.53)	14.5 (8.50)
PC aa C36:1 (μmol/L)	54.2 (18.3)	53.4 (17.5)	54.6 (16.5)	56.2 (16.9)	54.0 (18.5)	55.4 (15.1)	55.7 (15.6)	54.3 (22.9)	57.5 (15.0)
PC aa C38:3 (μmol/L)	50.8 (19.6)	53.4 (17.5)	53.0 (18.0)	53.4 (19.1)	51.3 (19.7)	52.2 (15.5)	50.7 (14.5)	52.7 (16.8)	52.9 (16.7)
PC aa C40:5 (μmol/L)	10.5 (3.67)	10.0 (4.18)	10.5 (4.16)	10.6 (4.37)	11.5 (4.80)	11.2 (3.99)	11.2 (4.09)	11.3 (4.32)	11.4 (3.71)
SM C16:1 (μmol/L)	17.7 (4.70)	17.1 (4.95)	17.7 (4.25)	17.8 (5.75)	15.7 (4.30)	16.7 (4.30)	16.4 (4.00)	16.7 (3.40)	16.9 (4.60)
PC ae C34:3 (μmol/L)[3]	8.73 ± 2.53	9.01 ± 2.55	8.77 ± 2.24	9.16 ± 2.50	7.70 ± 2.38	8.03 ± 2.36	8.36 ± 2.28	8.17 ± 2.24	8.08 ± 2.31
PC ae C40:6 (μmol/L)	5.38 (1.60)	5.51 (1.87)	5.38 (1.81)	5.62 (2.06)	5.02 (1.64)	5.34 (1.56)	5.16 (1.90)	5.04 (1.52)	5.07 (1.48)
PC ae C42:5 (μmol/L)	2.32 (0.74)	2.46 (0.75)	2.35 (0.59)	2.44 (0.69)	2.17 (0.56)	2.30 (0.75)	2.24 (0.56)	2.20 (0.65)	2.17 (0.60)
PC ae C44:4 (μmol/L)	0.39 (0.13)	0.40 (0.15)	0.39 (0.12)	0.39 (0.13)	0.34 (0.11)	0.37 (0.12)	0.36 (0.10)	0.34 (0.11)	0.35 (0.10)
PC ae C44:5 (μmol/L)	1.75 (0.58)	1.81 (0.70)	1.74 (0.55)	1.86 (0.60)	1.60 (0.48)	1.76 (0.61)	1.69 (0.59)	1.70 (0.68)	1.62 (0.55)
Lyso-PC a C18:2 (μmol/L)[3]	34.3 ± 13.6	33.3 ± 12.6	32.9 ± 12.2	34.3 ± 13.4	39.7 ± 16.5	39.4 ± 14.1	40.4 ± 13.4	39.6 ± 15.5	40.1 ± 13.4
HDL cholesterol (mg/dL)	59.5 (18.7)	61.6 (19.8)	57.7 (18.1)	59.3 (19.2)	48.8 (13.7)	49.5 (14.2)	50.3 (15.7)	49.8 (19.2)	48.8 (17.9)
Triglycerides (mg/dL)	87.0 (53.9)	87.6 (49.3)	91.6 (61.5)	90.5 (58.0)	129 (115)	126 (111)	126 (93.6)	123 (97.1)	137 (111)

[1] a, acyl; aa, diacyl; ae, acyl-alkyl; CRP, C-reactive protein; EPIC, European Prospective Investigation into Cancer and Nutrition; GGT, γ-glutamyltransferase; PC, phosphatidylcholine; SHBG, sex hormone–binding globulin; SM, sphingomyelin.

[2] Median; IQR in parentheses (all such values).

[3] Values are means ± SDs.

[4] n = 122 postmenopausal women and 302 men.

TABLE 2
Multivariable-adjusted means (95% CIs) of biomarkers associated with type 2 diabetes by coffee categories of a subsample of the EPIC-Potsdam cohort in women and men[1]

	Women (n = 1032)					Men (n = 578)					
	>0 to <2 cups/d	≥2 to <3 cups/d	≥3 to <4 cups/d	≥4 cups/d	P-trend	>0 to <2 cups/d	≥2 to <3 cups/d	≥3 to <4 cups/d	≥4 to <5 cups/d	≥5 cups/d	P-trend
n (subcohort)	184	300	208	340		116	132	109	110	111	
CRP (mg/L)[2]	0.89 (0.75, 1.10)	0.82 (0.71, 0.93)	0.70* (0.60, 0.82)	0.70* (0.62, 0.79)	0.01	0.61 (0.48, 0.77)	0.71 (0.58, 0.88)	0.52 (0.42, 0.66)	0.60 (0.47, 0.75)	0.51 (0.40, 0.65)	0.17
Adiponectin (μg/mL)[2]	8.85 (8.30, 9.43)	8.80 (8.38, 9.24)	8.80 (8.30, 9.33)	9.39 (8.97, 9.83)	0.08	6.27 (5.74, 6.85)	5.57* (5.15, 6.03)	5.96 (5.47, 6.50)	5.67 (5.21, 6.19)	6.12 (5.59, 6.70)	0.88
GGT (U/L)[3]	12.6 (11.3, 14.1)	12.8 (11.8, 14.0)	12.3 (11.2, 13.6)	12.3 (11.4, 13.3)	0.57	28.0 (24.5, 32.3)	27.5 (24.3, 31.2)	24.8 (21.8, 28.3)	23.3 (20.5, 26.5)	23.7 (20.7, 27.2)	0.02
Fetuin-A (γg/mL)	277 (268, 286)	271 (264, 278)	271 (263, 279)	265* (259, 272)	0.06	263 (253, 274)	256 (247, 266)	263 (252, 273)	262 (251, 272)	267 (256, 278)	0.48
SHBG (nmol/L)[2,4]	54.2 (45.6, 64.4)	64.3 (54.4, 75.9)	50.6 (42.5, 60.3)	60.4 (54.0, 67.6)	0.63	33.8 (30.6, 37.3)	35.5 (32.6, 38.8)	37.6 (34.4, 41.2)	33.8 (30.7, 37.3)	35.7 (32.4, 39.3)	0.73
Phenylalanine[5]	0.01 (−0.14, 0.16)	0.001 (−0.11, 0.12)	0.02 (−0.12, 0.15)	−0.02 (−0.12, 0.09)	0.81	−0.02 (−0.21, 0.17)	0.26* (0.09, 0.43)	0.02 (−0.17, 0.21)	−0.04 (−0.22, 0.15)	−0.27 (−0.47, −0.08)	0.01
Glycine[5]	0.004 (−0.14, 0.14)	−0.12 (−0.22, −0.01)	0.01 (−0.11, 0.14)	0.09 (−0.01, 0.20)	0.06	0.02 (−0.17, 0.21)	0.08 (−0.09, 0.25)	−0.03 (−0.22, 0.16)	0.05 (−0.13, 0.24)	−0.14 (−0.34, 0.05)	0.27
PC aa C32:1[5]	0.16 (0.02, 0.30)	0.05 (−0.06, 0.16)	−0.11* (−0.24, 0.02)	−0.07* (−0.17, 0.04)	0.009	0.16 (−0.02, 0.34)	0.13 (−0.03, 0.28)	−0.05 (−0.22, 0.12)	−0.09 (−0.26, 0.08)	−0.18* (−0.36, 0.003)	0.003
PC aa C36:1[5]	0.10 (−0.04, 0.24)	−0.02 (−0.13, 0.09)	−0.06 (−0.19, 0.07)	−0.001 (−0.11, 0.10)	0.40	0.13 (−0.06, 0.31)	0.07 (−0.09, 0.23)	−0.05 (−0.23, 0.13)	−0.06 (−0.24, 0.12)	−0.11 (−0.29, 0.08)	0.05
PC aa C38:3[5]	0.08 (−0.05, 0.22)	0.03 (−0.08, 0.13)	−0.06 (−0.19, 0.07)	−0.03 (−0.13, 0.07)	0.17	0.04 (−0.14, 0.22)	0.07 (−0.10, 0.23)	−0.05 (−0.23, 0.12)	−0.03 (−0.21, 0.15)	−0.04 (−0.23, 0.14)	0.37
PC aa C40:5[5]	0.11 (−0.03, 0.25)	−0.04 (−0.15, 0.06)	−0.04 (−0.16, 0.09)	−0.0005 (−0.11, 0.10)	0.44	0.13 (−0.05, 0.32)	0.05 (−0.12, 0.21)	−0.08 (−0.26, 0.10)	−0.02 (−0.20, 0.16)	−0.10 (−0.29, 0.09)	0.09
SM C16:1[5]	0.04 (−0.10, 0.18)	−0.05 (−0.16, 0.05)	−0.004 (−0.13, 0.12)	0.03 (−0.07, 0.13)	0.73	−0.11 (−0.30, 0.09)	0.06 (−0.11, 0.24)	−0.03 (−0.22, 0.16)	−0.04 (−0.23, 0.15)	0.10 (−0.10, 0.30)	0.37
PC ae C34:3[5]	−0.11 (−0.25, 0.03)	−0.001 (−0.11, 0.11)	−0.05 (−0.18, 0.08)	0.09* (−0.01, 0.20)	0.04	−0.13 (−0.31, 0.06)	−0.03 (−0.20, 0.13)	0.08 (−0.11, 0.26)	0.05 (−0.13, 0.24)	0.04 (−0.15, 0.23)	0.18
PC ae C40:6[5]	−0.09 (−0.23, 0.05)	−0.04 (−0.15, 0.07)	−0.01 (−0.14, 0.12)	0.09* (−0.01, 0.19)	0.03	−0.06 (−0.25, 0.14)	0.04 (−0.13, 0.21)	0.11 (−0.08, 0.30)	−0.02 (−0.21, 0.17)	−0.08 (−0.28, 0.12)	0.74
PC ae C42:5[5]	−0.13 (−0.27, 0.02)	0.02 (−0.09, 0.13)	−0.08 (−0.21, 0.05)	0.10* (−0.01, 0.20)	0.04	−0.14 (−0.33, 0.05)	0.15* (−0.02, 0.32)	−0.02 (−0.20, 0.17)	0.07 (−0.11, 0.26)	−0.08 (−0.27, 0.12)	0.90
PC ae C44:4[5]	−0.09 (−0.23, 0.06)	0.02 (−0.09, 0.13)	−0.04 (−0.17, 0.09)	0.05 (−0.05, 0.16)	0.22	−0.07 (−0.26, 0.12)	0.09 (−0.08, 0.26)	0.01 (−0.17, 0.20)	0.01 (−0.17, 0.19)	−0.06 (−0.25, 0.13)	0.79
PC ae C44:5[5]	−0.08 (−0.22, 0.07)	0.03 (−0.08, 0.14)	−0.08 (−0.22, 0.05)	0.07 (−0.04, 0.18)	0.23	−0.12 (−0.31, 0.07)	0.16* (−0.01, 0.33)	−0.04 (−0.23, 0.14)	0.05 (−0.14, 0.23)	−0.07 (−0.26, 0.12)	0.92
Lyso-PC a C18:2[5]	0.003 (−0.14, 0.15)	−0.04 (−0.15, 0.07)	−0.05 (−0.18, 0.08)	0.07 (−0.04, 0.17)	0.33	−0.01 (−0.19, 0.18)	−0.07 (−0.24, 0.09)	−0.01 (−0.19, 0.18)	0.03 (−0.15, 0.22)	0.07 (−0.12, 0.26)	0.38
HDL cholesterol (mg/dL)[2]	58.7 (56.6, 60.9)	59.0 (57.4, 60.6)	57.4 (55.5, 59.3)	58.9 (57.3, 60.4)	0.65	47.1 (44.8, 49.4)	49.2 (47.1, 51.4)	49.9 (47.6, 52.4)	51.2* (48.8, 53.7)	49.0 (46.6, 51.5)	0.17
Triglycerides (mg/dL)[2]	96.0 (89.6, 103)	91.9 (87.3, 96.8)	91.2 (85.7, 97.0)	89.9 (85.6, 94.4)	0.17	146 (131, 163)	134 (121, 147)	138 (124, 154)	123* (111, 137)	126 (113, 141)	0.04

[1] Model adjusted for age at recruitment, smoking status (never, past, current smoker <20 units/d, or current smoker ≥20 units/d), alcohol intake (g/d), leisure-time sport activities (no sports, ≤4 h/wk, or >4 h/wk), biking (no biking, <2.5 h/wk, 2.5–4.9 h/wk, or ≥5 h/wk), hormone use in women (none, hormone replacement therapy, or oral contraceptive), education status (in or no training, vocational training, technical school, or technical college or university degree), caffeinated tea intake (mL/d), total energy intake (kcal/d), whole-grain bread intake (g/d), red meat intake (g/d), processed meat intake (g/d), BMI (kg/m^2), and waist circumference (cm). P-trend value reflects whether the mean of the biomarker significantly falls or rises across the coffee categories. a, acyl; aa, diacyl; ae, acyl-alkyl; CRP, C-reactive protein; EPIC, European Prospective Investigation into Cancer and Nutrition; GGT, γ-glutamyltransferase; PC, phosphatidylcholine; SHBG, sex hormone–binding globulin; SM, sphingomyelin. *$P < 0.05$. The P value reflects whether the value of the biomarker significantly differs from the reference value (lowest coffee category of >0–<2 cups/d).

[2] Values are geometric means.

[3] Values are exp (geometric mean).

[4] n = 122 postmenopausal women and 302 men.

[5] Box-Cox-transformed and z score standardized values.

TABLE 3
RRs (95% CIs) for type 2 diabetes by coffee categories in a case-cohort study embedded in the EPIC-Potsdam study in women and men[1]

	Women (n = 1178)				Men (n = 814)				
	>0 to <2 cups/d	2 to <3 cups/d	3 to <4 cups/d	≥4 cups/d	>0 to <2 cups/d	2 to <3 cups/d	3 to <4 cups/d	4 to <5 cups/d	≥5 cups/d
No. of cases	25	48	29	61	48	74	46	50	36
Multivariable-adjusted model[2]	1.00	1.08 (0.63, 1.86)	0.80 (0.42, 1.53)	0.78 (0.46, 1.33)	1.00	1.56 (0.91, 2.69)	1.23 (0.67, 2.28)	0.82 (0.45, 1.50)	0.40 (0.19, 0.81)
Additional adjustments									
CRP	1.00	1.03 (0.60, 1.76)	0.71 (0.36, 1.39)	0.75 (0.44, 1.27)	1.00	1.73 (0.99, 3.03)	1.42 (0.76, 2.66)	0.89* (0.48, 1.66)	0.44* (0.21, 0.91)
Adiponectin	1.00	1.06 (0.60, 1.88)	0.75 (0.38, 1.49)	0.95 (0.53, 1.69)	1.00	1.64 (0.95, 2.85)	1.28 (0.67, 2.42)	0.82 (0.44, 1.52)	0.41 (0.20, 0.84)
GGT	1.00	1.05 (0.61, 1.80)	0.81 (0.43, 1.54)	0.78 (0.46, 1.32)	1.00	1.60 (0.93, 2.77)	1.32 (0.71, 2.46)	0.87* (0.47, 1.60)	0.42* (0.21, 0.87)
Fetuin-A	1.00	1.08 (0.62, 1.87)	0.81 (0.43, 1.55)	0.83 (0.49, 1.41)	1.00	1.56 (0.91, 2.66)	1.22 (0.66, 2.24)	0.77 (0.42, 1.41)	0.36 (0.17, 0.73)
Liver markers[3] simultaneously	1.00	1.04 (0.60, 1.80)	0.82 (0.44, 1.56)	0.83 (0.49, 1.41)	1.00	1.60 (0.93, 2.74)	1.29 (0.69, 2.41)	0.82 (0.44, 1.51)	0.38 (0.18, 0.78)
Phenylalanine	1.00	1.05 (0.61, 1.80)	0.78 (0.41, 1.49)	0.76 (0.45, 1.30)	1.00	1.52 (0.87, 2.66)	1.44 (0.77, 2.70)	0.90 (0.49, 1.66)	0.49* (0.24, 1.00)
Glycine	1.00	1.08 (0.62, 1.88)	0.87 (0.45, 1.67)	0.83 (0.48, 1.42)	1.00	1.67 (0.98, 2.85)	1.27 (0.69, 2.31)	0.85 (0.47, 1.53)	0.39 (0.19, 0.78)
PC aa C32:1	1.00	1.08 (0.63, 1.88)	0.83 (0.43, 1.58)	0.81 (0.47, 1.37)	1.00	1.62 (0.93, 2.80)	1.28 (0.68, 2.40)	0.88 (0.48, 1.62)	0.43 (0.21, 0.87)
PC aa C36:1	1.00	1.08 (0.62, 1.86)	0.80 (0.42, 1.53)	0.77 (0.45, 1.32)	1.00	1.59 (0.92, 2.74)	1.26 (0.68, 2.35)	0.84 (0.46, 1.53)	0.41 (0.20, 0.84)
PC aa C40:5	1.00	1.11 (0.64, 1.91)	0.81 (0.42, 1.55)	0.76 (0.44, 1.29)	1.00	1.63 (0.94, 2.82)	1.31 (0.70, 2.45)	0.85 (0.46, 1.56)	0.42 (0.21, 0.87)
PC ae C34:3	1.00	1.10 (0.64, 1.89)	0.79 (0.41, 1.52)	0.80 (0.47, 1.37)	1.00	1.67 (0.96, 2.90)	1.38 (0.75, 2.52)	0.93 (0.50, 1.71)	0.42 (0.21, 0.86)
PC ae C40:6	1.00	1.09 (0.63, 1.88)	0.82 (0.43, 1.56)	0.81 (0.47, 1.38)	1.00	1.55 (0.90, 2.66)	1.23 (0.67, 2.26)	0.82 (0.45, 1.49)	0.40 (0.20, 0.81)
PC ae C42:5	1.00	1.14 (0.66, 1.99)	0.88 (0.46, 1.70)	0.87 (0.51, 1.50)	1.00	1.56 (0.91, 2.68)	1.21 (0.66, 2.22)	0.84 (0.46, 1.53)	0.40 (0.20, 0.81)
HDL cholesterol	1.00	1.13 (0.66, 1.96)	0.75 (0.39, 1.41)	0.81 (0.48, 1.37)	1.00	1.69 (0.98, 2.93)	1.43 (0.78, 2.64)	0.96* (0.52, 1.77)	0.47* (0.23, 0.95)
Triglycerides	1.00	1.12 (0.65, 1.92)	0.75 (0.39, 1.47)	0.76 (0.44, 1.30)	1.00	1.61 (0.92, 2.79)	1.25 (0.68, 2.31)	0.88 (0.48, 1.60)	0.43 (0.22, 0.88)
Classic biomarkers reflecting dyslipidemia[4] simultaneously	1.00	1.18 (0.68, 2.05)	0.74 (0.38, 1.42)	0.80 (0.47, 1.36)	1.00	1.68 (0.97, 2.91)	1.39 (0.76, 2.56)	0.98* (0.53, 1.78)	0.48* (0.24, 0.97)
All biomarkers reflecting dyslipidemia[5] simultaneously	1.00	1.31 (0.75, 2.29)	0.85 (0.43, 1.67)	0.91 (0.53, 1.57)	1.00	1.72 (1.00, 2.98)	1.46 (0.78, 2.71)	1.05 (0.57, 1.95)	0.49 (0.24, 0.99)

[1] *Significant difference of β-coefficients obtained from the multivariable-adjusted model (reference) and the multivariable-adjusted model also adjusted for coffee-associated biomarkers. aa, diacyl; ae, acyl-alkyl; CRP, C-reactive protein; EPIC, European Prospective Investigation into Cancer and Nutrition; GGT, γ-glutamyltransferase; PC, phosphatidylcholine.

[2] Model stratified by age, adjusted for smoking status (never, past, current smoker <20 units/d, or current smoker ≥20 units/d), alcohol intake (g/d), leisure-time sport activities (no sports, ≤4 h/wk, or >4 h/wk), biking (no biking, <2.5 h/wk, 2.5–4.9 h/wk, or ≥5 h/wk), hormone use in women (none, hormone replacement therapy, or oral contraceptive), education status (in or no training, vocational training, technical school, or technical college or university degree), caffeinated tea intake (mL/d), total energy intake (kcal/d), whole-grain bread intake (g/d), red meat intake (g/d), processed meat intake (g/d), BMI (kg/m^2), and waist circumference (cm).

[3] Fetuin-A and GGT simultaneously as separate covariates.

[4] HDL cholesterol and triglycerides simultaneously as separate covariates.

[5] Acyl-alkyl-phosphatidylcholines C34:3, C40:6, and C42:5, diacyl-phosphatidylcholines C32:1, C36:1, and C40:5; HDL cholesterol; and triglycerides simultaneously as separate covariates.

DISCUSSION

In this prospective study of middle-aged men and women, coffee consumption was related to various serum metabolites in both sexes. In addition, coffee consumption was inversely associated with fetuin-A and CRP concentrations in women and with GGT and triglyceride concentrations in men. Coffee consumption showed an inverse trend with T2D risk in both sexes, which reached significance in men only. This inverse association in men was slightly attenuated after adjustment for lipid markers or phenylalanine in men.

HDL cholesterol and triglycerides are well-established risk markers for T2D (34). In men in our study, coffee consumption showed an inverse trend with triglycerides and appeared to be positively associated with HDL cholesterol. In line with these findings, 3 observational studies also reported inverse associations between coffee consumption and triglyceride concentrations (35–37). Furthermore, the strength of the inverse trend between coffee consumption and T2D risk in men in our study was significantly reduced after adjustment for classic biomarkers of dyslipidemia. In line with our findings, adjustment for total cholesterol and triglycerides attenuated the association of coffee consumption with T2D risk in a prospective cohort study in Swedish middle-aged women (9). These results indicate that a possible protective effect of coffee consumption on T2D risk might be mediated through a lowering of triglycerides. In Germany, where our study is set, the traditional method of coffee preparation is filtering (38), which mainly holds back the lipid-raising coffee oils (39). In accordance with this, a recent meta-analysis of randomized controlled trials demonstrated that only unfiltered, but not filtered, coffee intake increased triglyceride concentrations and that coffee intake had no impact on HDL cholesterol (3). The discrepancies of results from observational and intervention studies regarding coffee consumption and blood lipids may be attributable to intervention studies being characterized by a shorter period of exposure to coffee (3) or residual confounding in observational studies. Consequently, our findings indicate that dyslipidemia may be a pathway linking coffee consumption to decreased T2D risk, but further studies into the potential mediating role of blood lipids in this context are warranted.

In a nested case-control study including only postmenopausal women, the inverse association between coffee consumption and T2D risk was attenuated after adjustment for SHBG (10), suggesting a mediating role for SHBG. In our study, coffee consumption was not clearly associated with SHBG. Consequently, SHBG was not considered in the sequential mediator analysis in our study. Regarding short-term effects of coffee consumption, a recent intervention study also found no effect of coffee consumption on SHBG after 8 wk of intervention (8). Of note, contrary to our analysis, the above-mentioned nested case-control study was not adjusted for food items (10).

In our study, coffee consumption was inversely associated with fetuin-A in women and with GGT in men, 2 markers related to liver fat accumulation and liver function. Although adjustment for GGT in men led to a significant attenuation of the coffee-T2D relation, adjustment for GGT in men or fetuin-A in women did not have an appreciable effect on the inverse trend between coffee consumption and T2D risk. Still, decaffeinated coffee intake in particular led to a decrease of fetuin-A concentrations in an intervention study (5). Furthermore, experiments in mice suggest that coffee polyphenols lead to a reduced diet-induced liver fat accumulation (40). Consequently, a more favorable liver marker profile suggesting a lower liver fat accumulation may be a factor that could partly explain the association between coffee consumption and T2D risk.

Coffee consumption led to increased concentrations of adiponectin in several intervention studies (4–6). Similarly, coffee consumption was related to higher adiponectin concentrations among women in our study. However, our study did not have sufficient power to demonstrate that an inverse association between coffee consumption and T2D risk is indeed mediated by adiponectin.

Compared with current biomarkers, metabolites, which represent intermediates and end products of metabolic pathways, more rapidly reflect physiologic disorders (41). In the EPIC-Potsdam study, several serum metabolites, including amino acids and choline-containing phospholipids, were independently associated with the risk of T2D (17). In both sexes in our study, coffee consumption was associated with several metabolites, including amino acids and diacyl- and acyl-alkyl-phosphatidylcholines. Furthermore, a reduction of the inverse trend between coffee consumption and T2D was observed after adjustment for phenylalanine in men. A cross-sectional analysis in participants in the KORA (Cooperative Health Research in the Region Augsburg) study suggests associations of coffee consumption with certain acylcarnitines or sphingomyelins; however, in contrast to our study, no association was made with glycerophospholipids or amino acids (42). Discrepancies between results of this cross-sectional study and our study may be explained in part through different statistical methods and study populations: contrary to our analysis, the above-mentioned cross-sectional study used unadjusted correlation models, and analyses were restricted to male participants aged 55–79 y (42). To our knowledge, a plausible biologic mechanism of the association between coffee consumption and phenylalanine has not been suggested. Metabolism of lipid metabolites and biomarkers of dyslipidemia seems to be closely intertwined—for example, diacyl-phosphatidylcholines play an important role in the hepatic secretion of HDL cholesterol (43). In this study population, we previously found that phosphatidylcholines, in general, were positively correlated with plasma HDL cholesterol (17). However, acyl-alkyl-phosphatidylcholines were inversely correlated with plasma triglycerides, whereas diacyl-phosphatidylcholines were positively correlated with plasma triglycerides (17).

To our knowledge, this study is the first to investigate a wide-ranging number of biomarkers that reflect various metabolic pathways to determine potential mediators of the association between coffee consumption and T2D risk. However, a considerable part of the inverse association between coffee consumption and T2D risk in men was not explained after adjustment for potential mediators. Therefore, further pathways that were not investigated in the present analysis may underlie the association between coffee consumption and T2D risk; for example, it has been reported that coffee consumption may mediate concentrations of gut peptides (eg, glucagon-like peptide 1) involved in the regulation of insulin secretion (44).

Several limitations need to be considered. The present mediator analysis does not allow us to draw conclusions regarding causality but rather is considered hypothesis generating.

Unmeasured confounding between the mediator or exposure and the outcome may lead to biased effect estimates (45). To take this into account, we incorporated numerous confounders for the causal effect of the mediator as well as of the exposure on the outcome. We relied on self-reports for data on coffee drinking. However, data from the German part of the EPIC project suggest that the FFQ allows us to estimate coffee consumption reliably and in comparison with repeated 24-h recalls with a high relative validity (13). Our results are based on a single measurement of biomarkers, and thus, random measurement error may have resulted in an attenuation of the results toward the null (46). We used data of nonfasted and fasted participants for our analyses. Nevertheless, our sensitivity analysis, when restricted to fasted participants, led to similar results. Most important, our study had limited power to evaluate the effect of adjustment for potential mediators on the association between coffee and T2D risk, particularly among women.

In this prospective study of middle-aged men and women, coffee consumption was inversely related with a diacyl-phosphatidylcholine in both sexes and positively associated with certain acyl-alkyl-phosphatidylcholines in women. In addition, coffee consumption showed an inverse trend with liver markers in both sexes, with CRP in women and with triglycerides and phenylalanine in men. Still, these markers only partly explained the inverse association between long-term coffee consumption and T2D risk.

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