Effects of coffee consumption on subclinical inflammation and other risk factors for type 2 diabetes: a clinical trial[1–3]

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ABSTRACT

Background: Coffee consumption is associated with a decreased risk of type 2 diabetes. Suggested mechanisms underlying the association have included attenuation of subclinical inflammation and a reduction in oxidative stress.

Objective: The aim was to investigate the effects of daily coffee consumption on biomarkers of coffee intake, subclinical inflammation, oxidative stress, glucose, and lipid metabolism.

Design: Habitual coffee drinkers (n = 47) refrained for 1 mo from coffee drinking; in the second month they consumed 4 cups of filtered coffee/d and in the third month 8 cups of filtered coffee/d (150 mL/cup). Blood samples were analyzed by gas chromatography–mass spectrometry, bead-based multiplex technology, enzyme-linked immunosorbent assay, or immunonephelometry.

Results: Coffee consumption led to an increase in coffee-derived compounds, mainly serum caffeine, chlorogenic acid, and caffeic acid metabolites. Significant changes were also observed for serum concentrations of interleukin-18, 8-isoprostane, and adiponectin (medians: –8%, −16%, and 6%, respectively; consumption of 8 compared with 0 cups coffee/d). Serum concentrations of total cholesterol, HDL cholesterol, and apolipoprotein A-I increased significantly by 12%, 7%, and 4%, respectively, whereas the ratios of LDL to HDL cholesterol and of apolipoprotein B to apolipoprotein A-I decreased significantly by 8% and 9%, respectively (8 compared with 0 cups coffee/d). No changes were seen for markers of glucose metabolism in an oral-glucose-tolerance test.

Conclusions: Coffee consumption appears to have beneficial effects on subclinical inflammation and HDL cholesterol, whereas no changes in glucose metabolism were found in our study. Furthermore, many coffee-derived methylxanthines and caffeic acid metabolites appear to be useful as biomarkers of coffee intake. *Am J Clin Nutr* 2010;91:950–7.

INTRODUCTION

Coffee is one of the most commonly consumed beverages in the world with an average per-capita intake in Europe of 5 kg/y. In Finland, the consumption is >11 kg, which is the highest in the world. Recent prospective cohort studies suggest that coffee drinking may protect against type 2 diabetes mellitus (1–3). It has been hypothesized that attenuation of subclinical inflammation, reduction of oxidative stress, and favorable changes in lipid profiles are potential mechanisms underlying this association (2).

Because subclinical inflammation represents one important mechanism in the development of type 2 diabetes (4, 5), it is biologically plausible that antiinflammatory effects of coffee could support normal glucose metabolism (6–8). Coffee is a complex mixture of hundreds of chemicals, many of them with potentially immunomodulatory activities, which occur naturally or are formed during the roasting process. However, most components of coffee, such as phenolic acids, have not been systematically studied in this context (9). The main phenolic acids of coffee are chlorogenic acids, which are quinic acid esters of caffeic acid. Green coffee beans can contain as much as 10% of dry weight chlorogenic acids, and coffee is the major source of chlorogenic acids in human diet, although they also occur in fruit and vegetables (9). The daily intake of chlorogenic acids in coffee drinkers ranges from 0.5 to 1.0 g (10). So far, information about the bioavailability of these compounds, which exhibit potent antioxidant properties in vitro (11, 12) and likely also in vivo (13), is limited.

We hypothesized that coffee attenuates circulating concentrations of proinflammatory immune mediators, which have been shown to be risk factors for type 2 diabetes (4, 14). Currently available data are from observational studies that often found inverse associations between coffee consumption and immunologic risk factors for type 2 diabetes (6, 8, 15). One study reported opposite results, a direct association (15), and another reported no

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correlation between coffee consumption and systemic inflammation (16). Unfortunately, these observational studies are prone to confounding by nondocumented lifestyle factors so that sound conclusions on causation are not possible. To date, no data are available on the effects of coffee consumption on immunologic risk factors for type 2 diabetes from clinical trials. Therefore, the aims of the study were to investigate in a clinical trial *1*) the effect of increasing coffee doses on serum concentrations of markers of subclinical inflammation, oxidative stress, lipids, and glucose metabolism; *2*) the effect of insulin resistance on these effects; *3*) the association between increasing coffee metabolite concentrations and changes in immune mediator levels; and *4*) the use of circulating methylxanthine and polyphenol concentrations as biomarkers of coffee intake.

SUBJECTS AND METHODS

Study population

Coffee consumers were recruited by newspaper advertisements. Subjects younger than 65 y, free of type 2 diabetes (excluded by the results of an oral-glucose-tolerance test before inclusion into the study), but with an elevated risk of type 2 diabetes [ie, $\geq$13 points in the Finnish diabetes risk score (17)] were eligible for participation. Exclusion criteria were the use of blood glucose–lowering medication or drugs that interfere with glucose metabolism, a history of malignancies or thyroid or liver diseases, any other chronic diseases likely to interfere with study participation, a history of alcohol or drug abuse, and pregnancy or breastfeeding. The study has been conducted in accordance with the Declaration of Helsinki (1964), as amended in South Africa (1996) and written informed consent was obtained from all participants.

Study design

The study represents a single-blind (investigator), 3-stage clinical trial (number of clinical trial: ISRCTN12547806) that lasted for 3 mo. Subjects received 0, 3, or 5 packages with 500 g coffee/mo. Juhla Mokka branded coffee was provided by the Paulig Group (Helsinki, Finland). All trial coffee packages were stored at $\leq$10°C and were protected from light and moisture. Coffee was prepared with participants' coffee machines at home. The subjects brewed the coffee daily at home with their own coffee machine using paper filters. They were allowed to divide their daily coffee dose as it suited them best and to keep the brewed coffee in a thermos if it was impossible to prepare it freshly before drinking.

During the first month, participants refrained from drinking coffee, whereas in the second month they consumed 4 cups coffee/d (1 cup = 150 mL) and in the third month 8 cups/d. A detailed medical history, including family history of diabetes, alcohol intake, use of any medications, and coronary heart disease was recorded.

Analysis of metabolic indicators, coffee-derived compounds, and immune mediators

At the end of each treatment stage, serum and plasma samples were taken after an overnight fast for $\geq$8 h by inserting an intravenous cannula into the forearm (antecubital) vein. Coffee-derived compounds (caffeine and its metabolites theophylline, theobromine, paraxanthine, and the polyphenols caffeic acid and its metabolites dihydrocaffeic acid, ferulic acid, dihydroferulic acid, isoferulic acid, dihydroisoferulic acid, dihydro-3-coumaric acid, 3-(3,4-dimethoxyphenyl)propionic acid, 3,4-dimethylcaffeic acid, and 3-coumaric acid) were measured in plasma samples by HPLC and gas chromatography–mass spectrometry by modifications of previously published methods (18, 19). Plasma samples were hydrolyzed by an enzyme solution containing β-glucuronidase/sulfatase before analyses. Samples from each study participant were analyzed in the same run. The intraassay CV was $<$12% for all polyphenols and methylxanthines.

Plasma glucose concentrations were measured by using a hexokinase method (Abbott Laboratories, Abbott Park, IL). Serum concentrations of total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides were measured by using enzymatic assays on a Hitachi 912 analyzer (Roche Diagnostics, Mannheim, Germany). Serum concentrations of apolipoproteins A-I and B were assessed by immunonephelometric assays on a BN II analyzer (Dade Behring, Marburg, Germany). Samples from the same study participants were always analyzed in the same run. Intraassay variations were $<$5% for all these measurements.

Serum insulin concentrations were measured by using enzyme-linked immunosorbent assay (ELISAs) from Invitrogen (Karlsruhe, Germany). Serum adiponectin and leptin concentrations were measured by using ELISAs from Mercodia (Uppsala, Sweden). Serum concentrations of interleukin (IL)-6, IL-1 receptor antagonist (IL-1ra), and macrophage migration inhibitory factor (MIF) were measured by using Quantikine HS (IL-6) and Quantikine (IL-1ra, MIF) ELISAs from R&D Systems (Wiesbaden, Germany) as described previously (20, 21). Serum concentrations of IL-18 were quantified by using a bead-based multiplex assay on a Luminex 100 analyzer (Luminex Corporation, Austin, TX) as described (14). Fluorescent xMAP COOH microspheres were purchased from Luminex Corporation. Recombinant IL-18 and anti-IL-18 detection and capture antibodies were obtained from MBL (Nagoya, Japan). Serum concentrations of C-reactive protein (CRP) and serum amyloid A (SAA) assessed by an immunonephelometric assay on a BN II analyzer (Dade Behring). Serum concentrations of 8-isoprostane and nitrotyrosine as markers of oxidative stress were determined by using an enzyme immunoassay from Cayman Chemical (Ann Arbor, MI) and an ELISA from Hycult Biotechnology (Uden, Netherlands), respectively. Samples from the same study participants were always analyzed on the same plate or in the same run. Intraassay CVs for insulin, leptin, IL-6, IL-1ra, MIF, adiponectin, IL-18, CRP, SAA, 8-isoprostane, and nitrotyrosine were 2.6%, 2.0%, 5.4%, 1.2%, 4.4%, 5.0%, 7.0%, 2.5%, 4.9%, 9.4%, and 7.0%, respectively.

Statistics

The results are expressed as means $\pm$ SDs or median and interquartile ranges as specified. Differences in the concentrations of coffee-derived compounds or immune mediators (8 compared with 0 cups/d and 4 compared with 0 cups/d) were analyzed by Friedman's test and Dunn's multiple comparisons test (adjusted for the number of post tests; ie, $n = 2$ for comparisons of 8 compared with 0 and 4 compared with 0 cups of coffee/d for each variable). Analyses were done for the whole

study groups (primary analysis) and for predefined subgroups (study group subdivided by median of fasting insulin concentrations and homeostasis model assessment of insulin resistance (HOMA-IR; exploratory analyses). Associations between changes in coffee-derived compounds and test variable levels were assessed with Spearman correlation coefficients. The level of significance was 0.05. Statistical analyses were conducted by using GraphPad Prism (version 4; GraphPad Software Inc, La Jolla, CA) and SAS (version 9.1; SAS Institute, Cary, NC).

RESULTS

Habitual coffee drinkers were included in the study. Clinical characteristics of the participants are shown in **Table 1**. Most of the subjects were female (77%) and obese. Two subjects dropped out during the intervention and were not included in the analyses.

TABLE 1
Baseline characteristics

Variable	Study population (n = 47)
Sex [n (%)]	
Male	11 (23)
Female	36 (77)
Age (y)	54.0 ± 9.0[1]
BMI (kg/m^2)	29.2 ± 4.6
Waist circumference (cm)	98.1 ± 10.5
Systolic blood pressure (mm Hg)	140.5 ± 15.3
Diastolic blood pressure (mm Hg)	89.5 ± 9.3
Smoking [n (%)]	
Yes	2 (4)
No	45 (96)
Coffee consumption before the trial (cups/d)[2]	4.0 ± 1.7

[1] Mean ± SD (all such values).
[2] 1 cup = 150 mL.

Coffee consumption induces an increase in coffee-derived compounds in plasma

Plasma concentrations of coffee biomarkers were very low when participants abstained from coffee drinking, whereas coffee consumption led to increases in their concentrations. In detail, plasma concentrations of caffeine, paraxanthine, theobromine, theophylline and caffeic acid, dihydrocaffeic acid, ferulic acid, isoferulic acid, dihydroferulic acid, dihydroisoferulic acid, 3-(3-hydroxyphenyl)propionic acid, 3-(3,4-dimethoxyphenyl)propionic acid, 3,4-dimethylcaffeic acid, and 3-coumaric acid (**Table 2**) increased significantly after consumption of 4 or 8 cups coffee/d.

Coffee consumption affects serum concentrations of immune mediators, markers of oxidative stress, lipids, and glucose metabolism

The consumption of 8 cups coffee/d led, compared with the results after 4 wk of coffee abstinence, to a significant decrease of IL-18 and 8-isoprostane (both $P < 0.01$) and a significant increase in adiponectin ($P < 0.05$) (**Table 3**). No significant changes were found for IL-6, MIF, IL-1ra, leptin, CRP, SAA, and nitrotyrosine.

Increased concentrations of total cholesterol ($P < 0.01$), HDL cholesterol ($P < 0.001$), and apolipoprotein A-I ($P < 0.05$) were observed after coffee consumption (8 cups/d), whereas no significant changes were found for LDL cholesterol and triglycerides. In agreement with these data, there were significant decreases in the ratios of LDL to HDL cholesterol ($P < 0.05$) and of apolipoprotein B to apolipoprotein A-I ($P < 0.01$) after the consumption of 8 cups coffee/d.

No effects of coffee consumption were observed on fasting glucose, fasting insulin, and HOMA-IR. Moreover, 30-min and 2-h glucose and insulin values as well as the insulinogenic index derived from oral-glucose-tolerance tests did not change significantly by coffee consumption. Immunologic and metabolic changes were not paralleled by weight changes during the study

TABLE 2
Effect of coffee consumption on coffee-derived compounds in the study population (n = 47)[1]

	Coffee consumption		
Biomarker	0 cups/d	4 cups/d	8 cups/d
Caffeine (μmol/L)	0.13 (0.06, 0.17)	2.46 (1.53, 4.43)[2]	6.54 (2.79, 10.86)[2]
Paraxanthine (μmol/L)	0.07 (0.05, 0.09)	3.66 (2.08, 6.34)[2]	7.77 (4.43, 10.43)[2]
Theobromine (μmol/L)	0.09 (0.04, 0.16)	1.07 (0.79, 1.53)[2]	2.03 (1.29, 1.90)[2]
Theophylline (μmol/L)	0.05 (0.04, 0.07)	0.88 (0.54, 1.11)[2]	1.64 (1.00, 2.20) [2]
Caffeic acid (μmol/L)	18.3 (13.4, 30.4)	38.3 (25.2, 63.7)[3]	62.2 (40.6,102.9)[2]
Dihydrocaffeic acid (nmol/L)	17.2 (9.9, 25.5)	47.9 (16.6, 177.9)[4]	75.2 (29.4,214.6)[2]
Coumaric acid (nmol/L)	9.1 (6.6, 13.2)	26.4 (14.2, 47.7)[2]	58.5 (26.6, 97.4)[2]
Dihydro-3-coumaric acid (nmol/L)	97 (48, 262)	716 (125, 1865)[2]	1583 (485, 3333)[2]
Ferulic acid (nmol/L)	40.1 (31.0, 63.2)	55.1 (37.2, 107.1)	67.1 (46.1, 178.6)[2]
Isoferulic acid (nmol/L)	5.3 (3.6, 24.7)	23.5 (8.4, 74.3)[3]	49.8 (8.2, 152.2)[2]
Dihydroferulic acid (nmol/L)	51.6 (21.3, 95.7)	93.9 (45.6, 299.2)[3]	194.7 (48.8, 631.1)[2]
Dihydroisoferulic acid (nmol/L)	19.6 (6.7, 99.6)	56.5 (23.2, 295.3)	90.6 (38.7, 256.5)[3]
Dimethoxycinnamic acid (nmol/L)	24.0 (20.7, 34.3)	77.0 (48.2, 138.6)[2]	177.7 (89.4, 254.9)[2]
3-(3,4-Dimethoxyphenyl)-propionic acid (nmol/L)	31 (20, 46)	203 (114, 316)[2]	398 (227, 646)[2]

[1] All values are medians; 25th and 75th percentiles in parentheses. 1 cup of coffee = 150 mL.
[2–4] Significantly different from 0 cups coffee/d (Friedman's test and Dunn's multiple comparisons): [2]$P < 0.001$, [3]$P < 0.05$, [4]$P < 0.01$.

TABLE 3
Effect of coffee consumption on immune mediators, markers of oxidative stress, lipids, and glucose metabolism[1]

	Coffee consumption		
	0 cups/d	4 cups/d	8 cups/d
Markers of subclinical inflammation			
IL-6 (pg/mL)	1.09 (0.3, 1.75)	1.17 (0.75, 1.95)	1.24 (0.85, 1.77)
IL-18 (pg/mL)	118 (88, 162)	105 (86,153)	108 (80, 156)[2]
MIF (ng/mL)	10.5 (7.8, 14.7)	10.9 (8.2, 14.3)	10.7 (8.7, 12.7)
IL-1ra (pg/mL)	296 (208, 472)	315 (241, 480)	331 (261, 500)
Adiponectin (ng/mL)	7957 (6317, 10901)	7764 (6499, 11074)	8421 (6634, 11256)[3]
Leptin (ng/mL)	25.7 (13.9, 46.8)	26.7 (15.4, 47.9)	31.7 (18.2, 57.1)
CRP (mg/L)	1.24 (0.50, 1.89)	1.12 (0.72, 3.69)	1.32 (0.63, 2.40)
SAA (mg/L)	3.20 (1.90, 6.60)	3.50 (2.10, 8.80)	4.10 (2.80, 7.30)
Markers of oxidative stress			
8-Isoprostane (pg/mL)	80 (39, 152)	77 (29, 155)	67 (19, 140)[2]
Nitrotyrosine (nmol/L)	42 (5, 145)	43 (5, 161)	50 (5, 172)
Lipids			
Total cholesterol (mg/dL)	208 (191, 229)	204 (189, 228)	223 (197, 244)[2]
LDL cholesterol (mg/dL)	123 (108, 138)	120 (103, 140)	125 (104, 146)
HDL cholesterol (mg/dL)	48 (43, 63)	50 (45, 61)[2]	51 (44, 66)[4]
LDL:HDL cholesterol	2.46 (2.05, 3.09)	2.33 (1.91, 2.92)[3]	2.27 (1.83, 2.76)[3]
apo B (g/L)	1.10 (0.94, 1.25)	1.04 (0.92, 1.21)	1.05 (0.93, 1.25)
apo A-I (g/L)	1.59 (1.41, 1.79)	1.62 (1.44, 1.84)	1.66 (1.50, 1.87)[3]
apo B/apo A-I	0.68 (0.59, 0.85)	0.60 (0.56, 0.77)[3]	0.62 (0.53, 0.73)[2]
Triglycerides (mg/dL)	125 (92, 152)	120 (89, 160)	124 (94, 181)
Glucose metabolism			
Fasting glucose (mmol/L)	5.85 (5.44, 6.06)	5.76 (5.45, 6.14)	5.80 (5.47, 6.34)
30-min Glucose (mmol/L)	9.02 (8.13, 10.11)	8.99 (7.81, 10.01)	9.22 (7.97, 10.36)
2-h Glucose (mmol/L)	6.39 (5.36, 7.43)	6.58 (5.46, 7.33)	6.56 (5.34, 7.48)
Fasting insulin (μU/mL)	15.6 (10.7, 21.0)	14.7 (12.4, 20.4)	14.9 (11.5, 21.0)
30-min Insulin (μU/mL)	66.8 (49.6, 110.0)	70.9 (46.7, 102.7)	70.6 (54.6, 97.0)
2-h Insulin (μU/mL)	54.5 (38.3, 81.1)	54.1 (35.2, 72.1)	60.2 (35.4,78.9)
HOMA-IR[5]	3.93 (2.84, 5.17)	4.10 (2.99, 5.46)	4.22 (2.96, 5.75)
Insulinogenic index[6]	15.6 (10.4, 27.9)	17.7 (9.2, 33.0)	18.1 (11.1, 31.0)

[1] All values are medians; 25th and 75th percentiles in parentheses (n = 47). 1 cup of coffee = 150 mL. HOMA-IR, homeostasis model assessment of insulin resistance; MIF, macrophage migration inhibitory factor; CRP, C-reactive protein; apo, apolipoprotein; SAA, serum amyloid A; IL, interleukin; IL-1ra, IL-1 receptor antagonist.

[2–4] Significantly different from 0 cups of coffee (Friedman's test and Dunn's multiple comparisons test): [2]$P < 0.01$, [3]$P < 0.05$, [4]$P < 0.001$.

[5] Calculated as fasting plasma glucose (mmol/L) × fasting insulin (μU/mL)/22.5

[6] Calculated as (insulin at 30 min – fasting insulin)/(glucose at 30 min – fasting glucose) with insulin in μU/mL and glucose in mmol/L.

(82.5 ± 13.3, 82.3 ± 13.3, and 82.3 ± 13.1 kg after consumption of 0, 4, and 8 cups/d, respectively; $P = 0.44$).

Coffee-mediated effects are more pronounced in insulin-resistant individuals

Because we found in a previous study that metabolic predisposition had an effect on the immunologic reaction to a dietary stimulus (22), we performed exploratory analyses by dividing the study population along the median of HOMA-IR and assessed the effects of coffee consumption on the variables shown in Table 3, separately for subgroups with a HOMA-IR >3.93 or ≤3.93. A comparison of the data after consumption of 8 cups/d compared with that after 0 cups/d showed significant decreases in serum IL-18 and 8-isoprostane concentrations and in the ratio apolipoprotein B to apolipoprotein A-I, and significant increases in serum adiponectin, apolipoprotein A-I, and HDL cholesterol were observed in the subgroup with high insulin resistance, whereas only total cholesterol and HDL cholesterol increased in the subgroup with low insulin resistance (**Figure 1**). IL-1ra and CRP were increased after 4 cups/d compared with 0 cups/d in the group with low insulin resistance, but not after 8 cups/d compared with 0 cups/d (data not shown). However, there was no evidence of an interaction between coffee consumption and HOMA-IR subgroups ($P > 0.4$ for all aforementioned variables). The subgroup analyses showed no significant differences in any of the markers of glucose metabolism in either group.

Correlation between changes in coffee-derived compounds and inflammation-associated markers

In post hoc analyses, we found strong positive correlations between the increase in serum concentrations of coffee-derived compounds and changes in adiponectin concentrations (8 compared with 0 cups coffee/d). Significant correlations were observed for caffeine ($r = 0.442$, $P = 0.002$; **Figure 2**), paraxanthine ($r = 0.394$, $P = 0.006$), theophylline ($r = 0.421$, $P = 0.003$), and theobromine ($r = 0.374$, $P = 0.009$) Similar, but weaker

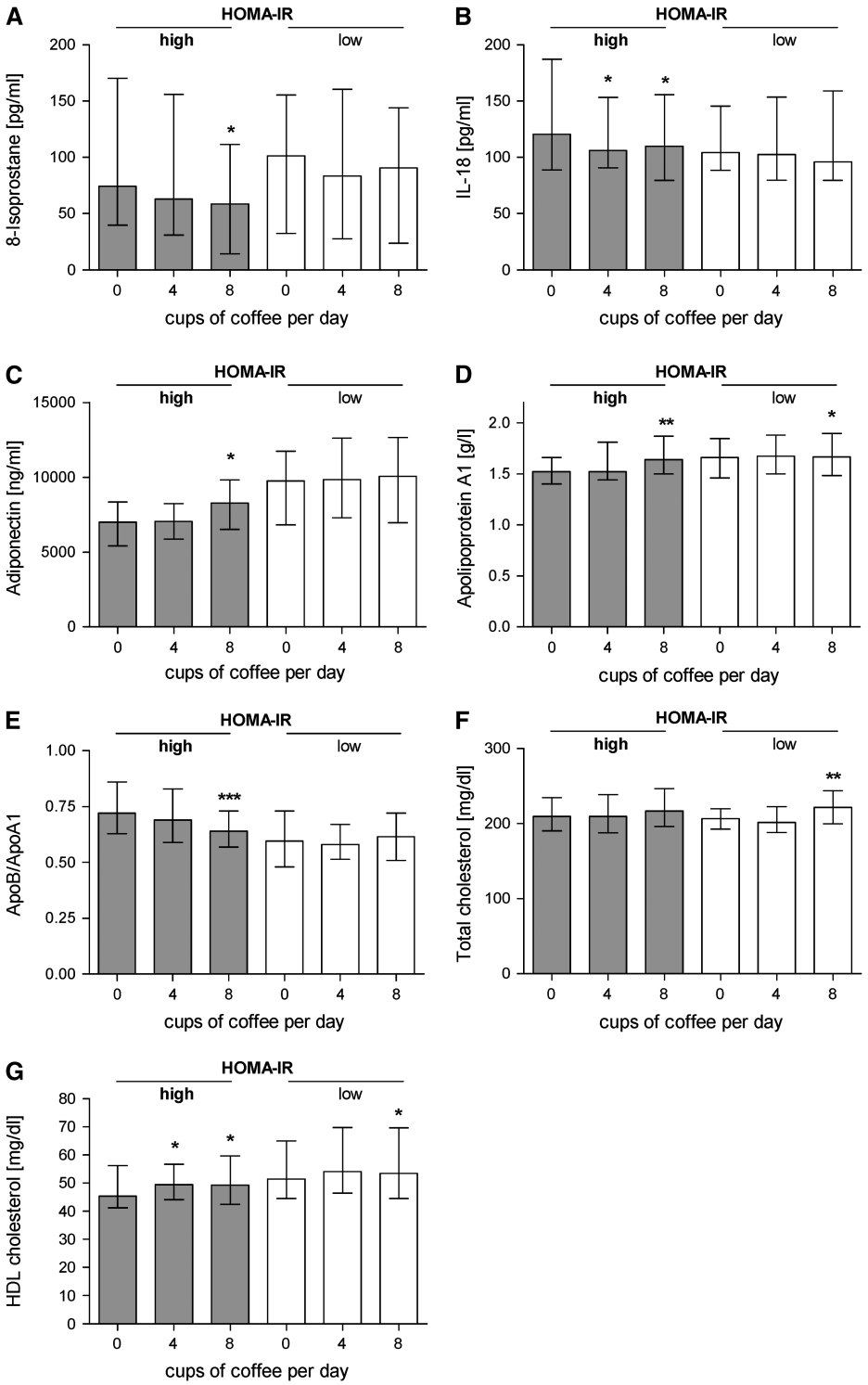

FIGURE 1. Influence of insulin resistance on the effect of coffee consumption on subclinical inflammation and lipids. Shown are median values and 25th and 75th percentiles of serum 8-isoprostane (A), interleukin-18 (IL-18; B), adiponectin (C), apolipoprotein A-I (D), ratio of apolipoprotein B to apolipoprotein A-I (ApoB/ApoA1; E), total cholesterol (F), and HDL cholesterol (G) in subgroups with high (>3.93; $n = 23$) and low (≤3.93; $n = 24$) values for homeostasis model assessment of insulin resistance (HOMA-IR). *,**,***Significantly different from 0 cups coffee/d in the respective subgroup (Friedman's test and Dunn's multiple comparisons test): $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. There was no evidence of an interaction between coffee consumption and insulin-resistance subgroup ($P > 0.4$ for all aforementioned markers).

associations were also observed for isoferulic acid ($r = 0.328$, $P = 0.025$) and dihydroisoferulic acid ($r = 0.323$, $P = 0.027$). Interestingly, negative correlations were found between differences in dihydrocaffeic acid ($r = -0.352$, $P = 0.015$), coumaric acid ($r = -0.477$, $P = 0.001$), 3-(3-hydroxyphenyl)propionic acid ($r = -0.488$, $P = 0.001$), isoferulic acid ($r = -0.289$, $P = 0.049$), dihydroferulic acid ($r = -0.304$, $P = 0.037$), dihydroisoferulic acid ($r = -0.373$, $P = 0.010$), and IL-1ra. All other

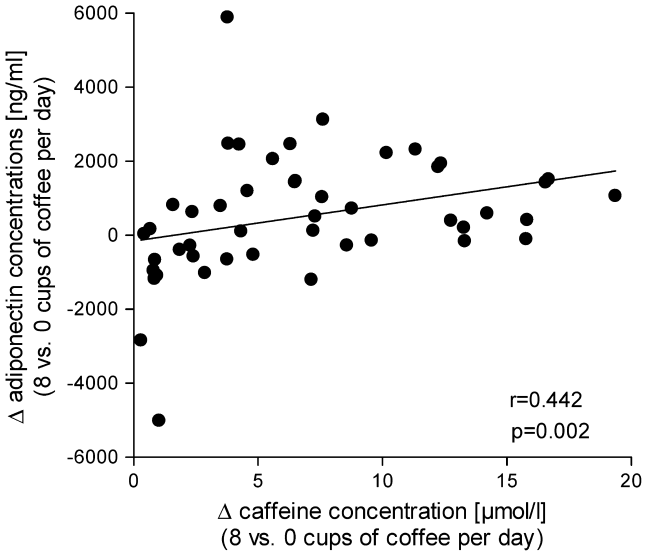

FIGURE 2. Correlation of differences in caffeine concentrations with differences in adiponectin concentrations. Shown are correlations ($n = 47$) of differences in serum adiponectin concentrations (change in adiponectin for 8 compared with 0 cups coffee/d) with differences in plasma concentrations of caffeine (change in caffeine for 8 compared with 0 cups coffee/d). Associations were determined with Spearman correlation coefficients.

inflammation-associated markers showed no correlation with most or all of the coffee-derived compounds (data not shown).

DISCUSSION

Our study represents the first intervention trial to investigate the antiinflammatory effect of coffee and to develop and test coffee-derived compounds in plasma as biomarkers of coffee intake during long-term coffee consumption. To date, validated biomarkers of coffee consumption have not been available. In our study, plasma concentrations of caffeine and its metabolites, caffeic acid and its metabolites, and 3-coumaric acid increased, which indicates that they may be useful as biomarkers of coffee intake. Many of these biomarkers are novel and have not been analyzed in plasma or serum previously. Previous studies have shown that caffeic acid, ferulic acid, dihydrocaffeic acid, and dihydroferulic acid glucuronides or sulfates appear in plasma after a single dose of coffee (23). The half-lives of the phenolic acids were reported to be rather short. Our study indicates that, during chronic consumption, the compounds are present in plasma for a longer time period than one would expect based on the short half-life after a single dose. This is not surprising, because most of them are slowly formed in the large intestine during metabolic reactions catalyzed by enzymes from intestinal microbes. Of the compounds measured here, the methylxanthines are likely the most selective ones as coffee biomarkers, because coffee is by far the most important source of caffeine in coffee-drinking adults. The phenolic acids are likely less selective as biomarkers, because they are also obtained from other foods of plant origin. However, coffee is also the most important dietary source of their precursors, ie, chlorogenic acid and caffeic acid (24).

We observed some changes in concentrations of inflammation-associated mediators in blood, which suggests an antiinflammatory consequence of coffee drinking. There was a significant decrease in circulating IL-18 concentrations, but no changes in systemic CRP or IL-6 concentrations. We previously identified higher concentrations of IL-18 as a risk factor for type 2 diabetes (14). The effect of coffee on CRP is controversial. One cross-sectional study described an association between coffee consumption and higher concentrations of inflammation markers (15). Other studies observed the opposite association. The Nurses' Health Study II reported an inverse correlation between coffee consumption and CRP, IL-6, soluble tumor necrosis factor-receptor 2, E-selectin, and soluble vascular adhesion molecule-1 [median increases from quintiles 1 to 5 (lowest to highest coffee consumption) of 51%, 3%, 9%, 32%, and 9%, respectively] in a population in which coffee consumption was relatively low (6). Lower CRP concentrations (−32% and –61% in different age groups) were also observed in coffee-consuming women in Japan (25), and a decrease in CRP and E-selectin of −10% and –3%, respectively, per 1 cup caffeinated coffee/d in diabetic women was found in the Nurses' Health Study I (7). Another association from observational studies is that of coffee consumption with higher systemic concentrations of the antiinflammatory and insulin-sensitizing adipokine adiponectin (8). Hypoadiponectinemia is closely associated with insulin resistance and risk of type 2 diabetes (26). Interestingly, adiponectin concentrations were higher in diabetic than in nondiabetic women (+28% compared with +12%, respectively) in a comparison of individuals who drank ≥4 cups caffeinated coffee/d and those who drank <1 cup/wk (8). We also observed a significant, but less pronounced increase in adiponectin concentrations in response to increasing coffee consumption (+6% in all study participants; +18% compared with +3% in individuals with high compared with a low HOMA-IR). This finding was confirmed in post hoc analyses by the correlation of increases in coffee metabolite concentrations with those of adiponectin (univariate associations without adjustment for potential confounding factors). The comparison of effect sizes in observational studies with our data indicates that observational studies may overestimate the effect of coffee consumption on subclinical inflammation. This could be due to residual confounding in these epidemiologic studies or to the fact that the duration of our trial (1 mo per stage) was short, and longer periods of coffee consumption may have stronger effects.

Our third finding relates to the antioxidative potential of coffee-derived compounds (27). Animal models showed that liver concentrations of the oxidative stress marker isoprostane increased after vitamin E depletion. This increase could be partly reversed by coffee or coffee components (28). Small intervention studies in men found an acute increase in plasma antioxidant capacity (13) or of resistance of LDL to oxidative modification (29) after coffee consumption. Another study observed an increase in plasma glutathione concentrations after consumption of 5 cups coffee/d for 1 wk (30). A fourth study compared the consumption of 0, 450, or 900 mL coffee/d for 3 wk and found significant changes in markers of oxidative stress, including plasma F2-isoprostanes (31). In the present study, we found a decrease in serum 8-isoprostane concentrations in response to 8 cups coffee/d for 1 mo. This finding was not significant in the subgroup with low insulin resistance, which may explain the lack of effect of coffee on isoprostane concentrations in the study by Mursu et al (31), which included healthy nonsmoking men only. The association between isoprostanes and other markers of oxidative stress with risk of diabetes and cardiovascular disease highlights the relevance of our finding (32).

The fact that we observed increases in total cholesterol, HDL cholesterol, and apolipoprotein A-I as well as a decrease in the ratios of LDL to HDL cholesterol and of apolipoprotein B to apolipoprotein A-I is more difficult to interpret. Previous studies often found increases in total and LDL cholesterol, but not in HDL cholesterol, in response to coffee consumption (33, 34 and references therein). However, these changes were more pronounced with boiled or unfiltered coffee (34); we used filtered coffee, which has less frequently been reported to raise total and LDL cholesterol. Although positive associations between consumption of filtered coffee and higher concentrations of HDL cholesterol and/or apolipoprotein A-I have been found in only a minority of studies (35, 36), we cautiously conclude from our data that there was at least no evidence of adverse effects of coffee on proatherogenic lipids in our study and that favorable effects on HDL cholesterol and apolipoprotein B should be investigated in future studies.

Finally, given the wealth of data from observational studies that indicate an inverse association between coffee consumption and type 2 diabetes (1–3), it is noteworthy that our study showed no effects of coffee on fasting and oral-glucose-tolerance-test–derived markers of glucose metabolism. We cannot exclude that the short duration of the present trial compared with coffee consumption over years in prospective studies could have contributed to this null result. Although we report herein favorable effects of coffee on IL-18 and adiponectin, which have been implicated in the risk of type 2 diabetes, the changes observed we observed were smaller than the differences between baseline concentrations of these markers in low- and high-risk individuals (14, 37–39).

Our study had several limitations and strengths. Our one-group study design without randomization, blinding of participants, and placebo control were limitations. Effects on the observed variables due to inclusion into the trial cannot be excluded. However, a strong argument in support of a molecular effect of coffee consumed is that we observed a good correlation between systemic concentrations of coffee metabolites and outcomes. Moreover, our study may have been too short to observe effects of coffee consumption on the development of type 2 diabetes that have been found in long-term observational studies. In this study we investigated the biological effects of coffee consumption on glucose and insulin metabolism. As strengths, it should be noted that we analyzed direct effects in an intervention trial, whereas previous data were mostly from cross-sectional or observational studies. In addition, the measurement of coffee constituents and metabolites allowed us to assess the compliance within the study. Finally, our study was based on multiple biomarkers that are associated with the risk of type 2 diabetes and/or cardiovascular disease.

In conclusion, coffee consumption appears to have favorable effects on some markers of subclinical inflammation and oxidative stress and to increase plasma concentrations of potential biomarkers of coffee intake. We found some evidence of beneficial effects on the lipid profile, but no effects on glucose metabolism. Because subclinical inflammation is a risk factor for type 2 diabetes, our results suggest one mechanism that could mediate the reduced risk of type 2 diabetes among individuals who habitually consume coffee for years.

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