C-Reactive Protein Partially Mediates the Inverse Association between Coffee Consumption and Risk of Type 2 Diabetes: the UK Biobank and Rotterdam Study Cohorts

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ABSTRACT

Background

We aimed to study the association between coffee consumption and risk of type 2 diabetes as well as the potential mediating role of inflammation.

Methods

Using two population-based cohorts, the UK-Biobank (UKB; n = 145,370) and the Rotterdam Study (RS; n = 7,172), we investigated associations of coffee consumption with incident type 2 diabetes, longitudinal changes in insulin resistance (HOMA-IR) and serum concentrations of inflammatory biomarkers and adipokines. We investigated biomarkers of inflammation as potential mediators of the association between coffee and type 2 diabetes, using causal mediation analysis. Models were adjusted for sociodemographic, lifestyle and health-related factors.

Results

During a median follow-up of 9.4 (RS) and 7.4 (UKB) years, 685 and 2,290 cases of type 2 diabetes occurred, respectively. One cup per day higher coffee consumption was associated with a 4-6% lower risk of type 2 diabetes in both RS (HR 0.94, 95% CI 0.90; 0.98) and UKB (HR 0.96, 95% CI 0.94; 0.98). Higher coffee consumption was also associated with lower HOMA-IR in RS (β -0.017, 95% CI -0.024; -0.010), and with lower CRP in RS (β -0.014, 95% CI -0.022; -0.005) and UKB (β -0.004, 95% CI -0.006; -0.002). Coffee-related changes in CRP mediated 3.4% (0.6%; 14.8%, RS) and 9.6% (5,6%; 24.0%, UKB) of the total effect of coffee on type 2 diabetes risk. Evidence for mediation was also found for adiponectin. Associations were generally stronger among women, never smokers, overweight individuals and consumers of ground coffee as compared to instant coffee.

Conclusions

We observed that the relation between higher coffee consumption and lower risk of type 2 diabetes is partly mediated by lower inflammation associated with coffee consumption.

INTRODUCTION

Coffee is one of the most frequently consumed beverages across the world.[1] It contains several bioactive compounds such as chlorogenic acids, caffeine and polyphenols, although the exact composition depends on the type of coffee and the preparation process used.[2] Given the popularity of coffee, its potential health effects have received significant attention. Coffee consumption has previously been linked to lower risk of cardiovascular and metabolic diseases.[3] Recent meta-analyses and reviews have concluded that coffee consumption is associated with a decreased risk of developing type 2 diabetes.[4–6] However, potential mechanisms underlying these associations are still being investigated.[7] In addition, higher coffee intake has been associated with lower concentrations of inflammatory markers, which may favorably affect cardiometabolic disease risk.[8]

Type 2 diabetes is partly considered an inflammatory disease, with a large number of studies reporting a positive association between markers of inflammation such as C-reactive protein (CRP) and type 2 diabetes risk.[9,10] Moreover, we previously demonstrated that many other inflammatory markers, including interleukin (IL) 13, IL-17 and extracellular newly identified receptor for advanced glycation end-products binding protein (EN-RAGE), were associated with insulin resistance and incident type 2 diabetes.[11] Adipokines, molecules secreted by adipose tissue, are also known to affect the inflammatory response, energy homeostasis and insulin resistance. Given that adiposity is a strong risk factor for type 2 diabetes, it is important to consider the role of adipokines in the association between inflammation and type 2 diabetes.

Studies on coffee consumption thus far have shown contradicting results with regard to its effects on inflammation.[12] This could be due to discrepancies between studies in the amount and type of coffee consumed, duration of the exposure to coffee and demographic characteristics of the study populations. Also, evidence regarding a potential mediating role of inflammatory status in the effect of coffee intake on type 2 diabetes risk is limited. Thus, the aim of this study was to firstly assess the association between coffee consumption and type 2 diabetes risk, and secondly to determine the potential role of inflammatory markers as mediators in this association through causal mediation analysis, among participants from two population-based prospective studies.

METHODS

Study Design

This study involves both prospective and cross-sectional analyses, and is embedded in two large population-based cohorts. These are the United Kingdom Biobank (UKB) and the Rotterdam Study (RS; Rotterdam, the Netherlands). UKB is a prospective cohort study that recruited 502,536 individuals aged 37 to 73 years. Participants were recruited at 22 research centers across England, Scotland and Wales, between April 2006 and December 2010.[13–15] Follow-up data for the longitudinal analyses were available, at the time of conducting the study, up to 27 September 2017. The RS is a population-based cohort study conducted in the district of Ommoord in Rotterdam, the Netherlands, consisting of three sub-cohorts.[16] For the first sub-cohort (RS-I), 7,983 participants aged 55 years or older were recruited in 1990. The second cohort (RS-II) started in 2000 with 3,011 new participants who had become 55 years of age or moved into the district. The third cohort (RS-III) was initiated with the inclusion of 3,932 new individuals aged 45 years or older in 2006. Follow-up examinations were performed every 3-5 years at the research center. The current study used baseline data from RS-I-3, RS-II-1, RS-III-1. For the prospective analyses, follow-up data in RS was completed up to 1 January 2012.

Assessment of coffee consumption

In UKB, baseline data on coffee consumption were collected at baseline through a self-administrated touch-screen food-frequency questionnaire (FFQ). This questionnaire included questions on the type of coffee (i.e. ground, instant or decaffeinated) consumed. Details on the questions and validation of the web-based questionnaire can be found elsewhere.[17,18] In RS, self-reported data on coffee consumption were obtained during home interviews among participants of sub-cohorts RS-I and RS-II, and through an FFQ for sub-cohort RS-III-1. Additional data on consumption of specific food groups among RS-I and RS-II participants were collected using a 170-item FFQ in which subjects were asked about the frequency and amount of foods consumed in the past. Participants of sub-cohort RS-III completed an updated and more extensive 389-item FFQ.

Assessment of inflammatory markers and adipokines

C-reactive protein (CPR, µg/mL) was measured in plasma samples collected at baseline and was analyzed using an immune-turbidimetric assay (Beckman Coulter AU5800) in UKB, and rate near-infrared particle immunoassay for high-sensitivity CRP (IMMAGE Immunochemistry System, Beckman Coulter) in RS. A random subset of 856 plasma samples from RS was sent to Rules-Based Medicine, Austin, Texas to measure specific inflammation biomarkers and adipokines: EN-RAGE (ng/ml), IL-13 (pg/mL), IL-17 (pg/

mL), IL-18 (pg/mL), IL-1 receptor antagonist (IL1ra, pg/mL), complement factor-H (CFH, ug/mL), complement-3 (C3, mg/mL), tumor necrosis factor receptor-2 (TNFRII, ng/mL), adiponectin (ug/mL) and leptin (ng/mL), using multiplex immunoassay. Intra-assay variability was < 4% and inter-assay variability was < 13%.

Ascertainment of insulin resistance and type 2 diabetes

In UKB, data on incident T2D was obtained through linkage with primary care data. Records were extracted for 45% of the cohort (n = 228,495). The end of coverage was May 2017 for Scotland, September 2017 for Wales and August 2017 for England. A detailed description of the linkage procedures is available on the website of the UKB. We defined incident type 2 diabetes as a diagnosis of ICD-10 (International Classification of Diseases, 10th revision) code E11. All participants with type 2 diabetes from primary care data and who were diagnosed before their UKB baseline assessment visit were excluded from the analyses. In RS cases of type 2 diabetes, plasma fasting levels of glucose and insulin were ascertained through follow-up using general practitioners' records, hospital discharge letters and glucose measurements from follow-up visits every 3-5 years. In both cohorts, type 2 diabetes was defined according to the WHO guidelines: fasting blood glucose ≥7.0 mmol/L, non-fasting blood glucose ≥11.1 mmol/L or use of blood glucose-lowering medication (RS). Information on blood glucose-lowering medication use was extracted from structured home interviews and linkage to pharmacy dispensing records. At baseline, over 95% of the RS population was covered by the pharmacies in the district. All potential incident cases of type 2 diabetes were independently adjudicated by two study physicians. In case of disagreement, consensus was reached by consulting an endocrinologist. In RS, glucose concentrations were measured in blood samples at baseline and at follow-up using the glucose hexokinase method within 1 week of sampling.[19] Insulin levels were determined through electrochemiluminescence immunoassay technology, using a Modular Analytics E170 analyzer (Roche Diagnostics GmbH, Germany). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as the product of serum glucose (mmol/L) and serum insulin (μU/L) divided by 22.5.

Covariates assessment

Variables considered as potential confounders included the demographic factors age and sex as well as ethnicity (in UKB only), measures of socioeconomic status (self-reported highest education level in RS and Townsend deprivation index[20] in UKB), lifestyle factors (smoking, quality of the diet, degree of physical activity, alcohol and tea intake) and cardiovascular risk factors (hypertension, body mass index (BMI) and serum lipids). Further details of the measurement procedures can be found elsewhere for RS and UKB.[16,21] Ethnicity in UKB was assessed at recruitment through self-reporting and

classified into 5 categories (white, mixed, south Asian, black, Chinese). Data on lifestyle factors were obtained through self-administered questionnaires. In both cohorts, smoking was categorized into never, former or current smoking. For physical activity assessment, we used the validated International Physical Activity Questionnaire in UKB and the Zutphen (RS-I and RS-II) and LASA (RS-III) questionnaires in RS. Physical activity was expressed in metabolic equivalent of task (MET) hours per week for both cohorts.[22–24] In UKB, self-reported data on tea (cups/day), alcohol (daily or almost daily; 3-4 times a week; once or twice per week; 1-3 times a month; special occasions only; and never) and food groups consumption were collected through the aforementioned FFQ at baseline. Furthermore, the FFQ also assessed the intake of 10 food items. These data were used to build a score expressing the adherence to the recommendations of the UK Eatwell Guide and the Food-Based Dietary Guidelines from the European Food Safety Authority).[25,26] Median intake of these foods was also recorded. In RS, food consumption was assessed using 170-item (RS-I and RS-II) and 389-item (RS-III) FFQs. A score (0–14) reflecting adherence to Dutch dietary guidelines was calculated by adding the scores for 14 components of the Dutch dietary guidelines, as described elsewhere.[27] In both cohorts, physical measurements and collection of blood samples were performed at the research centers. BMI was calculated as weight in kilograms divided by height in meters squared. Blood pressure was recorded as the mean of two consecutive measures. Total serum cholesterol (total-C) and serum high-density lipoprotein cholesterol (HDL-C) were measured in fasting blood samples, using the cholesterol oxidase-peroxidase (CHO-POD) enzymatic reaction and enzyme immune-inhibition methods, respectively (AU5800 chemistry analyzer, Beckman Coulter). Disease information was collected through self-administrated questionnaires and by general practitioner records. Definitions of hypertension and cardiovascular disease (CVD) cases can be found elsewhere for RS and UKB.[16,21]

Population for analyses

We excluded participants with missing data on coffee intake or CRP levels and lack of follow-up for type 2 diabetes. We also excluded participants with type 2 diabetes or CVD at baseline. Thus, the final sample size for analysis was 145,370 participants from UKB and 7,172 participants from RS. A subset of 846 individuals in RS had available data on additional inflammatory markers and adipokines. For a subset of 111,159 UKB participants, information was available on the type of coffee consumed (ground, instant, decaffeinated). Supplementary Figure 3.3.1 shows the study sample selection process in detail.

Statistical analyses

Analyses were performed separately in RS and UKB. For all analyses, coffee consumption was first analyzed continuously (i.e. per one cup per day increase) and subsequently in four categories: non-consumers (0 cups/day); 0.5-2 cups/day; 3-4 cups/day; and 5 or more cups/day. Non-consumers were used as the reference group. Proportional hazards regression was used to assess the association between coffee intake and incident type 2 diabetes. Results were reported as hazard ratios (HR) with corresponding 95% confidence intervals (CIs). The timescale in these regression models was follow-up time in years from baseline examination until incident type 2 diabetes, death, or withdrawal from the study, whichever came first. Linear mixed-effect models were used to study associations between coffee intake and changes in HOMA-IR over time RS participants for whom at least two measurements of HOMA-IR were available. HOMA-IR was transformed to the natural logarithmic scale to approximate a normal distribution. Random intercepts (per participant) and random slopes (for the time variable) were included in the models. Results were expressed as coefficients (β) with corresponding 95% CIs.

For the aforementioned analyses, two statistical models were designed. Model 1 was adjusted for age, sex, RS sub-cohort (RS only), highest attained level of education (RS only), deprivation index (UKB only), smoking, physical activity, diet quality score and daily alcohol and tea intake. Model 2 was additionally adjusted for BMI, hypertension, and serum total/HDL cholesterol ratio. In RS, alcohol intake in grams per day was transformed to the natural logarithmic scale. We conducted linear regression analyses to examine the cross-sectional associations between coffee intake and CRP in both cohorts, and leptin, adiponectin, EN-RAGE, TNFR-II, IL-17, IL-1RA, CHF, IL-18, IL-13, C3 in RS. Biomarkers were natural log-transformed. Results were expressed as coefficients (β) with corresponding 95% CIs.

The biomarkers significantly associated with coffee consumption were tested in a mediation analysis. We first estimated the associations between coffee consumption and biomarker (*a,* Figure 3.3.2); between biomarker and type 2 diabetes adjusted for coffee intake (*b*); and between coffee intake and type 2 diabetes adjusted for biomarker (*c´*). Next, we used the *mediation* package for the statistical software *R* to estimate the proportion of the total effect (*c*) of coffee on risk of type 2 diabetes that is mediated by coffee-related changes in the biomarker concentrations, by comparing the effect estimates of each association of coffee consumption with type 2 diabetes, under the assumption of sequential ignorability (i.e. the assumption that there is no unmeasured confounding).[28] Quasi-Bayesian confidence intervals were constructed for the estimated effects with 10,000 simulations. Results were expressed as proportion

mediated in percentage and corresponding 95% CIs. Ten-fold multiple imputation was performed to account for missing data on covariates. A two-sided p-value < 0.05 was considered statistically significant. All results correspond to the pooled results of the ten imputed datasets. Analyses were performed using R version 4.0.1 (R foundation for Statistical Computing, Austria).

RESULTS

Baseline characteristics

The mean age was 65.1 (SD 9.4) years among RS participants (n = 7,172), of whom 59.7% (n = 4,283) were female. In UKB (n = 145,370) the mean age was 55.2 (SD 8.1) years and 58.0% (n = 84,343) were female. During a median follow-up of 9.4 (IQR 4.5; 11.8) years in RS and 7.4 (IQR 6.8; 8.3) years in UKB, 685 and 2,290 incident type 2 diabetes cases occurred, respectively. Table 3.3.1 and Supplementary Tables 3.3.1 and 3.3.2 show more details on the characteristics of the participants.

Coffee consumption, insulin resistance and incident type 2 diabetes

After comprehensive adjustment for covariates (model 2), higher coffee intake was associated with lower type 2 diabetes risk in both RS (HR 0.94 per cup/day increase, 95% CI 0.90; 0.98) and UKB (HR 0.96, 95% CI 0.94; 0.99). Compared to non-consumers, those drinking ≥ 5 cups per day had a lower risk of developing type 2 diabetes (Table 3.3.2). This was observed for both RS (HR 0.62, 95% CI 0.45; 0.86) and UKB (HR 0.80. 95% CI 0.69; 0.93). Among RS participants, we also observed an association between higher coffee consumption and a longitudinal decrease in HOMA-IR levels (β -0.017, 95% CI -0.024; -0.010).

Coffee consumption and markers of inflammation

After comprehensive adjustment for covariates (model 2), higher coffee intake was associated with lower circulating levels of CRP in both RS (β -0.014 per cup per day increase, 95% CI -0.022; -0.005) and UKB (β -0.004, 95% CI -0.006; -0.002) (Table 3.3.3). Among participants from RS with data on additional inflammatory markers, we observed that higher coffee intake was associated with higher adiponectin concentrations (β 0.025, 95% CI 0.007; 0.042). Higher coffee intake was also associated with lower complement-3 and higher IL-18, but only in certain categories of coffee consumption as compared to non-consumers (Supplementary Table 3.3.3). No significant findings were observed for the other biomarkers. Results did not change after additional adjustment for CRP levels.

Table 3.3.1: Summary of baseline characteristics of the Rotterdam Study and UK Biobank participants, stratified by coffee consumption categories.

	Non-coffee drinkers	0.5-2 cups/day	3-4 cups/day	5 or more cups/day	Total
The Rotterdam Study					
n (%)	542 (7.6)	1,594 (22.2)	2,883 (40.2)	2,053 (28.6)	7,172
Age, mean (SD), years	62.1 (9.8)	68.4 (10.1)	65.8 (9.2)	62.3 (7.8)	65.1 (9.4)
Sex, (% women)	373 (68.8)	1,064 (66.8)	1,844 (61.8)	1,002 (48.8)	4,283 (59.7)
Smoking status					
Never smoker	252 (46.5)	652 (40.9)	994 (33.3)	453 (22.1)	2,351 (32.8)
Former smoker	209 (38.6)	730 (45.8)	1,939 (46.8)	839 (40.9)	3,174 (44.3)
Current smoker	81 (14.9)	212 (13.3)	593 (19.9)	761 (37.0)	1,647 (22.9)
Physical activity, median (IQR), MET-hours/week	61.9 (68.4)	71.3 (60.9)	60.8 (60.8)	70.5 (67. 6)	72.0 (63.4)
BMI, mean (SD), kg/m^2	26.8 (4.7)	26.7 (4.0)	26.9 (3.8)	27.1 (4.0)	26.9 (4.0)
CRP, median (IQR), ug/mL	1.40 (2.80)	1.27 (2.9)	1.50 (2.70)	1.36 (2.47)	1.5 (2.7)
Incident type 2 diabetes cases, n (%)	52 (9.6)	171 (10.7)	276 (9.6)	187 (9.1)	685 (9.6)
The UK Biobank					
n (%)	31,773 (21.9)	66,153 (45.5)	30,440 (20.9)	17,004 (11.7)	145,370
Age, mean (SD), years	53.9 (8.09)	55.8 (8.10)	55.5 (8.03)	54.5 (7.94)	55.2 (8.10)
Sex, (%) women	19,818 (62.4)	39,088 (59.1)	16,715 (54.9)	8,722 (51.3)	84,343 (58.0)
Smoking status, n (%)					
Never smoker	19,659 (61.9)	39,535 (59.8)	16,624 (54.6)	7,559 (44.4)	83,377 (57.4)
Former smoker	9,077 (28.6)	21,335 (32.3)	10,325 (33.9)	5,711 (33.6)	46,448 (32.0)
Current smoker	3,037 (9.6)	5,283 (8.0)	3,491 (11.5)	3,734 (22.0)	15,545 (10.7)
Physical activity, median (IQR), MET-hours/week	48.1 (46.8)	45.8 (43.7)	45.0 (43.3)	47.3 (45.9)	46.3 (44.4)
BMI, mean (SD), kg/m^2	26.2 (4.4)	25.9 (4.0)	26.6 (4.2)	27.1 (4.3)	26.3 (4.20)
CRP, median (IQR), ug/mL	1.15 (1.84)	1.03 (1.57)	1.08 (1.65)	1.76 (1.19)	1.08 (1.67)
Incident type 2 diabetes cases, n (%)	205 (1.6)	298 (1.2)	147 (1.1)	95 (1.4)	745 (1.3)

Table 3.3.2: Associations of coffee consumption with longitudinal measures of HOMA-IR and incident type 2 diabetes in the Rotterdam Study and with incident type 2 diabetes in the UK Biobank.

		The Rotterdam Study				UK Biobank	
		Longitudinal HOMA-IR[1] (n = 4,138)		Incident type 2 diabetes (n = 7,172)		Incident type 2 diabetes (n = 145,370)	
	Coffee (cups per day)	β (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Model 1	Per cup increase	-0.011 (-0.020; -0.002)	0.013	0.95 (0.91; 0.99)	0.033	0.98 (0.95; 0.99)	0.043
	0	ref		ref		ref	
	0.5-2	0.044 (-0.021; 0.109)	0.187	0.83 (0.60; 1.14)	0.253	0.90 (0.81; 0.99)	0.004
	3-4	-0.005 (-0.065; 0.054)	0.856	0.67 (0.50; 0.91)	0.011	0.84 (0.74; 0.96)	0.012
	≥ 5	-0.028 (-0.089; 0.033)	0.376	0.67 (0.49; 0.92)	0.014	0.88 (0.75; 1.01)	0.085
	p for trend		0.028		0.005		0.030
Model 2	Per cup increase	-0.017 (-0.024; -0.010)	< 0.001	0.94 (0.90; 0.98)	0.008	0.96 (0.94; 0.98)	0.001
	0	ref		ref		ref	
	0.5-2	0.020 (-0.033; 0.073)	0.465	0.79 (0.57; 1.09)	0.147	0.92 (0.83; 1.02)	0.124
	3-4	-0.022 (-0.071; 0.026)	0.366	0.64 (0.47; 0.87)	0.004	0.82 (0.72; 0.94)	0.004
	≥ 5	-0.064 (-0.114; -0.014)	0.012	0.62 (0.45; 0.86)	0.004	0.80 (0.69; 0.93)	0.005
	p for trend		< 0.001		0.001		0.001

Estimates are regression coefficients (β) from linear mixed models and hazard ratios (HR) from proportional hazards regression models with corresponding 95% confidence intervals (CI). Model 1: adjusted for age, sex, Rotterdam Study cohort (RS only), level of education (RS only), deprivation index (UKB only), ethnicity (UKB only), tea consumption, alcohol consumption, smoking status, physical activity and diet quality score; Model 2: additionally adjusted for body mass index, hypertension and serum total cholesterol/HDL ratio. There were n=685 cases of incident type 2 diabetes in the Rotterdam Study and n = 2,290 cases in the UK Biobank. [1]Variable was transformed using the natural logarithm.

Table 3.3.3: Associations of coffee consumption with CRP levels per categories of consumption

	Coffee (cups/day)	The Rotterdam Study (n = 7,172)		UK Biobank (n = 145,370)	
		β (95% CI)	P-value	β (95% CI)	P-value
Model 1	Per cup increase	-0.009 (-0.017; 0.000)	0.058	-0.001 (-0.003; 0.001)	0.128
	0	ref		ref	
	0.5-2	-0.030 (-0.096; 0.036)	0.371	-0.048 (-0.056; -0.039)	< 0.001
	3-4	-0.048 (-0.110; 0.014)	0.128	-0.039 (-0.049; -0.028)	< 0.001
	≥ 5	-0.067 (-0.131; -0.002)	0.043	-0.020 (-0.032; -0.008)	< 0.001
	p for trend		0.026		< 0.001
Model 2	Per cup increase	-0.014 (-0.022; -0.005)	0.002	-0.004 (-0.006; -0.002)	< 0.001
	0	ref		ref	
	0.5-2	-0.039 (-0.102; 0.023)	0.217	-0.048 (-0.056; -0.040)	< 0.001
	3-4	-0.064 (-0.123; -0.005)	0.034	-0.044 (-0.054; -0.034)	< 0.001
	≥ 5	-0.084 (-0.153; -0.030)	0.004	-0.033 (-0.045; -0.021)	< 0.001
	p for trend		0.001		< 0.001

Estimates are regression coefficients (β) from linear regression models with corresponding 95% confidence intervals (CI). Model 1: adjusted for age, sex, Rotterdam Study cohort (RS only), level of education (RS only), deprivation index (UKB only), ethnicity (UKB only), tea consumption, alcohol consumption, smoking status, physical activity, and diet quality score; Model 2: further adjusted for body mass index, hypertension and serum total cholesterol/HDL ratio. Transformation using the natural logarithm was used for CRP (μg/mL).

Mediation analyses

A schematic representation of the causal mediation analyses and results are shown in Figure 3.3.1 and Supplementary Table 3.3.4. In RS, the association between coffee intake and CRP levels was statistically significant ($a_{CRP\text{-}RS}$, β -0.014, 95% CI -0.022; -0.005), as well as the association between CRP and incident type 2 diabetes adjusting for coffee intake ($b_{CRP\text{-}RS}$, HR 1.17 per 1 unit increase in log-transformed CRP, 95% CI 1.04; 1.31). Similarly, coffee intake was associated with incident type 2 diabetes independent of CRP ($c'_{independent\ of\ CRP\text{-}RS}$, HR 0.94, 95% CI 0.90; 0.99). The mediation analysis in RS demonstrated that a significant proportion of the total effect of coffee consumption on risk of type 2 diabetes was mediated by coffee-related changes in CRP (proportion mediated 3.4%, 95% CI 0.6%; 14.8%). In UKB, we observed a significant inverse association between coffee intake and CRP ($a_{CRP\text{-}UKB}$, β -0.011, 95% CI -0.012; -0.009); and between CRP and type 2 diabetes incidence, adjusting for coffee consumption ($b_{CRP\text{-}UKB}$, HR 1.45, 95% CI 1.36; 1.53). The association between coffee consumption and type 2 diabetes incidence adjusting for CRP was also significant ($c'_{independent\ of\ CRP\text{-}UKB}$, HR 0.97, 95% CI 0.94; 0.99). In UKB, changes in CRP also significantly mediated [9.6%, 95% CI 5.7%; 24.0%] the total effect of coffee on risk of type 2 diabetes.

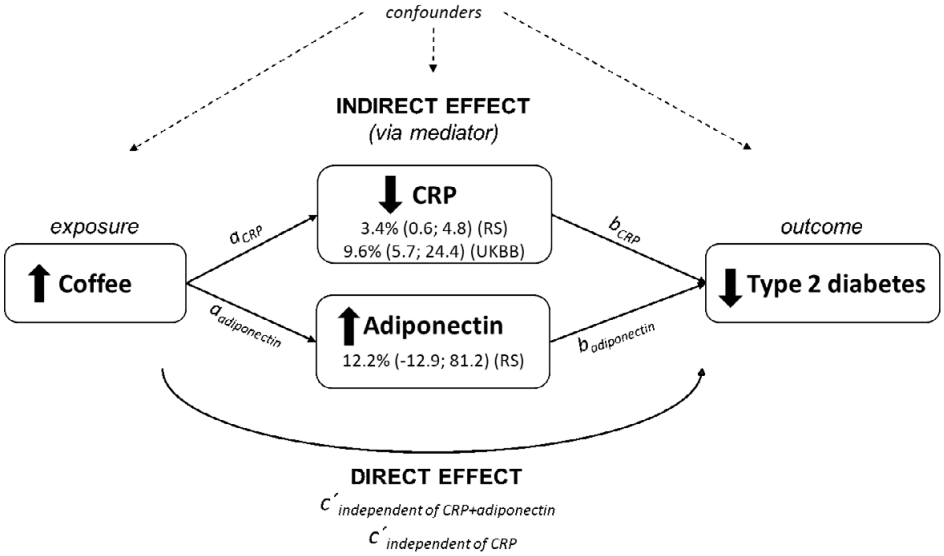


Figure 3.3.1. Schematic representation of the causal mediation analyses. These analyses estimated the proportion of the total effect of higher coffee intake (increase in one cup per day) on risk of type 2 diabetes that is potentially mediated by coffee-related changes in serum C-reactive protein levels (CRP) and adiponectin. In the figure, a_{CRP} and $a_{adiponectin}$ represent the potential effect of coffee consumption on log-CRP and log-adiponectin levels, respectively. $a_{CRP\text{-}RS}$: β = -0.014 (95% confidence interval -0.022; -0.005); $a_{CRP\text{-}UKB}$: β = -0.010 (-0.012; -0.007) and $a_{adiponectin\text{-}RS}$: β = 0.025 (0.007; 0.042). Furthermore, b_{CRP} and $b_{adiponectin}$ correspond to the effect of coffee-related changes in log-CRP and log-adiponectin levels on risk of type 2 diabetes, respectively, controlling for coffee consumption. $b_{CRP\text{-}RS}$: HR = 1.17 (1.04; 1.31); $b_{CRP\text{-}UKB}$: HR = 1.45 (1.36; 1.53); and $b_{adiponectin}$: HR = 0.58 (0.32; 0.83). c' relates to the effect of coffee consumption on type 2 diabetes risk that is exerted directly or via mediators other than CRP or adiponectin. $c'_{independent\ of\ CRP\text{-}UKB}$: HR = 0.97 (0.94; 0.99); $c'_{independent\ of\ CRP\text{-}RS}$: HR = 0.94 (0.90; 0.99); and $c'_{independent\ of\ CRP+adiponectin\text{-}RS}$: HR = 0.90 (0.80; 1.01). The analyses estimated that coffee-related changes in CRP levels may constitute a partial mediator mechanism in the effect of coffee intake on type 2 diabetes, with a proportion mediated of 3.4% (0.6%; 4.8%) and 9.6% (5.7%; 24.4%), among Rotterdam Study (RS) and UK Biobank (UKB) participants respectively. Adiponectin may also constitute a potential mediator. Dotted lines represent confounding among the exposure, mediators and outcome variables. Confounders considered were age, sex, education (RS only), deprivation index (UKB only), ethnicity (UKB only), tea consumption, alcohol consumption, smoking, physical activity, diet score, body mass index, hypertension and serum total cholesterol/high-density lipoprotein (HDL) ratio.

Adiponectin, complement 3 and IL-18 were also tested as potential mediators in RS, adjusting for CRP in each case. Evidence for mediation was found only for adiponectin, whose effect on type 2 diabetes incidence was independent of coffee consumption and CRP levels ($b_{adiponectin\text{-}RS}$, HR 0.58, 95% CI 0.32; 0.83). In this case, the direct effect of coffee consumption on type 2 diabetes did not remain significant after additional adjustment for adiponectin ($c'_{independent\ of\ CRP+adiponectin\text{-}RS}$, HR 0.90, 95% CI 0.80; 1.01). Estimates for complement-3 and IL-18 did not suggest they were part of the causal pathway (Supplementary Table 3.3.4).

Table 3.3.4: Causal mediation analyses for the association of coffee intake (cups/day) with type 2 diabetes by inflammatory markers in the UK biobank and Rotterdam Study

	a biomarker ~ coffee[1]		*b* type 2 diabetes ~ biomarker + coffee[1]		*c´* type 2 diabetes ~ coffee + biomarker[1]		Proportion mediated	
	β (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	Percentage (%) (95% CI)	p-value
UK Biobank								
CRP[2]	-0.011 (-0.012; -0.009)	< 0.001	1.45 (1.36; 1.53)	< 0.001	0.97 (0.94; 0.99)	0.003	9.6 (5.7; 24.4)	0.002
The Rotterdam Study								
CRP[2]	-0.014 (-0.022; -0.005)	0.002	1.17 (1.04; 1.31)	0.008	0.94 (0.90; 0.99)	0.010	3.4 (0.6; 4.8)	0.015
Adiponectin[2,3]	0.025 (0.007; 0.042)	0.006	0.58 (0.32; 0.83)	0.006	0.90 (0.80; 1.01)	0.079	12.2 (-12.9; 81.2)	0.066
C3[2,3]	-0.006 (-0.012; 0.001)	0.094	2.35 (0.78; 7.1)	0.129	0.89 (0.79; 0.99)	0.048	3.4 (-4.7; 26.0)	0.207
IL-18[2,3]	0.001 (-0.017; 0.019)	0.090	1.36 (0.88; 2.12)	0.170	0.89 (0.79; 0.99)	0.047	-0.1 (-15.9; 12.8)	0.894

Estimates are regression coefficients (β) from linear mixed models and hazard ratios (HR) from proportional hazards regression models with corresponding 95% confidence intervals (CI). [1]Regressions adjusted for age, sex, Rotterdam Study cohort (RS only), level of education (RS only), deprivation index (UKB only), ethnicity (UKB only), tea consumption, alcohol consumption, smoking status, physical activity, diet quality score, hypertension, body mass index and serum total cholesterol/HDL ratio. [2]Variable was transformed using the natural logarithm. [3]Additionally adjusted for CRP.

Additional analyses

The analyses for the association between coffee consumption and type 2 diabetes were repeated excluding cases of incident type 2 diabetes which occurred during the first 2 years of follow-up (RS n = 589; UKB n = 547). No major differences were observed in the effect estimates (Supplementary Table 3.3.5). We identified significant interaction between coffee intake and sex, age, smoking or BMI on some of the outcomes (Supplementary Table 3.3.6) and, therefore, conducted stratified analyses. Associations between coffee and CRP became slightly weaker with older age (p-interaction 0.046). Associations between coffee and type 2 diabetes, HOMA-IR and CRP were generally stronger among women and never smokers in both cohorts and among former smokers in RS. Results for former and current smokers were inconsistent across cohorts (Supplementary Tables 3.3.7 - 3.3.9). Those with a higher BMI (≥ 25 kg/m^2) showed stronger associations with lower CRP and HOMA-IR (Supplementary Table 3.3.10). Associations between coffee and type 2 diabetes and CRP were stronger among consumers of regular ground coffee, than among drinkers of instant or decaffeinated coffee (Supplementary Table 3.3.11).

DISCUSSION

In this study, we observed associations between higher coffee consumption and lower risk of type 2 diabetes. Additionally, higher coffee consumption was associated with lower longitudinal HOMA-IR and lower plasma CRP, as well as higher adiponectin and IL-18 concentrations. Furthermore, we found evidence of mediation of the association between coffee consumption and risk of type 2 diabetes by coffee-related changes in CRP and adiponectin levels.

Although findings were generally consistent between the RS and UKB, a few differences between the two cohorts were observed in the strength of associations or for certain categories of coffee intake. These differences might be due to dissimilar coffee consumption habits and characteristics of both study populations. Average coffee consumption was higher among individuals in the RS cohort as compared to those in UKB (modal consumption 0.5-2 cups/day versus 3-4 cups/day). Given these differences between the countries, and taking into account the notion that the United Kingdom is traditionally a tea-drinking nation, we also included tea consumption as a potential confounder in our models.[29] In addition to these differences, UKB participants were on average around ten years younger than RS participants.

Our findings confirm the evidence from several previous studies with regards to the protective effect of coffee consumption on type 2 diabetes risk.[4,5] Several previous studies suggested potential mechanisms explaining these observations. For instance, chlorogenic acid, a phenolic coffee compound, reduces intestinal glucose uptake and blood glucose levels. Furthermore, it has been hypothesized that the antioxidant capacity of some coffee compounds may reduce oxidative stress. Coffee consumption may also modulate adenosine receptor signaling and, in turn, insulin and glucagon signaling. Modulation of the microbiome content and diversity by coffee has also been suggested as an intermediate mechanism.[7,30]

We observed associations between higher coffee intake and lower plasma CRP, lower complement-3 and higher adiponectin concentrations as well as an association with IL-18 concentrations, but no significant association with the rest of the studied biomarkers. Ours is the first study to suggest that CRP and adiponectin might be in the causal pathway mediating the association of higher coffee consumption and lower risk of type 2 diabetes, although with opposite directions of effect, by providing evidence from causal mediation analyses. The small proportion (3.4% in RS) of the total effect of coffee consumption on risk of type 2 diabetes that appeared to be mediated by coffee-related changes in CRP, may suggest the existence of a more complex network of interplaying metabolic and inflammatory biomarkers and alternative indirect effects of coffee that were not investigated in this study, or for which our analyses were underpowered. For example, CRP is produced in response to increase in the concentrations of pro-inflammatory cytokines IL-6, IL-1 and IL-17 and recent evidence showed that coffee intake may be associated with a broader range of biomarkers of inflammation and metabolism beyond CRP alone, such as adiponectin.[31,32]

We found evidence that adiponectin mediates the effect of coffee intake on type 2 diabetes risk. Furthermore, when adiponectin was included as predictor of type 2 diabetes risk, the direct effect of coffee consumption (*c'*) was significantly attenuated. This might suggest that adiponectin could completely mediate the association. However, coffee is hypothesized to be involved in a more complex interplay of biomarkers, which is in agreement with our mediation findings on CRP.[32] Thus, a potential alternative explanation for the observed attenuation of coffee's direct effect may be the influence of a different pathway or the presence of another mediator in the opposite direction.[33] Moreover, since the analysis on adiponectin was performed in a smaller sample of participants with available data on specific biomarkers, issues with statistical power cannot be ruled out. A previous Mendelian randomization study found no consistent evidence for a causal association between genetically lower levels of serum adiponectin and higher risk of type 2 diabetes or higher fasting insulin.[34] There-

fore, our mediation analysis result on adiponectin must be interpreted cautiously. Whether adiponectin might constitute a biomarker reflecting another underlying causal mechanism needs additional research. Adiponectin has been shown to have anti-inflammatory and insulin-sensitizing effects.[35] Lower concentrations of serum adiponectin have been associated with obesity and insulin sensitivity. Thus, adiponectin has been suggested as a therapeutic target for diabetes and obesity.[35] Available evidence on coffee intake and adiponectin is contradictory.[32,36] Our findings indicate a significant dose-response association between coffee intake and higher adiponectin concentrations.

Furthermore, we showed a significant positive association between higher coffee consumption and plasma IL-18 in some categories of coffee consumption. Our results on plasma TNFRII and leptin contradict a recent observational study reporting a significant trend between higher coffee consumption and lower plasma TNFRII and leptin.[32] Our findings on IL1ra were in agreement with clinical trials that found no changes in plasma concentrations after coffee intervention.[37]

We observed some disparities in the strength of the association between higher coffee consumption and lower type 2 diabetes risk. This was also the case for the association between higher coffee consumption and lower CRP concentrations. These disparities were observed among participants with different smoking statuses as well as between the RS and UKB. Never smokers had a stronger association between coffee consumption and type 2 diabetes and CRP in both cohorts, as well as former smokers in RS. However, in UKB, the results among former and current smokers were inconsistent. These discrepancies might be explained by the differences in smoking habits between the two cohorts, as well as differing coffee consumption patterns. Smoking may play an important role increasing oxidative stress and the consequent inflammatory response.[38] A study reported that current smokers with long smoking duration and greater daily smoking quantity had elevated oxidative stress markers and lower antioxidant levels than never and former smokers, but that this is reversible after a long period of smoking cessation.[38] Future studies on coffee and health outcomes should explore to what extent current and former smoking may interfere with coffee´s antioxidant effects.

Detrimental effects of coffee, like raising serum lipids, are attributed to coffee oils, which could be removed from the coffee extract by using a filter.[39] A meta-analysis of clinical trials reported that the increment in total cholesterol was greater among patients in trials using unfiltered coffee as treatment.[39] In our study, ground coffee,

which is generally filtered, showed the strongest beneficial association with lower type 2 diabetes risk and CRP concentration.

Strengths of this study are the large sample size from two cohort studies and the longitudinal analyses of repeated measures of HOMA-IR in the RS specifically. Moreover, we made use of causal mediation analysis including time-to-event data on incident type 2 diabetes. An additional strength of our study is the comprehensive consideration of confounding factors. Among several other confounding factors, our models were additionally adjusted for smoking status, alcohol consumption and diet quality. Furthermore, we performed stratified analyses providing further insight into how the association between coffee consumption and type 2 diabetes differed between subgroups. There are also several limitations to our study. The analyses on specific inflammatory markers and adipokines had limited sample size, therefore conclusions from these analyses must be carefully considered. Additionally, our study did not assess differences between caffeinated and decaffeinated coffee. There is controversy regarding a potential effect of caffeine on inflammatory markers, as concluded by a recent systematic review of clinical trials.[12] Furthermore, we did not perform adjustment for sugar intake with coffee, although we did adjust for overall diet quality. In the mediation analyses, we used as the mediator CRP concentrations which were measured at the same time point as coffee intake. Ideally, CRP would have been measured some period of time after the assessment of coffee consumption. However, use of a cross-sectional analysis will not have introduced reverse causation since it is unlikely that circulating concentrations of CRP change coffee consumption behavior.

In conclusion, our findings from two large population-based cohort studies emphasize the association between higher coffee consumption and lower type 2 diabetes risk, as well as lower insulin resistance over time. Furthermore, we observed that higher coffee intake was associated with lower CRP concentrations. Ours is the first study to demonstrate that changes in CRP related to coffee consumption may mediate part of the inverse association between coffee consumption and type 2 diabetes.

REFERENCES

1 Gunter MJ, Murphy N, Cross AJ, Dossus L, Dartois L, Fagherazzi G *et al.* Coffee Drinking and Mortality in Ten European Countries – the EPIC Study. *Ann Intern Med* 2017; **167**: 236–247.

2 Ludwig IA, Clifford MN, Lean ME, Ashihara H, Crozier A. Coffee: biochemistry and potential impact on health. *Food Funct* 2014; **5**: 1695–1717.

3 Ding M, Bhupathiraju SN, Satija A, 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**: 643–659.

4 Carlstrom M, Larsson SC. Coffee consumption and reduced risk of developing type 2 diabetes: a systematic review with meta-analysis. *Nutr Rev* 2018; **76**: 395–417.

5 Poole R. Coffee consumption and health: umbrella review of meta-analyses of multiple health outcomes. *BMJ* 2017; **359, j5024**. doi:10.1136/bmj.j5024.

6 Grosso G, Godos J, Galvano F, Giovannucci EL. Coffee, Caffeine, and Health Outcomes: An Umbrella Review. *Annu Rev Nutr* 2017; **37**: 131–156.

7 Tsalamandris S. The Role of Inflammation in Diabetes: Current Concepts and Future Perspectives. *Eur Cardiol* 2019; **14**: 50–59.

8 Lopez-Garcia E, Dam RM, Qi L, Hu FB. Coffee consumption and markers of inflammation and endothelial dysfunction in healthy and diabetic women. *Am J Clin Nutr* 2006; **84**: 888–893.

9 Kanmani S, Kwon M, Shin MK, Kim MK. Association of C-Reactive Protein with Risk of Developing Type 2 Diabetes Mellitus, and Role of Obesity and Hypertension: A Large Population-Based Korean Cohort Study. *Sci Rep* 2019; **9**: 4573.

10 Liu C. Adiponectin, TNF-alpha and inflammatory cytokines and risk of type 2 diabetes: A systematic review and meta-analysis. *Cytokine* 2016; **86**: 100–109.

11 Brahimaj A. Novel inflammatory markers for incident pre-diabetes and type 2 diabetes: the Rotterdam Study. *Eur J Epidemiol* 2017; **32**: 217–226.

12 Paiva C. Consumption of coffee or caffeine and serum concentration of inflammatory markers: A systematic review. *Crit Rev Food Sci Nutr* 2019; **59**: 652–663.

13 Collins R. What makes UK Biobank special? *Lancet Lond Engl* 2012; **379**: 1173–1174.

14 Palmer LJ. UK Biobank: bank on it. *Lancet Lond Engl* 2007; **369**: 1980–1982.

15 Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J *et al.* UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med* 2015; **12**: e1001779.

16 Ikram MA. Objectives, design and main findings until 2020 from the Rotterdam Study. *Eur J Epidemiol* 2020; **35**: 483–517.

17 Bradbury KE, Young HJ, Guo W, Key TJ. Dietary assessment in UK Biobank: an evaluation of the performance of the touchscreen dietary questionnaire. *J Nutr Sci* 2018; **7**: e6.

18 Liu B, Young H, Crowe FL, Benson VS, Spencer EA, Key TJ *et al.* Development and evaluation of the Oxford WebQ, a low-cost, web-based method for assessment of previous 24 h dietary intakes in large-scale prospective studies. *Public Health Nutr* 2011; **14**: 1998–2005.

19 Neeley WE. Simple Automated Determination of Serum or Plasma Glucose by a Hexokinase/Glucose-6-Phosphate Dehydrogenase Method. *Clin Chem* 1972; **18**: 509–515.

20 Townsend P, Phillimore P, Beattie A. *Health and deprivation: Inequality and the north*. Croom Helm: London, 1988.

21 UK Biobank: Protocol for a large-scale prospective epidemiological resource. 2007.https://www.ukbiobank.ac.uk/wp-content/uploads/2011/11/UK-Biobank-Protocol.pdf (accessed 24 Sep2020).

22 Craig CL. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 2003; **35**: 1381–1395.

23 Caspersen CJ, Bloemberg BP, Saris WH, Merritt RK, Kromhout D. The prevalence of selected physical activities and their relation with coronary heart disease risk factors in elderly men: the Zutphen Study, 1985. *Am J Epidemiol* 1991; **133**: 1078–1092.

24 Stel VS, Smit JH, Pluijm SMF, Visser M, Deeg DJH, Lips P. Comparison of the LASA Physical Activity Questionnaire with a 7-day diary and pedometer. *J Clin Epidemiol* 2004; **57**: 252–258.

25 Cobiac LJ, Scarborough P, Kaur A, Rayner M. The Eatwell Guide: Modelling the Health Implications of Incorporating New Sugar and Fibre Guidelines. *PLoS One* 2016; **11, e0167859**: 16–35010.

26 EFSA Panel on Dietetic Products, Nutrition and Allergies. *Scientific Opinion on establishing Food-Based Dietary Guidelines*. European Food Safety Authority (EFSA), 2010.

27 Voortman T. Adherence to the 2015 Dutch dietary guidelines and risk of non-communicable diseases and mortality in the Rotterdam Study. *Eur J Epidemiol* 2017; **32**: 993–1005.

28 Tingley D, Yamamoto T, Hirose K, Keele L, Imai K. mediation: R Package for Causal Mediation Analysis. *J Stat Softw* 2014; **59**.

29 Nelson M, Poulter J. Impact of tea drinking on iron status in the UK: a review. *J Hum Nutr Diet* 2004; **17**: 43–54.

30 Pollack RM, Donath MY, LeRoith D, Leibowitz G. Anti-inflammatory Agents in the Treatment of Diabetes and Its Vascular Complications. *Diabetes Care* 2016; **39 Suppl 2**: 244–252.

31 Agrawal A, Cha-Molstad H, Samols D, Kushner I. Overexpressed nuclear factor-kappaB can participate in endogenous C-reactive protein induction, and enhances the effects of C/EBPbeta and signal transducer and activator of transcription-3. *Immunology* 2003; **108**: 539–547.

32 Hang D. Coffee consumption and plasma biomarkers of metabolic and inflammatory pathways in US health professionals. *Am J Clin Nutr* 2019; **109**: 635–647.

33 Zhang Z. Causal mediation analysis in the context of clinical research. *Ann Transl Med* 2016; **4**: 425.

34 Yaghootkar H. Mendelian randomization studies do not support a causal role for reduced circulating adiponectin levels in insulin resistance and type 2 diabetes. *Diabetes* 2013; **62**: 3589–3598.

35 Achari AE, Jain SK. Adiponectin, a Therapeutic Target for Obesity, Diabetes, and Endothelial Dysfunction. *Int J Mol Sci* 2017; **18**. doi:10.3390/ijms18061321.

36 Jacobs S. Evaluation of various biomarkers as potential mediators of the association between coffee consumption and incident type 2 diabetes in the EPIC-Potsdam Study. *Am J Clin Nutr* 2014; **100**: 891–900.

37 Ramakers BP. Circulating adenosine increases during human experimental endotoxemia but blockade of its receptor does not influence the immune response and subsequent organ injury. *Crit Care* 2011; **15, R3**. doi:10.1186/cc9400.

38 Zhou JF. Effects of cigarette smoking and smoking cessation on plasma constituents and enzyme activities related to oxidative stress. *Biomed Env Sci* 2000; **13**: 44–55.

39 Cai L, Ma D, Zhang Y, Liu Z, Wang P. The effect of coffee consumption on serum lipids: a meta-analysis of randomized controlled trials. *Eur J Clin Nutr* 2012; **66**: 872–877.

Supplemental Table 3.3.1: Detailed baseline characteristics of the Rotterdam Study participants by coffee intake categories

	Non-coffee drinkers	0.5-2 cups/day	3-4 cups/day	5 or more cups/day	Total
n, (%)	542 (7.6)	1,594 (22.2)	2,983 (40.2)	2,053 (28.6)	7,172
Age, mean (SD), years	62.1 (9.8)	68.4 (10.1)	65.8 (9.2)	62.3 (7.8)	65.1 (9.4)
Sex, % women	373 (68.8)	1064 (66.8)	1844 (61.8)	1002 (48.8)	4283 (59.7)
Education level					
Primary	63 (11.6)	221 (13.9)	338 (11.3)	207 (10.1)	832 (11.6)
Intermediate	211 (38.9)	644 (40.4)	1271 (42.6)	816 (39.7)	2939 (41.0)
Higher general	160 (29.5)	455 (28.5)	879 (29.5)	595 (29.0)	2086 (29.1)
University	108 (19.9)	274 (17.2)	495 (16.6)	435 (21.2)	1315 (18.3)
Tea consumption, n (%) consumers	486 (89.7)	1465 (91.9)	2704 (90.6)	1589 (77.4)	6241 (87.0)
Alcohol consumption, median (IQR), g/day	1.20 (0.00; 8.57)	3.57 (0.54; 10.25)	6.40 (0.71; 14.29)	6.43 (0.71; 15.0)	5.71 (0.54; 14.29)
Diet quality score, mean (SD)	7.1 (2.0)	6.9 (1.9)	6.8 (1.9)	6.3 (1.8)	6.7 (1.9)
Smoking status					
Never smokers	252 (46.50)	652 (40.9)	994 (33.3)	453 (22.1)	2351 (32.8)
Former smoker	209 (38.6)	730 (45.8)	1939 (46.8)	839 (40.9)	3174 (44.3)
Current smoker	81 (14.9)	212 (13.3)	593 (19.9)	761 (37.0)	1647 (22.9)
Physical activity, median (IQR), MET-h/week	61.4 (31.2; 97.9)	70.8 (43.1; 103.8)	74.1 (46.6; 107.5)	70.5 (37.2; 104.6)	72.0 (42.0; 105.1)
BMI, mean (SD), kg/m^2	26.8 (4.7)	26.7 (4.0)	26.9 (3.8)	27.1 (4.0)	26.9 (4.0)
Hypertension, n (%)	306 (56.5)	1046 (65.3)	1788 (60.0)	1099 (53.5)	4237 (59.1)
Total cholesterol, mean (SD), mmol/L	5.69 (1.04)	5.84 (1.03)	5.82 (0.97)	5.8 (0.97)	5.8 (0.99)
HDL cholesterol, median (IQR), mmol/L	1.37 (1.16; 1.68)	1.40 (1.16; 1.71)	1.40 (1.16; 1.67)	1.36 (1.13; 1.63)	1.38 (1.16; 1.67)
CRP median (IQR), µg/mL	1.40 (0.50; 3.30)	1.66 (0.70; 3.61)	1.50 (0.60; 3.30)	1.36 (0.57; 3.04)	1.50 (0.60; 3.30)
Incident type 2 diabetes cases, n (%)	52 (9.6)	171 (10.7)	276 (9.6)	187 (9.1)	685 (9.6)

Supplemental Table 3.3.2: Detailed baseline characteristics of the UK Biobank participants by coffee intake categories.

	Non-coffee drinkers	0.5-2 cups/day	3-4 cups/day	5 or more cups/day	Total
n, (%)	31,773 (21.9)	66,153 (45.5)	30,440 (20.9)	17,004 (11.7)	145,370
Age, mean (SD), years	53.92 (8.1)	55.8 (8.1)	55.5 (8.0)	54.5 (7.9)	55.2 (8.1)
Sex, (%) women	19,818 (62.4)	39,088 (59.1)	16,715 (54.9)	8,722 (51.3)	84,343 (58.0)
Deprivation index	-1.9 [-3.5; 0.8]	-2.4 [-3.8; -0.04]	-2.4 [-3.8; -0.1]	-2.1 [-3.6; 0.5]	-2.3 [-3.7; 0.2]
Ethnicity, n (%)					
White	29,367 (92.4)	63,504 (96.0)	29,810 (97.9)	16,750 (98.5)	139,431 (95.9)
Mixed	508 (1.6)	855 (1.3)	261 (0.9)	138 (0.8)	1,762 (1.2)
South Asian	1,155 (3.6)	1,045 (1.6)	201 (0.7)	65 (0.4)	2,463 (1.7)
Black	561 (1.8)	557 (0.8)	142 (0.5)	46 (0.3)	1,306 (0.9)
Chinese	182 (0.6)	195 (0.3)	26 (0.1)	5 (0.0)	408 (0.3)
Tea consumption, mean (SD), cups	4.54 (3.3)	3.8 (2.5)	2.5 (2.2)	1.9 (2.9)	3.48 (2.84)
Alcohol consumption, n (%), frequency	3.37 (1.6)	2.8 (1.4)	2.7 (1.4)	2.9 (1.5)	2.9 (1.5)
Daily or almost daily	4,299 (13.5)	13,512 (20.4)	7,131 (23.4)	3,493 (20.5)	28,435 (19.6)
3-4 times per week	5,801 (18.3)	17,032 (25.7)	8,149 (26.8)	3,980 (23.4)	34,962 (24.1)
1-2 times per week	8,513 (26.8)	18,355 (27.7)	8,133 (26.7)	4,458 (26.2)	39,459 (27.1)
1-3 times per month	4,226 (13.3)	7,429 (11.2)	3,101 (10.2)	2,032 (12.0)	16,788 (11.5)
Special occasions only	4,736 (14.9)	6,212 (9.4)	2,467 (8.1)	1,848 (10.9)	15,263 (10.5)
Never	4,198 (13.2)	3,613 (5.5)	1,459 (4.8)	1,193 (7.0)	10,463 (7.2)
Diet quality score (SD)	5.11 (1.81)	5.3 (1.8)	5.1 (1.8)	4.8 (1.8)	5.1 (1.8)
Smoking status, n (%)					
Never smokers	19,659 (61.9)	39,535 (59.8)	16,624 (54.6)	7,559 (44.4)	83,377 (57.4)
Former smoker	9,077 (28.6)	21,335 (32.3)	10,325 (33.9)	5,711 (33.6)	46,448 (32.0)
Current smoker	3,037 (9.6)	5,283 (8.0)	3,491 (11.5)	3,734 (22.0)	15,545 (10.7)

Supplemental Table 3.3.2: Detailed baseline characteristics of the UK Biobank participants by coffee intake categories. (continued)

	Non-coffee drinkers	0.5-2 cups/day	3-4 cups/day	5 or more cups/day	Total
Physical activity, median (IQR), MET-hours/week	32.0 (14.4; 65.1)	31.6 (14.9; 62.4)	31.1 (14.4; 62.2)	32.4 (14.9; 67.6)	31.6 (14.8; 63.1)
BMI, mean (SD), kg/m^2	26.7 (4.5)	26.3 (4.2)	26.9 (4.3)	27.4 (4.5)	26.6 (4.3)
Hypertension, n (%)	130 (0.4)	218 (0.3)	92 (0.3)	56 (0.3)	495 (0.3)
Total cholesterol, mean (SD), mmol/L	5.8 (1.1)	5.9 (1.1)	5.9 (1.1)	5.9 (1.1)	5.9 (1.1)
HDL cholesterol, median (IQR), mmol/L	1.4 (1..2; 1.7)	1.5 (1.2; 1.8)	1.45 (1.22; 1.72)	1.4 (1.2; 1.7)	1.4 (1.2; 1.7)
CRP median (IQR), µg/mL	1.3 (0.6; 2.7)	1.2 (0.6; 2.3)	1.18 (0.59; 2.41)	1.3 (0.7; 2.6)	1.2 (0.6; 2.5)
Incident type 2 diabetes cases, n (%)	599 (1.9)	979 (1.5)	431 (1.4)	281 (1.7)	2290 (1.6)

Supplemental Table 3.3.3. Associations between coffee intake and type 2 diabetes-related inflammatory markers and adipokines in RS (n = 846), with and without additional adjustment for CRP.

	Coffee (cups/day)	Multivariable adjusted (model 2)		Additional adjustment for CRP	
		β (95% CI)	p-value	β (95% CI)	p-value
Adiponectin (µg/mL)	Per cup increase	0.022 (0.003; 0.041)	0.023	0.023 (0.005; 0.041)	0.010
	0	ref		ref	
	0.5-2	0.093 (-0.065; 0.251)	0.247	0.068 (-0.078; 0.213)	0.362
	3-4	0.143 (-0.010; 0.296)	0.068	0.137 (-0.003; 0.278)	0.056
	≥ 5	0.162 (0.001; 0.323)	0.049	0.137 (-0.011; 0.285)	0.070
	Trend		0.029		0.020
Leptin (ng/mL)	Per cup increase	0.009 (-0.013; 0.032)	0.417	0.011 (-0.012; 0.033)	0.358
	0	ref		ref	
	0.5-2	-0.006 (-0.193; 0.181)	0.948	0.008 (-0.177; 0.194)	0.930
	3-4	0.027 (-0.154; 0.208)	0.770	0.035 (-0.144; 0.215)	0.699
	≥ 5	0.009 (-0.182; 0.199)	0.930	0.027 (-0.162; 0.215)	0.783
	Trend		0.729		0.644
EN-RAGE (ng/ml)	Per cup increase	0.009 (-0.013; 0.032)	0.417	0.011 (-0.012; 0.033)	0.358
	0	ref		ref	
	0.5-2	-0.006 (-0.193; 0.181)	0.948	0.008 (-0.177; 0.194)	0.930
	3-4	0.027 (-0.154; 0.208)	0.770	0.035 (-0.144; 0.215)	0.699
	≥ 5	0.009 (-0.182; 0.199)	0.930	0.027 (-0.162; 0.215)	0.783
	Trend		0.729		0.644
C3 (mg/mL)	Per cup increase	-0.004 (-0.011; 0.003)	0.282	-0.004 (-0.011; 0.003)	0.261
	0	ref		ref	
	0.5-2	-0.081 (-0.141; -0.021)	0.009	-0.068 (-0.126; -0.011)	0.021
	3-4	-0.078 (-0.137; -0.020)	0.008	-0.073 (-0.129; -0.017)	0.010
	≥ 5	-0.083 (-0.145; -0.022)	0.008	-0.071 (-0.130; -0.013)	0.017
	Trend		0.137		0.141
TNFR II (ng/mL)	Per cup increase	0.001 (-0.011; 0.014)	0.828	0.001 (-0.011; 0.013)	0.819
	0	ref		ref	
	0.5-2	0.042 (-0.059; 0.143)	0.411	0.054 (-0.046; 0.153)	0.292
	3-4	0.030 (-0.068; 0.128)	0.552	0.034 (-0.062; 0.131)	0.487
	≥ 5	0.028 (-0.075; 0.131)	0.590	0.039 (-0.062; 0.141)	0.448
	Trend		0.926		0.969

Supplemental Table 3.3.3. (continued)

	Coffee (cups/day)	Multivariable adjusted (model 2)		Additional adjustment for CRP	
		β (95% CI)	p-value	β (95% CI)	p-value
IL-17 (pg/mL)	Per cup increase	-0.005 (-0.022; 0.012)	0.591	-0.003 (-0.020; 0.014)	0.698
	0	ref		ref	
	0.5-2	0.034 (-0.104; 0.172)	0.627	0.032 (-0.105; 0.170)	0.647
	3-4	0.059 (-0.075; 0.192)	0.389	0.061 (-0.071; 0.194)	0.365
	≥ 5	0.016 (-0.125; 0.156)	0.828	0.018 (-0.122; 0.158)	0.804
	Trend		0.996		0.918
IL-1-RA (pg/mL)	Per cup increase	0.000 (-0.026; 0.025)	0.973	-0.005 (-0.030; 0.021)	0.717
	0	ref		ref	
	0.5-2	0.147 (-0.065; 0.358)	0.175	0.151 (-0.057; 0.359)	0.155
	3-4	0.143 (-0.062; 0.348)	0.171	0.132 (-0.069; 0.333)	0.198
	≥ 5	0.072 (-0.144; 0.287)	0.515	0.061 (-0.150; 0.273)	0.569
	Trend		0.718		0.528
CFH (µg/mL)	Per cup increase	0.012 (-0.006; 0.030)	0.179	0.011 (-0.007; 0.029)	0.231
	0	ref		ref	
	0.5-2	0.110 (-0.041; 0.262)	0.154	0.108 (-0.043; 0.259)	0.162
	3-4	0.094 (-0.053; 0.240)	0.212	0.087 (-0.059; 0.234)	0.242
	≥ 5	0.154 (-0.001; 0.308)	0.051	0.147 (-0.007; 0.301)	0.061
	Trend		0.121		0.149
IL-18 (pg/mL)	Per cup increase	0.004 (-0.015; 0.022)	0.691	0.001 (-0.017; 0.019)	0.904
	0	ref		ref	
	0.5-2	0.160 (0.009; 0.311)	0.038	0.161 (0.012; 0.310)	0.035
	3-4	0.175 (0.029; 0.322)	0.019	0.168 (0.023; 0.313)	0.023
	≥ 5	0.120 (-0.034; 0.274)	0.127	0.113 (-0.039; 0.265)	0.146
	Trend		0.716		0.874
IL-13 (pg/mL)	Per cup increase	0.010 (-0.001; 0.021)	0.074	0.010 (-0.001; 0.021)	0.081
	0	ref		ref	
	0.5-2	-0.009 (-0.099; 0.082)	0.850	-0.013 (-0.103; 0.077)	0.779
	3-4	0.018 (-0.070; 0.105)	0.690	0.013 (-0.074; 0.101)	0.767
	≥ 5	0.023 (-0.069; 0.115)	0.621	0.019 (-0.073; 0.110)	0.692
	Trend		0.224		0.238

Model adjusted for age, sex, cohort, highest education level, tea consumption, alcohol consumption, smoking, physical activity, diet score, body mass index, hypertension, ratio total cholesterol/HDL and CRP. Abbreviations: EN-RAGE, Extracellular Newly identified Receptor for Advanced Glycation End-products binding protein; C3, complement compound 3; TNFR II, tumor Necrosis Factor Receptor 2; IL, interleukin; RA, receptor antagonist; CFH, complement factor H.

Supplemental Table 3.3.4. Causal mediation analyses for the association of coffee intake (cups per day) with type 2 diabetes by inflammatory markers in the UK Biobank (UKB) and Rotterdam Study (RS).

	a Biomarker ~ coffee[1]		*b* Type 2 diabetes ~ biomarker + coffee[1]		*c´* Type 2 diabetes ~ coffee + biomarker[1]		Proportion mediated	
	β (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	Percentage (95% CI)	p-value
UK Biobank								
CRP[2]	-0.011 (-0.012; -0.009)	<0.001	1.45 (1.36; 1.53)	<0.001	0.97 (0.94; 0.99)	0.003	9.6 (5.7; 24.4)	0.002
The Rotterdam Study								
CRP[2]	-0.014 (-0.022; -0.005)	0.002	1.17 (1.04; 1.31)	0.008	0.94 (0.90; 0.99)	0.010	3.4 (0.6; 4.8)	0.015
Adiponectin[2,3]	0.025 (0.007; 0.042)	0.006	0.58 (0.32; 0.83)	0.006	0.90 (0.80; 1.01)	0.079	12.2 (-12.9; 81.2)	0.066
C3[2,3]	-0.006 (-0.012; 0.001)	0.094	2.35 (0.78; 7.1)	0.129	0.89 (0.79; 0.99)	0.048	3.4 (-4.7; 26.0)	0.207
IL-18[2,3]	0.001 (-0.017; 0.019)	0.090	1.36 (0.88; 2.12)	0.170	0.89 (0.79; 0.99)	0.047	-0.1 (-15.9; 12.8)	0.894

[1]Models adjusted for age, sex, Rotterdam Study cohort (RS only), level of education (RS only), deprivation index (UKBB only), ethnicity (UKBB only), tea consumption, alcohol consumption, smoking status, physical activity, diet quality score, hypertension, body mass index and ratio serum total cholesterol/HDL. [2]Variables were transformed using the natural logarithm. [3]Additionally adjusted for CRP.

Supplemental Table 3.3.5. Associations between coffee intake and incident type 2 diabetes excluding cases of incident type 2 diabetes during the first 2 years of follow- upin the Rotterdam Study (RS) and the UK Biobank (UKB).

	Coffee (cups/day)	**Rotterdam Study (n = 7,076)**		**UK Biobank (n = 144,823)**	
		HR (95% CI)	p-value	HR (95% CI)	p-value
Model 1	Per cup increase	0.96 (0.91; 1.01)	0.089	0.96 (0.93; 0.98)	**0.002**
	0	ref		ref	
	0.5-2	0.96 (0.66; 1.39)	0.824	0.86 (0.76; 0.97)	**0.014**
	3-4	0.78 (0.55; 1.11)	0.168	0.81 (0.70; 0.94)	**0.005**
	≥ 5	0.76 (0.52; 1.03)	0.148	0.78 (0.78; 0.93)	**0.005**
	Trend		**0.028**		**0.002**
Model 2	Per cup increase	0.95 (0.90; 0.99)	**0.026**	0.94 (0.91; 0.96)	**<0.001**
	0	ref		ref	
	0.5-2	0.91 (0.63; 1.31)	0.608	0.88 (0.78; 0.99)	**0.032**
	3-4	0.73 (0.51; 1.04)	0.085	0.77 (0.66; 0.89)	**<0.001**
	≥ 5	0.70 (0.48; 1.02)	0.062	0.70 (0.59; 0.84)	**<0.001**
	Trend		**0.010**		**<0.001**

Model 1: adjusted for age, sex, Rotterdam Study cohort (RS only), level of education (RS only), deprivation index (UKB only), ethnicity (UKB only), tea consumption, alcohol consumption, smoking status, physical activity, and diet quality score; Model 2: additionally adjusted for BMI, hypertension and serum total cholesterol/HDL ratio. Natural logarithm transformation was used for CRP (µg/mL). There were n = 589 cases of incident type 2 diabetes in RS and n = 547 cases in UKB.

Supplemental Table 3.3.6. Interaction terms of coffee intake with covariates for the main analyses in the Rotterdam Study (RS) and UK Biobank (UKB).

	Rotterdam Study			**UK Biobank**	
	Type 2 diabetes	CRP[1]	HOMA-IR[1]	Type 2 diabetes	CRP[1]
Sex	0.999	0.222	0.041	0.650	<0.001
Age	0.981	0.046	0.774	0.991	0.161
Smoking					
Former	0.982	0.604	0.958	0.076	0.853
Current	0.305	0.002	0.602	0.914	<0.001
BMI	0.267	0.015	0.298	0.085	<0.001

Models adjusted for age, sex, Rotterdam Study cohort (RS only), level of education (RS only), deprivation index (UKB only), ethnicity (UKB only), tea consumption, alcohol consumption, smoking status, physical activity, diet quality score, body mass index, hypertension and serum total cholesterol/HDL ratio; corresponding to model 2 in the main analyses. [1]Variable transformed using the natural logarithm. There were n = 685 cases of incident type 2 diabetes in RS and n = 2,290 cases in UKB. Abbreviations: BMI, body mass index.

Supplemental Table 3.3.7. Associations of coffee intake with type 2 diabetes incidence, and log transformed CRP and HOMA-IR in the Rotterdam Study (RS) and the UK Biobank (UKB), stratified by sex.

	Rotterdam Study				UK Biobank			
	Women (n = 4,283)		Men (n = 2,889)		Women (n = 84,343)		Men (n = 61,027)	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Type 2 diabetes	0.93 (0.88; 0.99)	0.021	0.95 (0.88; 1.02)	0.135	0.96 (0.93; 1.00)	0.047	0.96 (0.93; 0.99)	0.005
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**
CRP[1]	-0.016 (-0.027; -0.005)	0.004	-0.006[3] (-0.020; 0.007)	0.340	-0.016[3] (-0.018; -0.013)	<0.001	-0.004[3] (-0.007; -0.002)	0.002
HOMA-IR[1,2]	-0.023[3] (-0.032; -0.014)	<0.001	-0.012[3] (-0.023; -0.001)	0.040	n/a	n/a	n/a	n/a

Models are adjusted for age, Rotterdam Study cohort (RS only), level of education (RS only), deprivation index (UKB only), ethnicity (UKB only), tea consumption, alcohol consumption, smoking status, physical activity diet quality score, body mass index, hypertension, and serum total cholesterol/HDL ratio. There were n = 405 incident cases of type 2 diabetes among women and n = 280 cases among men in RS, and n = 958 cases among women and n = 1,332 cases among men in UKB. [1]Variable was transformed using the natural logarithm. [2]Total sample size n = 2,463 women and n = 1,675 men. [3]Denotes associations for which interaction terms between coffee consumption and sex were statistically significant ($p < 0.05$).

Supplemental Table 3.3.8. Associations of coffee intake with outcomes type 2 diabetes incidence, CRP and HOMA-IR in the Rotterdam Study, stratified by smoking status.

	Never smokers (n = 2,336)		Former smokers (n = 3,161)		Current smokers (n = 1,637)	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Type 2 diabetes	0.91 (0.84; 0.99)	0.024	0.93 (0.87; 0.10)	0.035	1.00 (0.91; 1.08)	0.901
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**
CRP[a]	-0.019[3] (-0.033; -0.005)	0.010	-0.026 (-0.039; -0.012)	<0.001	0.010[3] (-0.007; 0.027)	0.239
HOMA-IR[a,b]	-0.013 (-0.026; -0.001)	0.035	-0.018 (-0.028; -0.007)	0.001	-0.021 (-0.037; -0.006)	0.007

Models are adjusted for age, sex, Rotterdam Study cohort, level of education, tea consumption, alcohol consumption, physical activity, diet quality score, body mass index, hypertension and serum total cholesterol/HDL ratio. There were n = 206 cases of incident type 2 diabetes among never smokers, n = 295 cases among former smokers and n = 178 cases among current smokers. [1]Variable was transformed using the natural logarithm. [2]Total sample never smokers n = 1,352; former smokers n = 1,902; current smokers n = 872. [3]Denotes associations for which interaction terms between coffee consumption and smoking category were statistically significant ($p < 0.05$).

Supplemental Table 3.3.9. Associations of coffee intake with outcomes type 2 diabetes incidence and CRP in the UK Biobank, stratified by smoking status.

	Never smoker (n = 83,169)		Former smoker (n = 46,294)		Current smoker (n = 15,495)	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Type 2 diabetes	0.90 (0.84; 0.96)	0.001	1.04 (0.97; 1.11)	0.284	0.90 (0.81; 0.10)	0.046
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**
CRP[1]	-0.012[2] (-0.016; -0.009)	<0.001	-0.011 (-0.016; -0.006)	<0.001	0.001[2] (-0.008; 0.010)	0.821

Models are adjusted for age, sex, deprivation index, ethnicity, tea consumption, alcohol consumption, physical activity, diet quality score, body mass index, hypertension and serum total cholesterol/HDL ratio. [1]Variable was transformed using the natural logarithm. There were n = 1,113 cases of incident type 2 diabetes among never smokers, n = 792 among former smokers and n = 375 among current smokers. [2]Denotes associations for which interaction terms between coffee consumption and smoking were statistically significant ($p < 0.05$).

Supplemental Table 3.3.10. Associations of coffee intake with the outcomes type 2 diabetes incidence, C-reactive protein (CRP) and homeostatic model of insulin resistance (HOMA-IR) in the Rotterdam Study (RS) and the UK Biobank (UKB), stratified according to body mass index.

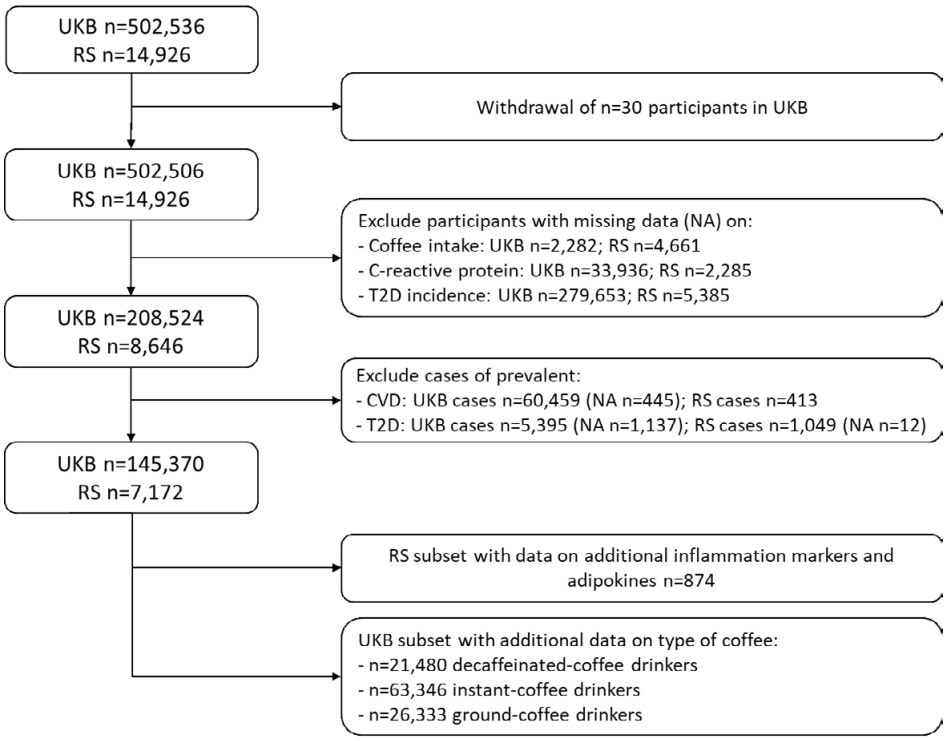
	Rotterdam Study				The UK Biobank			
	Normal weight (BMI <25) (n = 2,425)		Overweight or obese (BMI ≥25) (n = 4,699)		Normal weight (BMI <25) (n = 88,903)		Overweight or obese (BMI ≥25) (n = 56,072)	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Type 2 diabetes	0.84 (0.76; 0.93)	0.001	0.98 (0.93; 1.03)	0.369	0.86 (0.74; 1.00)	0.057	0.96 (0.92; 1.00)	0.084
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
CRP[a]	-0.003[2] (-0.018; 0.012)	0.706	-0.013[2] (-0.024; -0.003)	0.013	-0.005[2] (-0.009; -0.001)	0.021	-0.007[2] (-0.010; -0.003)	<0.001
HOMA-IR[a,b]	-0.010 (-0.022; 0.003)	0.128	-0.014 (-0.024; -0.005)	0.004	n/a	n/a	n/a	n/a

Models are adjusted for age, sex, Rotterdam Study cohort (RS only), level of education (RS only), deprivation index (UKB only), ethnicity (UKB only), tea consumption, alcohol consumption, smoking status, physical activity, diet quality score, hypertension and serum total cholesterol/HDL ratio. There were n = 128 cases of incident type 2 diabetes among RS participants with normal weight and n = 552 cases among those with overweight or obesity. In UKB, there were n = 196 cases of incident type 2 diabetes among those with normal weight and n = 2,083 among those with overweight or obesity. [1]Variable was transformed using the natural logarithm. [2]Denotes associations for which interaction terms between coffee consumption and BMI category were statistically significant (p<0.05).

Supplemental Table 3.3.11. Associations between coffee consumption and incidence of type 2 diabetes as well as serum C-reactive protein (CRP), stratified according to type of coffee consumed, in the UK Biobank (UKB).

	Decaffeinated coffee[1]		Instant coffee[2]		Ground coffee[3]	
	HR (95% CI)	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Type 2 diabetes	0.97 (0.93; 0.93)	0.130	0.96 (0.93; 0.99)	0.003	0.88 (0.83; 0.93)	<0.001
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**	**β (95% CI)**	**p-value**
CRP[4]	-0.009 (-0.013; -0.006)	< 0.001	-0.004 (-0.007; -0.002)	< 0.001	-0.026 (-0.030; -0.022)	< 0.001

Models are adjusted for age, sex, deprivation index, ethnicity, tea consumption, alcohol consumption, smoking status, physical activity, diet quality score, hypertension and serum total cholesterol/HDL ratio. [1]Total sample n = 53,253, of whom n=21,480 were decaffeinated-coffee drinkers. [2]Total sample n = 95,119, of whom n = 63,346 were instant-coffee drinkers. [3]Total sample n = 58,106 of whom n = 26,333 were ground-coffee drinkers. [4]Variable was transformed using the natural logarithm.



Supplemental Figure 3.3.1. Flow diagram of study sample selection. Abbreviations: Rotterdam Study, RS; UK Biobank, UKB; T2D, type 2 diabetes; cardiovascular disease, CVD.