Liver INTERNATIONAL
OFFICIAL JOURNAL OF THE INTERNATIONAL ASSOCIATION FOR THE STUDY OF THE LIVER

Liver International ISSN 1478-3223

CIRRHOSIS AND LIVER FAILURE

Coffee consumption in NAFLD patients with lower insulin resistance is associated with lower risk of severe fibrosis

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Keywords
diet – histology – HOMA – lifestyle – nonalcoholic steatohepatitis

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Received 26 July 2013
Accepted 27 October 2013

DOI:10.1111/liv.12379

Abstract

Background & Aims: Coffee has inverse relationships with both type 2 diabetes and hepatic fibrosis in patients with nonalcoholic fatty liver disease (NAFLD). Relationships were explored between coffee intake and insulin resistance (IR) with respect to NAFLD histologic severity. *Methods:* We analyzed data from 782 adults (≥18 years) in the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) from 2004 to 2008. IR was assessed using the HOMA-IR. We modeled associations between coffee intake and NAFLD histologic severity using multiple logistic regression; and interactions between coffee and IR on NAFLD histology were explored. *Results:* Among 782 participants, 38% (n = 295) were men, 12% (n = 97) were Latino, mean age (± standard deviation) was 48 ± 12 years. Median BMI was 33.5 kg/m^2 [interquartile range, 29.7–38.3] and median HOMA-IR was 4.3 [2.7–7.2]. Diabetes was present in 24% (n = 189). NASH was present in 79% (n = 616), and 25% (n = 199) had advanced fibrosis. The frequency of coffee intake (cups/day, cpd) was as follows: 0 cpd, n = 230 (29%); <1 cpd, n = 219 (28%); 1 to <2 cpd, n = 116 (15%); ≥2 cpd, n = 217 (28%). The effect of coffee on fibrosis varied with degree of IR (interaction P = 0.001). Coffee consumers with less IR, defined as HOMA-IR<4.3, had a lower odds of advanced fibrosis [OR = 0.64; 95% CI, (0.46–0.88), P = 0.001]. There was no protective effect of coffee on advanced fibrosis among individuals with higher HOMA-IR [OR = 1.06, 95% CI (0.87–1.28), P = 0.6]. *Conclusions:* Coffee intake is inversely associated with advanced fibrosis among NAFLD patients with lower HOMA-IR. Our findings warrant further investigation given the worldwide ubiquity of coffee intake.

Nonalcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease affecting up to 30% of the US adult population (1–4). NAFLD encompasses a histopathological spectrum ranging from bland steatosis to nonalcoholic steatohepatitis (NASH). NASH is characterized by hepatic inflammation, ballooning degeneration of hepatocytes and hepatic steatosis – with or without fibrosis. NASH is associated with decreased long-term survival, higher propensity for progression to advanced fibrosis and cirrhosis, and higher risk of hepatocellular carcinoma (HCC) (5, 6).

Although the pathophysiology of NAFLD remains incompletely defined, a number of clinical factors are intimately associated with this disease – obesity, insulin resistance, diabetes mellitus, hypertension and hyperlipidaemia. Lifestyle habits, such as fructose consumption, physical activity level, alcohol and tobacco use have also been studied in NAFLD and shown to impact disease severity (7–12). Further insights into relationships between NAFLD and other modifiable lifestyle factors may contribute to the development of intervention strategies for NAFLD.

Coffee consumption is a popular lifestyle choice worldwide; at least 50% of US adults consume coffee on a daily basis (13). Beneficial effects of coffee have been observed in multiple chronic medical conditions includ-

ing type 2 diabetes, Parkinson's disease, prostate cancer, hepatitis C (HCV), HCC, and more recently, NAFLD (14–33). Moreover, a large population-based study found that increasing coffee intake is associated with a modest decrease in all-cause mortality (31). Therefore, we hypothesized that coffee would be associated with decreased NAFLD histologic severity. We further hypothesized that the beneficial effects of coffee on NAFLD histologic severity would be modified by severity of insulin resistance.

Patients and methods

Research design and study population

This was a cross-sectional study using prospectively collected data from participants enrolled in the US multicentre collaborative research consortium known as the Nonalcoholic Steatohepatitis Clinic Research Network (NASH CRN) from 2004 to 2008 (34–36). The NASH CRN is a National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) funded consortium to study the pathogenesis of NAFLD and the determinants of disease progression and severity. We included in our analyses NASH CRN participants who were enrolled in either the NASH CRN Database or the PIVENS Trial (Pioglitazone vs. Vitamin E vs. Placebo for the Treatment of Non-Diabetic Patients with NASH; ClinicalTrials.gov Identifier: NCT00063622) (35, 36). Only baseline data at the time of enrollment into the NASH CRN and PIVENS trial were utilized so that the results were not impacted by their involvement in the NASH CRN related studies. All laboratory, dietary and hepatic histological data used for our analyses were concurrent, being obtained within 6 months of enrollment into the NASH CRN. Only those participants who were 18 years of age or older and had data available pertaining to both NAFLD liver histology and dietary data documenting coffee consumption were used for these analyses.

Informed consent was obtained in writing from all participants in the NASH CRN and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by all the institutional review boards at all NASH CRN participating sites.

NAFLD diagnosis

As part of the entry criteria, all patients enrolled in the NASH CRN must meet the following criteria: (i) documented absence of other aetiologies of chronic liver disease (viral, autoimmune, drug-induced, cholestatic, genetic); (ii) no clinically significant history of alcohol consumption, which was defined as: greater than 20 or 10 g/day for men and women, respectively, in the NASH CRN Database; or greater than 30 or 20 g/day for men and women, respectively, in the PIVENS Trial; and (iii) histologically confirmed NAFLD according to the NASH-CRN criteria (see below).

Coffee consumption

Dietary information was obtained using a validated dietary questionnaire (Block Food Frequency Questionnaire, version 1998), based upon self-reported typical eating habits over the past year (37). Coffee consumption was reported as average number of cups per day (cpd). Data collection with respect to coffee consumption in the Block Food Frequency Questionnaire was capped at a maximum of 5 cpd. No distinction was made between caffeinated and decaffeinated coffee in the Block Food Frequency Questionnaire.

Outcomes

The primary outcome of interest for the current investigation was advanced fibrosis based on liver histology, categorized as: none to moderate (≤Stage 2) or advanced (>Stage 2). A secondary outcome of interest was the presence versus absence of NASH histology.

Liver biopsy slides for all study participants were stained with hematoxylin and eosin and Masson's trichrome and were reviewed and scored centrally by the members of the NASH CRN Pathology Committee who were blinded to clinical data. The Nonalcoholic Fatty Liver Disease Activity Score (NAS) and stage of fibrosis were based on the histological scoring system for NAFLD developed by the NASH CRN (38). The NAS included a systematic assessment of the following histologic features: steatosis, hepatocyte ballooning, and lobular inflammation. The final diagnosis for each biopsy was determined by consensus of the NASH CRN Pathology Committee as follows: (i) definite steatohepatitis; (ii) definitely not steatohepatitis; and (iii) borderline steatohepatitis. Fibrosis on liver biopsy was staged from 0 to 4 as follows: 0 = none; 1a = mild zone 3 (central) perisinusoidal fibrosis; 1b = moderate zone 3 perisinusoidal fibrosis; 1c = periportal and portal fibrosis (zone 1 only); 2 = both zone 3 perisinusoidal and periportal or portal fibrosis; 3 = bridging fibrosis; and 4 = cirrhosis. Liver biopsy length (mm) was also recorded.

Covariates

Information regarding the following variables was also obtained for all individuals included in our analyses:

Demographics

Age, gender, self-reported race and ethnicity (categorized as Non-Latino, Latino, Caucasian, Black, Asian, American Indian, Native Hawaiian/Pacific Islander, and Multiracial), education level (categorized as less than

high school, Some College, and Advanced Degree) and annual income (categorized as <$30 000, $30 000–$49 999, ≥$50 000).

Anthropometrics

Height (meters, m), weight (kilograms, kg) and waist circumference (centimeters, cm) were measured at the time of enrollment into the NASH CRN. Body mass index (BMI) kg/m^2 was calculated for each participant.

Laboratory Data

Laboratory data were collected at the time of enrollment and included measurements of aspartate and alanine aminotransferases (AST and ALT, respectively), gamma-glutamyl transferase (GGT), serum albumin, total bilirubin, alkaline phosphatase, platelet count, lipid profile [including total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglycerides] and fasting insulin and fasting glucose levels. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from fasting glucose and insulin levels, using the following equation: [fasting insulin (μU/ml)] × [fasting glucose (mg/dl)]/405. The HOMA-IR was not calculated for individuals taking insulin or for individuals in whom fasting insulin data was not available.

Clinical data

Information regarding comorbidities, including diabetes mellitus and hyperlipidaemia were also collected.

Smoking, alcohol intake and physical activity

Participants' smoking history was documented and the number of pack-years smoked was recorded. Alcohol use was also documented and recorded as average number of drinks per month. Physical activity levels were calculated based upon self-reported leisure time physical activity using a questionnaire derived from the National Health and Nutrition Examination Survey (39–41). Using a standard reference for metabolic equivalent (MET) intensities for specific activities and the reported duration of each activity, a score for each individual's total physical activity, expressed as metabolic hours per week (MET hours/week) was generated (40).

Statistical analyses

Continuous data were reported as mean ± standard deviation (sd) or median [Interquartile range, IQR], as appropriate. Student's *t*-test, ANOVA, Kruskal Wallis and Mann Whitney *U* testing were applied for comparisons, as appropriate. Chi squared testing was used to compare categorical variables.

Associations between clinical characteristics and advanced fibrosis (>stage 2) were investigated using univariate and multivariate logistic regression models. Data were reported as odds ratios (OR) with 95% Confidence Intervals (95% CI). Stepwise logistic regression analysis was used to identify significant predictors of advanced fibrosis. The *P*-value for addition or elimination from the models was $P < 0.05$, and the models were forced to include terms for age, gender and ethnicity. Interactions between coffee intake and insulin resistance (HOMA-IR) with respect to fibrosis stage were also explored. For these analyses, the participant population was divided into two groups based on the population's median value for HOMA-IR, (<4.3 vs. ≥4.3), and multivariate logistic regression models were conducted for each of the two HOMA-IR groups, adjusting for variables selected from the stepwise logistic regression model.

Similar logistic regression analyses were also performed for the secondary outcome of presence versus absence of NASH histology on liver biopsy.

For all analyses, STATA 11.0 (College Station, TX, USA) was utilized. Nominal two-sided *P* values were used. We considered differences statistically significant when *P* values were less than 0.05. Statistical interactions were considered statistically significant when *P* values were less than 0.01.

Results

Patient characteristics

Characteristics of the overall population

Seven hundred and eighty-two adults had biopsy-proven NAFLD with complete coffee consumption history (Table 1). The mean age of the cohort was 48 (± 12) years and 38% ($n = 295$) were men. Eighty-four percent ($n = 633$) of participants self-identified as Caucasian, and 12% ($n = 97$) were Latino. The majority of participants were either overweight or obese with the median BMI of the overall patient population being 33.5 kg/m^2 [IQR 29.7–38.3] and median waist circumference of 108 cm [IQR 99–117]. Diabetes was present in 24% ($n = 189$) of participants and hyperlipidaemia in 56% ($n = 435$).

The median AST and ALT levels were 44 U/L [IQR, 32–64] and 62 U/L [IQR, 41–92] respectively. The median alkaline phosphatase and total bilirubin levels were 81 U/L [IQR, 66–103] and 0.7 mg/dl [IQR, 0.5–0.9] respectively. The HOMA-IR was calculated for individuals who were not taking exogenous insulin and who had the necessary data available for calculation of the HOMA-IR ($n = 769$). The median HOMA-IR for the population was 4.3 [IQR, 2.7–7.2].

The mean number of cups of coffee consumed per day (cpd) was 1.0 ± 1.2, mean number of pack-years smoked was 7.7 ± 16.1 and mean number of alcoholic drinks per month was 5.3 ± 11.6. NASH histology

Table 1. Characteristics of the Overall Study Population (n = 782)

Age, years (mean ± sd)	48 ± 12
Male, *n* (%)	295 (38%)
Latino, *n* (%)	97 (12%)
Race, *n* (%)	
Caucasian	633 (84%)
Black	20 (2.7%)
Asian	36 (4.8%)
American Indian	27 (3.6%)
Native Hawaiian/Pacific Islander	5 (0.7%)
Multiracial	32 (4.3%)
BMI, kg/m^2 (median, IQR)	33.5 [29.7–38.3]
Waist Circumference, cm (median, IQR)	108 [99–117]
Biopsy Length, mm (median, IQR)	17 [12–25]
NASH Histology (Definite or Probable), *n* (%)	616 (79%)
Cirrhosis, *n* (%)	59 (8%)
Fibrosis, *n* (%)	
≤Stage 2	583 (75%)
>Stage 2	199 (25%)
Steatosis, *n* (%)	
<5%	34 (4%)
5–33%	295 (38%)
34–66%	271 (35%)
>66%	182 (23%)
Lobular Inflammation, *n* (%)	
0	3 (0.4%)
<2 under 20×	399 (51%)
2–4 under 20×	290 (37%)
>4 under 20×	90 (12%)
Ballooning, *n* (%)	
None	259 (33%)
Few	206 (26%)
Many	317 (41%)
NAS, *n* (%)	
<5	400 (52%)
≥5	382 (48%)
Cups of Coffee/Day, cpd (mean ± sd)	1.0 ± 1.2
AST, U/L (median, IQR)	44 [32–64]
ALT, U/L (median, IQR)	62 [41–92]
Alkaline Phosphatase, U/L (median, IQR)	81 [66–103]
Total Bilirubin, mg/dl (median, IQR)	0.7 [0.5–0.9]
Gamma Glutamyl Transferase, U/L (median, IQR)	47 [29–82]
Glucose, mg/dl (median, IQR)	96 [86–110]
Insulin, μU/ml (median, IQR)	17.9 [11.8–27.5]
HOMA-IR (median, IQR)	4.3 [2.7–7.2]
Diabetes, *n* (%)	189 (24%)
Hyperlipidaemia, *n* (%)	435 (56%)
Alcohol, drinks/month (mean ± sd)	5.3 ± 11.6
Smoking, pack-years (mean ± sd)	7.7 ± 16.1
Physical Activity, METS/week (median, IQR)	115 [85–151]
Education Level, *n* (%)	
<High School	239 (31%)
Some College	293 (37%)
Advanced Degree	250 (32%)
Annual Income, *n* (%)	
<$30 000	158 (21%)
$30 000–$49 999	162 (21%)
≥$50 000	449 (58%)

(definite or probable) was present in 79% of participants (n = 616). Advanced fibrosis (>stage 2) was present in 25% (n = 199). Fifty-nine individuals (8%) had cirrhosis.

Participant characteristics by coffee consumption

To determine the impact of coffee on various metabolic parameters, participants were further characterized by their daily coffee consumption (Table 2). Twenty-nine per cent of participants (n = 230) drank 0 cpd; 28% (n = 219) drank <1 cpd; 15% (n = 116) drank 1 to <2 cpd; and 28% (n = 217) drank ≥2 cpd. Between these four groups, there was a statistically significant difference in age (P = 0.0001), with individuals drinking ≥2 cpd being older than those who drank fewer cpd. Fewer individuals who self-identified as Latino drank ≥2 cpd (P = 0.003). Additionally, AST levels were inversely related to daily coffee intake (P = 0.02). Number of pack-years smoked and number of alcoholic beverages consumed per month also increased with increasing coffee consumption (P = 0.0001 and P = 0.002 respectively).

Participant characteristics by advanced fibrosis

Comparisons of study participants categorized by presence or absence of advanced fibrosis (>stage 2) are demonstrated in Table 3. Compared with those without advanced fibrosis, individuals with advanced fibrosis had significantly higher AST, alkaline phosphatase and GGT levels (P < 0.0001, for all). Diabetes was more prevalent, and HOMA-IR was significantly higher, among individuals with advanced fibrosis (P < 0.0001 for both). The number of alcoholic beverages consumed per month was significantly lower among individuals with advanced fibrosis, whereas the number of pack-years smoked was significantly greater among individuals with advanced fibrosis (P < 0.0001 and P = 0.0004 respectively).

Univariate analyses for advanced fibrosis

To identify features associated with advanced fibrosis, univariate analyses were performed (Table 4). Age, BMI, waist circumference, liver biopsy length, AST, alkaline phosphatase, glucose, insulin, HOMA-IR, diabetes and smoking were significantly positively associated with advanced fibrosis. In contrast, male gender, Latino ethnicity, alcohol consumption, physical activity level, education level and annual income were significantly inversely associated with advanced fibrosis.

Interaction between coffee and HOMA-IR in increasing the odds of advanced fibrosis

The interaction between coffee intake and insulin resistance (HOMA-IR) with respect to odds of advanced

Table 2. Comparison of Population Characteristics by Coffee Intake*

	0 cpd (n = 230)	<1 cpd (n = 219)	1- < 2 cpd (n = 116)	≥ 2 cpd (n = 217)	P
Age, years	45 ± 12	45 ± 13	51 ± 12	53 ± 9	0.0001
Male, (%)	87 (38%)	91 (42%)	40 (34%)	77 (35%)	0.5
Latino, *n* (%)	25 (11%)	35 (16%)	22 (19%)	15 (7%)	0.003
Race, *n* (%)					0.03
Caucasian	119 (89%)	155 (76%)	91 (83%)	188 (87%)	
Black	4 (2%)	8 (4%)	2 (2%)	6 (3%)	
Asian	8 (4%)	16 (8%)	4 (4%)	8 (4%)	
American Indian	7 (3%)	12 (6%)	5 (5%)	3 (1%)	
Native Hawaiian/Pacific Islander	0 (0%)	4 (2%)	1 (1%)	0 (0%)	
Multiracial	6 (3%)	10 (5%)	6 (6%)	10 (5%)	
BMI, kg/m^2	34.2 [29.5–38.7]	33.7 [29.2–38.7]	33.0 [29.7–38.0]	33.3 [29.8–37.8]	0.8
Waist Circumference, cm	108 [99–119]	108 [98–116]	106 [98–117]	109 [99–118]	0.7
Biopsy Length, mm	17 [11–25]	19 [12–25]	17 [13–23]	16 [11–24]	0.4
NASH Histology (Definite or Probable), *n* (%)	178 (77%)	172 (79%)	96 (83%)	170 (78%)	0.7
Cirrhosis, *n* (%)	19 (8%)	16 (7%)	6 (5%)	18 (8%)	0.7
Fibrosis, *n* (%)					0.2
≤Stage 2	173 (76%)	170 (78%)	79 (69%)	156 (72%)	
>Stage 2	55 (24%)	48 (22%)	36 (31%)	60 (28%)	
AST, U/L	43 [32–62]	45 [31–65]	54 [35–73]	40 [31–58]	0.02
ALT, U/L	60 [42–88]	62 [39–95]	67 [44–104]	60 [40–91]	0.5
Alkaline Phosphatase, U/L	84 [67–106]	79 [67–100]	80 [67–97]	60 [40–91]	0.8
Total Bilirubin, mg/dl	0.6 [0.5–0.9]	0.7 [0.5–0.9]	0.7 [0.5–0.9]	0.6 [0.5–0.9]	0.3
Gamma Glutamyl Transferase, U/L	46 [29–75]	49 [29–83]	50 [33–91]	47 [29–77]	0.4
HOMA-IR	4.3 [2.6–7.7]	4.3 [2.5–6.8]	4.8 [3.2–7.1]	4.2 [2.7–7.0]	0.5
Diabetes, *n* (%)	53 (23%)	50 (23%)	24 (21%)	62 (29%)	0.3
Hyperlipidaemia, *n* (%)	127 (55%)	118 (54%)	59 (51%)	131 (60%)	0.3
Alcohol, drinks/month	3.7 ± 9.1	4.9 ± 11.5	6.3 ± 13.3	7.0 ± 12.9	0.002
Smoking, pack-years	4.6 ± 14.0	5.1 ± 11.2	10.3 ± 20.7	12.2 ± 18.4	0.0001
Physical Activity, METS/week	113 [88–146]	121 [91–161]	108 [80–143]	113 [78–149]	0.3
Education Level, *n* (%)					0.8
<High School	65 (28%)	70 (32%)	34 (29%)	70 (32%)	
Some College	92 (40%)	73 (33%)	47 (41%)	81 (37%)	
Advanced Degree	73 (32%)	76 (35%)	35 (30%)	66 (30%)	
Annual Income, *n* (%)					0.4
<$30 000	47 (21%)	51 (24%)	19 (17%)	41 (19%)	
$30 000–$49 999	44 (19%)	43 (20%)	33 (29%)	42 (20%)	
≥$50 000	135 (60%)	123 (57%)	62 (54%)	129 (61%)	

Unless otherwise noted, data presented are mean ± sd; or median [IQR].
*cpd, cups per day.

fibrosis was investigated. We found a statistically significant interaction between HOMA-IR and coffee intake with respect to increasing the odds of advanced fibrosis (P = 0.001). As a result of this significant interaction, we examined the effect of coffee intake on the odds of advanced fibrosis in multivariate modeling after stratifying by the degree of insulin resistance, as measured by HOMA-IR (see below). Specifically, we dichotomized the participant population into two groups based in insulin resistance using the population's median HOMA-IR value (<4.3 vs. ≥4.3) as the cut-off.

Multivariate analyses for advanced fibrosis

The results from multivariate logistic regression modeling for advanced fibrosis, which included an interaction term between coffee intake and HOMA-IR are demonstrated in Table 5. In the multivariate model, the following factors were significantly associated with increased odds of advanced fibrosis: age [OR 1.05, 95% CI (1.03–1.07), P < 0.0001], AST [OR 1.01, 95% CI (1.004–1.011), P < 0.0001], GGT [OR 1.003, 95% CI (1.003–1.005), P = 0.02], diabetes [OR 1.62, 95% CI (1.05–2.09), P = 0.03], smoking [OR 1.60, 95% CI (1.03–2.47), P = 0.04] and biopsy length [OR 1.03, 95% CI (1.01–1.05), P = 0.002]. Conversely, alcoholic beverage consumption [OR 0.98, 95% CI (0.96–0.99), P = 0.04] and coffee intake [OR 0.68, 95% CI (0.52–0.89), P = 0.005] were significantly associated with decreased odds for advanced fibrosis.

There was a significant interaction between coffee intake and HOMA-IR with respect to the odds of advanced fibrosis (P < 0.0001). Among individuals with less insulin resistance (HOMA-IR <4.3), increasing coffee intake was associated with decreased odds of advanced fibrosis, OR 0.64, 95% CI: 0.46–0.88, P = 0.001. How-

Table 3. Comparison of Population by Advanced Fibrosis (≤Stage 2 vs. >Stage 2)

	≤Stage 2	>Stage 2	*P*
Age, years (mean ± sd)	46.5 ± 12.2	54.0 ± 10.4	<0.0001
Male, *n* (%)	230 (40%)	62 (31%)	0.03
Latino, *n* (%)	82 (14%)	13 (7%)	0.004
Race, *n* (%)			0.04
Caucasian	464 (84%)	165 (85%)	
Black	14 (3%)	6 (3%)	
Asian	30 (5%)	6 (3%)	
American Indian	24 (4%)	2 (1%)	
Native Hawaiian/Pacific Islander	4 (1%)	1 (1%)	
Multiracial	18 (3%)	14 (7%)	
BMI, kg/m^2 (median, IQR)	33.2 [29.6–37.8]	34.6 [29.9–39.2]	0.05
Waist Circumference, cm (median, IQR)	108 [99–116]	110 [99–122]	0.010
Biopsy Length, mm (median, IQR)	17 [11–24]	19 [14–26]	0.004
Coffee (cpd)	0.9 ± 1.2	1.1 ± 1.2	0.1
AST, IU/L	41 [30–61]	53 [39–74]	<0.0001
ALT, IU/L	63 [41–92]	60 [43–90]	0.5
Alkaline Phosphatase, IU/L	79 [64–97]	90 [70–116]	<0.0001
Total Bilirubin, mg/dl	0.7 [0.5–0.9]	0.7 [0.5–1.0]	0.4
GGT (IU/L)	42 [28–72]	66 [45–107]	<0.0001
Glucose (mg/dl)	95 [86–108]	99 [86–119]	0.01
Insulin (μIU/ml)	17 [11–25]	22 [15–36]	<0.0001
HOMA-IR	3.9 [2.5–6.3]	5.9 [37–9.9]	<0.0001
HOMA, dichotomized, *n* (%)			<0.0001
<4.3	291 (51%)	57 (29%)	
≥4.3	277 (49%)	138 (71%)	
Diabetes, *n* (%)	111 (19%)	77 (39%)	<0.0001
Hyperlipidaemia, *n* (%)	319 (55%)	113 (57%)	0.7
Alcohol, drinks/month	5.8 ± 11.8	3.5 ± 10.5	<0.0001
Smoking, ≥ 10 pack-years	6.4 ± 15.0	11.5 ± 19.5	0.0004
Physical Activity, METS/week	118 [89–153]	108 [79–140]	0.008
Education Level, *n* (%)			0.02
<High School	164 (28%)	73 (37%)	
Some College	214 (37%)	76 (38%)	
Advanced Degree	200 (35%)	50 (25%)	
Annual Income, *n* (%)			0.01
<$30 000	106 (19%)	52 (27%)	
<$30 000–$49 000	113 (20%)	47 (24%)	
≥$50 000	349 (61%)	97 (49%)	

Unless otherwise noted, data presented are mean ± sd; or median [IQR].

ever, among individuals with greater insulin resistance (HOMA-IR ≥4.3), there was no significant association between coffee intake and odds of advanced fibrosis, OR 1.06, 95% CI: 0.87–1.28, $P = 0.6$, (Fig. 1).

Multivariate analyses for NASH histology

Our analyses of the secondary outcome of NASH histology found no significant associations between coffee consumption and odds of NASH histology (data not shown).

Discussion

In this study we demonstrate that coffee intake has a differential effect on the odds of advanced fibrosis in NAFLD and is dependent on underlying insulin resistance. Among individuals with less insulin resistance as indicated by a lower HOMA-IR (<4.3), increasing coffee intake is associated with decreased odds of advanced fibrosis. However, among individuals with more severe insulin resistance as indicated by a higher HOMA-IR (≥4.3), there was no significant effect of coffee intake on the odds of advanced fibrosis. The mechanisms underlying the differential effect of coffee intake on hepatic fibrosis are not immediately evident from our data. However, type 2 diabetes, a state of extreme insulin resistance, has been cross-sectionally associated with advanced fibrosis in patients with NAFLD (42). It is plausible that the detrimental effects of greater insulin resistance on hepatic fibrosis overshadow the beneficial effects of coffee on hepatic fibrosis. Specifically, many patients with greater insulin resistance are actually diabetic, and the preventive effects of coffee may be related to its effects on improving insulin sensitivity early on in the spectrum of progression of loss of function of

Table 4. Univariate Analyses for Risk of Advanced Fibrosis (≤Stage 2 vs. >Stage 2)

	Odds Ratio	95% CI	*P*
Age, years	1.06	[1.04–1.07]	<0.0001
Male	0.68	[0.49–0.97]	0.03
Latino	0.42	[0.23–0.78]	0.006
Race	1.03	[0.91–1.18]	0.6
BMI, kg/m^2	1.02	[1.00–1.05]	0.08
Waist, cm	1.02	[1.004–1.03]	0.006
Biopsy length, mm	1.03	[1.01–1.04]	<0.0001
Coffee, cpd	1.10	[0.96–1.24]	0.2
AST, IU/L	1.009	[1.004–1.012]	<0.0001
ALT, IU/L	0.99	[0.99–1.00]	0.4
Alk Phos, IU/L	1.01	[1.01–1.02]]	<0.0001
Total Bili, mg/dl	1.09	[0.73–1.61]	0.7
GGT (IU/L)	1.004	[1.002–1.006]	<0.0001
Glucose, (mg/dl)	1.01	[1.00–1.01]	0.004
Insulin, (μIU/ml)	1.02	[1.01–1.03]	<0.0001
HOMA-IR	1.06	[1.03–1.09]	<0.0001
Diabetes	2.66	[1.87–3.78]	<0.0001
Hyperlipidaemia	1.07	[0.77–1.47]	0.7
Alcohol, drinks/month	0.98	[0.96–0.99]	0.02
Smoking, ≥10 pack-years	2.01	[1.40–2.89]	<0.0001
Physical Activity, METS/ week	0.995	[0.992–0.998]	0.007
Education Level	0.61	[0.57–0.92]	0.006
Annual Income	0.74	[0.61–0.91]	0.003

Table 5. Multivariate Analyses for Risk for Advanced Fibrosis (≤Stage 2 vs. >Stage 2)

Covariate	Odds Ratio [95% CI]	*P*
Age (years)	1.05 [1.03–1.07]	<0.0001
Male	1.04 [0.69–1.58]	0.8
Latino	0.66 [0.33–1.31]	0.2
Waist Circumference (cm)	1.01 [0.99–1.03]	0.06
AST (U/L)	1.01 [1.004–1.01]	<0.0001
GGT (U/L)	1.003 [1.003–1.005]	0.02
Diabetes	1.62 [1.05–2.49]	0.03
Smoking (≥ 10 pack-years)	1.60 [1.03–2.47]	0.04
Alcohol (drinks/month)	0.98 [0.96–0.99]	0.04
Biopsy Length (mm)	1.03 [1.01–1.05]	0.002
Coffee (cpd)	0.68 [0.52–0.89]	0.005
HOMA-IR	1.004 [0.97–1.04]	0.8
Coffee × HOMA-IR†	1.07 [1.03–1.10]	<0.0001

†Interaction term between Coffee intake (cpd) and HOMA-IR.

pancreatic beta cell. One could further speculate that once a patient develops overt diabetes there may be limited or no benefits with coffee intake.

Aside from fibrosis, the daily coffee consumption did not affect any of the other histologic features associated with NASH. Interestingly, our data also demonstrate that the length of the liver biopsy was significantly and positively associated with the odds of advanced fibrosis. Biopsy length maintained its significant association with advanced fibrosis in multivariate analysis. These results

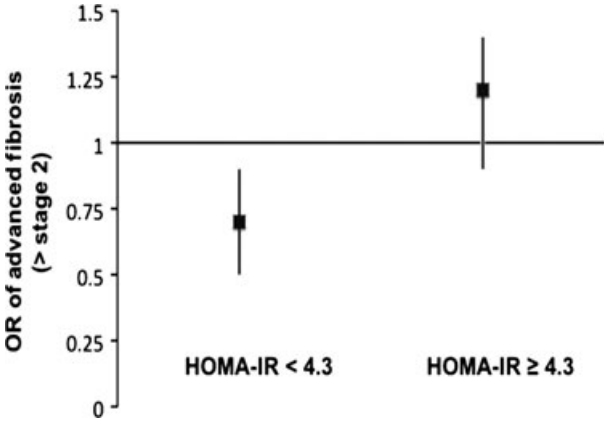

Fig. 1. Differential effect of coffee consumption on risk for advanced fibrosis by degree of insulin resistance (HOMA-IR <4.3 vs. HOMA-IR ≥4.3).

are consistent with the well-known sampling variability and sampling errors that may be associated with liver biopsies, and also underscore the need for the development of accurate and valid noninvasive markers for NASH and fibrosis in NASH (43).

There are only a few publications investigating the associations between coffee and/or caffeine consumption, and NAFLD; two of these studies incorporated hepatic histologic data (28–30, 32, 33). In a study of patients with biopsy-proven NASH, Molloy *et al.* demonstrated that individuals with stage 0-1 fibrosis consumed significantly greater amounts of coffee caffeine per day when compared with individuals with stage 2-4 fibrosis ($P = 0.035$) (28). These authors concluded that coffee may confer a decreased risk for advanced fibrosis and speculated that moderate coffee consumption may be a readily implemented adjunct to the management of patients with NASH. Anty *et al.* also conducted a study investigating the influence of coffee and caffeine consumption on hepatic fibrosis among bariatric surgery patients who underwent intra-operative hepatic wedge biopsy (29). These investigators found that the consumption of regular coffee, but not espresso, was independently associated with less fibrosis (< stage 2). The reasons for the differential effects of regular coffee and espresso on hepatic fibrosis found in their bariatric surgery population remain unclear, but these investigators speculated that the differences might be attributable to chemical alterations induced during the preparation of these two forms of caffeinated beverage.

It is not fully known what substances in coffee may be beneficial in the amelioration of severity of hepatic fibrosis, but it is known that coffee contains hundreds of components in addition to caffeine. These components include: phenolic compounds; chlorogenic acids; melanidins; maillard reaction products; lignans; ferulic acid; diterpenes; and vitamins and minerals. It is worth noting that chlorogenic acids have been previously demonstrated to have the following effects: anti-oxidant

properties; increased insulin sensitivity; decrease inflammation and decreased gluconeogenesis (44). However, the specific mechanisms by which coffee may exert any beneficial effects in NAFLD and other disease processes remain to be unraveled.

The strengths of our study include its large sample size with a substantial number of patients with biopsy-proven NASH, including a broad range of hepatic fibrosis severity. The data for this study were prospectively collected through the NASH CRN and included extensive clinical, laboratory and histologic information on study participants. The availability of these data allowed us to adjust for clinically relevant potential confounders in our multivariate analyses. The issue of confounding is particularly important in the context of dietary coffee intake as heavy coffee consumption may be strongly associated with other factors such as lack of exercise and/or cigarette smoking and, perhaps, with an overall less healthy lifestyle (14). Cigarette smoking has been demonstrated to be associated with increased risk for advanced fibrosis in NASH (7). Conversely, moderate alcohol consumption and vigorous physical activity levels have been demonstrated to be associated with lower risk for advanced fibrosis in NASH (8, 39). In our analyses, we found that tobacco and alcohol use in our patient population increased with increasing coffee intake. As such, we felt it was imperative to include these potential confounders in our multivariate models. Although we included a measure of physical activity in our univariate models, this variable did not contribute to the generation of the final multivariate models.

Our study also had some limitations including: a paucity of information regarding caffeinated versus decaffeinated coffee; truncation of coffee intake at a maximum of 5 cups per day in the Block Food Frequency Questionnaire; the self-reported nature of coffee drinking habits based on dietary recall; and lack of information on coffee cup size, type of coffee and strength of the brew. As has been described previously in studies investigating associations between dietary intake and disease processes, some measurement error is inevitable (45). In large-scale epidemiological studies, such as the NASH CRN, self-administered food frequency questionnaires, rather than exhaustive food diaries, are often used. Although food diaries may have the advantage of reducing reliance on memory of dietary intake, validation studies comparing food frequency questionnaires to food diaries have shown good agreement between the two (46, 47). We also note that, despite the absence of data pertaining to: exact serving size; type of coffee and strength of the brew in our analyses; we were able to demonstrate an effect of coffee on advanced fibrosis in NAFLD. However, the potential for misclassification in the amount of coffee consumed does limit the ability to draw valid conclusions from our data on the specific amounts of coffee needed to exert a beneficial effect.

In conclusion, we demonstrate that increasing coffee intake is associated with decreased odds of advanced fibrosis among patients with less insulin resistance. No beneficial effect of coffee intake was seen among patients with greater insulin resistance. The potential for beneficial effects of coffee intake among patients with chronic liver diseases is becoming more evident in the literature. However, longitudinal studies are needed to further investigate the impact of coffee consumption on hepatic fibrosis, and whether coffee may be a useful adjunct therapy in NAFLD.

Acknowledgements

Financial support: The Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) is supported by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (grants U01DK061718, U01DK061728, U01DK061731, U01DK061732, U01DK061734, U01DK061737, U01DK061738, U01DK061730, U01DK061713) and the National Institute of Child Health and Human Development (NICHD).

Several clinical centers use support from the National Center for Advancing Translational Sciences (NCATS) in conduct of NASH CRN Studies (grants UL1TR000439, UL1TR000077, UL1TR000436, UL1TR000150, UL1TR000424, UL1TR000006, UL1TR000448, UL1TR000040, UL1TR000100, UL1TR000004, UL1TR000423, UL1TR000058, UL1TR000067, UL1TR000454).

Conflicts of interest: The authors do not have any disclosures to report.

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