Increased Caffeine Consumption Is Associated with Reduced Hepatic Fibrosis

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Although coffee consumption has been associated with reduced frequency of liver disease, it is unclear whether the effect is from coffee or caffeine and whether there is an effect on hepatic fibrosis specifically. This study was undertaken to use a food-frequency instrument for dietary caffeine consumption to evaluate the relationship between caffeine intake and liver fibrosis. Patients undergoing liver biopsy completed a detailed caffeine questionnaire on three occasions over a 6-month period. Caffeine intake was compared between patients with mild and advanced liver fibrosis (bridging fibrosis/cirrhosis). Logistic regression was used to evaluate the association between caffeine consumption and hepatic fibrosis. One hundred seventy-seven patients (99 male, 104 white, 121 with chronic hepatitis C virus [HCV] infection) undergoing liver biopsy completed the caffeine questionnaire on up to three occasions. Results from repeated questionnaires were consistent. Daily caffeine consumption above the 75th percentile for the cohort (308 mg = approximately 2.25 cups of coffee equivalents) was associated with reduced liver fibrosis (odds ratio [OR], 0.33; 95% confidence interval [CI], 0.14-0.80; $P = 0.015$) and the protective association persisted after controlling for age, sex, race, liver disease, body mass index, and alcohol intake in all patients (OR, 0.25; 95% CI, 0.09-0.67; $P = 0.006$), as well as the subset with HCV infection (OR, 0.19; 95% CI, 0.05-0.66; $P = 0.009$). Despite a modest trend, consumption of caffeine from sources other than coffee or of decaffeinated coffee was not associated with reduced liver fibrosis. A reliable tool for measurement of caffeine consumption demonstrated that caffeine consumption, particularly from regular coffee, above a threshold of approximately 2 coffee-cup equivalents per day, was associated with less severe hepatic fibrosis. (HEPATOLOGY **2010;51: 201-209.**)

The potential beneficial health effects of caffeine are controversial. Despite a common perception that coffee consumption may have negative health consequences, a recent large population-based study found that increasing coffee intake actually led to a modest decrease in all-cause mortality, largely because of a reduced rate of cardiovascular death.[1] Similarly, increased caffeine, and specifically coffee consumption, has been associated with a lower prevalence of chronic liver disease. Two recent population-based studies (The National Health and Nutrition Examination Survey I and III) have reported that higher caffeine consumption (>2 cups/day) was associated with a lower risk of elevated alanine aminotransferase (ALT) levels and a lower risk of chronic liver disease.[2,3] In the analysis of the National Health and Nutrition Examination Survey III data, there was a 44%

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; CI, confidence interval; HCV, hepatitis C virus; OR, odds ratio.

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reduction in the risk of elevated ALT levels in persons who drank more than 2 cups of coffee per day compared with non-coffee drinkers. Additionally, a recent large cohort study of 330 patients with alcoholic and nonalcoholic cirrhosis showed a strong inverse relationship between coffee drinking (>4 cups/day) and elevated serum enzymes, especially in those who drank large quantities of alcohol.[4] This relationship was suggested in earlier studies, which found that coffee consumption was associated with lower serum gamma-glutamyl transferase and ALT levels.[5-9]

In addition to an association with liver enzyme elevation, coffee has been reported to reduce the risk of advanced liver disease and its complications. An Italian case-control study found that patients who presented to the hospital with decompensated cirrhosis were less likely to drink coffee than matched controls, and a Norwegian registry study reported that coffee consumption was associated with a lower risk of death of complications of cirrhosis.[10,11] In addition, many studies have shown an inverse relationship between coffee drinking and the risk of hepatocellular carcinoma.[12-15] The data were summarized in two recent meta-analyses and confirmed a protective effect of higher caffeine consumption with respect to hepatocellular carcinoma.[16,17]

From the data, it is difficult to discern how coffee may be playing a beneficial role in patients with liver disease. Coffee consumption appears to lower liver enzymes and has a protective effect against complications in patients with advanced disease. However, the relationship between coffee and progression of fibrosis has not been examined, and it is also unclear whether coffee itself or caffeine provides the beneficial effect. Hence, the aim of this study was to assess caffeine consumption accurately and to evaluate the association of coffee and caffeine intake with severity of fibrosis in patients with chronic liver disease. The results show that higher caffeine consumption is associated with milder fibrosis in patients with chronic liver disease, particularly those with chronic hepatitis C virus (HCV) infection.

Patients and Methods

Development of Caffeine Questionnaire. A questionnaire was developed using the format of the questionnaire used in the Nurses' Health Study to evaluate caffeine intake.[1] Questions were added for all possible sources of caffeine, and the period of assessment was increased from "during the past week" to "during the past month" (Appendix 1). Patients were asked to quantify the frequency and quantity of consumption of caffeine-containing products, including regular and diet carbonated soft drink beverages, regular coffee, decaffeinated coffee, black tea, green/Chinese tea, herbal tea, cocoa/hot chocolate; caffeine-fortified drinks; chocolate candies, and caffeine pills or caffeine-containing medications (list provided as Appendix 2). The frequency of consumption was quantified (as in the Nurses' Health Study questionnaire) as never, 1 to 3 per month, 1 per week, 2 to 4 per week, 5 to 6 per week, 1 per day, 2 to 3 per day, 4 to 5 per day, and 6 or more per day. To determine whether reporting of consumption patterns varied over time, participants were asked whether the amount of caffeine consumption had changed in the previous 6 months or in the previous 5 years. The questionnaire also assessed consumption of alcohol-containing beverages.

Patient Selection and Data Collection. From January 2006 to November 2008, all patients evaluated in the Liver Diseases Branch of the National Institutes of Health were asked to complete the questionnaire. Of these, only patients who had or were scheduled to undergo a liver biopsy for clinical indications within 6 months not receiving prescribed therapy for liver disease were included in the analysis. A visual aid including a can of soda, an 8-ounce (oz) cup of coffee, a chocolate bar, and a list of medications containing caffeine was presented to the patients to aid in filling out the questionnaire. The nurses administering the questionnaire were instructed not to comment on the possible effects of caffeine on liver disease. To ensure consistency of responses, participants were asked to complete the questionnaire three times over a period of 6 months, with an interval of at least 2 weeks between each questionnaire. Laboratory tests and body mass index (BMI) were obtained at the time of liver biopsy. Liver histology was scored using the modified Ishak scoring system for activity (histology activity index) and fibrosis by a hepatic pathologist (D.E.K.) who was blinded to the results of the caffeine questionnaire.[18]

Statistical Analysis. Total caffeine intake from foods and beverages (mg/day) was calculated by summing caffeine content based on estimates from the published literature on caffeinated cola (46 mg/can),[19] regular coffee (137 mg per 8-oz cup),[19] decaffeinated coffee (3 mg per 8-oz cup),[20,21] black tea (47 mg per 8-oz cup),[2,19] green tea (30 mg per 8-oz cup),[20,22] Chinese (oolong) tea (30 mg per 8-oz cup),[22] cocoa (6 mg per 8-oz cup),[20] caffeine-fortified drinks (71 mg per can),[20] candy chocolate bars (7 mg per 1 oz),[19] and caffeine pills (200 mg per pill)[23] (Table 1). Consistency of questionnaire responses was assessed using the Cronbach coefficient alpha, which is a measure of the internal consistency and reliability of a psychometric instrument.[24] The mean daily caffeine intake for each individual was calculated as the mean of total caffeine consumption from all completed questionnaires.

Table 1. Caffeine Content in Beverages and Foods

Beverage/Food	Caffeine Content [mg per standard unit of consumption]	Mean Units Consumed/ Day (±SD)	Proportion of Caffeine Consumed in Study Cohort
Regular coffee–8 oz	137	1.0 ± 0.1.4	71%
Decaffeinated coffee–8 oz	3	0.08 ± 0.31	0.1%
Caffeinated cola–12 oz	46	0.55 ± 0.99	13%
Caffeine-free cola–12 oz	0	0.18 ± 0.51	0
Black tea–8 oz	47	0.29 ± 0.74	7%
Green tea–8 oz	30	0.25 ± 0.66	4%
Herbal tea–8 oz	0	0.16 ± 0.57	0
Cocoa–8 oz	6	0.06 ± 0.24	0.2%
Caffeine-fortified drinks–1 Can	71	0.02 ± 0.10	0.6%
Candy chocolate bars–1 oz	7	0.21 ± 0.31	0.7%
Caffeine pill	200	0.03 ± 0.14	3%
Total	–		100%

Mean values and standard error of the mean are reported. Univariate and multivariate logistic regression analyses were performed to evaluate the association of caffeine intake with advanced liver fibrosis (bridging fibrosis/cirrhosis, Ishak fibrosis score ≥3).[18] Analyses were done for all patients studied as well as for those with HCV infection alone. Regression analysis was performed with caffeine intake as a continuous variable and dichotomized above and below the 75th percentile of mean caffeine intake for the cohort. The threshold of the 75th percentile for the cohort was selected *a priori.* Covariates with *P* values of 0.05 or less by univariate analysis were entered into multivariable models, and factors of clinical importance also were evaluated to exclude important confounding. To determine whether effects were related to caffeine or coffee consumption, the effects of caffeinated and decaffeinated coffee were compared. Statistical analyses were performed using STATA version 9.0, SAS version 9.1, and Prism version 4 software. A *P* value less than 0.05 was considered statistically significant.

Results

Patient Characteristics. All patients who underwent liver biopsy (n = 177) completed the caffeine questionnaire on at least one occasion. Ninety-nine (56%) were male; 104 (59%) white, 33 (19%) black, 34 (19%) Asian, and 6 (3%) Hispanic; the mean age was 51 years (range, 18-78), and the mean BMI was 27.5 ± 6.2 kg/m^2 (Table 2). Most patients (121/177; 68%) had chronic hepatitis C; the remaining patients had chronic hepatitis B (13%), delta hepatitis (3%), nonalcoholic steatohepatitis (11%), primary biliary cirrhosis (2%), or autoimmune hepatitis (3%). Baseline data from patients with HCV infection are shown in Table 3. On liver biopsy, 123 (69%) patients had mild fibrosis (42 with no fibrosis and 81 with portal fibrosis only), and 54 (31%) patients had advanced fibrosis (36 with bridging fibrosis and 18 with cirrhosis).

Estimated daily consumption of caffeine from food and beverages ranged from none to 1028 mg/day and averaged 195 mg/day, which is the equivalent of 1.4 cups of coffee per day. Fifty patients reported drinking no coffee. Of all caffeine consumed, 71% came from regular coffee (0.1% from decaffeinated coffee), 13% from caffeinated soda, 7% from black tea, 4% from green tea, 0.2% from cocoa, 0.6% from caffeine-fortified beverages, 0.7% from chocolate, and 3% from caffeine pills (Table 1). A second questionnaire was completed by 80% of patients and a third questionnaire by 56%, all within a 6-month period but separated by at least 2 weeks. Repeat administration of the questionnaire demonstrated consistent results, with a Cronbach coefficient alpha of 0.90 (Fig. 1).

Table 2. Daily Caffeine Consumption According to Patient Characteristics

	N	Mean Caffeine Intake [mg/day] (SEM)	Mean Caffeine [Coffee Cup Equivalents]	*P*-Value
Males	99	215 (23)	1.6	0.14
Females	78	168 (20)	1.2	
Race/ethnicity				
White	104	266 (23)	1.9	<0.0001
African American	33	98 (21)	0.7	
Asian	34	85 (16)	0.6	
Hispanic	6	110 (53)	0.8	
Age [years]				
18-45	51	139 (24)	1.0	0.053
46-55	66	234 (29)	1.7	
56-78	60	199 (26)	1.5	
BMI [kg/m^2]				
<25	65	201 (26)	1.5	0.81
25-30	57	204 (32)	1.5	
≥30	46	178 (29)	1.3	
HCV	121	212 (21)	1.5	0.10
Other liver diagnosis	56	156 (23)	1.1	
ALT <40 [U/L]	41	216 (39)	1.5	0.46
ALT ≥ 40 [U/L]	136	188 (17)	1.4	
HAI				
1-4	23	223 (35)	1.6	0.20
5-8	93	168 (22)	1.2	
>8	60	223 (30)	1.6	
Ishak fibrosis <3	123	212 (21)	1.5	0.043
Ishak fibrosis≥3	54	154 (19)	1.1	
Alcohol consumption				
Yes	70	176 (21)	1.3	0.35
No	107	207 (22)	1.5	

Table 3. Daily Caffeine Consumption According to Patient Characteristics in the HCV Cohort

	N	Mean Caffeine Intake [mg/day] (SEM)	Mean Caffeine [Coffee Cup Equivalents]	*P*-Value
Males	66	245 (31)	1.8	0.08
Females	55	172 (25)	1.3	
Race/ethnicity				
White	72	292 (30)	2.1	<0.0001
African American	29	98 (22)	0.7	
Asian	18	92 (21)	0.7	
Hispanic	2	76 (70)	0.6	
Age [years]				
18-45	19	151 (48)	1.1	0.44
46-55	48	223 (35)	1.6	
56-78	54	224 (30)	1.6	
BMI [kg/m^2]				
<25	39	257 (37)	1.9	0.41
25-30	39	196 (40)	1.4	
≥30	36	194 (35)	1.4	
ALT <40 [U/L]	29	241 (52)	1.8	0.44
ALT ≥ 40 [U/L]	92	203 (22)	1.5	
HAI				
1-4	14	208 (53)	1.5	0.98
5-8	58	209 (32)	1.5	
>8	49	217 (32)	1.6	
Ishak fibrosis < 3	84	241 (28)	1.8	0.033
Ishak fibrosis ≥ 3	37	146 (19)	1.1	
Alcohol consumption				
Yes	83	220 (26)	1.3	0.54
No	38	196 (33)	1.5	

White patients reported greater mean caffeine intake (mean ± standard error of the mean: 266 ± 23 mg/day) than African Americans (98 ± 21 mg/day, $P = 0.0001$) or Asians (85 ± 16 mg/day, $P < 0.0001$) from both coffee and other sources (Table 2). There was a trend toward higher caffeine intake among men than women but no correlation with BMI. In this cohort, more than half (60%) reported no alcohol intake, and only six (3%) consumed more than 10 g/day (range, 0–33 g/day).

Caffeine Intake and Severity of Liver Disease. The average daily caffeine intake was similar in patients with normal and elevated ALT levels (Table 2). In addition, there was no association between histological activity (histology activity index scores) and caffeine intake. However, greater daily caffeine consumption was associated with less severe fibrosis on liver biopsy (Table 2). Patients with Ishak fibrosis less than 3 had a mean caffeine intake of 212 ± 21 mg/day compared with 154 ± 19 mg/day in those with advanced fibrosis ($P = 0.043$). In patients with HCV infection, this difference was more pronounced (241 ± 28 mg/day versus 146 ± 19 mg/day; $P = 0.033$). Increasing mean caffeine intake as a continuous variable was associated with less severe fibrosis for those with HCV infection but not for the group as a whole. For each 67 mg caffeine intake (approximately one half cup of coffee), there was a 14% decrease in the odds of advanced fibrosis for patients with HCV infection (HCV: odds ratio [OR] per 67 mg caffeine, 0.86; 95% confidence interval [CI], 0.74-0.99; $P = 0.039$), but this association was not as strong in patients with other diagnoses (All: OR per 67 mg caffeine, 0.91; 95% CI, 0.81-1.02; $P = 0.098$).

To clarify the relationship between caffeine and fibrosis further, caffeine intake was categorized by quartile and dichotomized as above or below the 75th percentile for the entire cohort (308 mg/day; approximately 2.25 cups of coffee per day). Caffeine intake was also categorized into coffee-cup equivalents (0-1, 1-2, and >2 per day). Patients consuming more than 308 mg/day caffeine had lower odds of having advanced fibrosis (OR, 0.33; 95% CI, 0.14-0.80; $P = 0.015$) (Fig. 2). This effect was more pronounced in patients with HCV infection (OR, 0.22; 95% CI, 0.07-0.68; $P = 0.008$). Among patients with HCV, 30 of 84 (36%) with mild fibrosis consumed more than 308 mg caffeine per day, compared with only 4 of 37 (11%) with advanced fibrosis ($P = 0.005$). By multivariable logistic regression, after controlling for age, sex, race, BMI, liver disease diagnosis, and alcohol intake, the relationship between caffeine intake and reduced fibrosis persisted both for the group as a whole (OR, 0.25; 95% CI, 0.09-0.67; $P = 0.006$) and for those with HCV infection (OR, 0.19; 95% CI: 0.05-0.66; $P = 0.009$) (Fig. 2). Age also remained significant by multivariable analysis, with increasing age increasing the risk of advanced fibrosis (OR, 1.06; 95% CI: 1.02-1.10; $P = 0.001$). In keeping with the reduced fibrosis on liver biopsy, patients with greater caffeine consumption also had lower aspartate aminotransferase (51 versus 74 U/L; $P = 0.01$), alkaline phosphatase (66 versus 81 U/L; $P = 0.005$), and direct bilirubin (0.14 versus 0.19 mg/dL; $P = 0.006$) levels, and

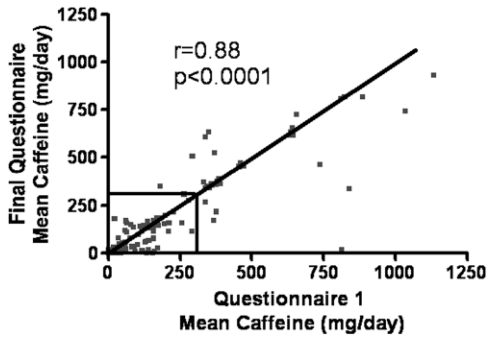

Fig. 1. Comparison of estimated daily caffeine intake between first and final completed questionnaire (2nd or 3rd) for each individual, demonstrating consistency of responses. Box indicates 308 mg caffeine consumption per day, the 75th percentile for the cohort. Responses above or below 308 mg/day were consistent between questionnaires in 96% of patients.

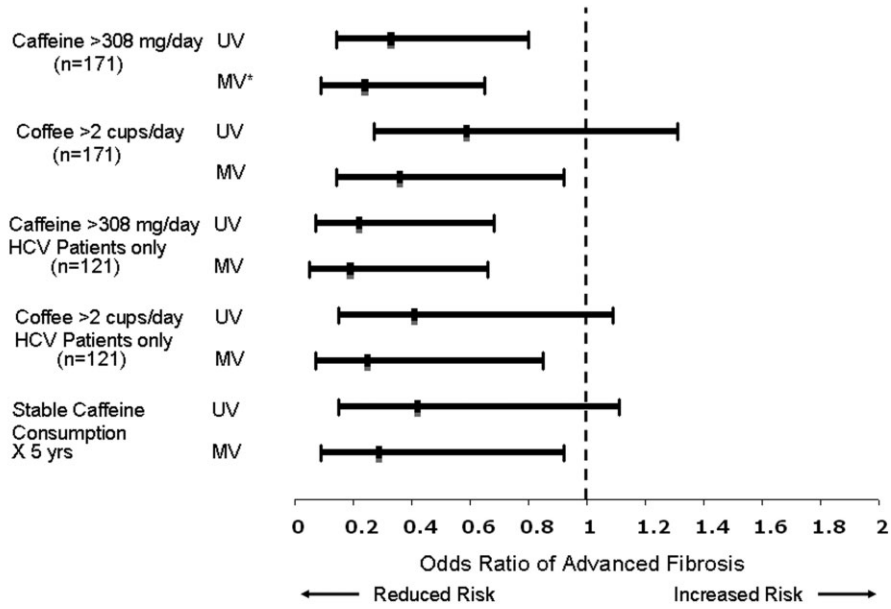

Fig. 2. Forest plot showing odds ratios with 95% confidence intervals for the association with advanced fibrosis. Results of univariate and multivariable logistic regression are shown for the association of each of the following with advanced hepatic fibrosis (Ishak ≥ 3): caffeine consumption above the 75th percentile for the cohort (308 mg/day), coffee consumption >2 cups per day, caffeine and coffee consumption for HCV patients only (n = 121), and caffeine consumption for patients reporting no change in caffeine intake in the past 5 years (n = 119). Multivariable odds ratios are adjusted for age, sex, liver disease diagnosis, BMI, race, and alcohol intake. Increased caffeine and coffee consumption are associated with a reduced risk of advanced fibrosis.

increased levels of serum albumin (3.99 versus 3.78 g/dL; $P = 0.005$).

Because white patients consumed greater than twice the amount of caffeine as nonwhite patients, the effect of race on the caffeine–fibrosis relationship was explored. Adjustment for race had no effect on the OR of advanced fibrosis for patients in the highest quartile of caffeine consumption (OR, 0.33; 95% CI, 0.13-0.83). The association between fibrosis and caffeine consumption above 308 mg/day was similar for white patients as for the group as a whole (OR, 0.30; 95% CI, 0.11-0.82; $P = 0.018$). A similar analysis for nonwhite patients revealed a nonsignificant protective association (OR, 0.62; 95% CI, 0.06-3.33; $P = 0.69$); however, only four (6%) nonwhite patients consumed more than 308 mg caffeine daily. When nonwhite patients were analyzed, using caffeine as either a continuous variable or above the 75th percentile for nonwhite patients only (130 mg/day), there was no apparent benefit to increasing caffeine intake and a nonsignificant trend toward an association with a greater risk of advanced fibrosis (OR, >130 mg/day, 1.49; 95% CI, 0.48-4.6; $P = 0.49$).

When caffeine intake was categorized by coffee-cup equivalents or compared by quartiles of consumption, there appeared to be a threshold effect on fibrosis. Greater than 2–coffee-cup equivalents of caffeine was associated with lower rates of advanced fibrosis (20%), but the protective association was not linear with similar rates of advanced disease among those consuming 0 to 1 (31%) and 1 to 2 (45%) coffee-cup equivalents of caffeine (Supporting Table 1). This pattern was again more pronounced in patients with HCV (>2 cups/day, 16%; 1-2 cups/day, 48%; <1 cup/day, 33%; $P = 0.035$) (Supporting Table 2). Similarly, although those in the highest quartile of caffeine consumption had a lower likelihood of advanced fibrosis when compared with all patients below this threshold, there was no increase in the odds of advanced fibrosis with increasing quartile of caffeine consumption, with patients in the 2nd and 3rd quartiles showing a trend toward more advanced fibrosis than those in the lowest quartile of caffeine intake, suggesting either a biphasic or a threshold effect. Once again, this pattern was more striking in those with HCV infection (Table 4). A similar threshold pattern was seen with alkaline phosphatase, aspartate aminotransferase and albumin levels but not with histology activity index, ALT, or other parameters of liver function. HCV RNA levels did not differ by caffeine consumption.

If the HCV cohort was considered in isolation, the 75th percentile of caffeine intake for the group was 345 mg/day. Consumption above this level was associated with a reduced likelihood of advanced fibrosis (OR, 0.19; 95% CI, 0.05-0.66; $P = 0.009$). By multivariable logistic regression, controlling for age, sex, race, BMI, and alcohol consumption, increased caffeine consumption was associated with a lower risk of advanced fibrosis (OR, 0.15; 95% CI, 0.04-0.60; $P = 0.007$). Increasing age was again associated with advanced fibrosis by multivariable analysis (OR, 1.07; 95% CI: 1.01-1.14; $P = 0.02$).

Most patients (85%) reported that their caffeine intake had not changed in the past 6 months, and 72% reported no change in the past 5 years. Of 26 patients who reported

Table 4. Odds of Advanced Fibrosis Based on Quartile of Caffeine Consumption

Caffeine Quartile	Proportion with Advanced Fibrosis	Odds Ratio of Advanced Fibrosis (95% CI)	Adjusted Odds Ratio of Advanced Fibrosis (95% CI)*	Odds Ratio of Advanced Fibrosis (95% CI)	Adjusted Odds Ratio of Advanced Fibrosis (95% CI)*
			All Patients		
1 (0-39 mg/day)	24%	1	1	1	1
2 (39-116 mg/day)	34%	1.60 (0.64–4.0)	1.26 (0.43–3.7)		
3 (116-308 mg/day)	48%	1.67 (1.07–2.6)	1.68 (0.95–3.0)		
4 (308-1028 mg / day)	16%	0.84 (0.59–1.2)	0.65 (0.38–1.1)	0.33 (0.14–0.80)	0.24 (0.09–0.65)
			HCV-Infected Patients		
1 (2.7-43 mg/day)	29%	1	1	1	1
2 (43-125 mg/day)	37%	1.42 (0.48–4.1)	0.96 (0.27–3.4)		
3 (125-345 mg/day)	47%	1.46 (0.86–2.5)	1.63 (0.82–3.3)		
4 (345-1028 mg/day)	25%	0.65 (0.40–1.0)	0.51 (0.27–0.98)	0.22 (0.07–0.68)	0.19 (0.05–0.66)

*Models adjusted for age, sex, race, body mass index, and alcohol consumption.

a change in caffeine intake in the previous 6 months, 5 (19%) had advanced fibrosis compared with 45 of 144 (31%) who reported no change ($P = 0.22$). Similarly, of 51 patients with a change in the past 5 years, 15 (29%) had advanced fibrosis, compared with 35 of 119 (29%) who reported stable caffeine intake ($P = 1.0$) (Fig. 2). Thus, a decrease or change in caffeine intake as assessed by this questionnaire did not appear to correlate with development of advanced fibrosis.

Association of Coffee with Fibrosis. To determine whether the association with fibrosis was related to caffeine or coffee, the effect of each component was evaluated separately. Caffeine consumption from sources other than coffee was not associated with reduced liver fibrosis in the population as a whole (OR per 67 mg of caffeine, 0.84; 95% CI, 0.60-1.17; $P = 0.30$) or in those with HCV infection (OR per 67 mg of caffeine, 0.78; 95% CI, 0.52-1.16; $P = 0.21$). Specifically, there was no relationship between caffeinated cola, green or black tea consumption, and fibrosis. Total caffeine consumption from coffee and noncoffee sources were not correlated ($P = 0.22$, $r^2 = 0.009$). After controlling for coffee consumption, the trend toward a protective association of increasing consumption of non–coffee-related caffeine on fibrosis remained nonsignificant. The mean consumption of caffeine restricted to coffee consumption was 152 ± 209 mg/day, with a 75th percentile of 270 mg/day. For all patients consuming greater than this amount, the multivariate adjusted OR of advanced liver disease was 0.39 (95% CI, 0.15-0.99; $P = 0.049$) and 0.26 (95% CI, 0.07-0.89; $P = 0.032$) for patients with HCV. For non–coffee related caffeine, the 75th percentile of consumption was 61 mg/day. There was a nonstatistically significant trend to suggest that consumption above this threshold was associated with a lower risk of advanced fibrosis. In addition to caffeine from coffee, increasing total cups of coffee (>2 cups of coffee daily) was associated with lower odds of advanced fibrosis (OR, 0.29; 95% CI, 0.09-0.92; $P = 0.036$) (Fig. 2). Furthermore, patients with advanced fibrosis reported drinking fewer cups of regular coffee per day (0.73 versus 1.3; $P = 0.06$), but similar amounts of decaffeinated coffee daily (0.10 versus 0.10; $P = 0.97$).

Discussion

A reliable tool for measurement of caffeine consumption was developed and used to demonstrate that caffeine intake above a threshold was associated with less severe fibrosis on liver biopsy. The protective association of caffeine was most pronounced in patients with HCV infection; however the number of patients with other liver diseases was relatively small (n = 56; 32%). In the HCV cohort, the protective association of caffeine on liver fibrosis remained significant whether evaluated as a continuous variable, categorized as coffee-cup equivalents, or dichotomized above or below the 75th percentile for the study population. After controlling for other factors known to affect fibrosis (age, sex, race, BMI, and alcohol consumption), the apparent protective effect of caffeine persisted. In keeping with the reduced fibrosis on liver biopsy, patients with greater caffeine consumption also had lower aspartate aminotransferase, alkaline phosphatase, and direct bilirubin and increased serum albumin

levels. Together these data suggest that increased caffeine consumption is associated with less advanced liver fibrosis.

Categorization of caffeine intake by coffee-cup equivalents or quartile suggested that the protective effect of caffeine may not be linear, and there appears to be a threshold effect. The proportion with advanced fibrosis and the mean liver test values were similar between patients consuming 0 to 1 and 1 to 2 coffee-cup equivalents of caffeine per day, but patients reporting greater than 2 coffee-cup equivalents of daily caffeine had a lower rate of advanced fibrosis and a trend toward lower aminotransferase levels and improved hepatic synthetic function (bilirubin, prothrombin time). Notably, when compared with patients in the lowest quartile of caffeine consumption, those in the 2nd and 3rd quartile showed a trend toward more advanced fibrosis (Table 4). Whether this truly implies that, at low levels of caffeine intake, there is a harmful effect to increasing caffeine consumption is hard to discern. The numbers of patients in each group were relatively small and after controlling for other factors, the apparent associations were not significant. This finding did, however, strengthen the suggestion that the potential beneficial effects of caffeine were not linear and that consumption above a threshold (approximately 2 coffee-cup equivalents per day) was necessary to have an effect on hepatic fibrosis. Clarification of whether there is a hepatoprotective threshold and whether the benefits plateau with further consumption will be important for understanding the biology and potentially for therapeutic recommendations.

Most previous studies of caffeine's health effects have focused largely on coffee consumption rather than total caffeine intake. The instrument developed for this study allowed for a relatively detailed breakdown of sources of dietary caffeine intake. However, for the purposes of analysis, it was necessary to assume that all caffeine sources of a given type contained equal amounts of caffeine irrespective of brand, the process of production, or other factors. The use of visual aids likely improved the reliability of estimates. Responses were consistent on repeat testing, suggesting that the instrument can provide reproducible results, and that caffeine consumption stays relatively constant over time, at least for the study period.

To tease apart whether the beneficial effects seen were related to caffeine or coffee intake, each component was evaluated individually. Consistent with previous reports, no beneficial effect was seen with green or black tea, caffeinated soda, or any other sources of caffeine.[5] However, a significant protective effect could have been missed because of small numbers; as 71% of total caffeine consumed came from coffee. Alternatively, if the beneficial effect of caffeine on fibrosis requires consumption above a threshold of daily caffeine, any benefit of non–coffee-related caffeine may have been inapparent because the absolute amount of caffeine consumed from sources other than coffee was relatively low (75th percentile: 61 mg from noncoffee sources versus 270 mg from coffee). The observation that the association with less advanced liver fibrosis was seen only with caffeinated coffee implies either that the benefit is derived from caffeine (all caffeine or only that in coffee) or possibly from a substance removed by the decaffeination process. Different decaffeinating procedures were not evaluated.

Race was an important effect modifier of the caffeine–fibrosis relationship. White patients consumed the most caffeine, and the protective association with advanced fibrosis was most apparent in this group. It is difficult to draw strong conclusions about the results in the nonwhite patients because of the relatively small numbers. The observation that nonwhite patients in the highest quartile of caffeine consumption for this group did not have lower odds of advanced fibrosis may simply be attributable to the fact that even the highest quartile in this group consumed much less caffeine than the apparent protective threshold.

Previous studies have shown that increased coffee consumption is associated with lower liver enzymes, reduced rates of liver cancer, and possibly even reduced hepatic decompensation and liver-related mortality.[2,4-11] The assumption has been that reduced fibrosis was attributable to a reduction in disease activity as reflected by serum aminotransferase levels. However, because most studies have relied on population surveys, liver histology was not evaluated, and the possible effects of coffee/caffeine on liver fibrosis had to be indirectly assessed. The distinction between anti-fibrogenic effects and protection against decompensation is important in understanding the underlying beneficial mechanism. With complete liver biopsy data on all 177 patients, across the spectrum of liver fibrosis, the data from this study suggest that the beneficial effect of caffeine is mediated through reduced rate of progression of fibrosis. However, the lack of association between caffeine intake and hepatic inflammation suggests that, rather than reducing fibrosis by minimizing ongoing inflammation, the protective effect of caffeine may be mediated through a direct anti-fibrogenic mechanism

Recent *in vitro* data suggest possible mechanisms by which coffee or caffeine may affect liver disease and specifically hepatic fibrogenesis. Studies in mice and rats as well as human hepatoma cell lines have shown that coffee and some of its major components (caffeine, cafestol, and kahweol) alter expression and activity of enzymes involved in xenobiotic metabolisms.[25-28] Inhibition of phase I enzymes and up-regulation of phase II enzymes such as glutathione-S-transferase have been reported,

both of which would favor reduced accumulation of toxic metabolites within hepatocytes.[27] Pretreatment with cafestol and kahweol protected mice from carbon tetrachloride hepatotoxicity by inhibiting cytochrome CYP 2E1, the enzyme responsible for carbon tetrachloride bioactivation.[29] With respect to caffeine specifically, Gressner and colleagues[30] recently reported that caffeine inhibits expression of connective tissue growth factor (CTGF) by interfering with transforming growth factor beta (TGFβ) signaling through the SMAD pathway.[30] Caffeine was also found to up-regulate peroxisome proliferator-activated receptor gamma (PPARγ) levels, which further reduce CTGF expression. Although these results from primary cell culture clearly need *in vivo* confirmation, inhibition of the transforming growth factor beta pathway is an attractive explanation for anti-fibrogenic effects attributed to caffeine.

It is important to consider potential confounding factors when interpreting the data from this study. The study was cross-sectional in nature, and caffeine consumption was estimated at the time of liver biopsy, despite the fact that any protective effect would likely occur over many years. Patients consuming the greatest amount of caffeine had less fibrosis on biopsy. Although it is tempting to conclude that caffeine has a protective effect on fibrogenesis, other explanations are also possible. Patients with more advanced liver fibrosis may have reduced their caffeine intake because of a presumption that caffeine may not be good for their health. Caffeine is metabolized by the liver, and therefore it is also possible that as hepatic function deteriorated, patients may have required less caffeine to achieve the same physiological effects, leading them to reduce their intake over time. By asking whether caffeine consumption patterns had changed in the past 6 months or 5 years, an attempt was made to discern whether patients with more advanced fibrosis were decreasing their caffeine intake. Most patients did not report a change in caffeine consumption patterns over time, but this is clearly an imperfect measure of this trend. Importantly, however, of patients reporting a change in intake over the past 5 years, there were similar numbers with and without advanced fibrosis, suggesting that worsening liver disease was not the impetus to alter consumption of caffeine. Other factors that may affect caffeine consumption such as socioeconomic status, education level, and recreational drug use, were also not considered in this analysis.

Conclusions

A useful instrument for a comprehensive evaluation of caffeine consumption was developed, which proved easy to use and highly reproducible. Caffeine consumption was associated with a lower risk of advanced liver fibrosis, particularly in patients with HCV infection; however, the data suggest that a beneficial effect requires caffeine consumption above a threshold of approximately 2 coffee-cup equivalents per day. The effect seemed to be most pronounced with caffeinated coffee as opposed to other caffeine-containing products. With accumulating data on the beneficial role of coffee and caffeine in liver disease, as well as the supporting *in vitro* data, it may now be time to consider a prospective study of coffee or caffeine on hepatic fibrogenesis.

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