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Coffee has hepatoprotective benefits in Brazilian patients with chronic hepatitis C even in lower daily consumption than in American and European populations

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ABSTRACT

The potential role of coffee as a hepatoprotective substance for chronic liver diseases has been widely discussed. Our main aim was to evaluate the effect of coffee intake regarding clinical, biochemical tests and liver biopsy data in treatment naïve patients with chronic hepatitis C. One hundred and thirty-six patients with chronic hepatitis C, diagnosed through liver biopsy, or by means of clinical, ultrasound or endoscopic signs of cirrhosis, were assessed by determination of biochemical tests, metabolic and morphological alterations. Food frequency was scrutinized by using a structured questionnaire. Coffee intake represented more than 90% of the total daily caffeine, and the 75th percentile was 4-Brazilian coffee-cup/day (≥255 mL/day or ≥123 mg caffeine/day). According to caffeine intake, patients were divided into two groups (< or ≥123 mg caffeine/day). Patients with higher ingestion of caffeine had lower serum levels of aspartate aminotransferase (× upper limit of normal) (1.8 ± 1.5 *vs* 2.3 ± 1.5, $p = 0.04$), lower frequencies of advanced (F3, F4) fibrosis (23.5% *vs* 54.5%, $p < 0.001$) and of histological activity grade (A3, A4) observed in liver biopsies (13.8% *vs* 36.9%, $p < 0.001$). By multivariate logistic regression, fibrosis was independently associated with caffeine intake (OR– 0.16; 95%CI – 0.03–0.80; $p = 0.026$), γ-glutamil transferase serum levels and morphological activity. But only fibrosis was associated with histological activity. In conclusion caffeine consumption greater than 123 mg/day was associated with reduced hepatic fibrosis. In addition, this study supports the assumption that coffee intake has hepatoprotective benefits for Brazilian patients with chronic hepatitis C, even in lower doses than that of American and European population intake.

Introduction

Hepatitis C virus (HCV) infects chronically 130–170 million of the world's population.[1] In Brazil, the prevalence of HCV infection ranges from 1 to 2%.[2] Evidence suggests that caffeine may have hepatoprotective properties. In addition to caffeine, coffee contains chlorogenic acid, which has antioxidant and antimutation activities,[3–5] and diterpenes (cafestol and kahweol) with anticarcinogenic properties.[6]

Coffee consumption, specifically with caffeine, has shown to be associated with a decreased risk of liver-associated

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enzyme elevations.[7–9] Also, many studies have reported an inverse relationship between coffee drinking and the risk of liver cirrhosis.[10–12] Some cohort[13–15] and case-control studies,[12,16–18] as well as two meta-analyses,[19,20] suggested an inverse relationship between coffee drinking and the risk of hepatocellular carcinoma. Two studies showed that regular coffee intake (above a threshold of 308 or 408 mg/day of caffeine) was associated with less severe hepatic fibrosis or with reduced histological activity in patients with chronic hepatitis C.[21,22] Another recent study reported that higher coffee intake had a protective effect on non-alcoholic fatty liver disease[23] and significant risk reduction for fibrosis among non-alcoholic steatohepatitis patients.[24]

Nowadays, the potential role of coffee as a hepatoprotective substance for chronic liver diseases has been widely discussed. Furthermore, the consumption of coffee by the Brazilian population has some peculiar characteristics in comparison with the European and American population. Thus, the main aim of this study was to evaluate the effect of coffee intake on clinical, biochemical and liver biopsy data in treatment naïve patients with chronic hepatitis C.

Materials and methods

Patients

One hundred thirty-six treatment naïve patients with chronic hepatitis C were selected from the clinic of Gastroenterology Division Federal University of Sao Paulo, Escola Paulista de Medicina, between January 2009 and December 2011. They were enrolled into the study if they met the following criteria: positivity for serum HCV RNA; liver biopsy or diagnosis of hepatic cirrhosis based on clinical, ultrasound or endoscopic characteristics as well as the absence of previous antiviral therapy for chronic hepatitis C. Patients using drugs containing caffeine, co-infected with hepatitis B virus (serum HBsAg positive) or human immunodeficiency virus, excessive alcohol consumption (ethanol dose $\geq$20 g/day for women and $\geq$40 g/day for men), concomitant liver diseases, decompensated diabetes mellitus and clinically or biochemically recognized systemic diseases were excluded from this study.

The study was approved by the ethics committee of São Paulo Hospital of UNIFESP. All participants received detailed information about the study and gave written informed consent according to the norms of the Helsinki declaration.

Laboratory tests

γ-Glutamil transferase (γGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined by an automated kinetic method. Lipid profile (total cholesterol, high-density lipoprotein cholesterol (HDL-C) triglycerides, low-density lipoprotein cholesterol (LDL-C) and fasting glucose were measured by an automated colorimetric method. Insulin concentration was determined by immunofluorometric assay and insulin resistance was calculated by homeostasis model assessment (HOMA-IR) using the following formula: fasting insulin (μU/mL) $\times$ fasting glycemia (mmol/L)/22.5.[25]

Liver histopathology

All liver biopsy specimens were fixed in formalin, embedded in paraffin and routinely processed for histological analysis. Histological scoring was performed according to the guidelines of the Brazilian Society of Pathology and Hepatology where the fibrosis score is similar to the Metavir classification, and inflammation semi-quantified as follows: grading of portal inflammation (0–4); interface hepatitis (0–4) and parenchymal necro-inflammatory lesions (0–4). The assessment of the inflammatory degree was done by the grade of interface hepatitis or with the sum of the portal inflammation, interface hepatitis and parenchymal lesion grade (histology activity index).[26]

Development of the caffeine questionnaire

Clinical interviews were performed by a dietitian using a structured questionnaire. A food frequency questionnaire was developed, using the format of the questionnaire used in the Health Professionals Follow-up Study Questionnaire,[27] to evaluate caffeine intake. Patients were asked to quantify the frequency and the quantity of consumption of caffeine-containing products, including regular and diet carbonated soft drink beverages, regular coffee, decaffeinated coffee, black or green tea, cocoa/hot chocolate, caffeine-fortified drinks or chocolate candies. Consumption frequency was quantified as daily, weekly, monthly, occasionally or never. This questionnaire included data about caffeine-containing medications, coffee consumption time, the filtration method (paper or cloth), quantity of coffee used per month and the number of family members who drink coffee.

Statistical analysis

Total caffeine intake from foods and beverages (mg/day) was calculated by summing up caffeine content based on estimates from the published literature: regular coffee (137 mg per 8-oz cup),[22,28,29] decaffeinated coffee (2 mg per 8-oz cup) (USDA), espresso coffee (64 mg per 1-oz cup) (USDA), instant coffee (63 mg per 8-oz cup) (USDA), green tea (30 mg per 7-oz cup),[30,31] chocolate candy bars (7.4 mg per 30 g).[29] Daily caffeine intake for each patient was calculated from total coffee intake according to the frequency questionnaire. Caffeine intake was dichotomized above and below the 75th percentile according to the threshold for the cohort of the previous study.[22]

Chi-square or Fisher's exact test was performed for categorical variables, Mann–Whitney for continuous variables (non-parametric test) and "*t*" Student test (categorical variables, parametric test). Spearman's correlation was performed to evaluate the level of correlation between the variables studied. Univariate analysis and logistic regression were performed to identify independent predictors.

Statistical analyses were carried out using the SPSS 10.0 for Windows (SPSS, Inc., Chicago, IL, USA), with the level of significance set at $p \leq 0.05$.

Table 1 – Baseline characteristics of the patients.

Variables	Total patients $n = 136$, 100%
Gender, male/female (%)	51.5/48.5
Age (years)	52 ± 13
Body mass index (kg/m²)	27 ± 4.6
ALT (× ULN)	2.2 ± 1.7
AST (× ULN)	2.2 ± 1.5
γGT (× ULN)	3.1 ± 3.2
Glucose, mg dL⁻¹	105.4 ± 38.2
HOMA-IR	2.6 ± 2.6
HDL cholesterol, mg dL⁻¹	45.1 ± 14.8
Triglycerides, mg dL⁻¹	99.3 ± 42.8
HCV genotype (%)	
1	67.6
2 and 3	32.4
Fibrosis score, (%)	
F0	8.8
F1	27.9
F2	16.2
F3	10.3
F4	36.8
Activity grade, (%)	
A0	8.8
A1	21.2
A2	38.1
A3	25.7
A4	6.2
Steatosis, (%)	
Yes	56.6
No	43.4
Caffeine consumption (mg/day)	112.1 ± 178.8
Lifetime caffeine consumption, (%)	
≥20 years	100%
Methods for coffee, (%)	
Paper filter	70.6
Cloth filter	29.4
Smoking, (%)	
Yes	29.4
No	70.6

Continuous data are expressed as mean ± standard deviation and categoric data are expressed as percentage. *Abbreviations*: ALT, alanine aminotransferase; AST, aspartate aminotransferase; (GT, (-glutamyl transferase; HOMA-IR, homeostasis model assessment of insulin resistance (fasting insulin [(U/mL] × fasting glycemia [nmol/L]/22.5); HDL cholesterol, high-density lipoprotein cholesterol; ULN, upper limit of normal.

Table 2 – Characteristics of the patients according to caffeine intake.

Parameters	Caffeine intake		*p*-Value
	<123 mg/day ($n = 102$)	≥123 mg/day ($n = 34$)	
Gender, male/female (%)	49/51	59/41	0.43
Age (years)	51.7 ± 12.6	51.1 ± 13.9	0.89
Body mass index (kg/m²)	27.1 ± 4.5	27.4 ± 5.5	1.0
ALT (× ULN)	2.2 ± 1.5	2.2 ± 2.1	0.41
AST (× ULN)	2.3 ± 1.5	1.8 ± 1.5	0.04
γGT (× ULN)	3.3 ± 3.3	2.9 ± 2.4	0.56
Glucose, mg dL⁻¹	105.3 ± 35.0	106.0 ± 47.5	0.26
HOMA-IR	2.6 ± 2.6	2.4 ± 2.6	0.39
HDL cholesterol, mg dL⁻¹	45.5 ± 14.9	43.8 ± 14.6	0.74
Triglycerides, mg dL⁻¹	99.1 ± 40.5	104.4 ± 50.0	0.51
HCV genotype, (%)			
1	67.6	66.7	0.99
2 and 3	32.4	33.3	
Fibrosis score, (%)			
F0	6.9	14.7	
F1	23.5	41.2	
F2	14.7	20.6	<0.001
F3	10.8	8.8	
F4	44.1	14.7	
Activity grade, (%)			
A0	7.1	13.8	
A1	17.9	31.0	
A2	38.1	38.0	<0.001
A3	28.6	17.2	
A4	8.3	0	
Steatosis, (%)			
Yes	45.7	37.5	0.562
No	54.3	62.5	
Methods for coffee, (%)			
Paper filter	31.4	29.4	0.88
Cloth filter	68.6	70.6	
Smoking, (%)			
Yes	28.4	32.4	0.65
No	71.6	67.6	

Continuous data are expressed as mean ± standard deviation and categoric data are expressed as percentage. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; γGT, γ-glutamyl transferase; HOMA-IR, homeostasis model assessment of insulin resistance (fasting insulin [μU/mL] × fasting glycemia [nmol/L]/22.5); HDL cholesterol, high-density lipoprotein cholesterol; ULN, upper limit of normal.

Results

Table 1 shows baseline characteristics of the patients. Seventy patients were men (51.5%), the mean age of the overall group was 52 ± 13 years and the mean body mass index (BMI) was 27 ± 4.6 kg/m² and HCV genotype 1 (67.2%) was predominant. Of the 136 patients, 113 patients were biopsied and 23 patients were considered to have liver cirrhosis on the grounds of clinical and endoscopic and/or ultrasound examination. Activity grades ≥3 and 4 and fibrosis stage ≥3 and 4 were present in 47.1% and 31.9% of patients, respectively. The average estimated daily consumption of caffeine from foods and beverages was 115.5 mg and of all the caffeine consumed, 96.6% came from regular coffee, 2.8% from caffeinated soft drinks, 0.4% from chocolate and 0.2% from tea.

The patients were divided into groups, according to the 75th percentile (123 mg of caffeine) (Table 2). The two groups did not present significant differences in demographic or anthropometric characteristics. However, significant reduction in AST serum level (× ULN) (1.8 ± 1.5 *vs* 2.3 ± 1.5, $p = 0.04$) was observed in the group with high caffeine ingestion. Caffeine intake related to greater than 4-Brazilian coffee-cup/day (≥255 mL/day or ≥123 mg caffeine/day) was also associated with lower frequencies of advanced fibrosis (F3, F4) (23.5% *vs* 54.5%, $p < 0.001$) and histological activity grade (A3, A4) observed in liver biopsies (13.8% *vs* 36.9%, $p < 0.001$) (Table 2).

Table 3 – Univariate and logist regression analysis of factors associated with fibrosis.

Parameters	Univariada *p value*	OR	95% IC	Multivariate *p value*
Caffeine consumption	0.004	0.16	0.03–0.80	0.026
Gender	0.036	0.36	0.10–1.27	0.111
Age	0.315			
AST (× ULN)	<0.001	2.06	0.40–10.52	0.387
ALT (× ULN)	0.012	0.98	0.20–4.71	0.982
γGT (× ULN)	<0.001	4.03	1.06–15.36	0.041
Cholesterol total	0.010	0.45	0.13–1.56	0.206
HOMA-IR	0.001	2.81	0.76–10.46	0.123
Activity grade	<0.001	12.22	3.28–45.54	<0.001
Steatosis	0.214			

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; γGT, γ-glutamyl transferase; HOMA-IR, homeostasis model assessment of insulin resistance (fasting insulin [μU/mL] × fasting glycemia [nmol/L]/22.5); ULN, upper limit of normal.

To clarify the effect of the caffeine intake on liver fibrosis and histological activity in the hepatitis C evolution, both univariate analysis and logistic regression were performed. In univariate analysis, advanced fibrosis (fibrosis stage ≥3 and 4) was significantly associated to caffeine intake ($p = 0.004$), gender ($p = 0.036$), AST serum level ($p < 0.001$), ALT serum level ($p = 0.012$), γGT serum level ($p < 0.001$), total cholesterol serum level ($p = 0.01$), HOMA-IR ($p = 0.001$) and histological activity ($p < 0.001$). By multivariate logistic regression, advanced fibrosis was independently associated with caffeine intake (OR = 0.16; 95% CI: 0.03–0.80; $p = 0.026$); γGT serum level (OR = 4.03; CI 95%: 1.06–15.36; $p = 0.041$) and histological activity (OR = 12.22; CI 95%: 3.28–45.54; $p < 0.001$) (Table 3).

A similar analysis for advanced histological activity grade observed in liver biopsies (activity grades ≥3 and 4) was performed. By univariate analysis, severe histological activity grade was significantly associated to caffeine intake ($p = 0.026$), AST serum level ($p = 0.001$), ALT serum level ($p = 0.037$), total cholesterol serum level ($p = 0.015$) and liver fibrosis ($p < 0.001$). However, by multivariate logistic regression, only liver fibrosis was associated with histological activity grade. No association could be found between histological activity and caffeine intake (Table 4).

Discussion

With the threshold of the 75th percentile to identify patients with greater caffeine intake, we found that these HCV patients presented lower levels of serum AST levels and lower frequency of advanced inflammatory activity and fibrosis degree when compared with those with lower caffeine intake. These findings are consistent with previous studies suggesting a liver protective effect of caffeine in patients with other liver diseases.[8,9,21,22,32,33] In order to assess the impact of caffeine ingestion on histological parameters, univariate and multivariate logistic regression were performed with the degree of fibrosis as a continuous variable, in order to clarify the real impact of caffeine in liver disease progression. In such an analysis, daily caffeine intake higher than 123 mg (4-Brazilian coffee-cup/day) stood out as an independent predictor of a lower risk of advanced fibrosis, together with serum γGT level and histological activity, two parameters previously described as independent risk factors for fibrosis. Although significant in univariate analysis, caffeine ingestion could not be associated with inflammatory activity, assessed by interface hepatitis or even by the histology activity index (data not shown), by logistic regression. It is possible that the exclusion of 17% of the patients from the cohort (those with overt cirrhosis and no liver biopsy performed) had reduced the strength of the analysis for the association between activity and caffeine intake, but the association between fibrosis degree and daily caffeine ingestion persisted even after the exclusion of these patients with overt cirrhosis.

Comparing this study with those previously published, it became clear that there is a difference between the pattern of daily caffeine ingestion between Brazilian, European and American patients with HCV chronic infection. Coffee intake accounts for more than 95% of the daily caffeine ingestion in our cohort while it has been reported to be around 70%

Table 4 – Univariate and logistic regression analysis of factors associated with activity grade.

Parameters	Univariada *p value*	OR	95% IC	Multivariate *p value*
Caffeine consumption	0.026	0.91	0.22–3.72	0.892
AST (× ULN)	0.001	1.22	0.24–6.21	0.812
ALT (× ULN)	0.037	1.24	0.29–5.29	0.777
Cholesterol total	0.015	0.52	0.18–1.56	0.246
Fibrosis	<0.001	13.21	3.74–46.74	<0.001
Steatosis	0.186			

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; γGT, γ-glutamyl transferase; ULN, upper limit of normal.

among American patients; in this study, the threshold of the 75th percentile was ≥123 mg caffeine/day (≥255 mL/day or 4-Brazilian coffee-cup/day), while other studies report a cut-off of 308 mg caffeine/day[22] or 407 mg caffeine/day.[21] These differences allowed us to demonstrate that a lower cut-off of caffeine intake has liver protective benefits. Protective effect of caffeine on liver was significant and evaluated as a continuous variable, categorized as coffee-cup equivalents, or dichotomized above or below the 75th percentile for the study population. 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. Approximately 4-Brazilian coffee-cup/day, was necessary to have an effect on fibrosis progression in these patients with chronic hepatitis C. Clarification of whether there is a hepatoprotective threshold and whether the benefits eventually plateau with further consumption will be important for understanding the biology and potentially for therapeutic recommendations. Furthermore, these results strongly support the assumption that coffee's caffeine has hepatoprotective benefits in Brazilian patients with chronic hepatitis C, since, in this cohort, coffee was almost the only source of caffeine intake.

Coffee contains a variety of chemical compounds, including caffeine, chlorogenic acid, quinides, trigonelline and lignin. The standardization of these compounds' concentration in a cup of coffee is difficult and the food frequency questionnaires are useful tools, but are subject to misclassification. To our knowledge, this is the first study able to examine the preparation and serving methods of coffee. For this reason, coffeine consumption *via* the number of consumed cups of coffee may correctly reflect the differences between the groups. Analyzing methods used to collect dietary data on coffee and caffeine intake in previous studies,[21,22,32,34] we observed that several authors had used only the food frequency questionnaire, which was self-administered or applied by the health-professional nurse. In the present study, in order to further investigate the dietary intake of coffee and caffeine, 24-h dietary recall was associated with a food frequency questionnaire and both applied by nutritionists. In professional practice, nutritionists are able to develop specific actions.[35] The food frequency questionnaire is the most widely used in population studies and more appropriate to classify individuals according to intake of foods or nutrients.[29]

Here, patients with higher caffeine intake had also lower AST levels. Similarly, a study enrolling Japanese patients with no previous liver dysfunction reported that coffee intake was inversely associated to AST and ALT serum concentrations, regardless of age, BMI, alcohol intake and smoking.[7] Another study identified lower ALT levels in patients with higher caffeine intake and it suggested that caffeine has an antioxidant inhibiting lipid peroxidation role and consequently, the formation of free radicals.[8] 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.[8,9,11,33,35]

The mechanisms underlying potential hepatoprotective effects of caffeine in patients with chronic hepatitis C remain to be determined. Caffeine is a purine alkaloid, acting through the antagonism of adenosine receptors A_1 and A_2.[23] Recent studies have demonstrated that adenosine, acting at A_2 receptors, stimulates hepatic stellate cell-mediated fibrosis of the liver[29,36–39] by increasing production of collagen I and III (the collagens present in scar tissue) *via* two distinct mitogen-activated protein kinase (MAPK)-dependent pathways, extracellular signal-regulated kinase 1/2 (ERK1/2) and p38MAPK, respectively.[40] Interestingly, caffeine, the most widely used drug in the world, mediates most of its pharmacological effects by non-selectively blocking adenosine receptors, including A_2 receptors, and can prevent hepatic fibrosis in animal models.[41,42] Several reports suggest caffeine and other constituents of coffee, as Kahweol and cafesterol, possess antioxidant properties.[43–45] Studies in mice and rats as well as with 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.[46,47] 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.[46] Pre-treatment with cafestol and kahweol protected mice from carbon tetrachloride hepatotoxicity by inhibiting cytochrome CYP 2E1, the enzyme responsible for carbon tetrachloride bioactivation.[48] With respect to caffeine specifically, a study reported that caffeine inhibits expression of a connective tissue growth factor (CTGF) by interfering with the transforming growth factor beta (TGFβ) signaling through the similar to mothers against decapentaplegic in drosophila (SMAD) pathway.[49] 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.[22]

In conclusion, a useful tool for a comprehensive evaluation of caffeine intake was applied and it indicated a beneficial effect, which requires caffeine intake above a threshold of approximately 4-Brazilian coffee-cup/day. In addition, our study showed that coffee intake has hepatoprotective benefits - an inverse association of the increased caffeine intake with a lower risk of advanced fibrosis - in treatment naïve Brazilian patients with chronic hepatitis C even in different doses from American and European intake.

Conflict of interest

The authors declare no conflicts of interest.

REFERENCES

1. Alter MJ. Epidemiology of hepatitis C virus infection. World J Gastroenterol. 2007;13:2436–41.
2. Martins AN, Arruda MB, Aleixo AW, et al. Prevalence of etravirine-associated mutations in clinical samples with genotypic resistance to nevirapine and efavirenz in Brazilian clinics. J Acquir Immune Defic Syndr. 2011;57:193–6.

3. Feng R, Lu Y, Bowman LL, Qian Y, Castranova V, Ding M. Inhibition of activator protein-1, NF-kappaB, and MAPKs and induction of phase 2 detoxifying enzyme activity by chlorogenic acid. J Biol Chem. 2005;280:27888–95.
4. Gomez-Ruiz JA, Leake DS, Ames JM. In vitro antioxidant activity of coffee compounds and their metabolites. J Agric Food Chem. 2007;55:6962–9.
5. Higdon JV, Frei B. Coffee and health: a review of recent human research. Crit Rev Food Sci Nutr. 2006;46:101–23.
6. Cavin C, Holzhauser D, Constable A, Huggett AC, Schilter B. The coffee-specific diterpenes cafestol and kahweol protect against aflatoxin B1-induced genotoxicity through a dual mechanism. Carcinogenesis. 1998;19:1369–75.
7. Ikeda M, Maki T, Yin G, et al. Relation of coffee consumption and serum liver enzymes in Japanese men and women with reference to effect modification of alcohol use and body mass index. Scand J Clin Lab Invest. 2010;70:171–9.
8. Ruhl CE, Everhart JE. Coffee and caffeine consumption reduce the risk of elevated serum alanine aminotransferase activity in the United States. Gastroenterology. 2005;128:24–32.
9. Tanaka K, Tokunaga S, Kono S, et al. Coffee consumption and decreased serum gamma-glutamyltransferase and aminotransferase activities among male alcohol drinkers. Int J Epidemiol. 1998;27:438–43.
10. Corrao G, Lepore AR, Torchio P, et al. The effect of drinking coffee and smoking cigarettes on the risk of cirrhosis associated with alcohol consumption. A case–control study, Provincial Group for the Study of Chronic Liver Disease. Eur J Epidemiol. 1994;10:657–64.
11. Corrao G, Zambon A, Bagnardi V, D'Amicis A, Klatsky A. Coffee, caffeine, and the risk of liver cirrhosis. Ann Epidemiol. 2001;11:458–65.
12. Gallus S, Tavani A, Negri E, La Vecchia C. Does coffee protect against liver cirrhosis? Ann Epidemiol. 2002;12:202–5.
13. Hu G, Tuomilehto J, Pukkala E, et al. Joint effects of coffee consumption and serum gamma-glutamyltransferase on the risk of liver cancer. Hepatology. 2008;48:129–36.
14. Inoue M, Yoshimi I, Sobue T, Tsugane S. Influence of coffee drinking on subsequent risk of hepatocellular carcinoma: a prospective study in Japan. J Natl Cancer Inst. 2005;97:293–300.
15. Shimazu T, Tsubono Y, Kuriyama S, et al. Coffee consumption and the risk of primary liver cancer: pooled analysis of two prospective studies in Japan. Int J Cancer. 2005;116:150–4.
16. Gelatti U, Covolo L, Franceschini M, et al. Coffee consumption reduces the risk of hepatocellular carcinoma independently of its aetiology: a case–control study. J Hepatol. 2005;42:528–34.
17. Montella M, Polesel J, La Vecchia C, et al. Coffee and tea consumption and risk of hepatocellular carcinoma in Italy. Int J Cancer. 2007;120:1555–9.
18. Tanaka K, Hara M, Sakamoto T, et al. Inverse association between coffee drinking and the risk of hepatocellular carcinoma: a case–control study in Japan. Cancer Sci. 2007;98:214–8.
19. Bravi F, Bosetti C, Tavani A, et al. Coffee drinking and hepatocellular carcinoma risk: a meta-analysis. Hepatology. 2007;46:430–5.
20. Larsson SC, Wolk A. Coffee consumption and risk of liver cancer: a meta-analysis. Gastroenterology. 2007;132:1740–5.
21. Costentin CE, Roudot-Thoraval F, Zafrani ES, et al. Association of caffeine intake and histological features of chronic hepatitis C. J Hepatol. 2011;54:1123–9.
22. Modi AA, Feld JJ, Park Y, et al. Increased caffeine consumption is associated with reduced hepatic fibrosis. Hepatology. 2010;51:201–9.
23. Gutierrez-Grobe Y, Chavez-Tapia N, Sanchez-Valle V, et al. High coffee intake is associated with lower grade nonalcoholic fatty liver disease: the role of peripheral antioxidant activity. Ann Hepatol. 2012;11:350–5.
24. Molloy JW, Calcagno CJ, Williams CD, Jones FJ, Torres DM, Harrison SA. Association of coffee and caffeine consumption with fatty liver disease, nonalcoholic steatohepatitis, and degree of hepatic fibrosis. Hepatology. 2012;55:429–36.
25. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412–9.
26. Gayotto LCC. Comitê SBP/SBH. Visão histórica e consenso nacional sobre a classificação das hepatites crônicas. Projeto do Clube de Patologia Hepática da Sociedade Brasileira de Patologia aprovado pela Sociedade Brasileira de Hepatologia. GED Gastroenterol Endosc Dig. 2000;19:137–40.
27. Health Professionals Follow-up Study Questionnaire. http://www.hsph.harvard.edu/hpfs/hpfs; 1998.
28. McCusker RR, Goldberger BA, Cone EJ. Caffeine content of specialty coffees. J Anal Toxicol. 2003;27:520–2.
29. Michels KB, Willett WC, Fuchs CS, Giovannucci E. Coffee, tea, and caffeine consumption and incidence of colon and rectal cancer. J Natl Cancer Inst. 2005;97:282–92.
30. Iso H, Date C, Wakai K, Fukui M, Tamakoshi A. The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults. Ann Intern Med. 2006;144:554–62.
31. Shimbo M, Nakamura K, Jing Shi H, et al. Green tea consumption in everyday life and mental health. Public Health Nutr. 2005;8:1300–6.
32. Freedman ND, Everhart JE, Lindsay KL, et al. Coffee intake is associated with lower rates of liver disease progression in chronic hepatitis C. Hepatology. 2009;50:1360–9.
33. Honjo S, Kono S, Coleman MP, et al. Coffee drinking and serum gamma-glutamyltransferase: an extended study of Self-Defense Officials of Japan. Ann Epidemiol. 1999;9:325–31.
34. Freedman ND, Curto TM, Lindsay KL, Wright EC, Sinha R, Everhart JE. Coffee consumption is associated with response to peginterferon and ribavirin therapy in patients with chronic hepatitis C. Gastroenterology. 2011;140:1961–9.
35. Willett W. Nutritional epidemiology. 2nd ed. New York: Oxford University Press; 1998.
36. Casiglia E, Spolaore P, Ginocchio G, Ambrosio GB. Unexpected effects of coffee consumption on liver enzymes. Eur J Epidemiol. 1993;9:293–7.
37. Chan ES, Montesinos MC, Fernandez P, et al. Adenosine A(2A) receptors play a role in the pathogenesis of hepatic cirrhosis. Br J Pharmacol. 2006;148:1144–55.
38. Hashmi AZ, Hakim W, Kruglov EA, et al. Adenosine inhibits cytosolic calcium signals and chemotaxis in hepatic stellate cells. Am J Physiol Gastrointest Liver Physiol. 2007;292:G395–401.
39. Sohail MA, Hashmi AZ, Hakim W, et al. Adenosine induces loss of actin stress fibers and inhibits contraction in hepatic stellate cells via Rho inhibition. Hepatology. 2009;49:185–94.
40. Che J, Chan ES, Cronstein BN. Adenosine A2A receptor occupancy stimulates collagen expression by hepatic stellate cells via pathways involving protein kinase A, Src, and extracellular signal-regulated kinases 1/2 signaling cascade or p38 mitogen-activated protein kinase signaling pathway. Mol Pharmacol. 2007;72:1626–36.
41. Peng Z, Fernandez P, Wilder T, et al. Ecto-5′-nucleotidase (CD73)-mediated extracellular adenosine production plays a critical role in hepatic fibrosis. FASEB J. 2008;22:2263–72.
42. Tofovic SP, Salah EM, Jackson EK, Melhem M. Early renal injury induced by caffeine consumption in obese, diabetic ZSF1 rats. Renal Failure. 2007;29:891–902.
43. Lee C. Antioxidant ability of caffeine and its metabolites based on the study of oxygen radical absorbing capacity and

inhibition of LDL peroxidation. Clin Chim Acta. 2000;295:141–54.
44. Ozercan IH, Dagli AF, Ustundag B, et al. Does instant coffee prevent acute liver injury induced by carbon tetrachloride (CCl(4))? Hepatol Res. 2006;35:163–8.
45. Scharf G, Prustomersky S, Huber WW. Elevation of glutathione levels by coffee components and its potential mechanisms. Adv Exp Med Biol. 2001;500: 535–9.
46. Cavin C, Marin-Kuan M, Langouet S, et al. Induction of Nrf2-mediated cellular defenses and alteration of phase I activities as mechanisms of chemoprotective effects of coffee in the liver. Food Chem Toxicol. 2008;46: 1239–48.
47. Higgins LG, Cavin C, Itoh K, Yamamoto M, Hayes JD. Induction of cancer chemopreventive enzymes by coffee is mediated by transcription factor Nrf2. Evidence that the coffee-specific diterpenes cafestol and kahweol confer protection against acrolein. Toxicol Appl Pharmacol. 2008;226:328–37.
48. Lee JH, Sul D, Oh E, et al. Panax ginseng effects on DNA damage, CYP1A1 expression and histopathological changes in testes of rats exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Food Chem Toxicol. 2007;45:2237–44.
49. Gressner OA, Lahme B, Rehbein K, Siluschek M, Weiskirchen R, Gressner AM. Pharmacological application of caffeine inhibits TGF-beta-stimulated connective tissue growth factor expression in hepatocytes via PPARgamma and SMAD2/3-dependent pathways. J Hepatol. 2008;49:758–67.