Caffeine is protective in patients with non-alcoholic fatty liver disease

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SUMMARY

Background
Non-alcoholic fatty liver disease (NAFLD), the hepatic manifestation of metabolic syndrome, is the most common cause of primary liver disease. Although recent studies have found that coffee drinking is protective against end stage chronic liver disease, there are scarce caffeine intake data in NAFLD specifically.

Aim
To investigate the effects of dietary behaviour in NAFLD patients, using four continuous cycles of the National Health and Nutrition Examination Surveys (NHANES 2001–2008).

Methods
Using data from four continuous cycles of NHANES, dietary intake questionnaires that list 62 nutrition components. Logistic regression was used to identify independent predictors of NAFLD among nutrition components after adjustment for potential clinical confounders. All analyses were run using SAS 9.1 and SUDAAN 10.0 (SAS Institute Inc., Cary, NC, USA).

Results
Of the 62 nutrient components used for the univariate analysis, 38% were significant (P-value <0.05) in NAFLD with caffeine consumption being higher in the control group (P-value <0.001). The multivariate analysis using demographics, clinical parameters and nutritional components found five factors independently associated with NAFLD [African American Race P-value <0.001); Male gender P-value <0.001); Obesity (BMI ≥ 30) P-value <0.001); Caffeine intake (mg) P-value <0.001) and total plain water consumption (g P-value ≤ 0.02)].

Conclusions
Our analysis shows that caffeine intake is independently associated with a lower risk for NAFLD suggesting a potential protective effect. These data necessitate further research to elucidate the mechanism by which caffeine can protect against NAFLD.
INTRODUCTION

Non-alcoholic fatty liver disease is currently one of the most common causes of elevated liver enzymes and chronic liver disease in the Western world.1–5 Attributed to the rapidly increasing rate of obesity, along with other components of metabolic syndrome, such as Type II diabetes, the prevalence of NAFLD is growing at an alarming rate in both adults and children.5,7 In the United States, it is estimated that 25–30% of the population is afflicted with NAFLD, 2–3% have NASH and of these, 10–15% develop cirrhosis.8–12

The general consensus is that obesity in the United States population is an important contributing factor in the increased incidence of NAFLD.4, 5 According to the National Health and Nutrition Examination Survey (NHANES) conducted between 2007 and 2008, the prevalence of obesity in adults reached 32.2% among men and 35.5% among women.13 Based on projected obesity rates, it is predicted that by the year 2025, 45–50% of the adult population will be obese, resulting in NAFLD related liver disease in over 25 million Americans.14

Despite the well established link between NAFLD and BMI,15 obese and overweight people are not the only ones who are at risk of developing NAFLD and NASH.16 The propensity of NAFLD to be a progressive disease suggests that early interventions could potentially prevent the more serious latter stage manifestations of this disease. These observations indicate that the development of NAFLD is indeed multi-factorial and other factors, such as dietary habits may play an important role in the development of NAFLD.

Although a number of nutritional studies have been performed to assess the interaction between diet and liver disease, caffeine intake has garnered a lot of attention. Studies done in the United States have shown that increased coffee intake is associated with a lower incidence of abnormal alanine aminotransferase (ALT) activity.17 In addition, studies from Europe and Japan have indicated an inverse relationship between coffee and levels of g-glutamyltransferase and aminotransferases in serum.18–24

Assessing end stage liver-specific outcomes, a large population based study in Norway found an inverse association between coffee consumption and liver cirrhosis.25 Furthermore, a study using the first National Health and Nutrition Examination Survey (1971–1975) found that coffee and tea drinking decreases the risk of clinically significant chronic liver disease as defined by death or hospitalisation due to CLD.17 Caffeine has also been implicated in hepatic fibrosis, as recently demonstrated by a study showing that regular coffee consumption, above a threshold of approximately two coffee-cup equivalents per day, was associated with less severe hepatic fibrosis.26 Recent studies have also suggested that coffee consumption may reduce the risk of developing hepatocellular carcinoma (HCC) in high-risk populations.27 In addition, research in the field of hepatitis C and treatment with peginterferon plus ribavirin found that high-level consumption of coffee (more than three cups per day) to be an independent predictor of improved virological response.28 Despite the increasing focus on the effects of coffee in the aetiology of severe liver disease, there is little data assessing the relationship between coffee consumption and NAFLD specifically.

Using recent U.S. population data, the aim of this study is to investigate the effects of dietary behaviour, specifically the nutrition components measured for NHANES participants, on the prevalence of NAFLD.

METHODS

Study population

The data for the study were obtained from four continuous cycles of the National Health and Nutrition Examination Surveys (NHANES) conducted between 2001 and 2008. The survey data were collected by the U.S. National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC) via household interviews, physical examination and laboratory tests. The survey cycles included into the study consisted of similar questionnaires and data collection methods. Demographic, clinical and laboratory parameters were transformed according to the provided guidelines to make the data comparable between the cycles.29

Inclusion and exclusion criteria used for this study were similar to previously published reports.4, 5 Furthermore definitions for obesity (BMI ≥30), Hypertension, hypercholesterolaemia and diabetes mellitus were similar to previously reported.4, 5 Finally, insulin resistance was defined as a homeostasis of model assessment score30 or HOMA) >3.0, elevated serum aminotransferases were defined as ALT ≥40 U/L or AST ≥37 U/L in men and ALT ≥31 U/L in women and elevated transferrin saturation was determined as 50% or higher.4, 5

We also divided participants to four major race or ethnic groups: non-Hispanic whites, non-Hispanic blacks, Hispanics, and ‘other,’ which included Aleut, Eskimo, American Indian, Asian or Pacific Islander.

In terms of tobacco and alcohol consumption, a positive smoking history was defined as on-going smoking or...
more than 100 cigarettes in the past 5 years.\(^4\), \(^5\) Alcohol intake was calculated according to self-reported data on the amount and frequency of alcohol consumption collected as a part of the Alcohol Use questionnaires.\(^4\), \(^5\) Excessive alcohol consumption was defined as >20 g/day for men and >10 g/day for women.

We used previously described definition for NAFLD.\(^2\), \(^4\), \(^5\), \(^31\), \(^32\) Specifically, in this study, NAFLD was defined as elevated serum aminotransferases without any indication of other causes of chronic liver disease such as viral hepatitis infection (defined as positive HCV RNA or HBsAg test), iron overload (transferrin saturation of 50% or higher) or excessive alcohol consumption as defined above. Similarly, controls were defined as participants without any evidence of chronic liver disease and normal liver enzymes.

**Nutrition data**

The dietary intake data collected as a part of the Dietary Recall Interview are used to estimate the types and amounts of foods and beverages (including all types of water) consumed during the 24-h period prior to the interview. Participants’ responses were used to estimate intakes of energy, nutrients and other food components from those foods and beverages.

The dietary interview component named What We Eat in America (WWEIA) had been conducted as a partnership between the U.S. Department of Agriculture (USDA) and the U.S. Department of Health and Human Services (DHHS). Daily aggregates of food energy and 62 nutrients/food components from all foods were calculated for NHANES data collection using USDA’s Food and Nutrient Database for Dietary Studies (FNDDS). The FNDDS includes comprehensive information that can be used to code individual foods and portion sizes reported by participants, and also includes nutrient values for calculating nutrient intakes.\(^33\) For this study, only First Day Interview data were used.

**Statistical analyses**

Sample weights were used to account for nonresponse and unequal selection probabilities for certain categories of the population. In addition to weighting on the basis of age, gender and ethnicity which is introduced to make the NHANES sample representative of the U.S. population, sampling weights calculated specifically for analysis of dietary recall interview components also accounted for disproportionately represented intakes on weekends. In addition, stratum and sampling units accounted for the survey design effects using Taylor series linearisation.

Continuous variables such as nutrients measured in grams or milligrams were compared using a \(t\)-test for a contrasted mean. When merging NHANES study cycles, appropriate selection of sampling weights and adjustment coefficients were applied according to the NHANES Analytic and Reporting Guidelines.\(^25\) The prevalence of various parameters, including demographic parameters and metabolic syndrome components, was compared between subjects with NAFLD and controls by the stratum-specific chi-squared test for independence. \(P\)-values of 0.05 or less were considered potentially statistically significant. Finally, logistic regression was used to identify independent predictors of NAFLD among nutrition components after adjustment for potential clinical confounders. All analyses were run using SAS 9.1 and SUDAAN 10.0 (SAS Institute Inc., Cary, NC, USA). The study was approved by the Inova Institutional Review Board.

**RESULTS**

**Study population**

Of the initial study population (41 658 participants from NHANES 2001 to 2008), 18 550 were considered eligible for the study. Of those, 1782 individuals (10.41 ± 0.37\%) fulfilled the definition of NAFLD and 16 768 were used as controls. The most relevant clinico-demographic differences between individuals with NAFLD and controls are noted in Table 1. As expected, individuals with NAFLD were less likely African American and more likely of Hispanic ethnicity, had higher rates of insulin resistance, hypercholesterolaemia and obesity.

**Nutrition and NAFLD**

Of the 62 nutrient components used for the study, 24 (38\%) were significantly different \((P < 0.05)\) between the NAFLD and control groups (Table 2). These included total protein, dietary fibre, both mono- and polyunsaturated fatty acids, total cholesterol together with a number of vitamins and microelements. Of the significantly different nutrients, the various types of fats and cholesterol were higher in the NAFLD cohort \((P < 0.05)\). Furthermore, the amounts of daily sodium, selenium and caffeine were also different between those with and without NAFLD \((P < 0.05)\).

In the multivariate analysis, where nutritional components were adjusted for demographic confounders such as age, gender, ethnicity and metabolic syndrome components, we found five independent predictors of NAFLD: African American race (OR (95\% CI) 0.520 (0.426–0.633), male gender (OR (95\% CI) 1.329
(1.132–1.562), obesity (BMI $\geq$ 30) (OR (95% CI) 2.087 (1.808–2.409), caffeine consumption (mg) (OR (95% CI) 0.999319 (0.998955–0.999684) and total plain water consumption (g) (OR (95% CI) 1.000065 (1.000008–1.000122) (Table 3).

**DISCUSSION**
Non-alcoholic fatty liver disease develops due to excessive fat accumulation in the liver, in the absence of significant alcohol use. NAFLD is thought to be the hepatic manifestation of metabolic syndrome and an early predictor of metabolic disorders amongst obese and normal-weight populations.\(^4\)\(^,\)\(^34\)\(^,\)\(^35\) The spectrum of NAFLD ranges from simple steatosis to non-alcoholic steatohepatitis (NASH). Although most cases of simple steatosis do not progress, 10–15% of steatohepatitis cases can progress to cirrhosis\(^36\)\(^,\)\(^37\) and subsequently into more serious conditions, such as HCC.\(^38\) The rising incidence of NAFLD observed in recent years necessitates a multidisciplinary approach to understanding the causative factors of this disease.

In this study, using the NHANES data collected between 2001 and 2008 using similar methodology for estimating daily nutrition intake, we assessed the nutritional contribution on the prevalence of NAFLD. As expected, individuals with NAFLD were less likely African American and more likely of Hispanic ethnicity, had higher rates of insulin resistance, hypercholesterolaemia and obesity. In addition, when metabolic syndrome components were included to the list of potential confounders for NAFLD, obesity was also found to be independently predictive of NAFLD.

Of the 62 nutrients assessed in the univariate analysis between the NAFLD and control groups, 24 (38%) were statistically different ($P$-value range = 0.0006–0.0493), with caffeine consumption being significantly higher in the control group without liver disease (165.19/C6 6.55 mg/⁄day vs. 188.29/C6 4.90 mg/⁄day, $P$-value = 0.0006). In addition to ranking in the top two in terms of statistical significance, caffeine was one of the only two compounds consumed independent from compounds one might encounter in regular food intake (Table 2). The inverse correlation between caffeine consumption and NAFLD gained further significance in our multivariate analysis as caffeine consumption was found to be one of the five independent predictors of NAFLD, remaining highly significant after adjustment for race, gender and metabolic syndrome components (Table 3).

### Table 1 | Demographic and clinical summary of the NAFLD cohort

<table>
<thead>
<tr>
<th>Demographic and clinical summary of the NAFLD cohort</th>
<th>NAFLD ($N=1782$)</th>
<th>Controls ($N=16768$)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N$</td>
<td>1782</td>
<td>16768</td>
<td>1</td>
</tr>
<tr>
<td>Prevalence, %</td>
<td>10.41 ± 0.37</td>
<td>89.59 ± 0.37</td>
<td>1</td>
</tr>
<tr>
<td>Caucasian, %</td>
<td>69.97 ± 2.43</td>
<td>72.61 ± 1.86</td>
<td>0.096</td>
</tr>
<tr>
<td>African-American, %</td>
<td>7.37 ± 0.98</td>
<td>11.51 ± 1.14</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hispanic, %</td>
<td>17.64 ± 1.86</td>
<td>11.50 ± 1.22</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Other ethnicities, %</td>
<td>5.02 ± 0.77</td>
<td>4.39 ± 0.40</td>
<td>0.425</td>
</tr>
<tr>
<td>Male, %</td>
<td>53.52 ± 1.81</td>
<td>47.73 ± 0.46</td>
<td>0.004</td>
</tr>
<tr>
<td>Age &lt;45, %</td>
<td>52.35 ± 2.09</td>
<td>51.11 ± 1.00</td>
<td>0.539</td>
</tr>
<tr>
<td>Age ≥45, &lt;55, %</td>
<td>21.60 ± 1.43</td>
<td>20.28 ± 0.55</td>
<td>0.394</td>
</tr>
<tr>
<td>Age ≥55, &lt;65, %</td>
<td>14.79 ± 1.25</td>
<td>13.62 ± 0.54</td>
<td>0.355</td>
</tr>
<tr>
<td>Age ≥65, %</td>
<td>11.26 ± 0.93</td>
<td>14.99 ± 0.56</td>
<td>0.0002</td>
</tr>
<tr>
<td>Obesity, %</td>
<td>46.73 ± 1.83</td>
<td>30.27 ± 0.74</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin resistance, %</td>
<td>56.32 ± 2.51</td>
<td>31.11 ± 1.00</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>8.72 ± 0.93</td>
<td>8.66 ± 0.35</td>
<td>0.948</td>
</tr>
<tr>
<td>Hypercholesterolaemia, %</td>
<td>80.23 ± 1.44</td>
<td>67.87 ± 0.61</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>32.43 ± 1.91</td>
<td>28.16 ± 0.70</td>
<td>0.021</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>25.02 ± 1.66</td>
<td>34.34 ± 0.99</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

NAFLD, non-alcoholic fatty liver disease.

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the prevalence of NAFLD and increase the plausibility that coffee may indeed offer some protection against the development of this condition. It is important to presume that caffeine intake may have hepatoprotective effects up-to to a certain threshold, after which no additional benefit can be gained. However, our data analysis was not able to establish a ‘safe’ threshold for caffeine intake.

Despite the scarcity of data on the exact mechanism by which coffee and its components impact the aetiology of liver disease, there are some promising hypotheses that may account for their metabolic actions. A recent cell culture study, using HepG2 and CaCo2 cells indicates that the UDP glucuronosyltransferase family of genes, thought to be proteins with indirect antioxidant, cytoprotective and genoprotective capabilities, are induced by coffee, independent of caffeine content, suggesting glucuronidation as a mechanisms for the protective and antioxidant effects of coffee.39 Previous studies focusing on fibrosis have shown that the methylxanthine caffeine, a large component of coffee, may inhibit the synthesis of connective tissue growth factor (CTGF/CCN2) in liver parenchymal and nonparenchym-

<table>
<thead>
<tr>
<th>Predictor</th>
<th>NAFLD</th>
<th>No NAFLD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>African American Race</td>
<td>0.520 (0.426–0.633)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Male gender</td>
<td>1.329 (1.132–1.562)</td>
<td>0.0007</td>
<td></td>
</tr>
<tr>
<td>Obesity (BMI ≥30)</td>
<td>2.087 (1.808–2.409)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Caffeine (mg) intake</td>
<td>0.999319 (0.998955–0.999684)</td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>Total plain water consumed (g)</td>
<td>1.000065 (1.000008–1.000122)</td>
<td>0.0254</td>
<td></td>
</tr>
<tr>
<td>BMI, body mass index; NAFLD, non-alcoholic fatty liver disease.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
mal cells by initiating the degradation of Smad2/3, consequently impairing the transforming growth factor β (TGF-β) signalling. As CTGF and TGF-β are both well known factors in fibrotic remodelling and carcinogenesis, this mechanism may explain the protective effects of coffee observed on fibrogenesis.

Shedding the most light on the impact of coffee on NAFLD specifically, however, are a series of studies assessing the impact of coffee on inflammatory cytokines. The first is a study investigating the effects of coffee on metabolic syndrome by looking at the gene expression in the liver and adipose tissues of mice that were fed a high-fat diet with added coffee, and finding a strong induction of anti-inflammatory responses in the coffee cohorts. A more recent study, of similar scope, assessed the effects of coffee on the risk of type II diabetes using spontaneously diabetic KK-A(y) mice and found that white adipose tissue mRNA levels of the inflammatory cytokines, MCP-1, IL-6, and TNF, in addition to adipose tissue MCP-1 and serum IL-6 concentrations in the coffee cohort were lower than the control group, resulting in lower hyperglycaemia and an improvement of fatty liver. In fact, recent animal studies have underlined the anti-oxidative and anti-inflammatory effects of coffee with regards to fat accumulation in general and steatohepatitis in particular.

Interestingly, a recent study reported the effects of adipocyte cytokine secretion on the development of NAFLD, in particular the increased levels of tumour necrosis factor-alpha (TNF-α), interleukin-8 (IL-8) and IL-6 observed between the NAFLD and control subjects. These data suggest that coffee, in addition to its anti-oxidant properties, may exert a suppressive effect on hyperglycaemia by improving insulin sensitivity, partly due to a reduction of inflammatory cytokine expression and subsequently, improving fatty liver.

Although far from conclusive, all of these data suggest a plausible mechanism which may explain the strong inverse correlation we found between caffeine intake and the propensity to develop NAFLD. Furthermore, it is evident from the available data that the effects of coffee on the aetiology of liver disease are indeed multi-factorial, necessitating detailed mechanistic studies to understand its exact impact.

In conclusion, using recent U.S. population data, we have identified caffeine as being one of the most significantly different nutrient components between the NAFLD and control cohorts. Our data indicate that caffeine may play a protective role in the development of NAFLD. Furthermore, our multivariate analysis highlighted caffeine consumption as one of five independent predictors of NAFLD after adjustment for important confounders. To the best of our knowledge, this is the first study showing a substantial inverse correlation between caffeine consumption and NAFLD specifically, in humans using population-based data. Despite the scarcity of mechanistic data on the protective effects of coffee in the aetiology of NAFLD, there are some very plausible hypotheses suggesting that coffee may have a suppressive effect on hyperglycaemia by improving insulin sensitivity, partly due to a reduction of inflammatory cytokine expression. The impact of coffee and its components on NAFLD in particular and liver disease in general, need to be studied in greater detail.

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Caffeine is protective in patients with NAFLD


37. de Alwis NM, Day CP. Non-alcoholic fatty liver disease: the mist gradually clears. *J Hepatol* 2008; 48 (Suppl. 1): S104.


43. Choi EY, Park SY, Cho YO. Freeze-dried instant coffee can promote the activities of antioxidant enzymes and induce weight loss but also aggravate the plasma cholesterol profile in rats. *Nutrition* 2011; 27: 1202–5.

