

## Consumption of Fructose Rich Industrialized Beverages in Patients with Biopsy-Proven Non-alcoholic Fatty Liver Disease: a Cross-Sectional Study

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### ABSTRACT

**Introduction:** Non-alcoholic fatty liver disease (NAFLD) ranges from simple steatosis to nonalcoholic steatohepatitis (NASH) and liver fibrosis. Recently, consumption of high fructose corn syrup (HFCS) has been associated with NAFLD development.

**Objective:** The aim of this study was to investigate the relationship between consumption of HFCS and NAFLD associated metabolic factors and disease progression.

**Methods:** This cross-sectional study included 51 patients with biopsy-proven NAFLD who underwent biochemical tests, anthropometrical assessment and full-day dietary evaluation including industrialized beverages quantification.

**Results:** Individuals were 80% female, with  $54 \pm 12$  years old, 96% with central obesity, 75% with insulin resistance or diabetes mellitus and were separated according to industrialized beverage intake:  $< 7$  and  $\geq 7$  coups/week (*i.e.*, daily). Daily consumption of HFCS was associated with obesity ( $P = 0.04$ ), hypertriglyceridemia ( $P = 0.05$ ), higher serum triglycerides ( $P = 0.03$ ) and VLDL ( $P = 0.01$ ). There was a significant correlation ( $R = 0.29$ ;  $P = 0.04$ ) between consumption of industrialized beverages and increased serum triglycerides. We found no association between daily HFCS intake and NASH diagnosis or presence of fibrosis.

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**Conclusion:** Excessive consumption of HFCS in industrialized beverages was associated with obesity, hypertriglyceridemia and high levels of blood triglycerides in patients with NAFLD.

### KEYWORDS

Nonalcoholic fatty liver disease; nonalcoholic steatohepatitis; fructose; high fructose corn syrup.

### ABBREVIATION

- A: grade of disease activity.
- ALP: alkaline phosphatase.
- ALT: alanine aminotransferase.
- AST: aspartate aminotransferase.
- BMI: body mass index.
- DM: diabetes mellitus.
- F: grade of fibrosis.
- GGT: gamma-glutamyltransferase.
- HDL: high-density lipoprotein.
- HOMA: homeostasis model assessment.
- IB: industrialized beverages.
- MS: metabolic syndrome.
- NAFLD: Nonalcoholic fatty liver disease.
- NASH: nonalcoholic steatohepatitis.
- VLDL: very low-density lipoprotein.
- WC: waist circumference.

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a clinicopathological condition that includes a spectrum ranging from simple steatosis, characterized by accumulation of triglycerides in the liver parenchyma, to non-alcoholic steatohepatitis (NASH), characterized by associated inflammation and ballooning degeneration, with potential progression to fibrosis and cirrhosis<sup>1,2</sup>.

NAFLD is considered the most common liver disease in Western countries, with estimated prevalence of 25%<sup>3</sup>. The disease is strongly associated with obesity, diabetes mellitus (DM) and metabolic syndrome (MS).

Prevalence of NAFLD has been increasing in the past decades, concomitantly with increasing of industrialized dietary habits and added sugar consumption, including fructose, as observed in the Western diet<sup>4</sup>. Fructose is the main carbohydrate responsible for the increase in calorie intake in typical Western diet and is consumed in the form of sucrose (50% fructose) and high fructose corn syrup (42% to 55% fructose). Before the 1900s, the North Americans consumed approximately 15 g of fructose per day (4% of total calories), mainly by fruits and vegetables. In 1994 the intake of fructose went up 55 g per day (10% of total calories)<sup>5</sup>.

High fructose corn syrup is a sweetener largely utilized by the industry due to its capacity to prolong products shelf life and decrease its cost. It is commonly present in industrialized beverages (IB).

The association between excessive consumption of this sweetener and its impact on hepatic steatosis is controversial in current literature. Two metanalysis did not find association between fructose intake and liver steatosis, suggesting that the overall calorie intake is the primary associated factor<sup>6,7</sup>. However, one recent metanalysis of four cross-sectional studies supported the association between fructose consumption and steatosis<sup>8</sup>. In human models, only one study has investigated the association between fructose intake and hepatic fibrosis, showing that a consumption higher than 7 cups a week of IB is associated with lower steatosis grade and higher fibrosis stage<sup>9</sup>.

The objective of this study was to investigate the relationship between ingestion of high fructose corn syrup present in IB and NAFLD associated metabolic factors, nutritional aspects and histological disease progression.

## MATERIAL AND METHODS

### *Subjects and Study Design*

This cross-sectional study was developed at the Nonalcoholic Fatty Liver Disease Outpatient Clinic of Hospital das Clínicas da Universidade Federal de Minas Gerais, Brazil. During a 12 months period, patients were prospectively considered for inclusion based on selection criteria.

The study was approved by the Research Ethic Committee of the Institution and all the participants signed a free informed consent form. All subjects were evaluated for NAFLD diagnosis and 51 patients with biopsy-proven NAFLD were included. Other causes of associated liver disorder were excluded, such as alcoholic liver disease (ingestion of > 20 g/day of ethanol for men and > 10 g/day of ethanol for women during a period exceeding one year), hepatitis B and C (presence of hepatitis B surface antigen and antibodies against hepatitis C virus, respectively), autoimmune liver diseases, Wilson's disease, alpha-1-antitrypsin deficiency and hereditary hemochromatosis. Patients who had previous gastric or jejunoileal bypass or were taking drugs that induced hepatic steatosis within the last six months were also excluded.

### *Histopathological Evaluation*

Pathology specimens were reviewed by a single liver pathologist who was blinded to the patients' clinical information. Simple steatosis and NASH were diagnosed based on the criteria proposed by the Fatty Liver Inhibition of Progression algorithm<sup>2,10</sup>. Grade of disease activity (A from A0 to A4) was calculated by addition of grades of ballooning and lobular inflammation according to Bedossa et al 2014. Liver fibrosis was evaluated according to Kleiner et al<sup>10,11</sup> and patients were categorized into two groups: none or initial fibrosis (*i.e.*, F0-F1– no fibrosis and portal fibrosis without septa, respectively) and significant fibrosis (*i.e.*, F ≥ 2 – portal fibrosis with few septa, fibrosis/bridge septa between the central and portal veins, and cirrhosis). The overall histological severity of disease was also evaluated according to activity and fibrosis by splitting the subjects in two groups: mild disease (A < 2 and F < 2) and significant disease (A > 2 and/or F > 2). None of the patients had significant weight loss between the biopsy and the beginning of the study.

### *Clinical and Laboratory Evaluation*

MS was defined according to the criteria of the International Diabetes Federation: presence of central obesity (waist circumference [WC] ≥ 90 cm for men and ≥ 80 cm for women), in addition to at least two of the following factors: serum triglycerides ≥ 150 mg/dl, HDL cholesterol < 40 mg/dl for men or < 50 mg/dl for women, fasting glucose level ≥ 110 mg/dl and arterial hypertension (arterial blood pressure ≥ 130/85 mmHg)<sup>12</sup>.

Body weight was measured using a mechanical Filizzola® scale with the capacity of 150 kg in 100 g demarcations. Height was measured using the scale stadiometer. The body mass index (BMI) was calculated by dividing weight by squared height and classified according to the World Health Organization's criterion, which defines underweight as BMI < 18.5 kg/m<sup>2</sup>, eutrophic as BMI between 18.5 and 24.9 kg/m<sup>2</sup>, overweight as BMI between 25 and 29 kg/m<sup>2</sup> and obese as BMI ≥ 30 kg/m<sup>2</sup>.

WC was measured by the standard technique (*i.e.*, using an inelastic two-meter anthropometrical tape and performing the measurement in the midpoint between the last rib and the iliac crest)<sup>13</sup>.

Body composition of lean and fat mass was evaluated using an electric bioimpedance analyser (*Biodynamics*® model 450 version 5.1).

According to the American College of Sports Medicine and the American Heart Association criteria<sup>14</sup>, patients were classified as physical activity practicing when they practiced physical activity for 30 minutes at least five times a week. Otherwise, they were classified as sedentary.

The following laboratory exams were performed: serum concentrations of total and fractionated cholesterol, triglycerides, fasting glucose and insulin levels, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), uric acid, total and fractionated bilirubin, albumin and vitamin D. Homeostasis Model Assessment (HOMA) was used to evaluate insulin resistance and was calculated as fasting serum insulin ( $\mu\text{U/mL}$ )  $\times$  fasting plasma glucose ( $\text{mmol/L}$ )/22.5. All exams were performed at the institution laboratory, after 12 hours fasting.

### **Nutritional Assessment**

Three daily ingestion recalls were performed on different days: two weekdays and one weekend day. The questionnaire records information on the intake of food and drinks within the previous 24 hours; thus, it reflects recent consumption. The ingested amounts were reported using household measurements<sup>15</sup> and then were converted into grams and analysed according to the Brazilian food composition guides<sup>16,17</sup>. Intake calculations included: carbohydrates, proteins, lipids, monounsaturated fats, polyunsaturated fats, saturated fats and fibers, which were compared to the Brazilian Guideline for Diagnosis and Treatment of Metabolic Syndrome I<sup>18</sup>.

The consumption of high fructose corn syrup was analysed by the frequency of IB (*i.e.*, industrialized juices and soft drinks) weekly consumed and the number of cups ingested. The patients were classified into two categories: minimum to moderate consumption (1 to 7 cups per week) and daily consumption ( $\geq 7$  cups per week)<sup>9</sup>.

### **Statistical Analysis**

Statistical analysis was performed using SPSS software, version 22.0 (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to test for normal distribution. For the univariate analysis, the chi-square test or the Fisher's exact test were used for comparing dichotomous and categorical variables. The t-test was used for comparing means of normally distributed variables, and the Mann-Whitney U test was used for

comparing asymmetric distributed of variables. Correlations were analysed using the Pearson's or Spearman's correlation coefficient as appropriate.

## **RESULTS**

Fifty-one patients with biopsy proved NAFLD were included. The baseline characteristics are described here. Population was 80% female with  $54.4 \pm 12.3$  years old. The majority was obese (69%): 45% of class I obesity, 18% class II and 6% class III. Only 2% of the patients were eutrophic. Central obesity occurred in 96% of the patients. Approximately 75% presented with insulin resistance or DM, 72% with arterial hypertension and 86% with MS.

The IB mean weekly consumption was  $5.8 \pm 5.5$  cups, which represents 9.1% of total carbohydrate consumption. Mild to moderate ingestion of IB ( $< 7$  cups/week) was reported in 60.8% of the subjects, and daily consumption ( $\geq 7$  cups/week) in 39.2%.

Table 1 shows the nutritional and demographic characteristics of the individuals according to the amount consumption of IB. There was no difference of mean age, weight, BMI, WC, lean mass and fat percentage between the groups  $< 7$  cups/week and  $\geq 7$  cups/week.

There was a lower proportion of obesity in the group that consumed  $< 7$  cups/week (58.1%) compared to the group that consumed  $\geq 7$  cups/week (85%) ( $p = 0.04$ ). The frequency of sedentary individuals was similar between groups (48.4% vs 50%) ( $p = 0.99$ ).

Table 2 shows the characteristics of nutritional intake in relation to the consumption of IB. The carbohydrates dietary intake was similar between the two groups ( $< 7$  cups/week vs  $\geq 7$  cups/week), as well as the proportion of total carbohydrates related to added sugar ingestion. Dietary intake of lipids, monounsaturated and polyunsaturated fatty acids were also similar between groups.

Table 3 presents patients clinical characteristics according to the consumption of IB. Hypertriglyceridemia frequency is associated with IB daily consumption ( $P = 0.05$ ).

The serum biochemical profile according to consumption of IB is shown in Table 4. Daily processed beverages consumption is associated with higher VLDL serum levels ( $P = 0.01$ ) and triglycerides concentration ( $P = 0.03$ ). There is a positive correlation between the weekly consumption of IB and serum triglyceride values ( $R = 0.29$ ;  $P = 0.04$ ).

From liver biopsy analysis, 24% had simple steatosis, 76% had NASH and 55% presented liver fibrosis ( $F1 = 61\%$ ,  $F2 = 11\%$ ,  $F3 = 21\%$ ,  $F4 = 7\%$ ). Fibrosis was divided in: F0-F1 (78%) and significant fibrosis ( $F \geq 2 = 22\%$ ). There was no significant difference between pattern of IB consumption and NASH ( $P = 0.32$ ) or significant fibrosis ( $P = 0.14$ ).

**Table 1.** Demographic and nutritional profile of patients with NAFLD in relation to the consumption of industrialized beverages.

Variable	< 7 cups(n = 31)	≥ 7 cups(n = 20)	P value
Sex (n)%			0.43 <sup>A</sup>
Female	(26/31) 83.9%	(15/20) 71.4%	
Male	(5/31) 16.1%	(5/20) 28.6%	
Age (years) (mean ± SD)	56.3 ± 11.4	51.4 ± 13.3	0.16 <sup>B</sup>
Weight (Kg) (mean ± SD)	82.7 ± 16.4	83.3 ± 12.4	0.84 <sup>B</sup>
BMI (Kg/m <sup>2</sup> ) (mean ± SD)	31.8 ± 5.2	32.5 ± 3.1	0.54 <sup>B</sup>
Lean mass (%) (mean ± SD)	64.9 ± 5.7	63.0 ± 5.7	0.27 <sup>B</sup>
Fat mass (%) (mean ± SD)	35.1 ± 5.7	37.0 ± 5.7	0.27 <sup>B</sup>
WC (cm) (mean ± SD)	101.8 ± 22.6	105.6 ± 11.2	0.49 <sup>B</sup>

NAFLD: nonalcoholic fatty liver disease; SD: standard deviation; BMI: body mass index; WC: waist circumference; <sup>A</sup>: chi square; <sup>B</sup>: t test.

**Table 2.** Relationship between carbohydrate and lipids intake according to the consumption of processed drinks in patients with NAFLD.

Variable mean ± SD or median (IQ ratio)	Recommendation % of total energy	All(n=51)	< 7 cups (n = 31)	≥ 7 cups (n = 20)	P value
Carbohydrates	50-60%	50.8 ± 11.9	49.7 ± 12.3	52.3 ± 11.4	0.48 <sup>A</sup>
Added sugar (% of total carbohydrates)		5.3 ± 3.6	5.3 ± 4.1	5.4 ± 2.9	0.92 <sup>A</sup>
Industrialized beverages (% of total carbohydrates)		9.1 (2.1-14.5)	2.3 (1.8-7.4)	13.8 (11.1-19.7)	0.00 <sup>B</sup>
Lipids	25-35%	34.1 (27.2-39.0)	33.1 (23.3-39.1)	37.0 (28.0-39.0)	0.72 <sup>B</sup>
Saturated fatty acids	< 10%	8.3 ± 3.0	7.6 ± 2.6	9.3 ± 3.2	0.06 <sup>A</sup>
Polyunsaturated fatty acids	Up to 10%	7.9 (7.3-9.7)	8.1 (7.3-9.9)	7.6 (6.7-9.4)	0.21 <sup>B</sup>
Monounsaturated fatty acids	Up to 20%	11.4 ± 2.9	11 ± 2.4	11.9 ± 3.5	0.29 <sup>A</sup>

SD: standard deviation; IQ: interquartile; <sup>A</sup>: t-test; <sup>B</sup>: U test of Mann-Whitney.

The disease grade of activity was not related with daily IB intake either, even when analysed by mild vs significant disease ( $P = 0.68$ ) and nonsignificant vs significant disease ( $P = 0.69$ ).

## DISCUSSION

In this study we investigated the relationship between consumption of IB rich in high fructose corn syrup and nutritional, anthropometrical, laboratorial and histopathological characteristics of NAFLD biopsy-proven subjects. We ob-

served that daily consumption of these beverages is associated with increased serum triglycerides and VLDL cholesterol levels. The only anthropometrical aspect associated was obesity. There was no relationship between daily IB and bioimpedance variables, NASH diagnosis, disease activity or significant fibrosis in histological evaluation.

The impact of excessive IB consumption on NAFLD is still controversial in current literature. Two metaanalysis that included 13 trials with 260 healthy patients<sup>6</sup> and 21 interventional studies<sup>7</sup> did not find association between fructose in-

**Table 3.** Clinical characteristics and relationship with the consumption of industrialized beverages in patients with NAFLD.

Variable	Population		< 7 cups (n=31)		≥ 7 cups (n=20)		P value
	N	%	N	%	N	%	
DM	22	43.1	14	45.2	8	40	0.71
IR	16	31.4	10	32.3	6	30	0.86
DM or IR	38	74.5	24	77.4	14	70	0.55
Hypercholesterolemia	34	66.7	20	64.5	14	70	0.68
Low HDL	33	64.7	19	61.3	14	70	0.52
Hypertriglyceridemia	30	58.8	15	48.4	15	75	0.05
Hypertension	37	72.5	24	77.4	13	65	0.33
MS	44	86.3	27	87.1	17	85	0.83

DM: diabetes mellitus; IR: insulin resistance; MS: metabolic syndrome; Analysis performed by chi square test.

**Table 4.** Serum biochemical profile according to the consumption of processed drinks by the patients with NAFLD.

Variable	All (n = 51)	< 7 cups (n = 31)	≥ 7 cups (n = 20)	P value
	mean ± SD or median (IQ ratio)			
Fasting glucose (mg/dL)	101 (89.0-144.0)	104 (89.0-172.0)	99 (90.0-123.5)	0.34 <sup>A</sup>
HOMA	4.4 (2.7-8.3)	5.3 (2.5-9.4)	4.2 (2.8-6.8)	0.61 <sup>A</sup>
D vitamin (ng/mL)	22 (19.0-28.6)	21 (18.5-32.0)	22 (20.0-25.0)	0.45 <sup>A</sup>
Insulin (U/mL)	18.4 ± 10.2	19.4 ± 11.4	26.7 ± 46.6	0.44 <sup>B</sup>
Total cholesterol (mg/dL)	196.7 ± 35.9	191.8 ± 37.6	211 ± 33.4	0.06 <sup>B</sup>
HDL (mg/dL)	43.4 ± 12.4	46.5 ± 11.1	44.1 ± 24.8	0.44 <sup>B</sup>
VLDL (mg/dL)	39.3 ± 22.1	30.5 ± 11.9	44.1 ± 24.9	0.01 <sup>B</sup>
LDL (mg/dL)	114.2 ± 29.3	114.7 ± 34.6	123.1 ± 21.2	0.33 <sup>B</sup>
Triglycerides (mg/dL)	194.7 ± 104.5	155.4 ± 62.1	216.7 ± 118.2	0.03 <sup>B</sup>
AST (x RV)	1.1 ± 0.7	1.1 ± 0.7	1.1 ± 0.7	0.82 <sup>B</sup>
ALT (x RV)	1.0 ± 0.9	0.9 ± 0.5	1.2 ± 1.2	0.12 <sup>B</sup>
GGT (x RV)	0.7 ± 0.5	0.7 ± 0.5	0.7 ± 0.5	0.94 <sup>B</sup>
ALP (x RV)	0.8 ± 0.4	0.8 ± 0.5	0.9 ± 0.2	0.56 <sup>B</sup>
Uric acid (mg/dL)	5.9 ± 1.4	5.4 ± 1.4	6.1 ± 1.3	0.11 <sup>B</sup>
Albumin (g/dL)	4.4 ± 0.2	4.3 ± 0.4	4.3 ± 0.3	0.97 <sup>B</sup>

SD: standard deviation; HOMA: homeostatic model assessment; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transferase; ALP: alkaline phosphatase; RV: reference value; <sup>A</sup>: U test of Mann-Whitney; <sup>B</sup>: t test.

take and hepatic steatosis, suggesting that the primary factor responsible for lipid accumulation in hepatocytes is probably related to overall calorie intake, and not specifically fructose. However, one recent meta-analysis of four cross-sectional studies supported the association between fructose intake and NAFLD development<sup>8</sup>. Besides that, few studies have analysed biopsy-proven NAFLD and only one investigated the association between IB consumption and liver fibrosis histologically in human models<sup>9</sup> as we did.

Previous data suggest that dietary imbalances play a role in NAFLD development and progression<sup>19</sup>, although specific NAFLD dietary recommendations are not consensual in the literature<sup>20</sup>. In our study, we also investigated the dietary characteristics related to excessive IB intake. Concerning macronutrients, carbohydrates and lipids intake were similar in the groups that consumed < 7 or ≥ 7 cups/week of IB, although daily lipid intake exceeded the recommended<sup>18</sup> in patients who ingested ≥ 7 cups/week of IB.

In the present study, approximately 9% of the total carbohydrates ingested were in the form of IB with high fructose corn syrup. In the previous years, the consumption of industrialized drinks in the United States has grown to approximately 8 to 9% of total consumed carbohydrates<sup>21</sup>, similarly to the present study. Other authors have reported the relationship between increased consumption of carbohydrates and the risk of hepatic inflammation, but the source of the consumed carbohydrates was not reported<sup>22</sup>. In this study, we described the carbohydrates source, but we did not find any association between its intake and NASH diagnosis.

The consumption of IB was demonstrated as an independent predictive factor for the development of overweight and obesity in otherwise healthy individuals<sup>23</sup>, and also for weight gain and type 2 DM development in women<sup>24</sup>. Furthermore, studies show that high loads of fructose diminish the hepatic content of adenosine triphosphate, which can cause a decrease in both cellular insulin binding and receptors expression, leading to insulin resistance<sup>25</sup>. A recent meta-analysis demonstrated that intake of IB results in 30% increased risk of developing type 2 DM<sup>26</sup>. Besides that, fructose can also increase *de novo* lipogenesis and reduce the fatty acids oxidation, which may cause hepatic lipid accumulation<sup>25</sup>. It is thought that short-term overfeeding with fructose decreases hepatic insulin sensitivity and increases liver fat content<sup>27</sup>.

Zelber-Sagi et al.<sup>28</sup> observed that NAFLD patients consumed IB two-times the amount consumed by non-NAFLD subjects. Moreover, this consumption was associated with increased risk of developing NAFLD. Recently, it was demonstrated that a reduction in fructose consumption improved in steatosis and insulin sensitivity<sup>29</sup>. Given the findings, it is an open question whether the improvement was due to fructose intake reduction or to total calories reduction<sup>6,7,30</sup>.

Our study was limited by the small sample size, since we only included patients with biopsy-proven NAFLD. Another limitation was the high proportion of NASH subjects in the sample, because in our institution liver biopsy is performed in potentially progressive NAFLD predicted by clinical criteria<sup>1</sup>. Also, the 24-hour food recall method presents an inherent difficulty, since patients tend to underestimate the amount of ingested portions. Moreover, Brazilian guidelines do not provide the amount of fructose contained in food and drink; thus, it was not possible to quantify consumption in grams per day.

Fructose intake and its relationship with NAFLD progression is still controversial in current literature, which reinforces the need to conduct other studies, preferably prospective, that better evaluate the causal role of fructose in the development of NAFLD. In addition, more data is necessary to evaluate whether or not a diet with low fructose content could improve the manifestations of the MS and liver histology in individuals with NAFLD.

## CONCLUSION

The excessive consumption of high fructose corn syrup in IB seems to be associated with obesity, hypertriglyceridemia and high blood levels of triglycerides and VLDL cholesterol in patients with NAFLD. No associations were found between fructose intake and NASH diagnosis or fibrosis presence.

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