Research Article

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Industrial, not fruit fructose intake is associated with the severity of liver fibrosis in genotype 1 chronic hepatitis C patients

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Background & Aims: Unhealthy food intake, specifically fructose, has been associated with metabolic alterations and with the severity of liver fibrosis in patients with non-alcoholic fatty liver disease. In a cohort of patients with genotype 1 chronic hepatitis C (G1 CHC), we tested the association of fructose intake with the severity of liver histology.

Methods: Anthropometric and metabolic factors, including waist circumference (WC), waist-to-hip ratio (WHR), dorso-cervical lipohypertrophy and HOMA were assessed in 147 consecutive biopsy-proven G1 CHC patients. Food intake, namely industrial and fruit fructose, was investigated by a three-day structured interview and a computed database.

All biopsies were scored by an experienced pathologist for staging and grading (Scheuer classification), and graded for steatosis, which was considered moderate-severe if $\geqslant$20%. Features of non-alcoholic steatohepatitis (NASH) in CHC were also assessed (Bedossa classification).

Results: Mean daily intake of total, industrial and fruit fructose was 18.0 ± 8.7 g, 6.0 ± 4.7 g, and 11.9 ± 7.2 g, respectively. Intake of industrial, not fruit fructose, was independently associated with higher WHR ($p = 0.02$) and hypercaloric diet ($p < 0.001$). CHC patients with severe liver fibrosis ($\geqslant$F3) reported a significantly higher intake of total (20.8 ± 10.2 *vs.* 17.2 ± 8.1 g/day; $p = 0.04$) and industrial fructose (7.8 ± 6.0 *vs.* 5.5 ± 4.2; $p = 0.01$), not fruit fructose (12.9 ± 8.0 *vs.* 11.6 ± 7.0; $p = 0.34$). Multivariate logistic regression analysis showed that older age (OR 1.048, 95% CI 1.004–1.094, $p = 0.03$), severe necroinflammatory activity (OR 3.325, 95% CI 1.347–8.209, $p = 0.009$), moderate-severe steatosis (OR 2.421, 95% CI 1.017–6.415, $p = 0.04$), and industrial fructose intake (OR 1.147, 95% CI 1.047–1.257, $p = 0.003$) were independently linked to severe fibrosis. No association was found between fructose intake and liver necroinflammatory activity, steatosis, and the features of NASH.

Conclusions: The daily intake of industrial, not fruit fructose is a risk factor for metabolic alterations and the severity of liver fibrosis in patients with G1 CHC.

Introduction

The clinical course of chronic hepatitis C (CHC) is strongly driven by the progression of liver fibrosis, occurring as a consequence of virus-dependent mechanisms of tissue damage [1], with the ultimate development of cirrhosis and its complications. In addition to conventional risk factors associated with the severity of fibrosis (necroinflammation, age, time of HCV infection, alcohol intake, viral coinfections [2]), recent evidence has suggested a relevant role of metabolic factors; along this line, obesity, insulin resistance (IR) [3], steatosis [4], post-menopausal status [5] and low vitamin D levels [6,7] have been associated with advanced disease.

In the complex interplay between liver and metabolic factors, recent studies in the setting of non-alcoholic fatty liver disease (NAFLD) have suggested that selective food intake might modulate the severity of liver disease. Specifically, observational studies highlighted the association between a high intake of saturated fatty acids, a high n-6/n-3 polyunsaturated fatty acid ratio, a low intake of fiber and a high intake of fructose [8] and the risk of non-alcoholic steatohepatitis (NASH), the more severe expression of NAFLD. The role of fructose consumption was associated with the development of IR, fatty liver, and hepatic damage in experimental studies [9], and a clinical study confirmed that a diet rich in fructose-sweetened beverages was also associated with increased insulin resistance [10]. From a clinical point of view, soft-drink consumption favors the development of obesity among children, adolescents and adults [11–13], and is associated with the features of the metabolic syndrome and the severity of liver fibrosis in patients with biopsy proven NAFLD [14].

Keywords: Fructose; Chronic hepatitis C; Liver fibrosis.
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Abbreviations: HCV, hepatitis C virus; G1, genotype 1; CHC, chronic hepatitis C; WC, waist circumference; DCL, dorso-cervical lipohypertrophy; WHR, waist-to-hip ratio.

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Very few data exist on dietary intake and liver disease progression in chronic HCV infection; a recent study did not find any link between dietary fructose and hepatic fibrosis, as assessed by FibroSURE, in HCV-infected males [15].

We tested the association of specific food intake, in particular of fructose intake, with the severity of liver disease in a cohort of patients with genotype 1 (G1) CHC.

Materials and methods

Patients

One hundred and forty-seven consecutive patients with G1 CHC, recruited at the Gastrointestinal & Liver Unit of the University Hospital in Palermo were included in the study. All had a histological diagnosis of CHC (any degree of fibrosis, including cirrhosis) on a liver biopsy and alcohol consumption <20 g/day in the last year or more, evaluated by a specific questionnaire. We excluded patients with advanced cirrhosis (Child-Pugh B and C), hepatocellular carcinoma, other causes of liver disease (autoimmune liver disease, Wilson's disease, hemochromatosis, α1-antitrypsin deficiency), excessive alcohol consumption or active i.v. drug addiction, HIV or HBV co-infection, previous treatment with antiviral therapy or the use of steatosis-inducing drugs (corticosteroids, valproic acid, tamoxifen, amiodarone).

The study was performed in accordance with the principles of the Declaration of Helsinki and its appendices, and with local and national laws. Approval was obtained from the hospital Institutional Review Board and Ethics Committee, and written informed consent was obtained from all patients.

Clinical and laboratory assessment

Clinical and anthropometric data were collected at the time of liver biopsy. BMI was calculated on the basis of weight in kilograms and height (in meters), and patients were classified as normal weight (BMI, 18.5–24.9 kg/m^2), overweight (BMI, 25–29.9), or obese (BMI $\geqslant$30). Waist circumference (WC) was measured at the midpoint between the lower border of the rib cage and the iliac crest. Hip circumference was measured at the widest point between hip and buttocks. Waist-to-Hip ratio (WHR) was defined as WC (cm) divided by HC (cm). Dorso-cervical lipohypertrophy (DCL) was defined as a clinically manifest "hump" at the base of the neck. The diagnosis of type 2 diabetes was based on the revised criteria of the American Diabetes Association, using a value of fasting blood glucose $\geqslant$126 mg/dl on at least two occasions [16]. In patients with a previous diagnosis of type 2 diabetes, current therapy with insulin or oral hypoglycemic agents was documented.

A 12-h overnight fasting blood sample was drawn at the time of liver biopsy for biochemistry (ALT, total, HDL- and LDL-cholesterol, triglycerides, glucose, and insulin) and platelet count. Insulin resistance (IR) was determined by the homeostasis model assessment (HOMA) method, as [17]: Insulin resistance (HOMA-IR) = Fasting insulin (μU/mL) × Fasting glucose (mmol/L)/22.5. HOMA-IR has been validated in comparison with the euglycemic/hyperinsulinemic clamp technique in both diabetic and non-diabetic patients [18].

All patients were also tested for HCV-RNA (RT-PCR homemade; limit of detection: 12 IU/ml) at the time of liver biopsy. Genotyping was performed by INNO-LiPA, HCV II, Bayer.

Assessment of food intake

Within ten days from liver biopsy, patients completed a three-day weighed food record, detailing all food and fluids consumed over two weekdays and one weekend day. They were provided with kitchen scales accurate to 0.1 g and a paper diary to register the three-day food intake. The diary was adapted by a dietitian to this specific study, in order to facilitate a correct recording of all foods and drinks consumed during the test period. Patients were also encouraged to include the brand names of foods and drinks, the recipes they used to prepare meals, and to provide details on cooking method. Food records were coded and analyzed by a research dietitian using the Mètadieta program (Me.Te.Da., San Benedetto del Tronto (AP), Italy); fructose content was calculated using records reported at www.health-diet.us/fructose/. The daily intake of industrial and fruit fructose was separately calculated: industrial fructose included any amount of fructose derived from food sources containing high fructose corn syrup (beverages like soft drink and fruit juices, processed foods like fast-food especially when enriched by industrial sauce), while fruit fructose entailed only whole fruit sources. Any inconsistency in records was checked extensively with patients during either outpatient visits and/or telephone recall before analysis. The diet was defined hypercaloric if contained >2000 kcal in men or >1800 kcal in women.

Liver biopsy

Slides of liver biopsy were coded and read by one pathologist (D.C.), who was unaware of patients' identity and history. A minimum length of 15 mm of biopsy specimen or the presence of at least 10 complete portal tracts was required for diagnosis [19]. Biopsies were classified according to Scheuer numerical scoring system [20]. The percentage of hepatocytes containing macrovescicular fat was determined for each 10x field. An average percentage of steatosis was then determined for the entire specimen. Steatosis was assessed as the percentage of hepatocytes containing fat droplets (minimum 5%) and evaluated as a continuous variable. According to the classification of Bedossa *et al.* [21], histological features of NASH in CHC were also assessed. In particular, NASH was defined according to a score made by addition of clarification/ballooning (0 absent, 1 mild, 2 significant) and perisinusoidal fibrosis semiquantitation (0 absent, 1 mild, 2 significant); a score of $\geqslant$3 was considered diagnostic for NASH [21]. Steatosis was classified as absent-mild when <20%, or moderate-severe when $\geqslant$20%.

Statistics

Continuous variables were summarized as mean ± SD, and categorical variables as frequency and percentage. Student's *t*-test and the Chi-square test were used when appropriate. Multiple linear regression analysis was performed to identify the variables independently associated with total, industrial and fruit fructose intake (continuous dependent variable). As candidate risk factors, we selected age, gender, BMI, WC and HC, WHR, DCL, diabetes, baseline ALT, platelet count, triglycerides, total, HDL and HDL cholesterol, blood glucose, insulin, HOMA score, Log_{10} HCV RNA levels, steatosis, necroinflammatory activity score, fibrosis, and features of NASH. For statistical purposes, the values of WHR were entered multiplied by 10 in the regression.

Multiple logistic regression models were used to assess the relationship of steatosis, necroinflammatory activity, presence of features of NASH and fibrosis to the demographic, anthropometric, dietary, metabolic, and histological characteristics of CHC patients. In the first model, the dependent variable was moderate-severe steatosis ($\geqslant$20%) coded as 1 *vs.* 0 (<20%); in the second model the dependent variable was severe necroinflammatory activity (grading 3) coded as 1 *vs.* 0 (grading 0–2); in the third model the dependent variable was the presence of features of NASH coded as 1 (present) *vs.* 0 (absent); in the fourth model the dependent variable was severe fibrosis (F3–F4) coded as 1 *vs.* 0 (F0–F2). As candidate risk factors, we selected the same independent variables included in the linear model and added dietary factors as additional independent variables.

Variables associated with the dependent variable at univariate analysis (probability threshold, $p \leqslant 0.10$) [22,23] were included in the multivariate regression models. To avoid the effect of colinearity, diabetes, IR, HOMA score, blood glucose levels, and insulin levels, as well as BMI, WC, HC, and WHR were not included in the same multivariate model. Regression analyses were done using Proc Logistic, Proc Reg, and subroutine in SAS (SAS Institute, Inc., Cary, North Carolina, USA) [24].

Results

Patient features and histology

The baseline features of the 147 CHC patients included in the study are shown in Table 1. The majority of them were overweight or obese, DCL was observed in 46% of cases, diabetes in 11%. Mean values of total cholesterol, HDL cholesterol, and triglycerides were within the normal range, while mean HOMA was high (3.25). One patient out of five had fibrosis $\geqslant$3 by Scheuer score, with a high prevalence of severe necroinflammation (grading 3). Steatosis $\geqslant$5% was observed in 77 patients (52.3%), even if of moderate/severe grade ($\geqslant$20%) in 23 cases only (13.6%). According to Bedossa *et al.* [21], 19 patients had a score