Research Article

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Serum uric acid concentrations and fructose consumption are independently associated with NASH in children and adolescents

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Background & Aims: Recent research has suggested that dietary fructose intake may increase serum uric acid (UA) concentrations. Both UA concentration and fructose consumption maybe also increase in NAFLD. It is not known whether dietary fructose consumption and UA concentration are independently associated with non-alcoholic steatohepatitis (NASH). Our aim was to investigate the factors associated with NASH in children and adolescents with proven NAFLD, and to test whether UA concentrations and fructose consumption are independently associated with NASH.

Methods: Obese children with NAFLD were studied (n = 271). NASH was diagnosed by a NAFLD activity score $\geqslant$5 and the fatty liver inhibition of progression (FLIP) algorithm. Fructose consumption (g/day) was assessed by food frequency questionnaire, and UA (mg/dl) was measured in serum. Binary logistic regression with adjustment for covariates and potential confounders was undertaken to test factors independently associated with NASH.

Results: NASH occurred in 37.6% of patients. Hyperuricaemia (UA $\geqslant$5.9 mg/dl) was present in 47% of patients with NASH compared with 29.7% of non-NASH patients (p = 0.003). Both UA concentration (OR = 2.488, 95% CI: 1.87–2.83, p = 0.004) and fructose consumption (OR = 1.612, 95% CI 1.25–1.86, p = 0.001) were independently associated with NASH, after adjustment for multiple (and all) measured confounders. Fructose consumption was independently associated with hyperuricaemia (OR = 2.021, 95% CI: 1.66–2.78, p = 0.01). These data were confirmed using the FLIP algorithm.

Conclusions: Both dietary fructose consumption and serum UA concentrations are independently associated with NASH. Fructose consumption was the only factor independently associated with serum UA concentration.

Lay summary: Currently, it is not known whether dietary fructose consumption and uric acid (UA) concentration are linked with non-alcoholic steatohepatitis (NASH) in children and adolescents. Our aim was to test whether UA concentrations and fructose consumption are independently associated with NASH in children and adolescents with proven non-alcoholic fatty liver disease (NAFLD). We show that both dietary fructose consumption and serum UA concentrations are independently associated with NASH and fructose consumption was independently linked with high serum UA concentrations.

Keywords: Uric acid; Fructose consumption; Non-alcoholic fatty liver disease (NAFLD); Non-alcoholic steatohepatitis (NASH); Obesity; Fructose; Child; Diet; Adolescent; Hyperuricemia.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is now largely regarded as the hepatic manifestation of metabolic syndrome (MetS) and NAFLD represents the most frequent chronic liver disease in children in Western countries [1]. NAFLD begins with the development of liver lipid accumulation and the condition progresses over time with the development of liver inflammation and fibrosis (non-alcoholic steatohepatitis or NASH). Although it was initially thought that NAFLD was a relatively harmless condition in children and adolescents, recent evidence shows that NASH occurs in this young population [2]. The development of NASH may markedly affect life expectancy and quality of life in affected individuals and therefore it is crucial to understand the risk factors for NASH in children and adolescents in order to design effective interventions which can be used safely to treat this young group of patients.

Nutritional, metabolic and genetic factors contribute to the development of NAFLD and NASH is also an important independent risk factor for extra hepatic diseases such as type 2 diabetes (T2DM) and cardiovascular disease [3]. Risk factors for liver disease progression and the development of NASH are: oxidative stress, systemic inflammation and insulin resistance [4]. Several studies in adults, have shown that hyperuricaemia is associated with insulin resistance (IR), T2DM, MetS and NAFLD but whether

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hyperuricaemia is associated with NAFLD in the paediatric population is uncertain [5,6].

Recent data suggest a correlation between serum uric acid (UA) concentrations and increased consumption of sugary drinks [7] containing fructose and glucose as the disaccharide sucrose or sugar. An increased dietary intake of fructose may be important in the pathogenesis of NAFLD through induction of *de novo* lipogenesis, inflammation, and IR [8]. In the intestine, fructose intake alters the gut microbiome and enhances endotoxin translocation into the portal circulation via increased permeability of tight junctions [9]. In the liver, fructose is rapidly metabolized, consuming adenosine triphosphate (ATP), which may result in increased adenosine monophosphate (AMP) and inosine monophosphate (IMP) and conversion of IMP to UA [10].

Since it is plausible that dietary fructose intake and UA concentrations are potential risk factors for liver disease progression in NAFLD, the aim of our study was to investigate the factors associated with NASH in children and adolescents with proven NAFLD, and test whether UA concentrations and fructose consumption are independently associated with NASH. Additionally, because fructose consumption may increase UA concentrations, we tested whether fructose consumption was independently associated with UA concentrations in this population with NAFLD.

Patients and methods

Anthropometrical and biochemical measurements

Overweight/obese children and adolescents (defined by body mass index [BMI]) with NAFLD, who were referred to the "Hepatometabolic Department" of the "Bambino Gesù" Children's Hospital, from January 2012 to November 2014 provided the data for the current study. In all patients, liver fat was initially identified by ultrasonography using established criteria, a bright hepatic echo pattern compared to echo response of the right kidney [11]. Other causes of steatosis, were excluded in all subjects, including alcohol intake ($\geqslant$140 g/week), total parenteral nutrition, and the use of drugs known to induce steatosis (e.g., valproate, amiodarone or prednisone). Patients with marked recent weight gain, diabetes and known genetic causes of dyslipidemia were excluded. Viral hepatitis (A, B, C, cytomegalovirus and Epstein-Barr virus), autoimmune or metabolic liver diseases, alpha-1-antitrypsin deficiency, Wilson's disease, and celiac disease were also ruled out by appropriate tests. Patients with systemic diseases, genetic syndromes, or chronically treated with drugs, were also excluded from the study.

Anthropometric and clinical parameters (weight, height, BMI, waist circumference [WC] and blood pressure) were measured in all children using standardized methods. The BMI z-score (SDS) was calculated according to BMI reference tables from the World Health Organization (WHO): overweight was defined by + 1 SD and obesity by + 2 SDs, bearing in mind that the z-score is the deviation of the BMI value for an individual from the mean value of the reference population, divided by the standard deviation for the reference population [12]. Lipid profile (total cholesterol, LDL-cholesterol and triglycerides), UA and liver function tests (LFTs: aspartate (AST) and alanine (ALT) aminotransferases, gamma-glutamyl-transpeptidase, bilirubin, albumin and international normalized ratio) were measured by standard methods. Moreover, in all children over the age of ten years, an oral glucose tolerance test was performed, as already described in the WHO recommendations [13,14].

Liver biopsy

According to the recent recommendation of the Hepatology Committee of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), all patients in the current study underwent liver biopsy in order to exclude other diseases, or to assess severity of liver disease (suspected by clinical and laboratory evaluation: marked and persistent hypertransaminasemia, hepatosplenomegaly, or the presence of a high paediatric NAFLD fibrosis index [PNFI]) [15,16]).

Liver biopsies were performed in all children using an automatic core biopsy device (Biopince, Amedic, Sweden) with an 18-G needle, 150 mm long, and the ability to cut tissue with lengths of up to 33 mm with precision [17]. Biopsies were at least 18 mm in length and were assessed by a single liver pathologist who was unaware of the patients' clinical and laboratory data. Biopsies were routinely processed (formalin-fixed, paraffin-embedded) and analysed by different staining. The main histological features, commonly described in NAFLD/NASH, including steatosis, inflammation (portal and lobular), hepatocyte ballooning, and fibrosis were scored according to the Scoring System for Non-Alcoholic Fatty Liver Disease developed by the NIH-sponsored NASH Clinical Research Network (CRN) [18]. Steatosis was graded on a 3-point scale: grade 0 = steatosis involving <5% of hepatocytes; grade 1 = steatosis involving up to 33%; grade 2 = steatosis involving 33–66%; and grade 3 = steatosis involving >66%. Lobular inflammation was graded on a 3-point scale: grade 0 = no foci; grade 1 = less than two foci per 200x field; grade 2 = 2–4 foci per 200x field; grade 3 = more than 4 foci per 200x field. Hepatocyte ballooning was graded from 0 to 2: 0 is none, 1 is a few balloon cells, 2 is many/prominent balloon cells. The stages of fibrosis were quantified on a 4-point scale: stage 0 = no fibrosis; stage 1 = perisinusoidal or periportal (1a = mild, zone 3, perisinusoidal; 1b = moderate, zone 3, perisinusoidal; 1c = portal/periportal); stage 2 = perisinusoidal and portal/periportal; stage 3 = bringing; and stage 4 = cirrhosis.

The presence of NASH was defined according to the NAFLD activity score (NAS). Cases with NAS of 5 or greater were diagnosed as NASH.

The fatty liver inhibition of progression (FLIP) algorithm, conceived by Bedossa *et al.*, for the diagnosis of NASH was also used to test the robustness of the results obtained with the NAS. The FLIP algorithm is another histological classification for diagnosing NASH and is based on the semi-quantification of three features: steatosis, ballooning and lobular inflammation as evaluated according to the steatosis, activity and fibrosis (SAF) score [19]. The FLIP classification uses steatosis as a criterion for entry into the weighted algorithm for hepatocellular ballooning and lobular inflammation. For any biopsy with at least steatosis grade 1, the algorithm includes nine possibilities for diagnosing NASH [20]. The presence of at least one component of the three characteristics (steatosis, ballooning, lobular inflammation) defines NASH.

Assessment of dietary fructose consumption

A food frequency questionnaire (FFQ) was administered to all patients who underwent liver biopsy, as previously reported [21]. Briefly, the frequency intake of a particular food was defined as follows: 'every day of the week', 'sometimes', and 'never'. The questionnaire included numerous subsections (breakfast, morning snack, lunch, afternoon snack, dinner, etc.) that examined the intake of specific foods and portions. The daily intake of all dietary components, for each patient was calculated using the food composition database (FCDBs), shown in the book of LARN – IV Edition, published by the National Italian Institute of Food Research and Nutrition (INRAN) and Italian Society of Human Nutrition (SINU). This database was used to assess the intake of fructose consumption per day (INRAN) [22].

The written informed consent was obtained from each patient included in the study and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (as revised in Seoul, Korea, October 2008) and was approved by the local Ethics Committee for our Hospital (ID Prot. 323/12-OPBG).

Statistical analysis

Statistical analyses were performed with STATISTICA (version 2010, Chicago, IL, USA). Normally distributed data are described as mean with standard deviations (SDs) and non-normally distributed data are expressed as median and interquartile ranges (IQRs). Categorical variables were analysed using χ^2 tests. Pearson's and Spearman's correlation coefficient tests were used to test univariate associations between exposures and outcomes. Binary logistic regression was used to test associations between NASH/non-NASH as the outcome and UA concentration, fructose consumption and age, sex, anthropometric and biochemical parameters as exposures. NASH was diagnosed by a NAS score $\geqslant$5 (= 1) and Non-NASH for NAS <5 (= 0), in the binary logistic regression analysis (and the regression model was repeated using NASH diagnosed by the FLIP algorithm). Subsequently, binary logistic regression was performed to investigate the association between fructose as the exposure and hyperuricaemia (UA $\geqslant$5.9 mg/dl or UA <5.9 mg/dl) [23] as the outcome; with other covariates and potential confounders as exposures in the model. Logistic regression analyses were undertaken using SPSS (IBM SPSS Statistics for Windows, version 20.0, Armonk, NY).

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Results

Anthropometric, biochemical and fructose consumption characteristics of children and adolescents with NAFLD

We included in the present study 271 consecutive obese adolescents with NAFLD (155 males, mean age 12.5 years) who underwent liver biopsy. In our population, 37.6% (n = 102) of patients had NAS ⩾5 (NASH) and 62.4% (n = 169) did not have NASH (NAS <5). Table 1 shows the differences in anthropometric and biochemical characteristics between the NAS ⩾5 and non-NASH (NAS <5) groups. Subjects in the NAS ⩾5 group had higher WC, transaminase levels, total cholesterol, triglyceride and UA concentrations and also fructose consumption. Furthermore, the NAS ⩾5 group showed higher significant TNF-α values compared with the NAS <5 group. There were no differences between the groups for IL-6 and IL-1β concentrations.

Dietary behaviour

The FFQ showed that breakfast was the meal that was most likely to be skipped in our population. 143 (52.76%) children never ate breakfast, 70 (25.8%) ate breakfast infrequently (sometimes) and 58 (21.4%) ate breakfast regularly (ever day). Milk was consumed at breakfast by all children. Morning and afternoon snacks were regularly consumed by 257 (94.8%) and 241 (88.9%) of children, respectively. The most consumed morning snacks were crackers, pizza and salty food, an evening snack consisted of biscuits, yogurt or other snacks. Lunch and dinner were regularly consumed by all patients. The foods eaten every day were cereals 127 (46.8%), vegetables 116 (42.8%) and fruit 108 (39.8%), whilst the foods consumed at least 1–2 time per week were meat 249 (91.8%), fish 131 (48.3%) and eggs 121 (44.6%).

Ninety percent of children ate vegetables, such as green salads and tomatoes one or more times per day. Eighty-nine percent reported drinking sodas and soft drinks one or more times a week. All children consumed extra virgin olive oil, at least 5–10 ml day. Table 1 shows the differences in fructose consumption (gr/day) and carbohydrate consumption (g/day) between the two groups with NAFLD (NAS ⩾5 *vs.* NAS <5).

Histological features of NAFLD

Table 2 describes the histological differences of patients stratified by NAS. One-hundred and two (37.6%) children had NASH (NAS ⩾5) and 169 (62.4%) were classified as non-NASH (NAS <5).

Table 1. Daily dietary consumption of fructose and carbohydrate and anthropometric and biochemical parameters in children and adolescents with NAFLD, stratified by NAFLD activity score (NAS).

	NAS ⩾5 (n = 102)	NAS <5 (n = 169)	*p* value
Age (yr)[1]	11.4 (10.4–13.3)	11.6 (9.8–13)	0.68
Sex (F/M %)[3]	44/58 (43/57)	56/103 (33/67)	0.52
Weight (median; IQR)[1]	68 (50–75)	66 (49–71)	0.55
BMI, kg/mq (mean, SD)[2]	27.2 (4.3)	26.1 (5.1)	0.61
WC, cm (mean, SD)[2]	90.3 (9.1)	85.8 (11.6)	0.01*
z-BMI (mean, SD)[2]	2.8 (1.2)	2.2 (1.5)	0.66
AST, UI/L (median; IQR)[1]	55 (32–65)	42 (28–53)	0.001*
ALT, UI/L (median; IQR)[1]	65 (36–110)	58 (35–78)	0.02*
Uric acid, mg/dl (median; IQR)[1]	6.6 (4–7.2)	5.1 (4.2–6.5)	0.05*
Hyperuricemia, (uric acid >5.9 mg/dl) (%)[3]	47%	29.5%	0.003*
Total cholesterol, mg/dl (median; IQR)[1]	166 (149–195)	156 (131–176)	0.02*
LDL cholesterol, mg/dl (median; IQR)[1]	108 (76–113)	90 (70–108)	0.22
HDL cholesterol, mg/dl (median; IQR)[1]	43 (33–48)	46 (38–49)	0.81
Triglycerides, mg/dl (median; IQR)[1]	103 (78–146)	91 (70–110)	0.05*
Fasting plasma glucose, mg/dl (median; IQR)[1]	84 (75–92)	82 (76–87)	0.64
Fasting plasma gluc-120' (median; IQR)[1]	113 (100–130)	112 (101–127)	0.85
Fasting insulin, mU/L (median; IQR)[1]	16 (9–19)	14 (10–20)	0.61
Insulin -120 minute mU/L (median; IQR)[1]	104 (44–139)	93 (76–136)	0.09
HOMA-IR (mean, SD)[2]	2.99 (2.1)	2.7 (1.67)	0.81
SBP, mmHg (mean, SD)[2]	112 (11.9)	111.4 (10.3)	0.65
DBP, mmHg (mean, SD)[2]	69.5 (8.2)	67.2 (9.6)	0.32
Fructose, g/day (median; IQR)[1]	70.4 (53–85)	52.6 (38–73)	0.002*
Carbohydrates, g/day (median; IQR)[1]	234 (123–432)	227 (128–370)	0.62
TNF-α, (ng/ml) (median; IQR)[1]	59.8 (10–112)	43.4 (12–91)	0.04*
IL-6, (pg/ml) (median; IQR)[1]	29.3 (13–55)	26.2 (8–49)	0.22
IL-1β, (pg/ml) (median; IQR)[1]	14.3 (7–22)	13 (6–21)	0.76

BMI, body mass index; WC, waist circumference; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HOMA-IR, homeostasis model assessment of insulin resistance; SBP, systolic blood pressure; DBP, systolic blood pressure, TNF-α, Tumor necrosis factor-α; IL, interleukin.
p-value unpaired Mann-Witney U test; Difference between proportions were tested using the Chi-Square Test.
[1] Normally distributed date described as mean, standard deviations (SDs).
[2] Non-normally distributed data expressed as median and IQRs.
[3] Prevalence of case (sex and hyperuricaemia – uric acid >5.9 mg/dl).
* $p < 0.05$.

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Table 2. Histological characteristics of children and adolescents with NAFLD (according to the Kleiner scoring system).[1]

Number (%)	NAS ⩾5 (n = 102)	NAS <5 (n = 169)
Steatosis		
0	2 (1.9)	17 (10)
1	31 (30.4)	59 (35)
2	23 (22.5)	75 (44.4)
3	46 (45)	18 (10.6)
Inflammation		
0	32 (31.4)	77 (45.5)
1	52 (51)	81 (48)
2	18 (17.6)	11 (6.5)
Ballooning		
0	48 (47)	88 (52)
1	26 (25.5)	55 (32.6)
2	28 (27.5)	26 (15.4)
NAS		
1		22 (13)
2		60 (35.5)
3		52 (30.5)
4		35 (21)
5	48 (47)	
6	37 (36.3)	
7	17 (16.7)	
Fibrosis		
0	34 (33.4)	45 (26.6)
1	46 (45)	111 (65.7)
2	11 (10.8)	10 (5.9)
3	11 (10.8)	3 (1.8)

NAS, NAFLD activity score.
The data are showed as prevalence case N(%).
[1] Prevalence of case.

Table 3. Logistic regression analysis testing the association between NAS ⩾5 as the outcome and fructose consumption and uric acid concentration, plus other factors as exposures.[1]

	Odds ratio (95% CI)	*p* value
BMI, kg/m^2	0.938 (0.80,0.99)	0.167
WC, cm	1.842 (1.11,1.95)	0.03*
Sex, (F/M%)	1.506 (0.77,3.23)	0.212
Fructose, g/day	1.612 (1.25,1.86)	0.001*
Uric acid, mg/dl	2.488 (1.87,2.83)	0.004*
ALT, IU/L	0.989 (0.77,1.10)	0.781
AST, IU/L	1.048 (0.99,1.12)	0.076
Fasting insulin, mU/L	0.754 (0.69,1.11)	0.264
Fasting glucose, mg/dl	0.787 (0.66,1.21)	0.247
HOMA-IR	3.21 (1.9, 5.72)	0.024*
Cholesterol, mg/dl	1.01 (0.89,1.07)	0.950
Triglyceride, mg/dl	1.208 (1.1,1.58)	0.048*
SBP, mmHg	0.999 (0.92,1.12)	0.654
DBP, mmHg	0.971 (0.93,1.08)	0.587
TNF-α, ng/ml	1.213 (0.98,1.36)	0.468
IL-6, pg/ml	1.041 (0.97,1.21)	0.742
IL-1β, pg/ml	1.242 (0.93,1.92)	0.482

The Odd Ratio is statistically significant with $p < 0.05$.
[1] Model of logistic regression between NASH as the outcome and UA concentration, fructose consumption and age, sex, anthropometric and biochemical parameters as exposures. NASH was diagnosed by a NAS score ⩾5 (= 1) and non-NASH for NAS <5 (= 0).
* $p < 0.05$.

Table 4. Univariate associations between both uric acid concentration and fructose consumption with anthropometric and biochemical parameters.[1]

	Uric acid (mg/dl)		Fructose (g/day)	
	r	*p* value	r	*p* value
BMI, kg/m^2	0.23	0.05	0.10	0.81
WC, cm	0.11	0.12	0.22	0.05
Sex, (F/M,%)	0.10	0.77	0.04	0.59
Fructose, g/day	0.52	0.04	1	
Uric acid, mg/dl	1		0.52	0.04
ALT, IU/L	0.06	0.45	0.21	0.03
AST, IU/L	0.04	0.56	0.12	0.11
Fasting insulin, mU/L	0.33	0.03	0.16	0.09
Fasting glucose, mg/dl	0.02	0.27	0.09	0.82
HOMA-IR	0.47	0.02	0.35	0.01
Cholesterol, mg/dl	0.09	0.85	0.10	0.43
Triglyceride, mg/dl	0.28	0.04	0.37	0.02
SBP, mmHg	0.01	0.79	0.18	0.59
DBP, mmHg	0.07	0.88	0.12	0.32
Carbohydrates, g/day			0.04	0.54
TNF-α, ng/ml	0.31	0.04	0.27	0.04
IL-6, pg/ml	0.17	0.23	0.24	0.05
IL-1β, pg/ml	0.14	0.16	0.14	0.11

The Correlations are statistically significant with $p < 0.05$.
[1] Pearson's and Spearman's correlations between UA and fructose with the clinical and laboratories values.

The NAS ⩾5 group had higher levels of steatosis (S3 = 45% *vs.* 10.6%), inflammation (17.6% *vs.* 6.5%) and fibrosis (F2-F3 = 21.6% *vs.* 7.7%) compared to the non-NASH group. To test the independence of associations between NAS and anthropometric, biochemical parameters and fructose consumption, we undertook regression analysis with NASH/non-NASH as the binary outcome (Table 3). This analysis showed that the following factors were independently associated with NAS ⩾5: WC, HOMA-IR, triglycerides, fructose consumption (OR = 1.612, 95% CI: 1.25–1.86, $p = 0.001$) and UA (OR = 2.488, 95% CI: 1.87–2.83, $p = 0.004$).

To validate the NAS findings obtained from classifying patients into NASH and non-NASH, we also stratified patients into NASH and non-NASH groups using the FLIP algorithm. The FLIP algorithm classified 19 (7%) patients as not having NAFLD, 156 (57.56%) had NAFLD, and 96 (35.42%) had NASH (Supplementary Table 1). We repeated the logistic regression analysis shown in Table 3, to determine which factors were independently associated with NASH determined by the FLIP algorithm. These data (Supplementary Table 2) showed that WC, HOMA-IR, triglyceride concentration, fructose consumption and UA were independently associated with NASH and the data were very similar to that obtained with the NAS.

Table 4 shows univariate correlations between anthropometric and biochemical parameters with both UA concentrations and fructose consumption. These analyses showed that UA concentration was positively correlated with fructose consumption and UA concentration was also correlated with BMI, HOMA-IR, fasting insulin, triglycerides and TNF-α concentrations. Consumption of fructose was correlated with WC, HOMA-IR, ALT, triglycerides, IL-6 and TNF-α concentration. Conversely, fructose consumption was not correlated with daily carbohydrate intake.

Because the univariate analyses showed correlations between fructose consumption and UA concentration, we tested whether fructose consumption was independently associated with hyperuricaemia in regression analysis (Table 5). These data show that fructose consumption was independently associated with UA concentration (OR = 2.021, 95% CI: 1.66–2.78, $p = 0.01$).

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Table 5. Logistic regression analysis testing associations between hyperuricaemia[**] as the outcome and fructose consumption and other factors as exposures.

	Odds ratio (95% CI)	*p* value
BMI, kg/m^2	1.299 (1.09,1.71)	0.04[*]
WC, cm	1.011 (0.85,1.13)	0.77
Sex, (F/M,%)	1.031 (0.66, 1.45)	0.82
Fructose, g/day	2.021 (1.66, 2.78)	0.01[*]
ALT, IU/L	1.002 (0.95, 1.10)	0.69
AST, IU/L	1.003 (0.99,1.05)	0.30
Fasting insulin, mU/L	2.104 (1.28, 2.89)	0.04[*]
Fasting glucose, mg/dl	0.960 (0.88, 1.01)	0.39
HOMA-IR	2.126 (1.55, 5.70)	0.04[*]
Cholesterol, mg/dl	1.002 (0.98, 1.01)	0.88
Triglyceride, mg/dl	1.021 (0.96, 1.10)	0.76
SBP, mmHg	1.011 (0.96, 1.11)	0.55
DBP, mmHg	0.99 (0.96, 1.02)	0.09
TNF-α, ng/ml	1.232 (1.13,1.98)	0.03[*]
IL-6, pg/ml	1.081 (0.88,1.21)	0.08
IL-1β, pg/ml	1.132 (0.99, 1.46)	0.53
Carbohydrates, g/day	1.03 (0.87,1.22)	0.16

The Odd Ratio is statistically significant with $p < 0.05$.

[*] $p < 0.05$.

[**] UA concentration $\geqslant 5.9$ mg/dl.

Discussion

Our novel data shows that in children and adolescents with NAFLD, serum UA concentration and dietary fructose consumption are independently associated with NASH, using two different histological scoring systems for classifying patients as having NASH. Furthermore, fructose consumption was independently associated with hyperuricaemia and hyperuricaemia occurred more frequently in patients with NASH, than in patients who did not have NASH. In each of the regression models, we were able to adjust for a range of potential confounders. That we are able to show the associations are independent of a comprehensive range of factors, gives confidence that these associations are unlikely to be due to confounding. Additionally, we demonstrate these findings in a considerable number of children who all underwent liver biopsy (n = 271 children and adolescents); the findings are also biologically plausible, and thus it seems reasonable to conclude that the results are not due to chance, bias (or confounding as mentioned above).

Numerous studies have shown that high UA levels are associated with metabolic syndrome and NAFLD but to date, to the best of our knowledge, no studies have tested the independence of associations between UA concentrations, fructose consumption and NASH confirmed by biopsy [24,25]. There is a growing body of evidence that UA may have a role in NAFLD, and our data are consistent with studies that have identified hyperuricemia as an independent predictor of fatty liver disease [26]. In this cross-sectional study, the authors show that in adults, higher values of UA are associated with greater risk of NAFLD, both in obese (OR = 2.55, 95% CI: 1.87–3.50) and in non-obese subjects (1.69, 95% CI: 1.37–2.08), (p <0.05) [26]. Ouyang *et al.* correlated the hyperuricemia in hepatic steatosis with the elevated consumption of fructose in association with increased expression of fructokinase (KHK) in the liver [27]. It is known that the increased consumption of fructose induces an upregulation of expression of both Glut 5 and KHK. KHK, upregulated by the concentration of fructose, is also regulated by the intracellular production of UA [28]. Fructose is absorbed in the intestinal lumen, and is then transported to the liver, where it rapidly enters glycolysis and is phosphorylated to fructose-1-phosphate by KHK. The phosphorylation of fructose also stimulates adenosine monophosphate (AMP) deaminase to convert AMP in inosine monophosphate (IMP) and then IMP is converted to UA [11]. Patients with NAFLD, who have a history of high fructose exposure, have a high concentration of UA, because they show a higher hepatic ATP depletion in response to fructose. These studies suggest how high levels of UA may be linked to both fructose consumption and hepatic steatosis via upregulation of KHK [29].

In our study, fructose consumption was significantly higher in the NASH group compared with the non-NASH group (70.4 g/day *vs.* 52.6 g/day; $p = 0.002$). Additionally, hyperuricaemia was independently associated with fructose consumption, which is in accordance with several studies that have shown that UA concentrations are related to excessive consumption of fructose [30]. Numerous studies have demonstrated that hyperuricemia is associated with IR and is a feature of the MetS and NAFLD [31] and reassuringly our data shows that HOMA-IR was independently associated with hyperuricaemia, after adjustment for covariates and potential confounders. Huang *et al.* shown that hyperuricemia is associated with ALT, LDL-C, fasting glucose and NASH (NAS >5), but mostly was independently associated with greater odds of advanced lobular inflammation of NAFLD and progression to NASH [5]. Regarding this finding [5], recently it has become evident that UA is biologically active and can stimulate the production of inflammatory mediators and a high level of UA also inhibits the bioavailability of endothelial NO causing a reduction of the vasodilatation [32]. Thus, it is plausible that UA may influence risk of NASH by promoting liver inflammation and affecting liver microvascular responses.

There are strengths and limitations to our study that should be considered. We have studied 271 children and adolescents who have undergone liver biopsy to assess the severity of NAFLD. All subjects have completed a dietary questionnaire to assess their fructose consumption and this assessment may not truly reflect all dietary consumption of fructose. However, any misclassification bias would tend to attenuate the strength of our findings, and would bias our results towards the null.

In conclusion, in a cohort of children and adolescents with a histological diagnosis of NAFLD and histological confirmation of NASH, we show for the first time that UA concentrations and dietary fructose consumption are independently and positively associated with NASH. Our data also show that dietary fructose consumption (and also HOMA-IR) were positively and independently associated with hyperuricaemia.

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Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Research Article

Authors' contributions

Prof Byrne and Prof. Nobili conceptualized and designed the study, contributed to the discussion and critically revised the manuscript; Dr. Alisi, Dr. De Vito, Dr. Scorletti and Dr. Villani conceptualized and designed the study, analysed data, interpreted results and drafted the manuscript; Dr. Mosca and Dr. Crudele enrolled patients, collected growth data and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2016.12.025.

References

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[1] Alisi A, Carpino G, Nobili V. Pediatric nonalcoholic fatty liver disease. Curr Opin Gastroenterol 2013;29:279–284.
[2] Kleiner DE, Makhlouf HR. Histology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in adults and children. Clin Liver Dis 2016;20:293–312.
[3] Byrne CD, Targher G. NAFLD: a multisystem disease. J Hepatol 2015;62:S47–S64.
[4] Yki-Järvinen H. Nutritional modulation of non-alcoholic fatty liver disease and insulin resistance. Nutrients 2015;7:9127–9138.
[5] Huang Q, Yu J, Zhang X, Liu S, Ge Y. Association of the serum uric acid level with liver histology in biopsy-proven non-alcoholic fatty liver disease. Biomed Rep 2016;5:188–192.
[6] Cardoso AS, Gonzaga NC, Medeiros CC, Carvalho DF. Association of uric acid levels with components of metabolic syndrome and non-alcoholic fatty liver disease in overweight or obese children and adolescents. J Pediatr (Rio J) 2013;89:412–418.
[7] Sullivan JS, Le MT, Pan Z, Rivard C, Love-Osborne K, Robbins K, et al. Oral fructose absorption in obese children with non-alcoholic fatty liver disease. Pediatr Obes 2015;10:188–195.
[8] Scorletti E, Calder PC, Byrne CD. Non-alcoholic fatty liver disease and cardiovascular risk: metabolic aspects and novel treatments. Endocrine 2011;40:332–343.
[9] Vos MB, Lavine JE. Dietary fructose in nonalcoholic fatty liver disease. Hepatology 2013;57:2525–2531.
[10] Cortez-Pinto H, Chatham J, Chacko VP, Arnold C, Rashid A, Diehl AM. Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study. JAMA 1999;282:1659–1664.
[11] Wang CC, Tseng TC, Hsieh TC, Hsu CS, Wang PC, Lin HH, et al. Severity of fatty liver on ultrasound correlates with metabolic and cardiovascular risk. Kaohsiung J Med Sci 2012;28:151–160.
[12] World Health Organization. WHO child growth standards: methods and development [monograph on the Internet]. Geneva (Switzerland): World Health Organization; 2006. Available from: http://www.who.int/child-growth/standards/technical_report/en/.
[13] Nobili V, Marcellini M, Devito R, Ciampalini P, Piemonte F, Comparcola D, et al. NAFLD in children: a prospective clinical-pathological study and effect of lifestyle advice. Hepatology 2006;44:458–465.
[14] World Health Organization (WHO), International Diabetes Federation (IDF). Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia. Report of a WHO/IDF Consultation, 2006.
[15] Vajro P, Lenta S, Socha P, Dhawan A, McKiernan P, Baumann U, et al. Diagnosis of nonalcoholic fatty liver disease in children and adolescents: position paper of the ESPGHAN Hepatology Committee. J Pediatr Gastroenterol Nutr 2012;54:700–713.
[16] Dezsőfi A, Baumann U, Dhawan A, Durmaz O, Fischler B, Hadzic N, et al. Liver biopsy in children: position paper of the ESPGHAN hepatology committee. J Pediatr Gastroenterol Nutr 2015;60:408–420.
[17] Pietrobattista A, Fruwirth R, Natali G, Monti L, Devito R, Nobili V. Is juvenile liver biopsy unsafe? Putting an end to a common misapprehension. Pediatr Radiol 2009;39:959–961.
[18] Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et alNonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–1321.
[19] Bedossa P, Poitou C, Veyrie N, Bouillot JL, Basdevant A, Paradis V, et al. Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. Hepatology 2012;56:1751–1759.
[20] Bedossa PFLIP Pathology Consortium. Utility and appropriateness of the fatty liver inhibition of progression (FLIP) algorithm and steatosis, activity, and fibrosis (SAF) score in the evaluation of biopsies of nonalcoholic fatty liver disease. Hepatology 2014;60:565–575.
[21] **Nobili V**, **Liccardo D**, Bedogni G, Salvatori G, Gnani D, Bersani I, et al. Influence of dietary pattern, physical activity, and I148M PNPLA3 on steatosis severity in at-risk adolescents. Gene Nutr 2014;9:32.
[22] Food Composition Table, available from http://nut.entecra.it/646/tabelle_di_composizione_degli_alimenti.html.
[23] Cho SM, Lee SG, Kim HS, Kim JH. Establishing pediatric reference intervals for 13 biochemical analytes derived from normal subjects in a pediatric endocrinology clinic in Korea. Clin Biochem 2014;47:268–271.
[24] Kanbay M, Jensen T, Solak Y, Le M, Roncal-Jimenez C, Rivard C, et al. Uric acid in metabolic syndrome: From an innocent bystander to a central player. Eur J Intern Med 2016;29:3–8.
[25] Lombardi R, Pisano G, Fargion S. Role of serum uric acid and ferritin in the development and progression of NAFLD. Int J Mol Sci 2016;17:548.
[26] Liu J, Xu C, Ying L, Zang S, Zhuang Z, Lv H, et al. Relationship of serum uric acid level with non-alcoholic fatty liver disease and its inflammation progression in non-obese adults. Hepatol Res 2016. http://dx.doi.org/10.1111/hepr.12734.
[27] Ouyang X, Cirillo P, Sautin Y, McCall S, Bruchette JL, Diehl AM, et al. Fructose consumption as a risk factor for non-alcoholic fatty liver disease. J Hepatol 2008;48:993–999.
[28] Lanaspa MA, Sanchez-Lozada LG, Cicerchi C, Li N, Roncal-Jimenez CA, Ishimoto T, et al. Uric acid stimulates fructokinase and accelerates fructose metabolism in the development of fatty liver. PLoS One 2012;7 e47948.
[29] Abdelmalek MF, Lazo M, Horska A, Bonekamp S, Lipkin EW, Balasubramanyam A, et al. Higher dietary fructose is associated with impaired hepatic adenosine triphosphate homeostasis in obese individuals with type 2 diabetes. Hepatology 2012;56:952–960.
[30] Bobridge KS, Haines GL, Mori TA, Beilin LJ, Oddy WH, Sherriff J, et al. Dietary fructose in relation to blood pressure and serum uric acid in adolescent boys and girls. J Hum Hypertens 2013;27:217–224.
[31] Sirota JC, McFann K, Targher G, Johnson RJ, Chonchol M, Jalai DI. Elevated serum acid levels are associated with non-alcoholic fatty liver disease independently of metabolic syndrome features in the United States. Liver ultrasound data from the National Health and Nutrition Examination Survey. Metabolism 2013;62:392–399.
[32] Crane JK, Mongiardo KM. Pro-inflammatory effects of uric acid in the gastrointestinal tract. Immunol Invest 2014;43:255–266.

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