Higher fructose intake is inversely associated with risk of nonalcoholic fatty liver disease in older Finnish adults\textsuperscript{1–4}

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ABSTRACT
Background: High fructose intake has been suggested to be a key factor that induces nonalcoholic fatty liver disease (NAFLD), but the evidence from large epidemiologic studies is lacking.
Objective: We examined the cross-sectional association between fructose intake and NAFLD by using the Fatty Liver Index (FLI) and the NAFLD liver fat score.
Design: The Helsinki Birth Cohort Study investigated 2003 Finnish men and women born in 1943–1944 in Helsinki who participated in a clinical health examination in the years 2001–2004. Trained study nurses measured weight, height, and waist circumference, and body mass index was calculated. Laboratory staff drew fasting blood for measurements of triglycerides and \( \gamma \)-glutamyl-transferase. The FLI and the NAFLD liver fat score were calculated on the basis of these measurements. Habitual fructose and other dietary intake over the past year were assessed by using validated and standardized 131-item food-frequency questionnaires. Data were analyzed in a cross-sectional manner by using logistic regression modeling with statistical software.
Results: In a model adjusted for age, sex, and energy intake, participants in the highest fructose intake quartile (range: 29.2–88.0 g/d) had lower risk of NAFLD assessed by using the FLI (OR: 0.56; 95% CI: 0.42, 0.75; \( P \text{-trend} < 0.001 \)) and NAFLD liver fat score (OR: 0.72; 95% CI: 0.53, 0.99; \( P \text{-trend} < 0.001 \)) than that of the lowest intake quartile (range: 2.2–15.2 g/d). This association remained after adjustment for educational attainment, smoking, physical activity, and other dietary variables only for the FLI (OR: 0.68; 95% CI: 0.47, 0.84; \( P \text{-trend} < 0.05 \)).
Conclusion: Our cross-sectional results did not support the current hypothesis that high intake of fructose is associated with a higher prevalence of NAFLD as assessed by using the FLI and NAFLD liver fat score. 

INTRODUCTION
Nonalcoholic fatty liver disease (NAFLD)\textsuperscript{5}, which is the hepatic manifestation of the metabolic syndrome (MetS) (1, 2), is the most common cause of chronic liver disease in the developed world with a prevalence ranging from 20% to 30% (3, 4). NAFLD is present when the hepatic fat content exceeds 5–10% of liver mass in the absence of other causes of steatosis such as other liver disorders, excessive alcohol intake, and use of hepatotoxic medication (5). The condition is closely associated with type 2 diabetes and obesity (6, 7) and, in addition, is an independent risk factor for cardiovascular disease (8). A continuum of conditions is included in the NAFLD diagnosis from simple steatosis via nonalcoholic steatohepatitis to fibrosis and cirrhosis (9).

The pathogenesis of NAFLD has been described by a “multi-hit” hypothesis, the key pathogenic mechanism of which is insulin resistance (1, 9). Hepatic lipid accumulation (ie, steatosis) represents the first hit, whereas several second hits (eg, inflammatory cytokines) are involved in the progression of the disease toward nonalcoholic steatohepatitis and beyond. Several factors have been implicated in the development of NAFLD and its progression, including carbohydrate intake. Fructose intake has received special attention, and several previous studies have linked high fructose intake with NAFLD (10). Fructose stimulates de novo lipogenesis in the liver that leads to the production of triglycerides, which, in turn, can be secreted as VLDLs. If this process is impaired, triglycerides can accumulate intracellularly and lead to steatosis (11). There have been some studies that indicated that fructose intake is increased in individuals with NAFLD, but the evidence has been inconsistent (12–14).

We have previously studied the prevalence of NAFLD in the Helsinki Birth Cohort Study (HBCS) from a developmental origins of health and disease perspective (15). In that study, we used an algorithm test, the NAFLD liver fat score and equation (16), to define NAFLD. We showed that a small body size during childhood was associated with adult NAFLD, especially in...
individuals who were obese as adults. The aim of the current study was to explore the associations between fructose intake and NAFLD.

SUBJECTS AND METHODS

The HBCS included 8760 participants who were born at Helsinki University Central Hospital as singletons between 1934 and 1944 and were still alive and resident in Finland in 1971 when every citizen was given a unique personal identification number. In the year 2000, cohort members were sent questionnaires that resulted in replies from 4515 subjects. Of respondents, a sample of 2902 individuals was derived by using random-number tables and invited to a clinical examination conducted in the years 2001–2004. Eventually, 2003 individuals participated in the health examination. The study was conducted according to the guidelines of the Declaration of Helsinki, and the Ethics Committee of the Hospital District of Helsinki and Uusimaa approved all procedures involving human subjects. Written informed consent was obtained from all participants.

At the study clinic, self-administered questionnaires were used to inquire about socioeconomic characteristics (eg, educational attainment), lifestyle factors (eg, smoking status), and current medication. The current use of antidiabetic medication and WHO criteria were used to define type 2 diabetes diagnosis (17). The MetS was defined according to harmonized criteria (18).

The Fatty Liver Index (FLI) (19) was calculated for all participants and was used to define the presence of NAFLD. The FLI is an algorithm test that consists of the following 4 variables: BMI (in kg/m²), waist circumference, fasting triglycerides, and fasting γ-glutamyl-transferase. In the original publication, an FLI >60 predicted NAFLD with a specificity of 86% and positive likelihood ratio of 4.3 against a diagnosis by using ultrasound. On the basis of this cutoff, a dichotomous variable was constructed with values >60 defined as a positive FLI. In the context of this publication, a positive FLI and the presence of NAFLD were used synonymously. We also calculated the NAFLD liver fat score (main outcomes, dichotomous) for all participants for reasons of comparison. This algorithm test is based on the following 5 variables: the presence of type 2 diabetes, presence of MetS, fasting insulin, serum aspartate aminotransferase (AST), and the AST:aminotransferase ratio.

At the clinic, trained study nurses measured height to the nearest 0.1 cm and weight to the nearest 0.1 kg. BMI was calculated by dividing weight by the square of height. Fasting blood samples were drawn and stored at −70°C before analysis. Blood samples included a standard 75-g oral-glucose-tolerance test. A hexokinase method (20) was used to measure plasma glucose; a 2-site immunometric assay (21) was used to measure plasma insulin; and photometric International Federation of Clinical Chemistry methods were used to measure aminotransferase, AST, and γ-glutamyl-transferase concentrations by using an Architect ci 82000 analyzer (Abbott Laboratories).

At the study site, participants completed a validated food-frequency questionnaire (FFQ) (22, 23), which was reviewed by a trained study nurse. The FFQ measured the habitual diet over the previous 12 mo. The consumption of each 128 FFQ item was reported by using 9 frequency categories that ranged from never or seldom to ≥6 times/d. The predefined sex-specific portion sizes for food items appeared as household and natural units (eg, glass and slice). A nutritionist entered data into the study database. The average daily intakes of foods, nutrients, and energy intakes were calculated by using the Finnish National Food Composition Database (Fineli) and in-house software (24).

From 2003 participants, those who reported ethanol intake ≥20 g/d (n = 244) were excluded. Because of current or previous liver disease, 80 subjects were excluded. These individuals were identified via cross-referencing with the national hospital discharge register and the national mortality register. We performed a search for International Classification of Diseases (ICD) codes related to liver disease in both registers (ICD 8–9: 070, 155, 291, 303, 570, 571, 572, and 573; ICD 10: B15–19, C22, F10, G62, K70–77, and K86). Of 80 excluded subjects, 71 individuals had an alcohol-related diagnosis, but no cases of explicit nonalcoholic steatosis or steatohepatitis were shown in either register. Furthermore, 54 subjects were excluded because of the use of medication associated with secondary hepatic steatosis according to American Association for the Study of Liver Diseases guidelines on the diagnosis and management of NAFLD (25). In addition, participants in this study were not allowed to have any missing values in main exposure (ie, dietary data) or outcome (NAFLD data) variables. Participants with an implausible energy intake (n = 8) and incomplete measurements for the FLI calculation (n = 6) were excluded. Final data included 1611 participants for the analysis.

We performed statistical analyses with the use of R statistical software version 2.15.1 (26). Because the test for the interaction between sex and outcome variables was P > 0.05, we conducted analyses together for men and women. All nutrient variables were adjusted for energy by using the residual method (27). We reported descriptive data by grouping individuals by intake of fructose as the mean (±SE) or percentage. A logistic regression analysis was used to calculate ORs and 2-sided 95% CIs for the FLI and NAFLD liver fat score (main outcomes, dichotomous) by energy-adjusted fructose intake quartiles (main explanatory variable). The selection of confounding variables was based on the current literature. Furthermore, the variable had to be associated with both the outcome and exposure in HBCS data to be included in the modeling (28). Model 1 was adjusted for age (continuous; y), sex (dichotomous; male or female), and intake of energy (continuous; kJ/d). Model 2 was further adjusted for smoking status (categorical: current smoker, former smoker, or never smoker) and leisure-time physical activity (categorical: no exercise, exercise 1–3 times/wk, or exercise ≥4 times/wk), and model 3 was further adjusted for dietary variables such as fiber (continuous; g/d), total fat intake (continuous; g/d), alcohol (continuous; g/d), and vitamin E (continuous; alpha-tocopherol equivalents in mg) intakes.

RESULTS

According to the FLI, 663 participants (44%) were classified as having NAFLD. Correspondingly, 511 participants (46%) were classified as having NAFLD when assessed by using the NAFLD liver fat score. The agreement between the 2 scores was 76.3% for a negative finding and 78.9% for a positive finding. Participants in the highest quartile of fructose intake were more likely women than men, had more years of education, and were less likely to be current smokers or physically inactive than those in the lowest fructose intake quartiles (Table 1).
Table 1
Participant characteristics according to intakes of fructose

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>403</td>
<td>403</td>
<td>402</td>
<td>403</td>
<td>—</td>
</tr>
<tr>
<td>Fructose (g/d)</td>
<td>2.2–15.2</td>
<td>15.3–21.8</td>
<td>21.9–29.1</td>
<td>29.2–88.0</td>
<td>—</td>
</tr>
<tr>
<td>Fructose (g/d)</td>
<td>10.6 ± 1.0</td>
<td>18.6 ± 1.0</td>
<td>25.2 ± 1.0</td>
<td>38.1 ± 1.0</td>
<td>—</td>
</tr>
<tr>
<td>Women (%)</td>
<td>41</td>
<td>55</td>
<td>67</td>
<td>76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (y)</td>
<td>61.6 ± 0.2</td>
<td>61.7 ± 0.2</td>
<td>61.5 ± 0.2</td>
<td>61.6 ± 0.2</td>
<td>0.91</td>
</tr>
<tr>
<td>Educational attainment (y)</td>
<td>11.4 ± 0.2</td>
<td>12.0 ± 0.2</td>
<td>12.4 ± 0.2</td>
<td>12.6 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>38</td>
<td>17</td>
<td>18</td>
<td>13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Physically inactive participants (%)</td>
<td>16</td>
<td>9</td>
<td>9</td>
<td>10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.7 ± 0.2</td>
<td>27.8 ± 0.2</td>
<td>27.4 ± 0.2</td>
<td>27.5 ± 0.2</td>
<td>0.35</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>95.7 ± 0.6</td>
<td>95.4 ± 0.6</td>
<td>93.9 ± 0.6</td>
<td>94.2 ± 0.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>81</td>
<td>82</td>
<td>81</td>
<td>81</td>
<td>0.58</td>
</tr>
<tr>
<td>Hypertriglyceridemia (%)</td>
<td>32</td>
<td>32</td>
<td>26</td>
<td>26</td>
<td>0.06</td>
</tr>
<tr>
<td>Reduced HDL-cholesterol (%)</td>
<td>15</td>
<td>13</td>
<td>11</td>
<td>10</td>
<td>0.08</td>
</tr>
<tr>
<td>Elevated fasting glucose (%)</td>
<td>51</td>
<td>51</td>
<td>47</td>
<td>46</td>
<td>0.23</td>
</tr>
<tr>
<td>Patients with diabetes (%)</td>
<td>17</td>
<td>14</td>
<td>12</td>
<td>13</td>
<td>0.36</td>
</tr>
<tr>
<td>Metabolic syndrome (%)</td>
<td>54</td>
<td>54</td>
<td>47</td>
<td>49</td>
<td>0.08</td>
</tr>
<tr>
<td>NAFLD (FLI) (%)</td>
<td>48</td>
<td>44</td>
<td>38</td>
<td>34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NAFLD liver fat score (%)</td>
<td>35</td>
<td>32</td>
<td>29</td>
<td>28</td>
<td>0.06</td>
</tr>
<tr>
<td>Energy intake (kJ/d)</td>
<td>8970 ± 160</td>
<td>9230 ± 160</td>
<td>9420 ± 160</td>
<td>9110 ± 160</td>
<td>0.07</td>
</tr>
<tr>
<td>Fiber (g/d)</td>
<td>22.8 ± 0.4</td>
<td>26.1 ± 0.4</td>
<td>28.1 ± 0.4</td>
<td>32.4 ± 0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total fat (g/d)</td>
<td>82.2 ± 0.5</td>
<td>76.9 ± 0.5</td>
<td>73.6 ± 0.5</td>
<td>66.3 ± 0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alcohol (g/d)</td>
<td>13.9 ± 0.6</td>
<td>14.5 ± 0.6</td>
<td>13.2 ± 0.6</td>
<td>12.1 ± 0.6</td>
<td>0.22</td>
</tr>
<tr>
<td>Vitamin E (α-tocopherol equivalents; mg/d)</td>
<td>12.2 ± 0.2</td>
<td>13.2 ± 0.1</td>
<td>13.4 ± 0.1</td>
<td>13.3 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1 Values were adjusted for age and sex. All nutrient intakes were also adjusted for intake of energy by using the residual method (27). P values for trends were obtained from linear regression for continuous variables and logistic regression for binary variables. P-values were adjusted for age and sex. For nutrient intakes, P-values were additionally adjusted for intake of energy. FLI, fatty liver index; NAFLD, nonalcoholic fatty liver disease.

2 All values are ranges.
3 Mean ± SEM (all such values).
4 Systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg.
5 Triglyceride concentration ≥1.7 mmol/L.
6 HDL concentration <1.0 mmol/L (men) or <1.3 mmol/L (women).
7 Fasting glucose concentration ≥5.55 mmol/L.

Furthermore, waist circumference tended to decrease across the fructose intake quartiles. Participants in the highest quartile of fructose intake had also higher intakes of fiber and vitamin E but lower intake of fat.

In the model adjusted for age, sex, and intake of energy (model 1), intake of fructose was negatively associated with NAFLD when assessed by using the FLI or NAFLD liver fat score (Table 2). Subjects in the highest quartile of fructose intake were 28–44% less likely to have NAFLD than were those in the lowest intake quartile. After additional adjustment for lifestyle and nutrient intake variables (model 3), the association attenuated to 32% for the FLI but remained significant (95% CI: 0.47, 0.97; P-trend < 0.05). After further adjustments, the association did not remain for the NAFLD liver fat score (model 3; Table 2).

Discussion

To our knowledge, this study is among the first large-scale studies to report a cross-sectional association between fructose intake and NAFLD assessed by using the FLI and the NAFLD liver fat score. Participants with higher fructose intake were significantly less likely to have a positive FLI score independently of the participants’ age, sex, lifestyle, and various nutrients. When NAFLD was assessed by using the NAFLD liver fat score, no association between intake of fructose and NAFLD emerged.

Much of the evidence on the association of fructose intake and NAFLD has been based on animal studies that showed that high fructose intake increased lipogenesis and triglyceride concentration, lowered HDL cholesterol concentrations, and lead to NAFLD (29). However, the few human trials and small-scale (n < 500) observational studies have provided inconsistent results (12, 14). A meta-analysis of 16 trials did not find any effect on HDL cholesterol when other carbohydrates were exchanged for fructose (13), and higher intake of fructose did not change triglycerides concentrations in healthy individuals (13, 14). In contrast, higher fructose intake has been associated with a lower prevalence of steatosis and a higher fibrosis grade in older subjects (12). Our findings in a cohort of 1611 adult Finns supports the evidence that higher intake of fructose is not associated with a higher prevalence of NAFLD.

The conflicting results between animal studies, experimental human trials, and our findings may have stemmed from the different levels of exposure. Animal and human feeding studies have been criticized for providing supraphysiologic doses of fructose that have varied typically between intake of 60–220 g/d. In our cohort, eg, the median fructose intake was 20 g/d, and only 45 participants had fructose intake ≥60 g/d. Because
fructose is approximately one-half of the intake of sucrose, these numbers are in line with the sucrose intake in the general Finnish population, which is 54 g/d for men and 43 g/d for women (30). When sugars were compared with other macronutrients, an isocaloric high-fat diet increased liver fat and insulin concentrations more than did an isocaloric high-carbohydrate diet (31). Furthermore, in healthy individuals, overfeeding with fructose increased liver fat less than did overfeeding with fat (32). Overall, it seems that adverse changes related to fructose intake have been more due to excess caloric intake rather than sugar composition (33). This finding has been consistent in clinical trials conducted thus far (34). Our results, which adjusted fructose intake for the intake of energy, also suggest that, in an energy-controlled situation, fructose does not associate with an increased prevalence of NAFLD.

Main sources of fructose in the Finnish population are added table sugar, soft drinks, and fruit. Previous studies have established a strong association between soft-drink consumption and an increased prevalence of NAFLD (35, 36). Recently, Petta et al (37) examined 147 biopsy-proven NAFLD patients and showed that intake of industrial but not fruit-originated fructose was independently associated with adverse metabolic features as well as severity of liver fibrosis. These results imply that the source of fructose plays a significant role in inducing NAFLD. In our study, fructose that came from fruit or soft drinks was not separated, which may have confounded the results. In the study population, the proportion of men and women who consumed fruit (excluding fruit juices) was 54–80%, whereas the proportion who consumed soft drinks (excluding artificially sweetened soft drinks) was between 0 and 8%. In subjects who consumed soft drinks, median intake was 42 g/d (nonadjusted), whereas for fruit, the median intake was 230 g/d (nonadjusted). On the basis of this information, it could be assumed that the participants were more likely to receive more fructose from fruit than from soft drinks. Thus, in our analysis, the possible protective effect of fruit on NAFLD may have overcome the possibly harmful effect of soft drinks and NAFLD. Because of the complex nature of the diet, the effect of fructose source on the development of NAFLD requires additional investigation.

Strengths of our study included a relatively large sample size, validated and standardized measurements as well as laboratory analyses, and the careful control of confounding variables. The FFQ has been culturally adapted to the Finnish population (22), and the version of the FFQ used in this study has been validated against 3-d dietary records in the general Finnish population (22, 23). However, there were also some limitations. Our sample was not population-based, which limited the generalizability of our results. Because the study design was cross-sectional, no conclusions could be drawn about the causal relation between fructose intake and prevalence of NAFLD. Thus, we could not rule out the possibility that the prevalence of NAFLD leads to a lower consumption of fructose, eg, due to dietary counseling that NAFLD patients may have received. Furthermore, the FFQ’s validity to measure dietary intake in NAFLD patients has not been separately examined. An overunderestimation or underestimation of fructose-containing foods (eg, fruit and soft drinks) may have affected our results to some extent. Fructose can also be seen as a surrogate for a healthy or unhealthy lifestyle, which is probably associated with NAFLD. We carefully controlled the analysis for lifestyle factors such as physical activity and smoking, but some residual confounding could still have remained because measurements of these factors were based solely on questionnaires. Moreover, we may have failed to take some unknown factor into account (eg, a genetic factor) associated with fructose intake, which also could have confounded the results.

The NAFLD diagnosis was based on the FLI algorithm test rather than the gold standard liver biopsy, which limited conclusions of our study. A liver biopsy would not have been a feasible option in our study setting because it is time consuming, costly, and associated with a small but notable procedure-related morbidity and even cases of mortality (38). Alternative methods of diagnosing NAFLD include the use of ultrasound, computer tomography, and magnetic resonance...
imaging. However, these methods were not included in the HBCS protocol. In a previous publication, in which associations between early growth and NAFLD were studied (15), we used the NAFLD liver fat score and equation (16) to define NAFLD. For purposes of the current study, we also used the FLI because it is more widely used. The FLI was validated against an ultrasound in the original publication and has been used in several publications as a surrogate for NAFLD (39–41). An important limitation in the use of an algorithm test, rather than a more-objective method, was risk of misclassification. We are currently conducting a validation study of both algorithm tests against a diagnosis by using magnetic resonance spectroscopy on a small subset of the study population (n = 41). In the comparison of the 2 scores, the NAFLD liver fat score has had a better balance in sensitivity and specificity (NAFLD liver fat score: sensitivity of 85% and specificity of 71%; FLI: sensitivity of 61% and specificity of 86%). Because of a higher probability of detecting false positives, the FLI score may have incorrectly classified individuals with a healthy liver and their dietary habits to the NAFLD cohort, thereby skewing the true trend between fructose intake and NAFLD cases and leading to inconsistent results between the 2 scores. However, the level of agreement between the 2 scores was considered acceptable (76.3% for a negative finding and a 78.9% for positive finding).

In conclusion, high intake of fructose was not associated with higher prevalence of NAFLD assessed by using the FLI and NAFLD liver fat score. Because calculations of daily nutrient intakes did not separate whether the fructose came from natural or artificial sources, a major question about the possible different effects of these 2 sources on the development of NAFLD remains. Furthermore, because our study was cross-sectional and concerned older adult Finns born in Helsinki, population-based longitudinal studies are needed to confirm the causality of these associations.

The authors’ responsibilities were as follows—JGE: designed the HBCS and was responsible for the original study idea; SM and NEK: were responsible for dietary data collected in the HBCS and critically revised the manuscript; SS and NEK: participated in data handling; NK: performed statistical analyses; NK and SS: wrote the manuscript and had primary responsibility for the final content of the manuscript; and all authors: approved the final manuscript. None of the authors had a conflict of interest.

REFERENCES