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TITLE PAGE

Lower fructose intake may help protect against development of non-alcoholic fatty liver in obese adolescents

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Abbreviations: NAFL = non-alcoholic fatty liver, ALT = alanine transaminase, AST = aspartate transaminase, GGT= gamma glutamyl transpeptidase, BMI = body mass index.

ABSTRACT

Objectives: Although obesity is a major risk factor for non-alcoholic fatty liver (NAFL), not all obese individuals develop the condition, suggesting that other factors such as diet may also contribute to NAFL development. We evaluated associations between fructose and total sugar intake and subsequent diagnosis of NAFL in obese and non-obese adolescents in a population-based cohort.

Methods: Adolescents participating in the Western Australian Pregnancy Cohort (Raine) Study completed 3-day food records and body mass index measurement at age 14 years. At age 17 years, subjects underwent abdominal ultrasound to determine NAFL status. Multivariate logistic regression models were used to analyse associations between energy-adjusted fructose and total sugar intake and NAFL status. Food diaries and liver assessments were completed for 592 adolescents.

Results: Prevalence of NAFL at age 17 was 12.8% for the total group, and 50% for obese adolescents. Fructose intake did not significantly differ between adolescents with or without NAFL in our cohort as a whole. Among obese adolescents, those without NAFL had significantly lower energy-adjusted fructose intake at age 14 years compared with those with NAFL (mean±SD 38.8±19.8 g/day, vs. 55.7±14.4 g/day P=0.02). Energy-adjusted fructose intake was independently associated with NAFL in obese adolescents (OR=1.09, 95% CI: 1.01-1.19, p=0.03) after adjustment for confounding factors. Energy-adjusted total sugar intake showed less significance (OR=1.03, 95% CI: 0.999-1.07, p=0.06). No significant associations were observed in other BMI categories.

Conclusions: Lower fructose consumption in obese adolescents at 14 years is associated with a decreased risk of NAFL at 17 years. Fructose rather than overall sugar intake may be more physiologically relevant in this association.

Keywords: Raine study, diet, fatty liver, fructose, adolescent

INTRODUCTION

Non-alcoholic fatty liver (NAFL) is reported to be an important cause of abnormal liver tests in adults as well as children (1, 2), although pediatric NAFL is often under diagnosed (3). In the Western Australian Pregnancy (Raine) Cohort, a NAFL prevalence of 13% in the 17 year follow-up has been reported (4), similar to previous reports in US (17%) (5) and Australian teenagers (10%) (6). Development of NAFL in adolescents may differ from that of adults, due in part to the metabolic disturbances that occur during periods of active growth such as puberty (7). Like adults, adolescents who are obese are more likely to have NAFL (4). Obesity is recognized as one of the major risk factors, while intestinal bacterial flora, hormones and dietary factors are also suggested to influence pediatric NAFL (3).

In relation to dietary risk factors for NAFL, fructose intake has been found to be 2-3 times higher in adults with NAFL disease compared to controls in a small US study (8), and intake was associated with increased fibrosis within a group of NAFL disease patients (9). On the other hand, in the latter study, fructose was also associated with decreased hepatic steatosis, adding to the controversial nature of this topic. Fructose is found in foods including fruit juice and sweeteners such as high fructose corn syrup. It is also found in conjunction with glucose in the form of sucrose, commonly known as table sugar. Sugar sweetened soft drinks, commonly manufactured using either high fructose corn syrup (55% fructose and 45% glucose) or sucrose (50% fructose and 50% glucose) have previously been associated with an increased risk of NAFL disease (10). Simple sugars, incorporating monosaccharides glucose, fructose and galactose, and disaccharides sucrose, lactose and maltose, have been linked with chronic disease through markers of heart disease, obesity, and poor diabetes control (11). However, for a given amount, fructose may have more effect on pathogenesis of NAFL compared to sugar as a whole, due to the ability of fructose to stimulate hepatic triglyceride synthesis (12). Unlike glucose, virtually all fructose is metabolized in the liver

and is more readily converted to fatty acids. Fructose also has the unique ability to raise uric acid, suggesting it may have a different role in metabolic disease than other sugars (13). Further, the unique relationship between fructose and uric acid also includes other metabolic derangement, such as inducing metabolic syndrome and hypertension, beyond what is observed with sucrose alone (14, 15).

However the relationship between fructose and NAFL requires further investigation, as there are limited large population-based studies published in the area of fructose and NAFL in adolescence. In addition, the link between fructose and NAFL in higher risk groups, such as in those who are obese, needs clarification. Furthermore, previously published studies have been cross-sectional in design and have not been able to identify whether fructose consumption is associated with future NAFL. We aimed to investigate intake of fructose at 14 years of age in relation to NAFL at 17 years of age, to determine whether this relationship differs according to obesity status, and to investigate whether fructose was more predictive of NAFL status than total sugar intake.

MATERIALS AND METHODS

Subjects

Subjects were adolescents participating in the population-based Western Australian Pregnancy Cohort (Raine) Study which is a longitudinal birth cohort (16). The Raine Study began with pregnant women enrolled through the public antenatal clinic at King Edward Memorial Hospital and nearby private clinics in Perth, Western Australia from 1989-1991, with babies subsequently followed up at regular intervals. This study reports on associations between dietary data from the 14-year follow-up (mean±SD age 14.0±0.2 y) and NAFL status at the 17-year follow-up (17.0±0.2 y). NAFL assessment was not performed at the 14-year follow-up. Derivation of the study group for this research is illustrated in Figure 1. The

study was approved by the ethics committees of King Edward Memorial Hospital and Princess Margaret Hospital for Children. Adolescents and their parent or guardian gave informed written consent.

Assessment of dietary intake

Raine Study adolescents who attended the 14-year follow-up assessment at the Telethon Institute for Child Health Research in Perth, Western Australia, were given instructions and a 3-day food record booklet to complete. Metric measuring cups and spoons were provided to assist with quantification of serve sizes and parental assistance was allowed if required. Adolescents were asked to indicate whether the food record for each of the three days represented their usual eating habits. A dietitian checked each record as it was returned, and clarified any potentially vague or missing details with adolescents over the telephone (17). Data were analysed using FoodWorks (Professional Version 4.00, Xyris Software, Brisbane). Total sugars were calculated as the sum of free monosaccharides and disaccharides. As FoodWorks does not generate fructose values, a customised fructose database was incorporated for foods with at least 0.1 g of carbohydrate per 100 g. Fructose values were sourced from the Food Standards Australia New Zealand database (18), the University of Minnesota Nutrition Coordinating Centre (19) and the Canadian Nutrient File (20). A total fructose value (g) per 100 g was calculated as: free fructose (g) per 100 g + ½ sucrose (g) per 100 g. Some foods that could not be matched to an individual food were matched on an ingredient basis. In total 99.7% of foods containing at least 0.1 g of carbohydrate per 100 g were allocated fructose values (21).

Assessment of NAFL and liver enzymes

A liver investigation comprised of liver ultrasound and liver enzyme assessment was first included in the Raine Study at the 17-year follow-up and was not done as part of the study

before this time. Based upon a self-report questionnaire completed by the participant and parent, no participants had been diagnosed with NAFL prior to age 17. Liver ultrasounds by trained ultrasonographers were used in the 17-year follow-up to determine the absence or presence of NAFL. A Siemens Antares ultrasound machine was used with a CH 6-2 curved array probe (Sequoia, Siemens Medical Solutions, Mountain View, CA) using a standardized protocol (22). Ultrasound images were interpreted by a specialist radiologist with fatty liver diagnosed according to the schema developed by Hamaguchi and colleagues which has 92% sensitivity and 100% specificity for the diagnosis (22). Adolescents with sonographic fatty liver and a weekly alcohol intake of less than 140 g for males and 70 g for females over the previous 12 months were classified as having NAFL (23). Adolescents completed a self-report questionnaire at the 17-year follow-up regarding their alcohol intake over the previous year (24). Liver enzymes alanine transaminase (ALT), aspartate transaminase (AST) and gamma glutamyl transpeptidase (GGT) were measured from blood samples using standard laboratory methods in a centralised laboratory.

Assessment of body mass index category

Body mass index (BMI) (weight in kilograms divided by height in metres squared) was calculated at the 14-year follow-up. Weight was measured to the nearest 100 g, using a Wedderburn Digital Chair Scale, and height was measured to the nearest 0.1 cm with a Holtain Stadiometer. Standard adolescent criteria were used to classify participants into BMI categories of underweight, normal weight, overweight, and obese (25, 26): *i)* underweight, BMI less than or equal to 16.41 kg/m^2 in males and 16.88 kg/m^2 in females, *ii)* normal weight, BMI of between 16.42–22.6 kg/m^2 in males and 16.89–23.3 kg/m^2 in females, *iii)* overweight, BMI of between 22.7–27.5 kg/m^2 in males and 23.4–28.5 kg/m^2 in females and *iv)* obese, BMI $\geq$27.6 kg/m^2 in males and $\geq$28.6 kg/m^2 in females.

Potential confounding variables

Information relating to maternal age, maternal education, family structure, and family income was obtained by parental report. An estimate of aerobic fitness was obtained using the Physical Working Capacity (PWC 170) test, which measures power output (watts) on a bicycle ergometer at a heart rate of 170 beats per minute (27). The Tanner stages of pubic hair development was used to assess puberty across both genders (28, 29). In a privately completed questionnaire, adolescents were asked to select their corresponding developmental stage from a set of standard drawings depicting Tanner stages two (sparse) to five (adult). Stage one was omitted as an option as this corresponds to a pre-pubescent period, defined as younger than 10 years of age. Gender, dietary fibre and saturated fat intakes were also considered as potential confounding factors.

Statistical analysis

Nutrient intakes were adjusted for energy using the residuals method (30) and described using means ± SD. The residuals method takes into account differences in energy requirements among the adolescents, and values were standardized by the mean nutrient intake for the average total energy intake in the study population. Fructose and total sugar intakes are energy-adjusted unless otherwise specified. One way ANOVA was used to analyse fructose and total sugar intakes over BMI categories. Independent t-tests, Mann-Whitney tests, chi-square tests or Fisher's exact tests were used to investigate associations between potential confounding factors and NAFL. Correlations between intakes and liver enzymes were analysed using Spearman's rho as liver enzyme data was not normally distributed. Multivariate logistic regression models were used to analyse associations between dietary intake and NAFL status, and linear regression was used for liver enzymes. Liver enzyme variables were log transformed to create a more normal distribution.

Independent t-tests were used to examine differences in liver enzymes and NAFL status. Analyses were performed using the Statistical Package for Social Sciences for Windows, Rel. 18.0.0 2011 (Chicago: SPSS Inc) and statistical significance was set at $P<0.05$.

RESULTS

Subjects

Differences between adolescents who were included and excluded in dietary analysis have been reported previously, with those participating in dietary assessment more likely to have a lower BMI and waist circumference, older mothers or higher family income (31). The prevalence of overweight and obesity in the cohort was 17% and 5%, respectively. Liver enzymes at age 17 years, aerobic fitness, family status, family income and daily dietary intake of carbohydrates and total sugars at age 14 differed significantly by BMI category (Table 1).

NAFL

The prevalence of NAFL in the group at age 17 years was 12.8% (n= 76/592). There were no significant differences in puberty stage between NAFL and non-NAFL groups (P=0.979). Rates of NAFL differed across the four BMI categories, with obese adolescents having the highest prevalence of NAFL at 50% (Table 2). ALT was significantly higher in adolescents with NAFL compared to those without (log values used for statistical analysis 3.10 vs 2.90, respectively; raw values 28.0 IU/L vs 20.2 IU/L), although no significant differences were observed with AST or GGT. Also, there were no significant differences in liver enzymes by NAFL status across BMI category.

Fructose and total sugar intake and NAFL diagnosis

Fructose intake observed in the present study did not significantly differ between adolescents with or without NAFL when our cohort was analysed as a whole group. Fructose intake was similar across all BMI subgroups, including obese adolescents with NAFL; the notable exception was that obese adolescents without NAFL consumed less fructose compared to all other subgroups. In obese individuals, fructose intake at age 14 was significantly lower in adolescents without NAFL at age 17 compared to adolescents with NAFL (38.8±19.8 g vs 55.7±14.4 g, respectively; P=0.01) (Figure 2). Among underweight individuals (n=39), fructose intake was higher in those with NAFL (70.9±3.3 g vs 53.7±19.1 g); however, this was not statistically significant (p=0.2). Fructose intake did not significantly differ between adolescents with or without NAFL as a total group or in subgroups of normal weight or overweight (Table 2). Total sugar, carbohydrate, fat and protein intakes were not significantly different by NAFL status. As a total group, fructose and total sugar intake were significantly correlated with liver enzymes ALT (both r=-0.13, P<0.01) and fructose with GGT (r=-0.09, P=0.03). AST was not significantly associated with either dietary factor.

Multivariate regression models

Gender was the only confounding factor identified that was significantly associated or showed borderline significance with both NAFL status and fructose intake, with females more likely to have NAFL (P=0. 041) and higher fructose intakes (independent samples t-test, P=0.066). Although participants from single parent families were more likely to have NAFL (P=0.016), this family characteristic was not significantly associated with fructose intake (P= 0.672), so it was not included as a confounding factor. Family income, maternal age and education, energy-adjusted saturated fat intake, dietary fibre and aerobic fitness at age 14 were not significantly associated with NAFL status at age 17 and were not included as confounding factors.

After adjustment for gender, there were no significant associations between intakes of fructose or sugar for the study cohort as a whole, or in the separate groupings of underweight, normal weight and overweight adolescents in relation to NAFL status (Table 3). However, in obese adolescents, higher fructose intake was associated with significantly increased odds of NAFL – for every one gram increase in energy adjusted fructose, odds of NAFL increased by 9% (OR=1.09, 95% CI: 1.01-1.19). Total sugar intake was also positively associated with risk of NAFL, approaching significance (OR=1.03, 95% CI: 0.999-1.07).

Subjects were categorised according to whether they were obese or not to investigate whether an interaction existed with fructose intake. The interaction variable 'obesity status × energy-adjusted fructose' was significant in the multivariate model (P=0.032) indicating that fructose was associated differently with NAFL risk depending on obesity status (non-obese as reference, OR=1.07, 95% CI 1.01-1.15). We further divided subjects into four groups on the basis of their obesity status and fructose intake (fructose groups divided at median intake): 1) non-obese and low fructose, 2) obese and low fructose, 3) non-obese and high fructose, and 4) obese and high fructose. Compared to group 1, groups 2 (P=0.018) and 4 (P<0.001) showed significantly increased risk of NAFL (Table 4).

In linear regression models adjusted for gender, ALT was the only liver enzyme significantly associated with fructose intake for the group as a whole (β=-0.003, 95% CI: -0.005-0.000, P=0.02). By BMI category, significant associations were observed for the overweight group for ALT (β =-0.007, 95% CI: -0.012- -0.002, P=0.006) and AST (β=-0.003, 95% CI: -0.006-0.000, P=0.032), but not GGT or any other BMI categories.

DISCUSSION

To our knowledge, this is the first study to highlight the potential role of fructose in the development of NAFL within obese adolescents in a prospective population based cohort**,** with the caveat that NAFL status at baseline assessment was not known and there is a large exclusion of subjects from the population-based cohort due to food intake not being available. Obese adolescents without NAFL reported a markedly lower energy-adjusted fructose intake compared to the other BMI-category groups, while the mean intake in the obese NAFL group was similar to intakes in the other BMI-category groups (with or without NAFL) (Figure 2). These results suggest that lower than usual fructose intake may be protective against the development of NAFL in obese adolescents, as opposed to a high fructose intake causing an increased risk of NAFL. As those who are obese are more predisposed to chronic disease, intake of fructose at lower rather than usual amounts may required to prevent NAFL. Factors other than fructose intake may be more important contributors to NAFL development in adolescents who are not classified as obese.

Adolescents who were in the higher half of fructose consumers and who were also obese at age 14 years were much more likely to have NAFL at 17 years compared to non-obese adolescents in the lower half of fructose consumers. In adolescents classified as obese at age 14 years, each gram of daily fructose consumed at this age was associated with a 9% increased risk of NAFL at age 17 years. Similarly, each gram of daily total sugar intake was associated with a 3% increased risk, although this finding did not reach statistical significance. Mean daily intakes of these nutrients by adolescents in the obese group (on an energy-adjusted basis) were 47 g and 118 g, respectively. Our findings indicate that an increase in daily fructose ingestion of 10% (4.7 g) by obese adolescents corresponds to an increase in risk of NAFL diagnosis of 50% (OR 1.50, 95% CI: 1.05-2.27). To put this into

perspective, in Australia a 330 ml can of sugar sweetened soft drink supplies approximately 17.5 g of fructose.

We found associations with fructose and NAFL in the obese group, but not in other BMI categories. At 14 years of age, obese teenagers who were not diagnosed with NAFL at 17 years consumed diets significantly lower in fructose than those who were not diagnosed with NAFL (Table 2). Although a number of factors have been identified as increasing the risk of NAFL, obesity is considered to be the most dominant risk factor (32). Our results also showed that family structure, in terms of whether the adolescent was residing in a one or two parent family, was also predictive of NAFL. This suggests that socioeconomic and environmental factors may also predispose adolescents to NAFL.

Obesity contributes to an overabundance of circulating free fatty acids and to insulin resistance. When the rate of lipid input to the liver through fatty acid uptake and insulin-stimulated *de novo* triglyceride synthesis exceeds the rate of lipid output through fatty acid oxidation and triglyceride secretion, steatosis develops (33). Although a major risk factor, not all people with obesity develop NAFL, with one third of obese adults having less than 5.5% hepatic fat as determined by magnetic resonance spectroscopy (34). This may be a result of preferential subcutaneous adipose distribution, genetics or lifestyle factors, particularly differences in diet.

Our study suggests that dietary fructose could be one of the differences contributing to whether or not an obese adolescent develops NAFL, supporting the results of previous studies on fructose and NAFL. Rats fed fructose-enriched diets develop NAFL with greater hepatic steatosis and inflammation compared to rats fed a fat-enriched diet (35, 36). In human studies, adult NAFL patients report an increased consumption of fructose compared

to controls (8, 37), and fructose intake has been shown to be associated with increased fibrosis and increased hepatic inflammation, although hepatic steatosis was reduced (9). Soft drink, a major source of fructose, has also been associated with increased risk of NAFL (38), and independently of metabolic syndrome diagnosis (39).

Further, our results suggest that the amount of fructose consumed may be more relevant to NAFL than the total amount of sugars. This implies that there may be something unique about the contribution of fructose to the development of NAFL, over and above dietary sugars in general. Fructose metabolism is restricted to the liver through the glycolytic pathway, while the majority of glucose enters the systemic circulation (40). Unlike glucose, phosphorylation of fructose is specific for fructose and not rate limiting. This can contribute to an increased formation of triglycerides when large amounts of fructose are consumed compared to glucose, as demonstrated in a feeding study in children (41). This study also showed that children with NAFL showed an increased sensitivity to fructose compared to children without NAFL (41). Fructose may also interact with nuclear transcription factors (sterol response element binding protein-1c) leading to alterations in the expression of genes involved in liver lipogenesis and glycolysis (42). In addition, liver injury in NAFL may be promoted by fructose through depleting hepatic energy stores (43) and causing bacterial overgrowth and increased intestinal permeability, resulting in movement of bacterial endotoxins into the portal plasma which may initiate hepatic inflammation (42, 44). Our observations of fructose intake and an increased risk of NAFL were limited to subjects who were obese. As obesity increases the predisposition to hepatic fat accumulation via increased lipid influx and *de novo* lipogenesis, ingestion of fructose may in some individuals, be sufficient to overwhelm the liver's capacity to cope with the extra lipid burden and consequently result in hepatic fat accumulation. It is also possible that obese individuals may be more vulnerable to the effects of fructose. For example, they may have a reduced ability

to recover from hepatic ATP depletion, as reduced hepatic ATP stores have been shown to be more prevalent in overweight and obese healthy subjects than in lean subjects (45).

We also observed inverse associations between liver enzymes and fructose in this study. This was an unexpected result, as enzymes are generally expected to be raised in people who have NAFL. In our group as a whole, only ALT was elevated in adolescents with NAFL and when investigated by BMI category, only in overweight adolescents. The lack of a relationship in this regard in the obese group may indicate that the effect of fructose intake on development of NAFL is independent of the presence or absence of raised liver enzymes, or that minimal hepatic inflammatory pathology existed between the groups. Another possibility is that liver enzymes provide little diagnostic or prognostic value in NAFL, given that they can fluctuate and most patients with histologically proven NAFL have liver enzymes in the normal range (47).

An important strength of the study was the detailed dietary assessment, consisting of a comprehensive customised fructose database (21) and the use of 3-day food records, shown to be appropriate for use in a pediatric population (49). There may be some bias in nutritional assessment due to underreporting or reduction of food intake during the days of conducting food records (50), however we attempted to reduce this risk by a dietitian following up records as they were returned to clarify any potentially omitted items. The current gold standard for NAFL diagnosis is liver biopsy, however this is an invasive procedure that is not suited to a large cohort study (51). Ultrasound correlates closely with degree of hepatic steatosis on liver biopsy in children and is considered to be an excellent technique to use for non-invasive diagnosis and estimation of hepatic steatosis in a younger population (52). Although we utilized a validated and standardized imaging protocol for the diagnosis of NAFL, the sensitivity of ultrasound falls with minor hepatic steatosis or with increasing BMI

and thus it is possible some cases of NAFL were not diagnosed. Where funding permits, future studies of NAFL in this age group should consider the use of hepatic magnetic resonance imaging or spectroscopy (MRI/MRS) as a preferred tool over ultrasound. These procedures can more accurately quantify fat in the liver and determine presence of NAFL (53, 54). In addition, data on the onset of puberty and steatosis may provide information potentially useful for investigation into the mechanisms responsible for fructose-associated NAFL in obese adolescents.

The 17-year follow-up was the first to assess NAFL in the Raine Study. As no standardized formal testing for NAFL was performed at age 14 years, we do not know whether the cases of NAFL identified in this study developed between 14 and 17 years of age, or before this time. Although no subjects reported a NAFL diagnosis in the 14-year follow-up, it is possible that some adolescents had undiagnosed NAFL at this time. The lack of diagnoses may reflect local medical practices of when or if to pursue the diagnosis of NAFL in a child, rather than the true frequency of NAFL at this time. However, as they did not know they had NAFL, their diets would not have changed for that purpose, suggesting the dietary associations observed would still be valid. Sampling bias may also exist, as the obese group in our study was relatively small (n=28) due to the Raine Study being a population-based cohort. In addition, overall study numbers for this analysis were limited because 36% of adolescents who received the 3-day food record did not complete it. Our findings suggest future research is warranted into clinical cohorts or larger population based cohorts to further study the relationship we observed in obese individuals.

A smaller study of children in the United States did not find significant associations with fructose, however reported sugar-sweetened beverage consumption was relatively low in this group which may contribute to the difference in findings (55). Sugar sweetened soft drinks

were the largest overall individual contributor to fructose intake in the Raine cohort, and were consumed by 60% of the Raine Study adolescents on at least one of the three days studied in the food diaries (21). As soft drinks do not supply the same nutritional value as other fructose contributors such as fruit, lower consumption of these beverages would be an effective strategy to decrease fructose intake in adolescents.

Dietary treatment for NAFL tends to focus on weight reduction, however it is not yet clear which type of diet would be more beneficial for prevention and treatment beyond the effects of weight loss (39) and there is a lack of specific guidelines pertaining to diet (46). Our findings support the concept that dietary fructose intake, in excess of that normally consumed in fruits and vegetables, may alter hepatic metabolism in favour of lipogenesis (7), although only in adolescents who are classified as obese. Overall, results of this study indicate that further research is warranted into the potentially protective role of a lower fructose diet in the development of NAFL in obese adolescents.

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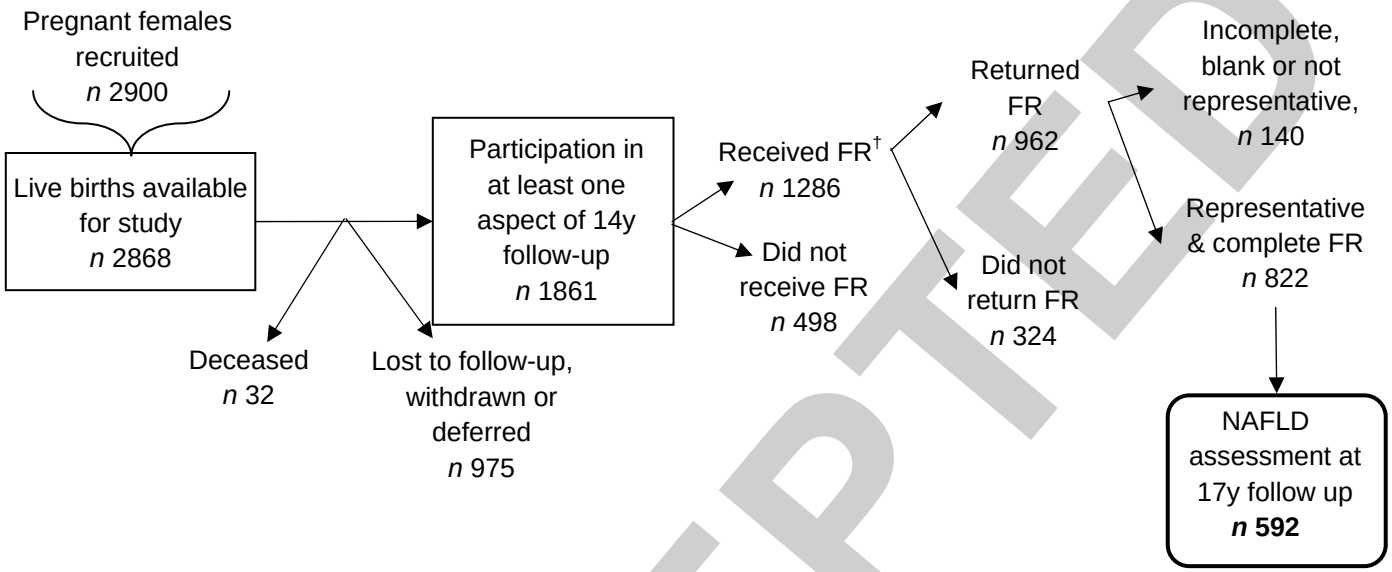

Figure 1: Derivation of study sample from overall Western Australian Pregnancy Cohort (Raine) Study, Perth, Western Australia. †Food records (FR) and accompanying measuring utensils and instructions were handed out only to subjects who attended the in-person follow-up session at the Telethon Institute for Child Health Research in Perth, Western Australia. NAFLD = non-alcoholic fatty liver disease.

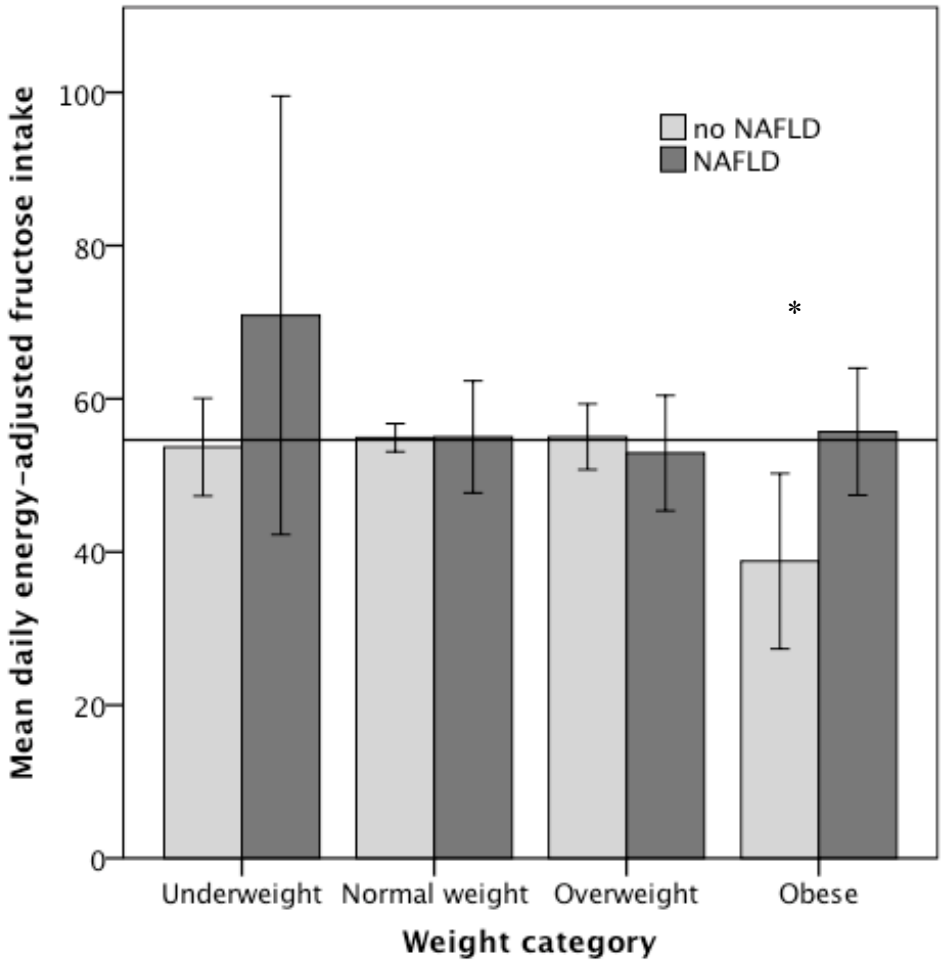

Figure 2: Mean daily fructose intake across body mass index (BMI) category [a] at 14 years in relation to non-alcoholic fatty liver disease (NAFLD) status at 17 years. The line represents the mean intake for the group as a whole (54 g). Error bars represent 95% confidence intervals.

[a] Standard adolescent criteria were used to classify participants into BMI categories of underweight, normal weight, overweight, and obese at 14 years [24, 25].
*NAFLD group significantly different to non-NAFLD group, P<0.05

Table 1: Characteristics of study cohort at 14 years by BMI category (n=592)

Characteristic (mean ± SD)	BMI category [a]				P value [b]
	Underweight (n=39)	Normal weight (n=424)	Overweight (n=101)	Obese (n=28)	
BMI (kg/m^2) [c]	15.9 ± 0.7	19.7 ± 1.7	25.0 ± 1.6	31.4 ± 3.2	<0.01
Age (y) [c]	14.0 ± 0.1	14.0 ± 0.2	14.0 ± 0.2	14.0 ± 0.2	0.71
Gender (% female)	56.4%	46.0%	49.5%	35.7%	0.36
Liver enzymes [d]					
Alanine transaminase (IU/L)	16.2 ± 5.2	20.2 ± 11.6	23.3 ± 12.1	34.0 ± 20.2	<0.01[b]
Aspartate transaminase (IU/L)	23.0 ± 3.4	25.2 ± 8.4	24.8 ± 7.2	30.4 ± 17.9	0.12[b]
Gamma glutamyl transpeptidase (IU/L)	12.8 ± 3.4	14.1 ± 8.3	15.0 ± 7.5	21.4 ± 12.9	<0.01[b]
Aerobic fitness (watts) [c]	87 ± 19	111 ± 29	117 ± 28	124 ± 36	<0.01
Family status (% single parent) [c]	13.2%	14.3%	20.8%	42.9%	<0.01
Family income per annum, $AUD (%<35 001) [c]	26.3%	15.9%	23.0%	51.9%	<0.01
Daily dietary intakes [c]					
Energy (MJ)	8.9 ± 2.0	9.5 ± 2.5	9.3 ± 2.4	9.0 ± 2.5	0.13
Carbohydrate (g)	266 ± 68	287 ± 83	279 ± 84	255 ± 77	0.03
Total sugars (g)	122 ± 48	133 ± 54	127 ± 51	112 ± 48	0.03
Fructose (g)	50.9 ± 20.3	54.8 ± 24.5	53.4 ± 24.9	44.6 ± 21.3	0.06
Protein (g)	80.0 ± 21.1	89.7 ± 26.5	88.4 ± 26.1	91.2 ± 27.4	0.06
Fat (g)	80.0 ± 20.1	82.2 ± 24.6	79.4 ± 24.1	81.2 ± 27.4	0.63

[a] Standard adolescent criteria were used to classify participants into BMI categories of underweight, normal weight, overweight, and obese [24, 25].
[b] K-W test as not normally distributed (otherwise ANOVA for continuous variables)
[c] at 14 year follow-up
[d] at 17 year follow-up

Table 2: Differences in daily intake of fructose and sugar at 14 years according to body mass index (BMI) category and non-alcoholic fatty liver disease (NAFLD) status at 17 years.

BMI Category [a] and NAFLD Status [b]	Energy-adjusted fructose intake (g/day)		Energy-adjusted total sugar intake (g/day)	
	mean±SD	P value	mean±SD	P value
Underweight (n=39)				
Without NAFLD (n=37/95%)	53.7 ±19.1	0.22	128 ± 35	0.25
With NAFLD (n=2/5%)	70.9 ± 3.2		158 ± 7	
Normal weight (n=424)				
Without NAFLD (n=392/92%)	54.9 ± 18.6	0.98	133 ± 35	0.76
With NAFLD (n=32/8%)	55.0 ± 20.3		131 ± 33	
Overweight (n=101)				
Without NAFLD (n=73/72%)	55.0 ± 18.4	0.61	132 ± 36	0.65
With NAFLD (n=28/28%)	52.9 ± 19.4		128 ± 31	
Obese (n=28)				
Without NAFLD (n=14/50%)	38.8 ± 19.8	0.02	107 ± 37	0.11
With NAFLD (n=14/50%)	55.7 ± 14.4		129 ± 31	

[a] Standard adolescent criteria were used to classify participants into BMI categories of underweight, normal weight, overweight, and obese at 14 years [24, 25].
[b] NAFLD status as determined by ultrasound at 17 years[23].

Table 3: Odds ratio estimates from multivariate logistic regression models of daily fructose and sugar intake at 14 years and risk of NAFLD at 17 years, total and separately for each body mass index (BMI) category[a]

Category [a]	Dietary factor (energy-adjusted g/day)	OR [b] (95% CI)	P	Nagelkerke r^2 [c]
Total (n=592)	Fructose	1.00 (0.99-1.01)	0.977	0.013
	Sugar	1.00 (0.99-1.01)	0.731	0.013
Underweight (n=39)	Fructose	1.03 (0.95-1.08)	0.461	0.218
	Sugar	1.02 (0.97-1.08)	0.375	0.242
Normal weight (n=474)	Fructose	1.00 (0.98-1.02)	0.954	0.031
	Sugar	1.00 (0.99-1.01)	0.765	0.031
Overweight (n=101)	Fructose	0.99 (0.97-1.02)	0.633	0.005
	Sugar	1.00 (0.99-1.01)	0.655	0.005
Obese (n=28)	Fructose	1.09 (1.01-1.19)	0.030	0.522
	Sugar	1.03 (0.999-1.07)	0.059	0.427

[a] As defined by standard adolescent criteria at 14 years [24, 25].
[b] Models adjusted for gender.
[c] Nagelkerke r^2 represents how well the model explains the variability of the outcome, and ranges from 0 to 1, with larger values indicating a better fit [50].

Table 4: Odds ratio estimates from multivariate logistic regression models of risk of NAFLD in adolescents, where adolescents (n=592) are grouped by energy-adjusted fructose intake (median split) and BMI category (non-obese vs obese)[a], and the reference group is non-obese, low fructose (n=277)

Category	OR [b] (95% CI)	P	Nagelkerke r^2 [c]
Non-obese, low fructose (n=277)	Reference	-	0.125
Obese, low fructose (n=19)	3.53 (1.24-10.04)	0.018	
Non-obese, high fructose (n=287)	0.73 (0.43-1.24)	0.241	
Obese, high fructose (n=9)	66.42 (7.93-556.57)	<0.001	

[a] As defined by standard adolescent criteria at 14 years [24, 25].
[b] Models adjusted for gender, family structure.
[c] Nagelkerke r^2 represents how well the model explains the variability of the outcome, and ranges from 0 to 1, with larger values indicating a better fit [50].