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Fructose Intake during Childhood: Food Sources and its Association with Cardiometabolic Health in Adolescence

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Aos meus pais,

Ao meu irmão,

Ao João.

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Abstract

Background/objectives: The consumption of sugar-sweetened beverages has been associated with worse cardiometabolic health in adults. Even though studies in children have linked both total sugar and the consumption of sugar-sweetened beverages with higher obesity risk, the literature on the relationship between fructose intake and overall cardiometabolic health in young populations is sparse. Because fructose can be found in various foods, from fruits to sugar-sweetened beverages, it is of the utmost importance to consider the main food sources when studying its association with cardiometabolic health.

The main objective of this thesis was to understand the relationship between fructose intake during childhood and cardiometabolic health risk in adolescence, considering the main food sources of fructose intake in the diet.

Design: This study includes 8647 children who participated in the Generation XXI birth cohort. At 4-, 7-, 10 and 13 years old, sociodemographic data, dietary intake, anthropometrics, and blood samples were collected. Dietary intake was collected at 4, 7 and 10 years old, using 3-day food diaries. Fructose intake was assessed in g/day, and clusters of fructose intake by its food sources were also estimated using hierarchical cluster analysis with the complete-linkage method and Euclidean distance. At 13 years old, two clusters of cardiometabolic risk were previously estimated: 1) a cluster of metabolically healthy adolescents (with average values of z-score of waist-circumference, Homeostatic Model Assessment for Insulin Resistance, high-density lipoprotein cholesterol (HDL-C), triglycerides and systolic blood pressure) (MHC); and 2) a cluster of metabolically unhealthy adolescents (values above the average for all risk factors and below the average for HDL-C) (MUC). Multivariable logistic regression models were used to

investigate the association between fructose intake at 4-,7- and 10- years old and cardiometabolic risk clusters at 13 years old (adjusted for maternal educational level, mother's age at birth, BMI before pregnancy, smoking and alcohol drinking during pregnancy, birth weight, being primiparous, and gestational age).

Results: Three clusters of fructose intake by its food sources were obtained: 1) a cluster of children with a high daily intake of fructose from fresh fruits and vegetables and low intake of fructose from soft drinks named "High Fruits and Vegetables and Low Soft Drinks" ("High F&V and Low SD"); 2) a cluster of children with low intake of fructose from soft drinks and fresh fruits and average intake of fructose from fresh vegetables, named "Low Soft Drinks and Fresh Fruits and Average intake of Fresh Vegetables" ("Low SD&F and Average Veg") (Reference); and a final group of children with a high fructose intake from soft drinks and a low fructose intake from fresh fruits and vegetables consumption called "High Soft Drinks and Low Fresh Fruits and Vegetables" ("High SD and Low F&V"). At 4 years old, the mean fructose intake was 14.8 g/day, increased until the 7 years of age and decreased at 10 years (7 years old: 17.0 g/day; 10 years old: 15.8 g/day). Fresh fruits (54.9 %) and vegetables (14.1 %) were the main sources of fructose in the diet at all ages, followed by soft drinks at 4 and 10 years old and nectars at 7 years old. At the age of 4, children classified in the "High SD and Low F&V" cluster had a higher risk of being metabolically unhealthy [OR = 1.84, (95% CI = 1.01, 3.34)] when compared to those classified in the "Low SD&F and Average Veg" cluster, even after accounting for the total energy intake and selected confounders . There was no significant association between the clusters of fructose intake by its food sources at 7 and 10 years and cardiometabolic health risk.

Conclusions: This study found that a higher intake of fructose from soft drinks but not from fruits and vegetables in 4-year-old children was associated with a higher risk of being metabolically unhealthy in adolescence. This study reinforces that soft drinks should be discouraged from early periods in the life course.

Keywords

Fructose, Cardiometabolic Health, Childhood, Cohort studies

Resumo

Contexto/objetivos: O consumo de bebidas açucaradas tem sido associado a uma pior saúde cardiometabólica em adultos. Apesar de os estudos terem associado o açúcar total e o consumo de bebidas açucaradas a um maior risco de obesidade em crianças, a literatura sobre a relação entre a ingestão de frutose e a saúde cardiometabólica geral em populações jovens é escassa. Uma vez que a frutose pode ser encontrada em vários alimentos, desde frutas a bebidas açucaradas, é da maior importância considerar as principais fontes alimentares quando se estuda a sua associação com a saúde cardiometabólica.

O principal objetivo desta tese foi compreender a relação entre o consumo de frutose durante a infância e o risco cardiometabólico na adolescência, considerando as principais fontes alimentares de frutose na dieta.

Metodologia: Este estudo inclui 8647 crianças que participaram na coorte de nascimento da Geração XXI. Aos 4, 7 e 10 e 13 anos de idade, foram recolhidos dados sociodemográficos, consumo alimentar, antropometria e amostras de sangue. A ingestão alimentar foi recolhida aos 4, 7 e 10 anos de idade, utilizando diários alimentares de 3 dias. A ingestão de frutose foi avaliada em g/dia, e os grupos de fontes alimentares de frutose foram também estimados utilizando a análise hierárquica de clusters com o método de ligação completa e a distância euclidiana. Aos 13 anos de idade, foram previamente estimados dois grupos de risco cardiometabólico: 1) um cluster de adolescentes metabolicamente saudáveis (com valores médios de z-score da circunferência da cintura, *Homeostatic Model Assessment for Insulin Resistance*, colesterol de lipoproteína de alta densidade (HDL-C), triglicerídeos e pressão arterial sistólica) (MHC); e 2) um cluster de adolescentes metabolicamente não saudáveis (valores acima da média para todos

os fatores de risco e abaixo da média para o HDL-C) (MUC). Foram utilizados modelos de regressão logística multivariável para investigar a associação entre o consumo de frutose aos 4, 7 e 10 anos de idade e os grupos de risco cardiometabólico aos 13 anos de idade (ajustados para o nível de escolaridade materna, idade da mãe à nascença, IMC antes da gravidez, consumo de tabaco e álcool durante a gravidez, peso à nascença, ser primípara e idade gestacional).

Resultados: Obtiveram-se três clusters de ingestão de frutose segundo as suas fontes alimentares: 1) um grupo de crianças com um elevado consumo diário de frutose proveniente de frutas e hortícolas frescos e um baixo consumo de frutose proveniente de refrigerantes, designado por "Elevado consumo de fruta e hortícolas frescos e baixo consumo de refrigerantes" ("High F&V and Low SD "); 2) um grupo de crianças com um baixo consumo de frutose proveniente de refrigerantes e fruta fresca e um consumo médio de frutose proveniente de hortícolas frescos, designado por "Baixo consumo de refrigerantes e fruta fresca e consumo médio de hortícolas frescos" ("Low SD&F and Average Veg") (Referência); e um último grupo de crianças com uma elevada ingestão de frutose proveniente de refrigerantes e uma baixa ingestão de frutose proveniente do consumo de fruta e hortícolas frescos, designado por "Elevada ingestão de refrigerantes e baixa ingestão de frutas e hortícolas frescos" ("High SD and Low F&V"). Aos 4 anos de idade, o consumo médio de frutose foi de 14,8 g/dia, tendo aumentado até aos 7 anos de idade e diminuído aos 10 anos (7 anos: 17,0 g/dia; 10 anos: 15,8 g/dia). Os frutos frescos (54,9%) e os vegetais (14,1%) foram as principais fontes de frutose na alimentação em todas as idades, seguidos dos refrigerantes aos 4 e 10 anos e dos néctares aos 7 anos. Aos 4 anos de idade, as crianças classificadas no grupo "High SD and Low F&V" tinham um risco mais elevado de não serem

metabolicamente saudáveis [OR = 1,84, (95% CI = 1,01, 3,34)] quando comparadas com as classificadas no grupo "Low SD&F and Average Veg", mesmo depois de ajustar para a ingestão energética total e confundidores selecionados.. Não se verificou qualquer associação entre os grupos de ingestão de frutose aos 7 e 10 anos e o risco para a saúde cardiometabólica.

Conclusões: Este estudo verificou que uma maior ingestão de frutose proveniente de refrigerantes, mas menor ingestão através de fruta e hortícolas, em crianças de 4 anos, está associada a um maior risco de serem metabolicamente não saudáveis na adolescência. Este estudo reforça a ideia de que os refrigerantes devem ser desencorajados, desde períodos precoces no curso de vida.

Palavras-Chave

Frutose, Saúde Cardiometabólica, Infância, Estudos de Coorte

Index

List of Abbreviations

AMP - Adenosine Monophosphate

BMI – Body Mass Index

CVD – Cardiovascular Disease

F&V – Fruits and Vegetables

HDL-C – High-Density Lipoprotein Cholesterol

High F&V and Low SD - High Fruits and Vegetables and Low Soft Drinks

High SD and Low F&V - High Soft Drinks and Low Fresh Fruits and Vegetables

Low SD&F and Average Veg - Low Soft Drinks and Fresh Fruits and Average Fresh Vegetables

MHC - Metabolically Healthy Children

MUC - Metabolically Unhealthy Children

SSB - Sugar-Sweetened Beverages

Figures Index

Tables Index

Introduction

Dietary fructose is consumed in its simple monosaccharide form and/or as a constituent of the disaccharide sucrose[1]. It is found naturally in fruits, vegetables, and honey[2].

Absorption and Metabolism of Dietary Fructose

After ingested, fructose is mostly absorbed passively from the intestinal lumen into the enterocyte membrane by a fructose-specific hexose transporter, GLUT5 (**Figure 1**)[3, 4], whereas GLUT2 transports into the blood vessels. Similarly, glucose and galactose are also transported by the GLUT2, competing for the same transporter[4].

Fructose absorption increases when it is combined with glucose or when it is ingested as sucrose, maximising this effect when glucose and fructose are ingested in the same amounts[5]. Fructose uptake can also increase with chronic consumption of it[5, 6]. However, the intestinal ability to absorb fructose reaches a saturation point[4]. When fructose is not absorbed, it can create an osmotic load on the distal intestine and colon, contributing to intestinal symptoms[4].

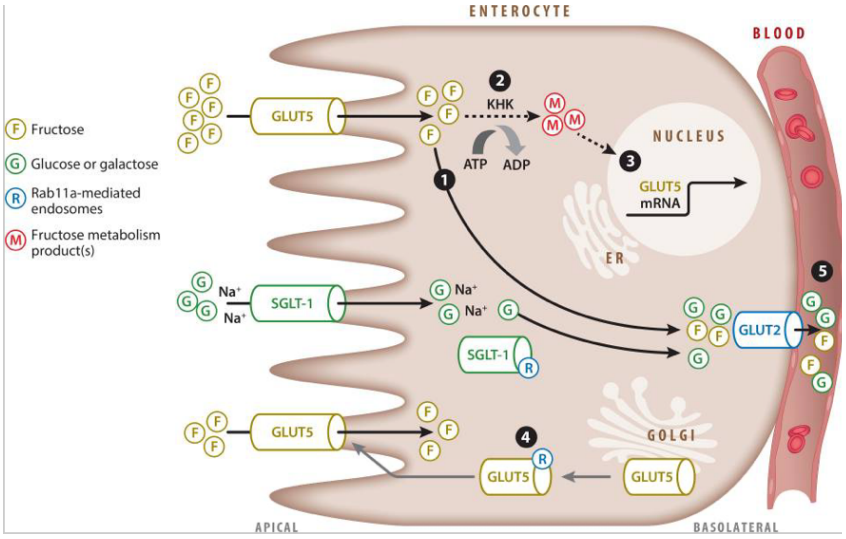

Figure 1: Intestinal fructose transport across the small intestinal epithelia. Reproduced from Ferraris et al., 2018 [2]

After absorption, fructose reaches the liver through the portal circulation and is converted into fructose-1-phosphate, which is then metabolised to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate[4]. In opposition to glucose uptake and glycolysis, which are influenced by insulin metabolism, these primary steps in fructose metabolism seem unregulated and bypass hormonal control[7].

Because liver fructolysis is unlimited, fructose loads can induce enlargement of the hexose and triose-phosphate pools, which are potential substrates for glycolysis, glycogenesis, gluconeogenesis, lipogenesis and oxidative phosphorylation[4]. This uncontrolled metabolism generates glycerophosphate and acetyl-CoA, predecessors of triglycerides[2]. Higher levels of circulating triglycerides, in the form of low-density lipoproteins (LDL), activate *de novo* lipogenesis and promote intrahepatic lipid accumulation[4]. This may lead to non-

alcoholic fatty liver disease, which in turn is strongly associated with visceral fat, diabetes and lipid disorders.[8].

Moreover, fructose phosphorylation may augment uric acid production via the activation of Adenosine Monophosphate (AMP) deaminase by leading to the catabolism of AMP into inosine monophosphate and uric acid.[2, 4] Furthermore, uric acid production through fructose intake may also occur because of the stimulation of purine synthesis[4]. Increased uric acid has been linked to oxidative stress and fat accumulation[8]. In addition, hyperuricemia has been independently correlated with developing type 2 diabetes in adults[2, 9, 10] and hypertension in adolescents already with type 2 diabetes[2, 11].

Chronic high-fructose intake may also cause persistent hyperinsulinemia and, consequently, insulin resistance over time. However, the mechanism behind this relationship is still unclear since fructose does not stimulate insulin secretion directly[12]. **Figure 2** elucidates some of the effects of high fructose uptake in multiple organs, leading to cardiometabolic dysfunction.

Fructose Intake at the Population Level and Main Food Sources in the Diet

A study from the National Health and Examination Survey (NHANES), evaluating fructose consumption in American children and teenagers from 1988 to 1994, showed that the average daily intake of fructose was 44.9 g/day at 2-5 years old and 72.8 g/day at 12-18 years old, respectively[13]. At 2-5 years, the main sources of fructose were fresh fruits and fruit juices, followed by sugar-sweetened beverages (SSB) and grains (including breads and cereals, as well as cakes, pies, snacks). On the other hand, at 6-18 years old, the main food source of fructose

was SSB (representing 45% of total fructose intake), grains, and lastly, fruits and fruit juices[13].

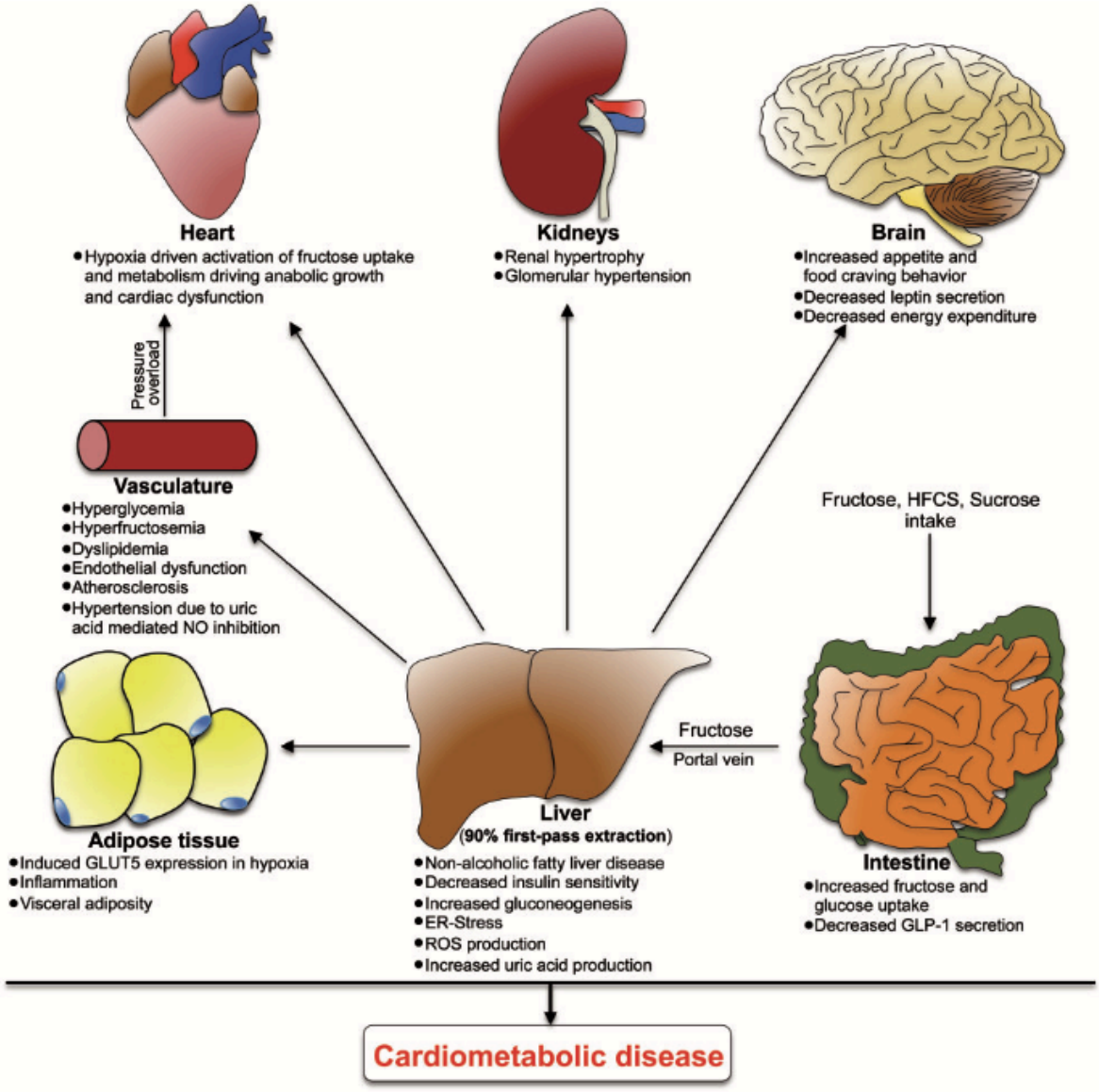

Figure 2: Effect of increased fructose uptake on multiple organs.

Reproduced from Mirtschink et al., 2018.[5]

Later, also from NHANES but using data from 1999-2004, children between 4 and 6 years old had a mean fructose intake of 43 g/day, while 7- to 10-year-old children consumed 51 g/day[14]. Adolescents and young adults (19-22 years old)

from both sexes showed the highest daily total fructose intake within the whole population[14]. Similar to the previous study, the primary food sources of fructose among children and teenagers were SSB, excluding infants and toddlers (1-3 years old), to whom fruits and fruit products were the main contributors[14].

In the Dutch National Food Consumption Survey 2007-2010, the main food sources of fructose in children and adolescents were also SSB, followed by dairy products, juices, fruit, cake, and cookies[15]. SSB accounted for 30% and 26% of the fructose consumption in boys and girls, respectively[15]. In boys, the mean daily fructose intake increased with age (7-8 years: 58 g/day; 9-13 years: 61 g/day; 14-18 years: 63 g/day), whereas in girls, the higher mean intake of fructose was found at 7-8 years (56 g/day) and 9-13 years (57 g/day), decreasing to 52 g/day at 14-18 years[15].

Findings from the DONALD study, an ongoing cohort of German children, analyzed time and age trends of fructose intake between 1990 and 2016 based on biomarker's excretion[16]. Over time, even though dietary records reported an increase in total sugar intake from childhood to adolescence, fructose excretion decreased[16]. Nevertheless, a slight increase in fructose excretion occurred during adolescence (8.5-16.5 years), suggesting that dietary habits may change during this period, and adolescents consume more fructose-rich foods[16].

Therefore, evidence suggests that during childhood, fructose intake seems to be higher in adolescence[13-16], with SSB being the major contributor[13-15].

Fructose Intake and Cardiometabolic Health

In a systematic review and meta-analysis including 31 controlled feeding trials reviewing the effect of fructose on body weight, fructose showed no overall effect on body weight in isocaloric trials (637 participants); however, excessive fructose in hypercaloric trials (119 participants) led to body weight increases (mean difference of 0.53 kg (95% CI 0.26-0.79)[17]. This suggested that total energy intake may also influence the relationship between fructose and risk factors of cardiometabolic health. Indeed, evidence has suggested that not only the source but also the total energy intake may influence whether fructose impacts CV health or not. In adults, two other systematic reviews and meta-analyses of controlled trials suggested that energy and certain food sources appeared to be mediators of the effect of fructose on non-alcoholic fat liver disease markers[18], inflammatory markers[19] and uric acid[20]. The excess of energy provided by SSB leads to an increase in intrahepatocellular lipids and an increase in ALT[18]. However, the evidence was of low quality because most studies were short-term[18], limiting conclusions.

In fact, results are still contradictory regarding the influence of fructose on glycemic control. In a systematic review and meta-analysis, habitual consumption of SSB, artificially sweetened beverages and fruit juices in the United States and United Kingdom were associated with a greater incidence of diabetes type 2, independently of adiposity[21]. On the other hand, a systematic review and meta-analyses of 155 intervention studies indicated that most food sources of fructose-containing sugars did not damage glycemic control when there was an energy-matched substitution for other macronutrients[22]. However, this same review also suggested that when SSB added excessive energy, a harmful effect on glycemic control was found [22].

Indeed, most studies focusing on the relationship between fructose and cardiometabolic health examined isolated food sources of fructose, particularly the consumption of SSB [23, 24]. A review systematic review and meta-analysis of 13 prospective studies concluded that consuming fructose-rich SSB was associated with the development of metabolic syndrome in adults [23]. However, the adverse association with metabolic syndrome did not seem to extend to other food sources of fructose[23], as the authors also suggested that the consumption of yoghurt and fruits might be protective regarding metabolic syndrome development[23]. Similar to the previous study, a protective link between yoghurt and fruits and CV disease was described in a systematic review and meta-analysis of prospective studies, as well as for breakfast cereals [24]. However, the benefits of fruit did not seem unlimited as there was no benefit when the consumption was more than 400 g/day[24].

When the dose is considered, another study found that even small doses of SSB were associated with a higher risk of cardiometabolic disease and mortality[24].

Additionally, studies have looked into the association between fructose and other risk factors, like uric acid[20], blood lipids[25] and inflammation[19]. Ayoub-Charette et al., in a systematic review and meta-analysis of controlled feeding trials, suggested that fructose from SSB increased uric acid, while fructose from 100% fruit juice had the contrary effect[20]. They concluded that to maintain uric acid levels, it appeared that food source was more important than energy control. However, some of the trials were short-term[20], limiting conclusions. Moreover, SSB increased C Reactive Protein[19].

Regarding lipid profile, a systematic review and meta-analysis of controlled feeding trials showed that fructose did not increase postprandial triglycerides on an isocaloric exchange with other carbohydrates, but postprandial triglycerides increased when consumed in the context of a hypercaloric diets[25].

Most evidence in children concerns total sugar[26] and fructose intake from SSB[2, 27] in relation to cardiometabolic health. A WHO meta-analysis of 5 prospective cohort studies in children showed that after a 1-year follow-up, a higher consumption of SSB was associated with a 55% higher risk of becoming overweight or obese compared to those with the lowest intake[27]. In addition, a review suggested that consuming fructose-rich SSB was associated with developing pediatric insulin resistance[2]. This relationship may be explained by higher central adiposity, which may be due to excessive fructose consumption over time[2].

Literature is sparse on the association linking fructose intake with cardiometabolic dysfunctions already in childhood. Thus, more studies are needed to assess this issue. Considering that fructose can be naturally found in some foods like fruits, which are also rich in other beneficial nutritional compounds, and in some foods like SSBs, becomes of the utmost importance to consider the main food sources of fructose when studying its relationship with cardiometabolic health.

Objectives

The main objective of this thesis was to understand the relationship between fructose intake during childhood and cardiometabolic health risk in adolescence, considering fructose intake from the main food sources in the children's diet, using data from the Generation XXI birth cohort.

Methodology

1. *Study design and participants*

Generation XXI is an ongoing prospective population-based birth cohort (Porto, Portugal, 2005–2006), described elsewhere in detail[28, 29]. Participants were recruited between April 2005 and August 2006 from all public maternities of the Metropolitan area of Porto, Portugal. At baseline, 8495 mothers and 8647 newborns were enrolled (participation proportion of 91%). Within 72 hours after delivery, structured questionnaires collected demographic and socioeconomic information, lifestyle factors, obstetric history, anthropometric measurements, and personal disease history through face-to-face interviews conducted by trained interviewers. The cohort was then re-evaluated at 4 (2009-2011: participation proportion of 86%), 7 (2012-2014: participation proportion of 80%), 10 years old (2015-2017: participation proportion of 76%) and 13 years old (2018-2020: participation proportion of 54%, interrupted earlier due to COVID-19).

2. *Ethical Approval*

The University of Porto Medical School/S. João Hospital Centre Ethics and the Portuguese Data Protection Authority Ethics provided ethical approval. All study phases complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki. Written informed consent was obtained from all participating children's parents/legal caregivers.

3. *Dietary Data*

At 4, 7, and 10 years old, dietary intake was assessed by 3-day food diaries in non-consecutive days (2 weekdays and 1 weekend). The children's main caregiver was instructed to describe all the food and drinks consumed, including any specific commercial brand names, and the quantity consumed in grams, units, or household measures. If the child had meals outside of the home without a parent present, the caregiver responsible during the day reported those meals. Meals prepared at home were documented with comprehensive recipe information, including ingredients and the preparation methods.

The food items reported during the 4-, 7- and 10-year follow-up using the software Food Processor SQL (2004-2005 EHSA Research, Salem, Oregon)[30] were matched to their corresponding counterpart in the eAT24 software[31].

After nutrient conversion, daily energy intake (kcal) and nutrient intake (g/day) were estimated for each participant.

Fructose values were extracted from EUROFIR – FoodExplorer, and added to the central food composition table, at specific item level and using the respective FoodEx2 code. The absolute values were converted according to their proportion to the total carbohydrates of the food item in our composition database. For recipes, this nutrient was calculated based on the fructose values of each single ingredient according to its proportion in the recipe.

4. Cardiometabolic Health-Related Parameters

At 13 years old follow-up, a clinical examination was performed by trained staff. Anthropometric measurement included the assessment of weight measured with minimum clothes and without shoes on a digital scale. Height was measured

with children in an upright position with a fixed stadiometer. Body Mass Index (BMI) z-score was calculated according to the World Health Organization growth charts[32]. Waist circumference was measured according to standard procedures[33].

Blood samples were collected after an overnight fast as part of the follow-up evaluation at 13 years. Enzymatic methods were used to obtain serum concentration of high-density lipoprotein cholesterol (HDL-C), triglycerides and glucose, whereas insulin was measured by electrochemiluminescence immunoassay. The formula used for calculating the Homeostasis Model Assessment Insulin Resistance (HOMA-IR) was: fasting glucose (mmol/L) * fasting insulin (mU/L) / 22.5. Age- and sex-specific z-scores for waist circumference, HDL-C, triglycerides, and HOMA-IR were calculated using the sample of Generation XXI.

Diastolic and systolic blood pressure were measured after children had been sitting for at least five minutes, and the measurement was taken three times using a digital blood pressure monitor. The mean of the diastolic and systolic blood pressure from the last two values was taken into account. Age-, sex- and height-specific z-scores were determined for diastolic and systolic blood pressure, according to the American Academy of Pediatrics[34].

Two clusters of cardiometabolic risk were previously estimated (Marinho AR et al., manuscript submitted): 1) a cluster of metabolically healthy adolescents (with average values of cardiometabolic risk factors) (MHC); and 2) a cluster of metabolically unhealthy adolescents (values above the average for all risk factors and below the average for HDL-C) (MUC).

5. Covariates

Potential covariates were selected, such as maternal educational level (number of schooling years categorized into <12, 12-14 and >14 years), mother's age at birth (in years), Body Mass Index (BMI) before pregnancy (kg/m2), pregnancy smoke (yes/no), alcohol intake (yes/no), birth weight (g), and being first born (yes/no) and gestational age (weeks).

6. Study Sample

Dietary intake, considering complete information from 3-day food diaries, was collected at 4 (2493 children), 7 (3587 children) and 10 years old (2855). At 13 years, 2415 children had information on cardiometabolic health patterns. At each evaluation, the final sample included children with information on dietary intake and cardiometabolic health: 1287 children were considered at 4, 1979 children at 7 and 1814 children at 10 years old.

7. *Statistics analysis*

The studied sample was compared with the remaining cohort using the chi-square test for categorical variables and two-sided Student's t-tests for continuous.

The clusters of fructose intake by its food sources were estimated using hierarchical cluster analysis with the complete-linkage method and Euclidean distance with data from daily fructose intake (g/day) from the main food sources. To define the proper number of clusters, the Bayesian Information Criterion was computed.

Using multiple imputations by chained equations (n=20) missing data on dietary intake, cardiometabolic parameters at 13 years old and covariates were imputed, assuming missing at random[35, 36]. Thus, the main analyses were performed, including 8647 children.

Multivariable logistic regression was used to investigate the associations between fructose intake (in g/day) or the three clusters of fructose by its food sources and the two cardiometabolic health clusters, adjusted for the following selected covariates: mother's educational background, sex, mother's age at birth, body mass index before pregnancy, smoke and alcohol ingestion during pregnancy and being firstborn, and gestational age. Interaction terms between sex and clusters of fructose intake by its food sources on the measured associations was tested, and as no significant interactions were found, the analyses were not stratified by sex.

SPSS (Statistical Package for the Social Sciences) version 27 for MacBook and R statistical software (The R Project for Statistical Computing, Vienna, Austria), version 4.0.0 for Windows software were used to the analysis, using the packages stats[37], *mclust*[38] and *mice*[39].

Results

Sample's Characteristics

Maternal and children's characteristics are shown in Table 1. Our sample was mainly composed by males. At 4 years old, those included in the sample were more likely to have older mothers (30.4 vs 28.7 years old), with a higher educational level (35.2% vs 22.1% with a university degree), were non-smokers during pregnancy (16.2% vs 24,7% non-smokers), and children had a higher birthweight (3226 g vs 3135 g) and higher time of gestation (38.8 vs 38.4

gestational weeks – values at 4 years old. (Supplementary Table 1). These characteristics had similar values at 7 and 10 years old (Supplementary Table 2 and 3).

Table 1: Characteristics of the participants at 4, 7 and 10 years old from the Generation XXI birth cohort.

	Included at 4 years (n= 1287)		Included at 7 years (n= 1979)		Included at 10 years (n= 1814)	
	Missing (n)	Mean (SD) / % (n)	Missing (n)	Mean (SD) / % (n)	Missing (n)	Mean (SD) / % (n)
Maternal characteristics						
Age at delivery, years	0	30.4 (4.8)	0	30.5 (4.8)	0	30.5 (4.9)
Educational level at baseline	7		8		11	
< 10 years	-	36.7 (470)	-	36.8 (726)	-	37.3 (672)
10-12 years	-	28.1 (360)	-	28.6 (563)	-	28.3 (510)
> 12 years	-	35.2 (450)	-	34.6 (682)	-	34.4 (621)
Pre-pregnancy BMI, kg/m^2	95	23.9 (4.0)	136	24.1 (4.2)	123	23.9 (4.1)
Smoking during pregnancy	12		26		20	
No	-	83.8 (1069)	-	86.3 (1685)	-	86.6 (1554)
Yes	-	16.2 (206)	-	13.7 (268)	-	13.4 (240)
Alcohol drinking during pregnancy	92		114		105	
No	-	86.9 (1038)	-	87.2 (1626)	-	87.0 (1486)
Yes	-	11.1 (157)	-	12.8 (239)	-	13.0 (223)
Children's characteristics						
Sex	0		0		0	
Female	-	48.9 (629)	-	47.3 (936)	-	48.3 (877)
Male	-	51.1 (658)	-	52.7 (1043)	-	51.7 (937)
First born	36	60.0 (750)	51	59.8 (1152)	47	58.6 (1035)
Birthweight, g	0	3226 (455)	0	3233 (450)	0	3229 (453)
Gestational age, weeks	0	38.8 (1.4)	0	38.8 (1.4)	0	38.8 (1.4)

Figure 3 shows the main food sources of fructose intake at the three follow-up evaluations. At all ages, fresh fruits (54.9 %), fresh vegetables (14.1 %) were the main sources of fructose intake in the diet, followed by soft drinks (at 4 and 10 years old) and nectars (at 7 years old).

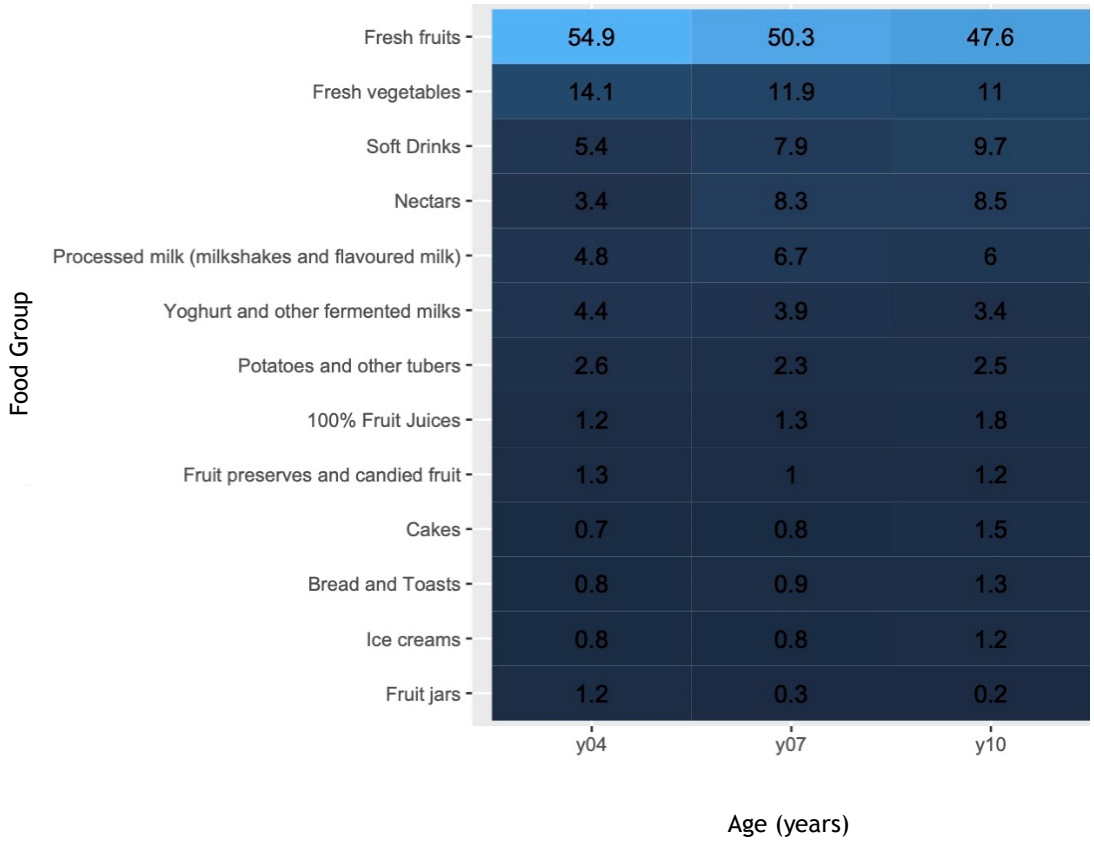

Figure 3: Main sources of fructose in the diet (%) in the Generation XXI birth cohort.

Characteristics of children's dietary intake are presented in Table 2. At 4, 7 and 10 years old, the mean intake of fructose was 14.8 g/day, 17.0 g/day and 15.9 g/day, respectively; and mean total energy intake was 1592 kcal/day, 1767 kcal/day and 1914 kcal, respectively. Children in the MHC had a higher intake of fructose (g/day) and a smaller energy intake.

Table 2: Total energy (kcal), fructose intake (g) and clusters of fructose by its food sources in the total sample and by clusters of cardiometabolic risk in the Generation XXI birth cohort.

		At 13 years	
	Total sample	**Metabolically Healthy Children**	**Metabolically Unhealthy Children**
	Mean (SD) / % (n)	**Mean (SD) / % (n)**	**Mean (SD) / % (n)**
***Dietary Intake at 4 years* (n= 1287)**			
Energy Intake, kcal	1592 (283)	1586 (284)	1609 (281)
Fructose Intake, g/d	14.8 (6.1)	15.1 (6.1)	14.1 (5.9)
Clusters of fructose food sources			
High F&V and Low SD	26.6% (342)	27.5% (265)	23.7% (77)
Low SD&F and Average Veg	69.1% (889)	69.1% (665)	68.9% (224)
High SD and Low F&V	4.4% (56)	3.3% (32)	7.4% (24)
***Dietary Intake at 7 years* (n= 1979)**			
Energy Intake, kcal	1767 (299)	1777 (301)	1736 (293)
Fructose Intake, g/d	17.0 (7.1)	17.2 (7.1)	16.6 (7.2)
Clusters of fructose food sources			
High F&V and Low SD	28.5% (565)	28.8% (427)	27.9% (138)
Low SD&F and Average Veg	63.5% (1257)	63.9% (949)	62.3% (308)
High SD and Low F&V	7.9% (157)	7.3% (109)	9.7% (48)
***Dietary Intake at 10 years* (n= 1814)**			
Energy Intake, kcal	1913 (388)	1926 (382)	1871 (404)
Fructose Intake, g/d	15.8 (6.8)	15.9 (6.8)	15.5 (7.0)
Clusters of fructose food sources			
High F&V and Low SD	22.2% (403)	22.4% (308)	21.6% (95)
Low SD&F and Average Veg	68.4% (1241)	68.7% (944)	67.5% (297)
High SD and Low F&V	9.4% (170)	8.9% (122)	10.9% (48)

Fructose Intake and Cardiometabolic Health Risk Clusters

Table 3 shows the association between total fructose intake (g/day) at 4, 7 and 10 years old and the cardiometabolic risk in adolescence. In the crude model, total fructose intake at 4 years old (g/day) was associated with a lower risk of being part of MUC at 13 years old. However, after accounting for confounders and total energy intake, this association was no longer significant (OR= 0.92; 95% CI 0.66-1.00, p-value=0.05)

Table 3: Association between total fructose (per each 10 g/day) at 4, 7 and 10 years old and cardiometabolic health clusters at 13 years, (imputed sample n=8647)

	Metabolically Unhealthy Children at 13 years
Total Fructose, g/d	**OR (95% CI)**
4 years	
Model 1, 10g/day	**0.82 (0.66-1.00)**
Model 2, 10g/day	0.90 (0.66-1.10)
Model 3, 10g/day	0.82 (0.66-1.00)
7 years	
Model 1, 10g/day	0.90 (0.82-1.00)
Model 2, 10g/day	0.90 (0.82-1.10)
Model 3, 10g/day	1.00 (0.90-1.22)
10 years	
Model 1, 10g/day	1.00 (0.82-1.10)
Model 2, 10g/day	1.00 (0.90-1.10)
Model 3, 10g/day	1.10 (0.82-1.22)

Model 1 – Crude Model; **Model 2** – Adjusted for confounders (mother's educational background, sex, mother's age at birth, body mass index before pregnancy, smoking and alcohol ingestion during pregnancy, being first born and gestational age); **Model 3** – Model 2 + adjusted for total energy intake at each follow-up.

Fructose Intake by its Food Sources Clusters at 4, 7, 10 and cardiometabolic outcomes at 13 years old

Three clusters of fructose intake by its food sources were obtained: 1) a cluster of children with a high daily intake of fructose from fresh fruits and vegetables and low intake of fructose from soft drinks named "High Fruits and Vegetables and Low Soft Drinks" ("High F&V and Low SD"); 2) a cluster of children with low intake of fructose from soft drinks and fresh fruits, and average intake of fructose intake from fresh vegetables, named "Low Soft Drinks and Fresh Fruits and Average intake of Fresh Vegetables" ("Low SD&F and Average Veg")

(Reference); and a final group of children with a high intake of fructose from soft drinks and low intake of fructose from fresh fruits and vegetables consumption called “High Soft Drinks and Low Fresh Fruits and Vegetables” (“High SD and Low F&V”).

Table 4 shows the association between the clusters of fructose intake by its food sources at 4 years old and the two clusters of cardiometabolic health risk. The group of children characterized by a high intake of fructose from soft drinks and low intake of fruits and vegetables “High SD and Low F&V“ at 4 years old was associated with a higher risk of being metabolically unhealthy in adolescence, even after adjusting for confounders and total energy intake (Model 3: OR = 1.84, 95% CI = 1.01 to 3.34).

Table 4: Association between clusters of fructose intake by its food sources at 4, 7, and 10 years and cardiometabolic health clusters at 13 years, (imputed sample n=8647)

	Metabolically Unhealthy Children at 13 years		
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**
	Dietary intake at 4 y	**Dietary intake 7 y**	**Dietary intake at 10 y**
Model 1			
Low SD&F and Average Veg	Ref.	Ref.	Ref.
High F&V and Low SD	0.89 (0.65-1.22)	1.03 (0.85-1.25)	1.01 (0.78-1.32)
High SD and Low F&V	**2.01 (1.14-3.53)**	1.32 (0.99-1.76)	1.31 (0.94-1.81)
Model 2			
Low SD&F and Average Veg	Ref.	Ref.	Ref.
High F&V and Low SD	0.95 (0.66-1.35)	1.12 (0.90-1.39)	1.10 (0.81-1.48)
High SD and Low F&V	**1.89 (1.03-3.46)**	1.20 (0.88-1.65)	1.15 (0.80-1.64)
Model 3			
Low SD&F and Average Veg	Ref.	Ref.	Ref.
High F&V and Low SD	0.92 (0.64-1.33)	1.12 (0.91-1.39)	1.11 (0.91-1.37)
High SD and Low F&V	**1.84 (1.01-3.34)**	1.29 (0.91-1.81)	1.27 (0.95-1.71)

Model 1 – Crude Model; **Model 2** – Adjusted for confounders (mother's educational background, sex, mother's age at birth, body mass index before pregnancy, smoking and alcohol ingestion during pregnancy, being first born and gestational age); **Model 3** – Model 2 + adjusted for total energy intake.

Discussion

This study showed that 4-year-old children classified in the cluster characterized by high fructose intake from SSB and low intake of fruits and vegetables - "High SD and Low F&V" - were associated with a higher risk of being metabolically unhealthy at 13 years old when compared to those in the "Low SD&F and Average Veg" cluster, even after adjusting for confounders. These findings are corroborated by previous systematic reviews and meta-analyses among adults[23, 24]. To the best of our knowledge, there are no studies in children linking specifically fructose from food sources with cardiovascular health. The mechanisms explaining the relationship found between fructose intake from SSB and cardiometabolic health are sparse and controversial. One explanation might be the increased energy intake and the reduced satiety effect of an SSB compared to solid food with the same energetic value[26], the absence of dietary fiber in its composition[40] and lack of mastication[41, 42] might reduce the satietogenic effect of SSB when compared to fresh fruit and fresh vegetables, for example.

At 7 and 10 years old, the associations of the cluster 'High SD and Low F&V' with cardiometabolic outcomes did not reach statistical significance; however, they consistently maintained the same direction as the one found at 4 years old. This lack of significance may be attributed to the fact that as children got older, the consumption of soft drinks increased up to adolescence, and the variability might have diminished. In our study, the main sources of fructose in the diet at 4

years old were: fresh fruit (54.9 %), fresh vegetables (14.1 %) and soft drinks (5.4 %). As children got older, the contribution of fresh fruits, fresh vegetables, yoghurt and fermented milk to the total fructose intake decreased. On the other hand, the contribution of nectars, processed milk, and SSB increased. Despite this increase in SSB intake in our sample, no significant associations were found between fructose intake from SSB at 7 and 10 years old and cardiometabolic outcomes at 13 years. Even though studies have suggested some tracking of dietary patterns during childhood[43, 44], our study suggested that early ages, particularly the 4 years of age, might be a sensitive period for the influence of fructose, particularly when consumed from SSB, on cardiometabolic health in adolescence.

When considering total fructose, we found an inverse marginal association between total fructose intake at 4 years old (g/day) and the risk of being metabolically unhealthy. This could be explained by the differential influence of the different sources of fructose. In our population, fresh fruits and vegetables were the main sources of fructose, and despite the lack of significance, children classified in the cluster of high intakes of fructose from fruits and vegetables were associated with a lower risk of being metabolically unhealthy (OR= 0.92, 95% CI= 0.64 to 1.33). Depending on the food source, these opposite effects of fructose may have nulled the overall effect of the nutrient. This reinforces the need for studies to focus on the food source of a nutrient when conducting a nutrient approach in nutritional epidemiology. The significance of the food matrix and the food sources become notably important, particularly when studying sugars. In fact, research has yielded contradictory findings regarding the association between the types of sugars and the risk of obesity[45]. A meta-analysis of randomized trials and prospective studies elucidates that sugar consumption is a

factor in body weight in *ad libitum* diet, and instead of the metabolism of sugars, energy balance was the cause of changes in body weight [45]. Studying the isolated effects of a single nutrient presents challenges since nutrients are not ingested in isolation. In addition, factors like the food matrix or nutrient interactions (antagonistic or synergetic) affect how a nutrient is absorbed or metabolized. The same nutrient in a different matrix may yield different effects on health. Fardet et al.[41] studied, in a narrative review of epidemiological studies, the difference between un/minimal processed, processed, and ultra-processed fruits and found that the less processed the food is, the greater the alkalizing, antioxidant, and satietogenic effects[46].

In our study population, we found a lower percentage of unhealthy food sources contributing to fructose intake than other studies addressing food sources of fructose[13-15]. Also, the population's mean of total fructose intake (at 4 years old: 14.8 g/day; at 7 years old: 17.0 g/day and at 10 years old: 15.9 g/day) was lower than the mean fructose intake in the Dutch children (43 g/day from 4 to 6 years old and 51 g/day from 7 to 10 years old)[15] and American children (56 and 58 g/day at 7-8 years and 57 and 61 g/day at 9-13 years, girls and boys respectively)[14]. Even though this could have diminished the variability of intake between individuals and limited the possibility of finding associations, our findings become particularly concerning given the positive association found between fructose intake from SSB and cardiometabolic risk[13-15].

Our study has many strengths. First, it uses data from an ongoing birth cohort, with a large number of participants, which allowed to account for many potential confounders, even though residual confounding cannot be discarded.

Moreover, due to the prospective design, it is possible to assume a temporal relationship between the exposure and the outcome. However, we cannot discard the possibility of selection bias: more educated and with higher socioeconomic levels tend to participate more in long-term studies. This selection bias may have been enhanced by the fact that only a subsample reported dietary intake. However, differences were likely because of large sample size rather than to substantial differences between participants (Cohen's effect size values were <0.35).

Another major strength of our study was the use of a 3-day food diary to collect dietary intake, which is considered a valid method to assess the intake of macronutrients at the individual level, especially in children. Moreover, the possibility of estimating fructose intake considering the food sources that contribute to a nutrient intake instead of studying only the nutrient *per se* also constitutes an advantage.

Another limitation of this thesis was the possibility of social desirability bias since parents may omit foods not acceptable at young ages. Even though fructose values were extracted from EUROFIR, a reliable European source of food composition, we only included fructose in its free form, meaning that the fructose in sucrose was not included in our analysis.

Conclusions

In conclusion, these findings highlight the importance of limiting the consumption of SSB from early periods in the life course. On the other hand, the consumption of fruits and vegetables should be promoted. More studies are

needed to understand which amount of fructose is considered excessive, particularly in young populations. Further studies should focus on the food source of the nutrient when considering a nutrient approach.

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Attachments

Attachments Index

Attachment A

Attachment A: Supplementary Table 1 - Comparison between included and non-included subjects in the study population (Generation XXI birth cohort), at 4 years.

	4 years				
	Included n=-1287		Non-Included n= 7360		P-value
	Missing (n)	Mean (SD) / % (n)	Missing (n)	Mean (SD) / % (n)	
Maternal characteristics					
Age at delivery, years	0	30.4 (4.8)	6	28.7 (5.7)	<0.001
Educational level at baseline	7		49		<0.001
< 10 years	-	36.7 (470)	-	51.4 (3758)	
10-12 years	-	28.1 (360)	-	26.5 (1940)	
> 12 years	-	35.2 (450)	-	22.1 (1613)	
Pre-pregnancy BMI, kg/m^2	95	23.9 (4.0)	634	23.9 (4.3)	0.92
Smoking during pregnancy	12		105		<0.001
No	-	83.8 (1069)	-	75.3 (5465)	
Yes	-	16.2 (206)	-	24.7 (1790)	
Alcohol drinking during pregnancy	92		383		0.04
No	-	86.9 (1038)	-	88.9 (6204)	
Yes	-	11.1 (157)	-	11.1 (773)	
Children's characteristics					
Sex	0		0		0.92
Female	-	48.9 (629)	-	49.0 (3608)	
Male	-	51.1 (658)	-	51.0 (3752)	
First born	36	60.0 (750)	134	58.5 (4228)	0.34
Birthweight, g	0	3226 (455)	1	3135 (550)	<0.001
Gestational age, weeks	0	38.8 (1.4)	21	38.4 (2.0)	<0.001

Attachment B

Attachment B: Supplementary Table 2 - Comparison between included and non-included subjects in the study population (Generation XXI birth cohort), at 7 years.

	7 years				
	Included n=-1979		Non-Included n= 6668		P-value
	Missing (n)	Mean (SD) / % (n)	Missing (n)	Mean (SD) / % (n)	
Maternal characteristics					
Age at delivery, years	0	30.5 (4.8)	6	28.6 (5.8)	<0.001
Educational level at baseline	8		48		<0.001
< 10 years	-	36.8 (726)	-	52.9 (3502)	
10-12 years	-	28.6 (563)	-	26.2 (1737)	
> 12 years	-	34.6 (682)	-	20.9 (1381)	
Pre-pregnancy BMI, kg/m^2	136	24.1 (4.2)	593	23.8 (4.3)	0.03
Smoking during pregnancy	26		91		<0.001
No	-	86.3 (1685)	-	73.7 (4849)	
Yes	-	13.7 (268)	-	26.3 (1728)	
Alcohol drinking during pregnancy	114		361		0.03
No	-	87.2 (1626)	-	89.0 (5616)	
Yes	-	12.8 (239)	-	11.0 (691)	
Children's characteristics					
Sex	0		0		0.08
Female	-	47.3 (936)	-	49.5 (3301)	
Male	-	52.7 (1043)	-	50.5 (3367)	
First born	51	59.8 (1152)	119	58.4 (3826)	0.30
Birthweight, g	0	3233 (450)	1	3124 (559)	<0.001
Gestational age, weeks	0	38.8 (1.4)	21	38.4 (2.1)	<0.001

Attachment C

Attachment C: Supplementary Table 3 - Comparison between included and non-included subjects in the study population (Generation XXI birth cohort), at 10 years.

	10 years				
	Included n=-1814		Non-Included n= 6833		P-value
	Missing (n)	Mean (SD) / % (n)	Missing (n)	Mean (SD) / % (n)	
Maternal characteristics					
Age at delivery, years	0	30.5 (4.9)	6	28.6 (5.7)	<0.001
Educational level at baseline	11		45		<0.001
< 10 years	-	37.3 (672)	-	52.4 (3556)	
10-12 years	-	28.3 (510)	-	26.4 (1790)	
> 12 years	-	34.4 (621)	-	21.2 (1442)	
Pre-pregnancy BMI, kg/m^2	123	23.9 (4.1)	606	23.9 (4.3)	0.49
Smoking during pregnancy	20		97		<0.001
No	-	86.6 (1554)	-	73.9 (4980)	
Yes	-	13.4 (240)	-	26.1 (1756)	
Alcohol drinking during pregnancy	105		370		0.01
No	-	87.0 (1486)	-	89.1 (5756)	
Yes	-	13.0 (223)	-	10.9 (707)	
Children's characteristics					
Sex	0		0		0.53
Female	-	48.3 (877)	-	49.2 (3360)	
Male	-	51.7 (937)	-	50.8 (3473)	
First born	47	58.6 (1035)	123	58.8 (3943)	0.89
Birthweight, g	0	3229 (453)	1	3127 (557)	<0.001
Gestational age, weeks	0	38.8 (1.4)	21	38.4 (2.1)	<0.001

Attachment D

Attachment D: Supplementary Table 4: Association between clusters of fructose according to the food source at 4, 7 and 10 years and cardiometabolic health clusters at 13 years, (complete-case sample).

	Metabolically Unhealthy Children at 13 years					
	OR (95% CI)		OR (95% CI)		OR (95% CI)	
	n	Dietary intake at 4 y	n	Dietary intake at 7 y	n	Dietary intake at 10 y
Model 1	1287		1979		1814	
Low SD&F and Average Veg		Ref.		Ref.		Ref.
High F&V and Low SD		0.86 (0.64-1.16)		1.00 (0.79-1.25)		0,98 (0.75-1.28)
High SD and Low F&V		**2.23 (1.28-3.86)**		1.36 (0.94-1.95)		1.25 (0.87-1.79)
Model 2	1081		1697		1564	
Low SD&F and Average Veg		Ref.		Ref.		Ref.
High F&V and Low SD		0.93 (0.66-1.32)		1.05 (0.81-1.37)		0.99 (0.74-1.34)
High SD and Low F&V		1.63 (0.85-3.13)		1.15 (0.75-1.74)		1.21 (0.81-1.83)
Model 3	1081		1697		1564	
Low SD&F and Average Veg		Ref.		Ref.		Ref.
High F&V and Low SD		0.92 (0.64-1.30)		1.04 (0.77-1.40)		1.04 (0.77-1.40)
High SD and Low F&V		1.59 (0.82-3.06)		1.36 (0.90-2.07)		1.36 (0.90-2.07)

Model 1 – Crude Model; **Model 2** – Adjusted for confounders (mother's educational background, sex, mother's age at birth, body mass index before pregnancy, smoke and alcohol ingestion during pregnancy, being first born and gestational age); **Model 3** – Model 2 + adjusted for total energy intake.

Attachment E

Attachment E - Manuscript to be published.

Title: Association between Fructose Intake and its Food Sources at preschool-aged Children and Cardiometabolic Health in adolescence: Findings from the Generation XXI Birth Cohort

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Abstract

Background/objectives: Total sugar and sugar-sweetened beverages consumption have been linked with higher obesity risk, but the literature on the relationship between fructose intake, particularly considering the main food sources, and cardiometabolic health in young populations is sparse.

The main objective was to understand the relationship between fructose intake during childhood and cardiometabolic health risk in adolescence, considering the main food sources fructose intake.

Methods: This study includes 8647 children from the Generation XXI birth cohort. Dietary intake was collected using 3-day food diaries. Fructose intake was assessed in g/day, and clusters of fructose intake by its food sources were estimated. At 13 years old, two clusters of cardiometabolic risk were previously estimated. Multivariable logistic regression models were used to investigate the association of fructose intake at 4-,7- and 10- years old and the clusters of fructose intake by its food sources with cardiometabolic risk clusters at 13 years old.

Results: Three clusters of fructose intake by its food sources were obtained: 1) "High Fruits and Vegetables and Low Soft Drinks"; 2) "Low Soft Drinks and Fresh Fruits and Average intake of Fresh Vegetables" (Reference); 3) "High Soft Drinks and Low Fresh Fruits and Vegetables". At the age of 4, children classified in the "High Soft Drinks and Low Fresh Fruits and Vegetables" cluster had a higher risk of being metabolically unhealthy [OR = 1.84, (95% CI = 1.01, 3.34)] when compared to those classified in the reference group. There was no significant association between the clusters of fructose intake at 7 and 10 years and cardiometabolic health risk.

Conclusions: This study supports that a higher intake of fructose from soft drinks but not from fruits and vegetables in 4-year-old children was associated with a higher risk of being metabolically unhealthy in adolescence. This study reinforces that soft drinks should be discouraged from early periods in the life course.

Keywords

Fructose, Cardiometabolic Health, Childhood, Cohort studies

Introduction

Dietary fructose is consumed in its simple monosaccharide form and/or as a constituent of the disaccharide sucrose[1].

Fructose absorption increases when it is combined with glucose or when it is ingested as sucrose, maximising this effect when glucose and fructose are ingested in the same amounts[2]. However, the intestinal ability to absorb fructose reaches a saturation point[3]. When fructose is not absorbed, it can create an osmotic load on the distal intestine and colon, contributing to intestinal symptoms[3].

After absorption, fructose reaches the liver through the portal circulation and is converted into fructose-1-phosphate, which is then metabolised to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate[3]. In opposition to glucose uptake and glycolysis, which are influenced by insulin metabolism, these primary steps in fructose metabolism seem unregulated and bypass hormonal control[4].

Because liver fructolysis is unlimited, fructose loads can induce enlargement of the hexose and triose-phosphate pools, which are potential substrates for glycolysis, glycogenesis, gluconeogenesis, lipogenesis and oxidative phosphorylation[3]. This uncontrolled metabolism generates glycerophosphate and acetyl-CoA, predecessors of triglycerides[5]. Higher levels of circulating triglycerides, in the form of low-density lipoproteins (LDL), activate *de novo* lipogenesis and promote intrahepatic lipid accumulation[3]. This may lead to non-alcoholic fatty liver disease, which in turn is strongly associated with visceral fat, diabetes and lipid disorders.[6].

Among adults, in a systematic review and meta-analysis including 31 controlled feeding trials reviewing the effect of fructose on body weight, fructose showed no overall effect on body weight in isocaloric trials (637 participants); however, excessive fructose in hypercaloric trials (119 participants) led to body weight increases (mean difference of 0.53 kg (95% CI 0.26-0.79)[7].

Not only the source but also the total energy intake may influence whether fructose has an impact on CV health or not. In adults, two other systematic review and meta-analysis of controlled trials suggested that energy and food sources appear to be mediators of the effect of fructose and non-alcoholic fat liver disease markers[8], inflammatory markers[9] and uric acid[10]. The excess of energy provided by SSB lead to an increase in intrahepatocellular lipids and an increase in ALT (less relevant)[8]. However, evidence was of low quality because most studies were short-term[8], limiting conclusions.

In children, most evidence concerns total sugar[11] and consumption of SSB[5, 12] in relation to cardiometabolic health. A World Health Organization (WHO) meta-analysis of 5 prospective cohort studies in children showed that after a 1-year follow-up, a higher consumption of SSB was associated with a 55% higher risk of becoming overweight or obese versus those with the lowest intake[12]. In addition, a review suggested that consuming fructose rich SSB was associated with developing pediatric insulin resistance[5]. This relationship may be explained by central adiposity, which rises with fructose consumption[5].

Literature is sparse on the association linking fructose intake with cardiometabolic dysfunctions already in childhood. Thus, more studies are needed to assess this issue. Considering that fructose can be naturally found in some foods like fruits,

which are also rich in other beneficial nutritional compounds, or in some foods like SSBs, becomes of the utmost importance to consider the main food sources of fructose when studying its relationship with cardiometabolic health.

Objectives

The main objective of this study was to understand the relationship between fructose intake during childhood and cardiometabolic health risk in adolescence, analysing fructose intake from the main food sources in the children's diet, using data from the Generation XXI birth cohort.

Methodology

7. Study design and participants

Generation XXI is an ongoing prospective population-based birth cohort (Porto, Portugal, 2005–2006), described elsewhere in detail[13, 14]. Participants were recruited between April 2005 and August 2006 from all public maternities of the Metropolitan area of Porto, Portugal. At baseline, 8495 mothers and 8647 newborns were enrolled (participation proportion of 91%). Within 72 hours after delivery, structured questionnaires collected demographic and socioeconomic information, lifestyle factors, obstetric history, anthropometric measurements, and personal disease history through face-to-face interviews conducted by trained interviewers. The cohort was then re-evaluated at 4 (2009-2011: participation proportion of 86%), 7 (2012-2014: participation proportion of 80%), 10 years old (2015-2017: participation proportion of 76%) and 13 years old (2018-2020: participation proportion of 54%, interrupted earlier due to COVID-19).

8. Ethical Approval

The University of Porto Medical School/S. João Hospital Centre Ethics and the Portuguese Data Protection Authority Ethics provided ethical approval. All study phases complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki. Written informed consent was obtained from all participating children's parents/legal caregivers.

9. Dietary Data

At 4, 7, and 10 years old, dietary intake was assessed by 3-day food diaries in non-consecutive days (2 weekdays and 1 weekend). The children's main caregiver was instructed to describe all the food and drinks consumed, including any specific commercial brand names, and the quantity consumed in grams, units, or household measures. If the child had meals outside of the home without a parent present, the caregiver responsible during the day reported those meals. Meals prepared at home were documented with comprehensive recipe information, including ingredients and the preparation methods.

The food items reported during the 4-, 7- and 10-year follow-up using the software Food Processor SQL (2004-2005 EHSA Research, Salem, Oregon)[15] were matched to their corresponding counterpart in the eAT24 software[16].

After nutrient conversion, daily energy intake (kcal) and nutrient intake (g/day) were estimated for each participant.

Fructose values were extracted from EUROFIR – FoodExplorer. The absolute values were converted according to their proportion to the total carbohydrates of the food item in our composition database. For recipes, this nutrient was calculated

based on the fructose values of each single ingredient according to its proportion in the recipe.

10. Cardiometabolic Health-Related Parameters

At 13 years old follow-up, a clinical examination was performed by trained staff. Anthropometric measurement included the assessment of weight measured with minimum clothes and without shoes on a digital scale. Height was measured with children in an upright position with a fixed stadiometer. Body Mass Index (BMI) z-score was calculated according to the World Health Organization growth charts[17]. Waist circumference was measured according to standard procedures[18].

Blood samples were collected after an overnight fast as part of the follow-up evaluation at 13 years. Enzymatic methods were used to obtain serum concentration of high-density lipoprotein cholesterol (HDL-C), triglycerides and glucose, whereas insulin was measured by electrochemiluminescence immunoassay. The formula used for calculating the Homeostasis Model Assessment Insulin Resistance (HOMA-IR) was: fasting glucose (mmol/L) * fasting insulin (mU/L) / 22.5. Age- and sex-specific z-scores for waist circumference, HDL-C, triglycerides, and HOMA-IR were calculated using the sample of Generation XXI.

Diastolic and systolic blood pressure were measured after children had been sitting for at least five minutes, and the measurement was taken three times, using a blood pressure monitor. The mean of the diastolic and systolic blood pressure from the last two values was taken into account. Age-, sex- and height-specific z-scores were determined for diastolic and systolic blood pressure, according to the American Academy of Pediatrics[19].

Two clusters of cardiometabolic risk were previously estimated (Marinho AR et al., manuscript submitted): 1) a cluster of metabolically healthy adolescents (with average values of cardiometabolic risk factors) (MHC); and 2) a cluster of metabolically unhealthy adolescents (values above the average for all risk factors and below the average for HDL-C) (MUC).

11. Covariates

Potential covariates were selected, such as: maternal educational level (number of schooling years categorized into <12, 12-14 and >14 years), mother's age at birth (in years), BMI (Body Mass Index) before pregnancy (kg/m2), pregnancy smoke (yes/no), alcohol intake (yes/no), birth weight (g), and being first born (yes/no) and gestational age (weeks).

12. Study Sample

Dietary intake, considering complete information from 3-day food diaries, was collected at 4 (2493 children), 7 (3587 children) and 10 years old (2855). At 13 years, 2415 children had information on cardiometabolic health patterns. At each evaluation, the final sample included children with information on dietary intake and cardiometabolic health: 1287 children were considered at 4, 1979 children at 7 and 1814 children at 10 years old.

7. *Statistics analysis*

The studied sample was compared with the remaining cohort using the chi-square test for categorical variables and two-sided Student's t-tests for continuous.

The clusters of fructose intake by its food sources were estimated using hierarchical cluster analysis with the complete-linkage method and Euclidean

distance with data from daily intake of fructose intake (g/day) from the main food sources. To define the proper number of clusters, the Bayesian Information Criterion was computed.

Using multiple imputations by chained equations (n=20) missing data on dietary intake, cardiometabolic parameters at 13 years old and covariates were imputed, assuming missing at random[20, 21]. Thus, the main analysis were performed including 8647 children.

Multivariable logistic regression was used to investigate the associations between fructose intake (in g/day) or the three clusters of fructose by its food sources and the two cardiometabolic health clusters, adjusted for the following selected covariates: mother's educational background, sex, mother's age at birth, body mass index before pregnancy, smoke and alcohol ingestion during pregnancy and being firstborn, and gestational age. Interaction terms between sex and clusters of fructose intake by its food sources on the measured associations was tested, and as no significant interactions were found, the analyses were not stratified by sex.

SPSS (Statistical Package for the Social Sciences) version 27 for MacBook and R statistical software (The R Project for Statistical Computing, Vienna, Austria), version 4.0.0 for Windows software were used to the analysis, using the packages stats[22], *mclust*[23] and *mice*[24].

Results

Sample's Characteristics

Maternal and children's characteristics are shown in Table 1. Our sample was mainly composed by males. At 4 years old, those included in the sample were more likely to have older mothers (30.4 vs 28.7 years old), with a higher

educational level (35.2% vs 22.1% with a university degree), were non-smokers during pregnancy (16.2% vs 24,7% non-smokers), and children had a higher birthweight (3226 g vs 3135 g) and higher time of gestation (38.8 vs 38.4 gestational weeks – values at 4 years old. (Supplementary Table 1). These characteristics had similar values at 7 and 10 years old (Supplementary Table 2 and 3).

Figure 1 shows the main food sources of fructose intake at the three follow-up evaluations. At all ages, fresh fruits (54.9 %), fresh vegetables (14.1 %) were the main sources of fructose intake in the diet, followed by soft drinks (at 4 and 10 years old) and nectars (at 7 years old).

Characteristics of children's dietary intake are presented in Table 2. At 4, 7 and 10 years old, the mean intake of fructose was 14.8 g/day, 17.0 g/day and 15.9 g/day, respectively; and mean total energy intake was 1592 kcal/day, 1767 kcal/day and 1914 kcal, respectively. Children in the MHC had a higher intake of fructose (g/day) and a smaller energy intake.

Fructose Intake and Cardiometabolic Health Risk Clusters

Table 3 shows the association between total fructose intake (g/day) at 4 and the cardiometabolic risk in adolescence. In the crude model, total fructose intake at 4 years old (g/day) was associated with lower risk of being part of MUC at 13 years old, however after accounting for confounders and total energy intake, this association was no longer significant (OR= 0.92; 95% CI 0.66-1.00, p-value=0.05).

Fructose Intake by its Food Sources Clusters at 4, 7, 10 and cardiometabolic outcomes at 13 years old

Three clusters of fructose intake by its food sources were obtained: 1) a cluster of children with a high daily intake of fructose from fresh fruits and vegetables and low intake of fructose from soft drinks named “High Fruits and Vegetables and Low Soft Drinks” (“High F&V and Low SD”); 2) a cluster of children with low intake of fructose from soft drinks and fresh fruits, and average intake of fructose intake from fresh vegetables, named “Low Soft Drinks and Fresh Fruits and Average intake of Fresh Vegetables” (“Low SD&F and Average Veg”) (Reference); and a final group of children with a high intake of fructose from soft drinks and low intake of fructose from fresh fruits and vegetables consumption called “High Soft Drinks and Low Fresh Fruits and Vegetables” (“High SD and Low F&V”).

Table 4 shows the association between the clusters of fructose food sources at 4 years old and the two clusters of cardiometabolic health risk. The group of children characterized by a high intake of fructose from soft drinks and cakes at 4 years old was associated with a higher risk of being metabolically unhealthy in adolescence, even after adjusting for confounders and total energy intake (Model 3: OR = 1.84, 95% CI = 1.01 to 3.34).

Discussion

This study showed that 4-year-old children classified in the cluster characterized by high fructose intake from SSB and low intake of fruits and vegetables - “High SD and Low F&V” - were associated with a higher risk of being metabolically unhealthy at 13 years old when compared to those in the “Low SD&F and Average Veg” cluster, even after adjusting for confounders. These findings are corroborated by previous systematic reviews and meta-analyses among adults[25, 26]. In children, to the best of our knowledge no studies exist linking fructose from

food sources with cardiovascular health. The mechanisms explaining the relationship found between fructose intake from SSB and cardiometabolic health are sparse and controversial. One explanation might be the increased energy intake and the reduced satiety effect of an SSB compared to solid food with the same energetic value [11], the absence of dietary fiber in its composition [27] and lack of mastication[28, 29] might reduce the satietogenic effect of SSB when compared to fresh fruit and fresh vegetables, for example.

At 7 and 10 years old, the associations of the cluster 'High SD and Low F&V' with cardiometabolic outcomes did not reach statistical significance; however, they consistently maintained the same direction as the one found at 4 years old. This lack of significance may be attributed to the fact that as children got older, the consumption of soft drinks increased up to adolescence, and the variability might have diminished. In our study, the main sources of fructose in the diet at 4 years old were: fresh fruit (54.9 %), fresh vegetables (14.1 %) and soft drinks (5.4 %). As children got older, the contribution of fresh fruits, fresh vegetables, yoghurt and fermented milk to the total fructose intake decreased. On the other hand, the contribution of nectars, processed milk, and SSB increased. Despite this increase in SSB intake in our sample, no significant associations were found between fructose intake from SSB at 7 and 10 years old and cardiometabolic outcomes at 13 years. Even though studies have suggested some tracking of dietary patterns during childhood[30, 31], our study suggested that early ages, particularly the 4 years of age, might be a sensitive period for the influence of fructose, particularly when consumed from SSB, on cardiometabolic health in adolescence.

When considering total fructose, we found an inverse marginal association between total fructose intake at 4 years old (g/day) and the risk of being metabolically unhealthy. This could be explained by the differential influence of the different sources of fructose. In our population, fresh fruits and vegetables were the main sources of fructose, and despite the lack of significance, children classified in the cluster of high intake of fructose from fruits and vegetables were associated with a lower risk of being metabolically unhealthy (OR= 0.92, 95% CI= 0.64 to 1.33). Depending on the food source, these opposite effects of fructose may have nulled the overall effect of the nutrient. This reinforces the need for studies to focus on the food source of a nutrient when conducting a nutrient approach in nutritional epidemiology. The significance of the food matrix and the food sources become notably important, particularly when studying sugars. In fact, research has yielded contradictory findings regarding the association between the types of sugars and the risk of obesity[32]. A meta-analysis of randomized trials and prospective studies elucidates that sugar consumption is a factor in body weight in *ad libitum* diet, and instead of the metabolism of sugars, energy balance was the cause of changes in body weight[32]. Studying the isolated effects of a single nutrient presents challenges since nutrients are not ingested in isolation. In addition, factors like the food matrix or nutrient interactions (antagonistic or synergetic) affect how a nutrient is absorbed or metabolized. The same nutrient in a different matrix may yield different effects on health. Fardet et al.[33] studied, in a literature review of epidemiological studies, the difference between un/minimal processed, processed, and ultra-processed fruits and found that the less processed the food is, the greater the alkalizing, antioxidant, and satietogenic effects[33].

In our study population, we found a lower percentage of unhealthy food sources contributing to fructose intake than other studies addressing food sources of fructose[34-36]. Also, the population's mean of total fructose intake (at 4 years old: 14.8 g/day; at 7 years old: 17.0 g/day and at 10 years old: 15.9 g/day) was lower than the mean fructose intake in the Dutch children (43 g/day from 4 to 6 years old and 51 g/day from 7 to 10 years old)[34] and American children (56 and 58 g/day at 7-8 years and 57 and 61 g/day at 9-13 years, girls and boys respectively)[36]. Even though this could have diminished the variability of intake between individuals and limited the possibility of finding associations, our findings support the positive association found between fructose intake from SSB and cardiometabolic risk[34-36].

Our study has many strengths. First, it uses data from an ongoing birth cohort, with a large number of participants, which allowed to account for many potential confounders, even though residual confounding cannot be discarded. Moreover, due to the prospective design, it is possible to assume a temporal relationship between the exposure and the outcome. However, we cannot discard the possibility of selection bias: more educated and with higher socioeconomic levels tend to participate more in long-term studies. This selection bias may have been enhanced by the fact that only a subsample reported dietary intake. However, differences were likely because of large sample size rather than to substantial differences between participants (Cohen's effect size values were <0.35).

Another major strength of our study was the use of a 3-day food diary to collect dietary intake, which is considered a valid method to assess the intake of macronutrients at the individual level, especially in children. Moreover, the

possibility of estimating fructose intake considering the food sources that contribute to a nutrient intake instead of studying only the nutrient *per se* also constitutes an advantage.

Another limitation of this thesis was the possibility of social desirability bias since parents may omit foods not acceptable at young ages. Even though fructose values were extracted from EUROFIR, a reliable European source of food composition, we only included fructose in its free form, meaning that the fructose in sucrose was not included in our analysis.

Conclusions

In conclusion, these findings highlight the importance of limiting the consumption of SSB from early periods in the life course. On the other hand, the consumption of fruits and vegetables should be promoted. More studies are needed to understand which amount of fructose is considered excessive, particularly in young populations. Further studies should focus on the food source of the nutrient when considering a nutrient approach.

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Table 1: Characteristics of the participants with data on dietary intake at 4, 7and 10 years old and cardiometabolic parameters at 13 years old in Generation XXI (n=1287)

	Included at 4 years (n= 1287)		Included at 7 years (n= 1979)		Included at 10 years (n= 1814)	
	Missing (n)	Mean (SD) / % (n)	Missing (n)	Mean (SD) / % (n)	Missing (n)	Mean (SD) / % (n)
Maternal characteristics						
Age at delivery, years	0	30.4 (4.8)	0	30.5 (4.8)	0	30.5 (4.9)
Educational level at baseline	7		8		11	
< 10 years	-	36.7 (470)	-	36.8 (726)	-	37.3 (672)
10-12 years	-	28.1 (360)	-	28.6 (563)	-	28.3 (510)
> 12 years	-	35.2 (450)	-	34.6 (682)	-	34.4 (621)
Pre-pregnancy BMI, kg/m^2	95	23.9 (4.0)	136	24.1 (4.2)	123	23.9 (4.1)
Smoking during pregnancy	12		26		20	
No	-	83.8 (1069)	-	86.3 (1685)	-	86.6 (1554)
Yes	-	16.2 (206)	-	13.7 (268)	-	13.4 (240)
Alcohol drinking during pregnancy	92		114		105	
No	-	86.9 (1038)	-	87.2 (1626)	-	87.0 (1486)
Yes	-	11.1 (157)	-	12.8 (239)	-	13.0 (223)
Children's characteristics						
Sex	0		0		0	
Female	-	48.9 (629)	-	47.3 (936)	-	48.3 (877)
Male	-	51.1 (658)	-	52.7 (1043)	-	51.7 (937)
First born	36	60.0 (750)	51	59.8 (1152)	47	58.6 (1035)
Birthweight, g	0	3226 (455)	0	3233 (450)	0	3229 (453)
Gestational age, weeks	0	38.8 (1.4)	0	38.8 (1.4)	0	38.8 (1.4)

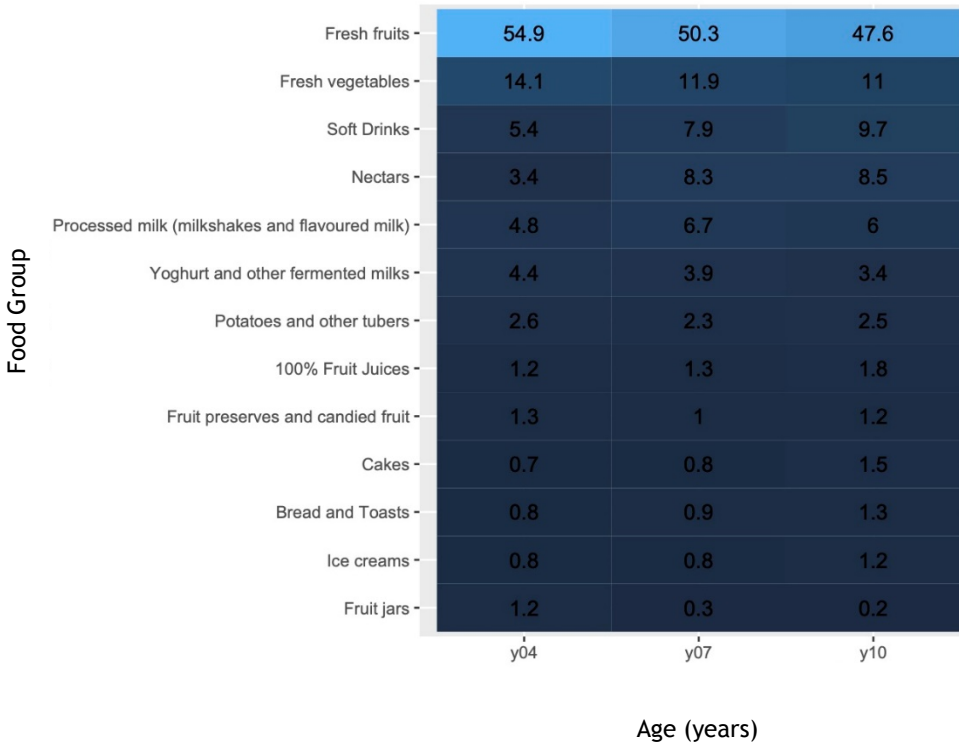

Figure 1: Main sources of fructose in the diet (%) in the study population (Generation XXI).

Table 2: Total energy and fructose intake in the total sample and by clusters of cardiometabolic risk.

		At 13 years	
	Total sample	**Metabolically Healthy Children**	**Metabolically Unhealthy Children**
	Mean (SD) / % (n)	**Mean (SD) / % (n)**	**Mean (SD) / % (n)**
***Dietary Intake at 4 years* (n= 1287)**			
Energy Intake, kcal	1592 (283)	1586 (284)	1609 (281)
Fructose Intake, g/d	14.8 (6.1)	15.1 (6.1)	14.1 (5.9)
Clusters of fructose food sources			
High F&V and Low SD	26.6% (342)	27.5% (265)	23.7% (77)
Low SD&F and Average Veg	69.1% (889)	69.1% (665)	68.9% (224)
High SD and Low F&V	4.4% (56)	3.3% (32)	7.4% (24)
***Dietary Intake at 7 years* (n= 1979)**			
Energy Intake, kcal	1767 (299)	1777 (301)	1736 (293)
Fructose Intake, g/d	17.0 (7.1)	17.2 (7.1)	16.6 (7.2)
Clusters of fructose food sources			
High F&V and Low SD	28.5% (565)	28.8% (427)	27.9% (138)
Low SD&F and Average Veg	63.5% (1257)	63.9% (949)	62.3% (308)
High SD and Low F&V	7.9% (157)	7.3% (109)	9.7% (48)
***Dietary Intake at 10 years* (n= 1814)**			
Energy Intake, kcal	1913 (388)	1926 (382)	1871 (404)
Fructose Intake, g/d	15.8 (6.8)	15.9 (6.8)	15.5 (7.0)
Clusters of fructose food sources			
High F&V and Low SD	22.2% (403)	22.4% (308)	21.6% (95)
Low SD&F and Average Veg	68.4% (1241)	68.7% (944)	67.5% (297)
High SD and Low F&V	9.4% (170)	8.9% (122)	10.9% (48)

Table 3: Association between total fructose (per each 10 g/day) at 4, 7 and 10 years old and cardiometabolic health clusters at 13 years, (imputed sample n=8647)

	Metabolically Unhealthy Children at 13 years
Total Fructose, g/d	**OR (95% CI)**
4 years	
Model 1, 10g/day	**0.82 (0.66-1.00)**
Model 2, 10g/day	0.90 (0.66-1.10)
Model 3, 10g/day	0.82 (0.66-1.00)
7 years	
Model 1, 10g/day	0.90 (0.82-1.00)
Model 2, 10g/day	0.90 (0.82-1.10)
Model 3, 10g/day	1.00 (0.90-1.22)
10 years	
Model 1, 10g/day	1.00 (0.82-1.10)
Model 2, 10g/day	1.00 (0.90-1.10)
Model 3, 10g/day	1.10 (0.82-1.22)

Model 1 – Crude Model; **Model 2** – Adjusted for confounders (mother's educational background, sex, mother's age at birth, body mass index before pregnancy, smoke and alcohol ingestion during pregnancy, being first born and gestational age); **Model 3** – Model 2 + adjusted for total energy intake at each follow-up.

Table 4: Association between clusters of fructose according to the food source at 4, 7, 10 and 13 years and cardiometabolic health clusters at 13 years, (imputed sample n=8647)

	Metabolically Unhealthy Children at 13 years		
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**
	Dietary intake at 4 y	**Dietary intake 7 y**	**Dietary intake at 10 y**
Model 1			
High F&V Low SD	0.89 (0.65-1.22)	1.03 (0.85-1.25)	1.01 (0.78-1.32)
High SD Low F&V	**2.01 (1.14-3.53)**	1.32 (0.99-1.76)	1.31 (0.94-1.81)
Model 2			
High F&V Low SD	0.95 (0.66-1.35)	1.12 (0.90-1.39)	1.10 (0.81-1.48)
High SD Low F&V	**1.89 (1.03-3.46)**	1.20 (0.88-1.65)	1.15 (0.80-1.64)
Model 3			
High F&V Low SD	0.92 (0.64-1.33)	1.12 (0.91-1.39)	1.11 (0.91-1.37)
High SD Low F&V	**1.84 (1.01-3.34)**	1.29 (0.91-1.81)	1.27 (0.95-1.71)

Model 1 – Crude Model; **Model 2** – Adjusted for confounders (mother's educational background, sex, mother's age at birth, body mass index before pregnancy, smoke and alcohol ingestion during pregnancy, being first born and gestational age); **Model 3** – Model 2 + adjusted for total energy intake. Reference group: Low SD&F; Average V.

Appendix

Supplementary Table 1 - Comparison between included and non-included subjects in the study population (Generation XXI), complete-case analysis, at 4 years.

	4 years				
	Included n=-1287		Non-Included n= 7360		P-value
	Missing (n)	Mean (SD) / % (n)	Missing (n)	Mean (SD) / % (n)	
Maternal characteristics					
Age at delivery, years	0	30.4 (4.8)	6	28.7 (5.7)	<0.001
Educational level at baseline	7		49		<0.001
< 10 years	-	36.7 (470)	-	51.4 (3758)	
10-12 years	-	28.1 (360)	-	26.5 (1940)	
> 12 years	-	35.2 (450)	-	22.1 (1613)	
Pre-pregnancy BMI, kg/m^2	95	23.9 (4.0)	634	23.9 (4.3)	0.92
Smoking during pregnancy	12		105		<0.001
No	-	16.2 (206)	-	75.3 (5465)	
Yes	-	83.8 (1069)	-	24.7 (1790)	
Alcohol drinking during pregnancy	92		383		0.04
No	-	11.1 (157)	-	88.9 (6204)	
Yes	-	86.9 (1038)	-	11.1 (773)	
Children's characteristics					
Sex	0		0		0.92
Female	-	48.9 (629)	-	49.0 (3608)	
Male	-	51.1 (658)	-	51.0 (3752)	
First born	36	60.0 (750)	134	58.5 (4228)	0.34
Birthweight, g	0	3226 (455)	1	3135 (550)	<0.001
Gestational age, weeks	0	38.8 (1.4)	21	38.4 (2.0)	<0.001

Supplementary Table 2 - Comparison between included and non-included subjects in the study population (Generation XXI), complete-case analysis, at 7 years.

	7 years				
	Included n=-1979		Non-Included n= 6668		P-value
	Missing (n)	Mean (SD) / % (n)	Missing (n)	Mean (SD) / % (n)	
Maternal characteristics					
Age at delivery, years	0	30.5 (4.8)	6	28.6 (5.8)	<0.001
Educational level at baseline	8		48		<0.001
< 10 years	-	36.8 (726)	-	52.9 (3502)	
10-12 years	-	28.6 (563)	-	26.2 (1737)	
> 12 years	-	34.6 (682)	-	20.9 (1381)	
Pre-pregnancy BMI, kg/m^2	136	24.1 (4.2)	593	23.8 (4.3)	0.03
Smoking during pregnancy	26		91		<0.001
No	-	86.3 (1685)	-	73.7 (4849)	
Yes	-	13.7 (268)	-	26.3 (1728)	
Alcohol drinking during pregnancy	114		361		0.03
No	-	87.2 (1626)	-	89.0 (5616)	
Yes	-	12.8 (239)	-	11.0 (691)	
Children's characteristics					
Sex	0		0		0.08
Female	-	47.3 (936)	-	49.5 (3301)	
Male	-	52.7 (1043)	-	50.5 (3367)	
First born	51	59.8 (1152)	119	58.4 (3826)	0.30
Birthweight, g	0	3233 (450)	1	3124 (559)	<0.001
Gestational age, weeks	0	38.8 (1.4)	21	38.4 (2.1)	<0.001

Supplementary Table 3 - Comparison between included and non-included subjects in the study population (Generation XXI), complete-case analysis, at 10 years.

	10 years				
	Included n=-1814		Non-Included n= 6833		P-value
	Missing (n)	Mean (SD) / % (n)	Missing (n)	Mean (SD) / % (n)	
Maternal characteristics					
Age at delivery, years	0	30.5 (4.9)	6	28.6 (5.7)	<0.001
Educational level at baseline	11		45		<0.001
< 10 years	-	37.3 (672)	-	52.4 (3556)	
10-12 years	-	28.3 (510)	-	26.4 (1790)	
> 12 years	-	34.4 (621)	-	21.2 (1442)	
Pre-pregnancy BMI, kg/m^2	123	23.9 (4.1)	606	23.9 (4.3)	0.49
Smoking during pregnancy	20		97		<0.001
No	-	86.6 (1554)	-	73.9 (4980)	
Yes	-	13.4 (240)	-	26.1 (1756)	
Alcohol drinking during pregnancy	105		370		0.01
No	-	87.0 (1486)	-	89.1 (5756)	
Yes	-	13.0 (223)	-	10.9 (707)	
Children's characteristics					
Sex	0		0		0.53
Female	-	48.3 (877)	-	49.2 (3360)	
Male	-	51.7 (937)	-	50.8 (3473)	
First born	47	58.6 (1035)	123	58.8 (3943)	0.89
Birthweight, g	0	3229 (453)	1	3127 (557)	<0.001
Gestational age, weeks	0	38.8 (1.4)	21	38.4 (2.1)	<0.001

Supplementary Table 4: Association between clusters of fructose according to the food source at 4, 7 and 10 years and cardiometabolic health clusters at 13 years, (complete-case sample).

	Metabolically Unhealthy Children at 13 years					
	OR (95% CI)		OR (95% CI)		OR (95% CI)	
	n	Dietary intake at 4 y	n	Dietary intake at 7 y	n	Dietary intake at 10 y
Model 1	1287		1979		1814	
Low SD&F and Average Veg		Ref.		Ref.		Ref.
High F&V and Low SD		0.86 (0.64-1.16)		1.00 (0.79-1.25)		0,98 (0.75-1.28)
High SD and Low F&V		**2.23 (1.28-3.86)**		1.36 (0.94-1.95)		1.25 (0.87-1.79)
Model 2	1081		1697		1564	
Low SD&F and Average Veg		Ref.		Ref.		Ref.
High F&V and Low SD		0.93 (0.66-1.32)		1.05 (0.81-1.37)		0.99 (0.74-1.34)
High SD and Low F&V		1.63 (0.85-3.13)		1.15 (0.75-1.74)		1.21 (0.81-1.83)
Model 3	1081		1697		1564	
Low SD&F and Average Veg		Ref.		Ref.		Ref.
High F&V and Low SD		0.92 (0.64-1.30)		1.04 (0.77-1.40)		1.04 (0.77-1.40)
High SD and Low F&V		1.59 (0.82-3.06)		1.36 (0.90-2.07)		1.36 (0.90-2.07)

Model 1 – Crude Model; **Model 2** – Adjusted for confounders (mother's educational background, sex, mother's age at birth, body mass index before pregnancy, smoke and alcohol ingestion during pregnancy, being first born and gestational age); **Model 3** – Model 2 + adjusted for total energy intake. Reference group: Low SD&F; Average V.

Fructose Intake during Childhood: Food Sources and its Association with Cardiometabolic Health in Adolescence

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