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# The effect of sugar-sweetened beverages on children's appetitive behaviours: a prospective approach from the Generation XXI birth cohort

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O presente trabalho foi elaborado no âmbito da coorte Geração XXI, desenvolvido no Departamento de Epidemiologia Clínica, Medicina Preditiva e Saúde Pública da Faculdade de Medicina do Porto e no Instituto de Saúde Pública da Universidade do Porto.

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Esta dissertação tem por base um manuscrito, no qual colaborei ativamente na formulação das hipóteses, análise e interpretação dos dados e fui responsável pela escrita das suas primeiras versões:

- The effect of sugar-sweetened beverages at 4 years of age on appetitive behaviours of 7-years-old children from the Generation XXI birth cohort.

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BMI: Body Mass Index

CEBQ: Children's Eating Behaviour Questionnaire

CEBQ-SR: Satiety Responsiveness CEBQ subdomain

CEBQ-SE: Slowness in Eating CEBQ subdomain

CEBQ-EF: Enjoyment of Food CEBQ subdomain

CEBQ-FR: Food Responsiveness CEBQ subdomain

CEBQ-FF: Food Fussiness CEBQ subdomain

CEBQ-DD: Desire for Drinks CEBQ subdomain

CEBQ-EOE: Emotional Overeating CEBQ subdomain

CEBQ-EUE: Emotional Undereating CEBQ subdomain

EIPAS: The Integrated Strategy for the Promotion of Healthy Eating

FFQ: Food frequency questionnaire

ICC: intraclass correlation coefficient

GLP-1: glucagon-like peptide-1

SSB: Sugar-sweetened beverages

P-CEBQ: Portuguese version of the Children's Eating Behaviour Questionnaire

PNPAS: National Programme for the Promotion of Healthy Eating

UK: United Kingdom

USA: United States of America

# Resumo

**Introdução e objetivo**

Os comportamentos alimentares desenvolvem-se cedo na vida e muitos fatores influenciam a regulação do apetite e as escolhas alimentares, incluindo fatores biológicos e ambientais. Comportamentos alimentares problemáticos, incluindo tanto a disinibição alimentar como a restrição alimentar, podem surgir durante a infância. Estes comportamentos têm sido associados a uma ingestão alimentar excessiva ou reduzida, generalizada ou de grupos específicos de alimentos, resultando num padrão alimentar de pior qualidade.

As bebidas açucaradas são consumidas em grande quantidade e têm sido fortemente associadas a caries dentárias e ganho de peso; atualmente são consideradas um dos fatores que mais contribuem para a obesidade infantil. Uma hipótese apontada é o facto de haver uma fraca compensação da energia fornecida sob formas líquidas, implicando um maior consumo energético total e uma potencial desregulação dos mecanismos da saciedade. Além disso é provável que a palatibilidade destas bebidas possa gerar sinais hedónicos que ultrapassam a regulação homeostática do apetite.

As bebidas açucaradas têm sido positivamente associadas com traços de disinibição alimentar e negativamente associadas com comportamentos de restrição, em estudos transversais. O efeito das bebidas açucaradas a longo prazo, especialmente nos comportamentos alimentares, tem sido pouco explorado.

Esta dissertação tem como objetivo estudar a associação entre o consumo de bebidas açucaradas aos 4 anos de idade e os comportamentos alimentares relacionados com o apetite em crianças de 7 anos.

**Métodos**

Os participantes são crianças da Geração XXI, uma corte de nascimentos de base populacional, recrutadas em todas as maternidades públicas do Porto, Portugal, em 2005-2006 (n=8647). As crianças e suas famílias foram acompanhadas em diferentes momentos; o presente estudo utiliza informação recolhida ao nascimento, aos 4 e aos 7 anos de idade (n=3917).

Os dados foram recolhidos presencialmente por entrevistadores treinados, utilizando questionários estruturados ou recuperados de registos médicos. O consumo de bebidas açucaradas foi avaliado por um questionário de frequência alimentar, previamente validado. Os comportamentos alimentares relacionados com o apetite foram avaliados através do Questionário de Comportamento Alimentar da Criança (CEBQ), auto-preechido pelo cuidador principal, previamente traduzido e testado nesta amostra, mostrando boas propriedades

psicométricas. Foram avaliados oito subdomínios relacionados com comportamentos alimentares distintos: Resposta à Saciedade (CEBQ-SR), Ingestão Lenta (CEBQ-SE), Prazer em Comer (CEBQ-EF), Resposta a Alimentos (CEBQ-FR), Seletividade Alimentar (CEBQ-FF), Desejo por Bebidas (CEBQ-DD), Sobre-ingestão Emocional (CEBQ-EOE) e Sub-ingestão Emocional (CEBQ-EUE). Esses oito subdomínios foram ainda agrupados em dois fatores, por análise de componentes principais: Restrição do apetite e Disinibição do apetite. O fator de Restrição do apetite relacionou-se com os subdomínios que medem sinais internos de saciedade e seletividade alimentar, principalmente CEBQ-FF, CEBQ-EF, CEBQ-SE e CEBQ-SR, e o fator de Desinibição do apetite relacionou-se a subdomínios que medem sinais externos e respostas emocionais em relação aos alimentos, principalmente CEBQ-FR, CEBQ-EOE, CEBQ-EUE e CEBQ-DD, ambos explicando 62% da variânicia total. A associação entre o consumo de bebidas açucaradas e as pontuações contínuas do CEBQ foi testada usando modelos lineares generalizados (coeficientes de regressão β). Para testar uma relação dose-resposta foi realizada uma regressão logística multinomial, utilizando os subdomínios do CEBQ divididos em tercis (o tercil inferior foi usado como referência) e *odds ratio* (OR) foram calculados para menor e maior consumo de bebidas açucaradas, bem como os respetivos intervalos de confiança de 95% (IC95%) com correção de Bonferroni. Os modelos foram testados em análise univariada e multivariada, utilizando como co-variáveis o sexo da criança, o índice de massa corporal materno antes da gravidez, a estrutura familiar e o tempo diário gasto com ecrãs aos 4 anos, a idade e escolaridade maternas (modelo 1) e todas as anteriores juntamente com o consumo materno de bebidas açucaradas aos 4 anos da criança (modelo 2).

**Resultados**

Em análise univariada, as crianças com maior consumo de bebidas açucaradas obtiveram uma pontuação maior, estatisticamente significativa, em Prazer em Comer (β=0,038, IC95% 0,001; 0,075), Resposta a alimentos (β=0,047, IC95% 0,010; 0,084), Sobre-ingestão Emocional (β=0,039, IC 95% 0,010; 0,069), Desejo por Bebidas (β=0,137, IC95% 0,100; 0,174) e com o fator de Disinibição do apetite (β=0,107, IC95% 0,061; 0,152).

Em análise multivariada (modelo 1), a maioria das associações perdeu a significância estatística; apenas o subdomínio Desejo por Bebidas (β=0,097, IC95% 0,059, 0,136) e o fator de Disinibição do apetite (β=0,076, IC 95% 0,029, 0,124) mantiveram a associação significativa com o consumo de bebidas açucaradas. A inclusão do consumo de bebidas açucaradas pela mãe aos 4 anos de idade da criança, diminuiu a magnitude destas associações, contudo permaneceram significativas (Desejo por Bebidas: β=0,075, IC95% 0,031; 0,119; fator de Disinibição do apetite: β=0,061, IC95% 0,007; 0,116).

As mesmas associações (modelo 2) foram testadas, mas de acordo com categorias crescentes de consumo de bebidas açucaradas e com pontuações crescentes nos subdomínios do CEBQ. O consumo de ≥1 porções de bebidas açucaradas por dia (comparado com uma menor ingestão) foi associado prospectivamente a uma maior probabilidade de estar nos tercis superiores das pontuações do CEBQ em Desejo por Bebidas (2º tercil: OR=1,262, IC95% 0,917, 1,736 ; 3º tercil: OR=1,345, IC 95% 1,048; 1,727) e no fator de Disinibição do apetite (3º tercil: OR=1,218, IC95% 0,934; 1,588). Não foram encontradas associações significativas com os subdomínios restantes de comportamento alimentar.

**Conclusões e considerações finais**

Um maior consumo de bebidas açucaradas aos 4 anos de idade foi associado a comportamentos de disinibição alimentar mais tarde na infância. Os fatores familiares tiveram um grande impacto na associação entre o consumo de bebidas açucaradas e os comportamentos alimentares. No presente estudo, o consumo de bebidas açucaradas pela mãe, bem como outras características maternas, tais como características sociodemográficas e o índice de massa corporal materno, tiveram um efeito considerável nas associações estudadas.

São necessários estudos longitudinais para compreender melhor os efeitos a longo prazo do consumo de bebidas açucaradas e o potencial efeito diferencial dos diversos tipos de bebidas açucaradas.

Enquanto isso, do ponto de vista da saúde pública, parece relevante, desde idades precoces, promover a ingestão de bebidas alternativas como a água e restringir a disponibilidade de outras opções açucaradas, consciencializando os pais e cuidadores da importância dessas exposições, uma vez que eles têm um papel fundamental na formação dos comportamentos alimentares das crianças. Estratégias eficazes, como comprar apenas alimentos saudáveis para casa, evitar estabelecimentos com opções pouco saudáveis, aprender a reconhecer a saciação, servir porções moderadas, fornecer exposições repetidas a novos e variados alimentos desde o início da vida, devem ser incentivadas.

Intervenções de saúde pública que criem ambientes alimentares mais saudáveis e melhorem a qualidade e acessibilidade a escolhas alimentares saudáveis para toda a população são essenciais. Simultaneamente, é uma necessidade urgente aprimorar a literacia alimentar dos pais e cuidadores, considerando o seu papel na ingestão alimentar das crianças e no estabelecimento de comportamentos alimentares.

**Palavras-chave**: pré-escolares; infância; comportamento alimentar; apetite, refrigerantes; estudos de coorte; CEBQ.

# Abstract

**Background and Objective**

Eating behaviours develop early in life and many factors influence the regulation of appetite and food choices, including biological and environmental factors. Problematic eating behaviours, including food approach and food avoidance traits, may arise during childhood. They have been associated with an excessive or reduced food intake in general or of specific food groups, resulting in poorer quality diets.

Sugar-sweetened beverages (SSB) are highly consumed and strongly associated with dental caries and weight gain in children; currently, they are considered one of the main contributors to childhood obesity. It has been hypothesized that this is due to poor energetic compensation provided by liquid forms, implying a greater total energy intake and a potential deregulation of satiety mechanisms. In addition, it is likely that the palatability of these beverages might generate hedonic signs that overcome the homeostatic regulation of appetite.

SSB consumption has been positively associated with food approaching-related traits and negatively associated with food avoidance behaviours, in cross-sectional studies. The long-term effects of SSB, especially on eating behaviours, are poorly studied.

This master thesis aims to investigate the association of SSB consumption at 4 years of age with appetitive behaviours of 7 years-old**.**

**Methods**

Participants are children from Generation XXI, a population-based birth cohort study assembled in all public maternity units from Porto, Portugal in 2005-2006 (n=8647). Children and their families were followed-up at different time moments; the current study uses complete data from children at birth, 4 and 7 years of age (n=3917).

Data was collected in face-to-face interviews by trained researchers, using structured questionnaires, or retrieved from medical records. SSB consumption was evaluated by a validated Food Frequency Questionnaire (FFQ). Appetitive behaviours were assessed using the Children's Eating Behaviour Questionnaire (CEBQ), self-reported by the main caregiver, and previously translated and tested in the current sample, showing good psychometric properties. Eight subdomains related to distinct eating behaviours were assessed: Satiety Responsiveness (CEBQ-SR), Slowness in Eating (CEBQ-SE), Enjoyment of Food (CEBQ-EF), Food Responsiveness (CEBQ-FR), Food Fussiness (CEBQ-FF), Desire for Drinks (CEBQ-DD), Emotional Overeating (CEBQ-EOE), and Emotional Undereating (CEBQ-EUE). These eight subdomains were further grouped into two factors, by principal component analysis: Appetite Restraint and Appetite Disinhibition. The Appetite Restraint factor was related to subdomains measuring internal cues of satiety and food fussiness, on which loaded

mostly CEBQ-FF, CEBQ-EF, CEBQ-SE, and CEBQ-SR, and the Appetite Disinhibition factor was related to subdomains measuring external food cues and emotional responses toward foods, on which loaded mostly CEBQ-FR, CEBQ-EOE, CEBQ-EUE, and CEBQ-DD, both explaining 62% of the total variance. The association between SSB and the CEBQ continuous scores was tested using generalized linear models (β regression coefficients). To test a dose-response relationship, multinomial logistic regression was run, using the CEBQ subdomains tertiles (the lower tertile was used as reference) and odds ratio (OR) were calculated for lower and higher SSB consumption, as well as the respective 95% confidence intervals (95%CI) with Bonferroni correction. Models were tested in univariate and multivariate analysis, using the following co-variables: child's sex, maternal Body Mass Index (BMI) before pregnancy, family structure and media screening time at 4 years-old, maternal age, maternal education (model 1) and the previous ones plus maternal consumption of SSB at age 4 of the child (model 2).

**Results**

In univariate analysis, children with a higher consumption of SSB scored significantly higher in Enjoyment of Food (β=0.038, 95%CI 0.001; 0.075), Food Responsiveness (β=0.047, 95%CI 0.010; 0.084), Emotional Overeating (β=0.039, 95%CI 0.010; 0.069), Desire for Drinks (β=0.137, 95%CI 0.100; 0.174), and in the Appetite Disinhibition composite factor (β=0.107, 95%CI 0.061; 0.152). In multivariate analysis (model 1), mostly associations were lost; only the Desire for Drinks subdomain (β=0.097, 95%CI 0.059, 0.136) and the Appetite Disinhibition factor (β=0.076, 95%CI 0.029, 0.124) remained statistically significantly associated with SSB. The inclusion of the consumption of SSB by mother at 4 years-old of the child, weakened these associations, but these remained significant (Desire for Drinks: β=0.075, 95%CI 0.031; 0.119; Appetite Disinhibition: β=0.061, 95%CI 0.007; 0.116).

The same associations (model 2) were tested but according to increasing categories of SSB consumption and scores of CEBQ subdomains. Consuming ≥1 serving per day of SSB (compared with a lower intake) was prospectively associated with a higher odds of being at the upper tertiles of the CEBQ Desire for Drinks scores (2$^{nd}$ tertile: OR=1.262, 95%CI 0.917, 1.736; 3$^{rd}$ tertile : OR=1.345, 95%CI 1.048; 1.727), and of the Appetite Disinhibition factor (3$^{rd}$ tertile: OR=1.218, 95%CI 0.934; 1.588). No significant associations were found with the remaining appetitive behaviours subdomains.

**Conclusions and Final Remarks**

A higher consumption of SSB at 4 years-old was associated with disinhibited eating behaviours later in childhood. Familial factors had a great impact on the association of children's SSB consumption with eating behaviours. In the current study, the mother's own

consumption of SSB and also other related characteristics, such as the socioeconomic background and weight status, had a considerable effect on the studied associations.

Longitudinal studies are necessary in order to understand better the long-term effects of SSB consumption, and the potential differential effect of the diverse types of SSB.

Meanwhile, from a public health point of view, it seems worthy, from early ages, to promote the intake of alternative drinks such as water and to restrict the availability of other sweetened options, making parents and caregivers aware of the importance of these exposures, once they have a pivotal role in shaping children's eating behaviours. Effective strategies, such as purchasing only healthy foods at home, avoiding unhealthy establishments, learning how to recognize fullness, serving moderate portions, provide repeated exposures to new and to variety of foods from early-life, should be encouraged.

Public health interventions that create healthier food environments and improve the quality and accessibility to healthy food choices for all the population are essential. Simultaneously, it is an urgent need the improvement of food literacy of the parents and caregivers, considering their role on children's food intake and on the establishment of eating behaviours.

**Keywords:** pre-schoolers; childhood; feeding behaviour; appetite, soft drinks; cohort studies; CEBQ.

# I. Introduction

## Eating Behaviours and Appetite

Eating behaviours may encompass a range of factors such as food preferences, food choices, willingness to taste, food intake, hedonic response, food acceptance and food neophobia [1, 2], while appetite is defined as the internal driving force for the search, choice, and ingestion of food [3]. Despite eating behaviours being commonly defined in a more social or psychological sense and the appetite in a more physiological way (the sensation of hunger), there is a narrow relationship between them, and both are influenced by internal (e.g. physiology) and external factors (e.g. social and cultural influences) [3, 4].

This work will focus mainly on behavioural aspects of appetite, not neglecting its biological interaction. Thus, to begin, the biological regulation of appetitive behaviours will be briefly described.

### *Regulation of eating behaviours and appetite*

Appetite regulation is a complex system, where the energy intake (i.e. food intake) and energy expenditure are expected to be balanced, resulting in energy homeostasis [4]. This homeostasis state is based on mechanisms of satiation (i.e. the sensation of fullness) and satiety (i.e. satisfaction between meals), being regulated by signals with many origins: sensory, cognitive, digestive, and hormonal [5].

The homeostatic system involves episodic and tonic processes that are related to short- and long-term regulation, respectively. Episodic signals are generated and synchronized by meal patterns (meal-to-meal regulation), resulting in an interaction between the gastrointestinal tract, their chemical components (such as cholecystokinin, ghrelin, etc), neural pathways and their receptors that contribute to the energy balance recognition by the brain. On the other hand, tonic signals are generated by the adipose tissue and are mainly dependent on leptin levels, contributing to appetite regulation in a day-to-day basis [4].

Along with these homeostasis processes, there is the hedonic system that involves numerous pathways, such as dopamine, glutamate, endocannabinoids pathways, etc., which are related to the limbic system. This system mediates the role that sensory pleasure plays in eating, i.e. the food intake purpose is not only to achieve energy homeostasis, but it is also related to a reward pathway. Hedonic signals can be stronger than the physiological needs and satiety signalling, resulting in an excessive food intake. In addition, regulation of appetite seems to be influenced by the type of food consumed, with evidence suggesting that palatable

foods (e.g. sugary and fatty snacks) can disrupt the appetite regulation, leading to an overcome of the homeostatic system by the hedonic system [6].

Researchers have proposed that weight gain can be related to disturbances in both homeostatic and hedonic systems. In addition, they do not operate independently [7], but share some sites of action, signals, and pathways [8]. It also has been described that gut-hormones, such as leptin and ghrelin, can regulate both pathways [8].

Beyond these biological processes, what people eat depends on many other factors, for instance culture, the socio-economic environment, availability. The determinants of food intake can be grouped into individual factors, social environment, physical environments and macro-level environments, as described by Story and colleagues (**Figure 1**) [9].

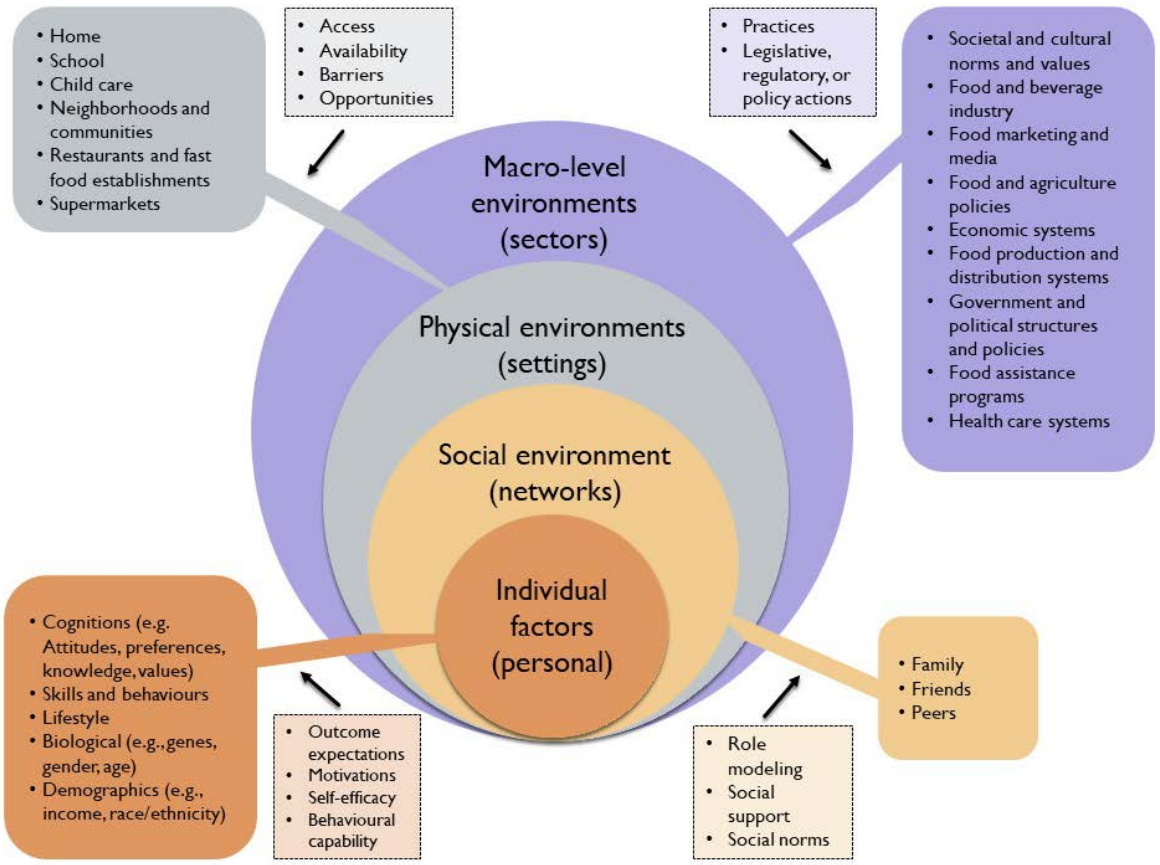


**Figure 1.** Framework of multiple influences on what people eat

[Adapted from Story *et al.* 2008 [9]].

As suggested, the eating behaviour is complex and results from an interaction of several factors embedded in different contexts. Understanding eating behaviours and their interactions is helpful to conduct studies in this field, as well as to design intervention plans.

Individual level factors are related to food choices and behaviours including cognitions, skills and behaviours, for example. These are able to impact food choices through one's expectations, motivations and self-efficacy. Besides these individual factors, environmental contexts also influence what a person eats, and this encompasses social, physical, and macro-level environments. The social environment comprises family, peers, community and may influence choices and eating behaviours by mechanisms like role modelling, social support, and social norms. The physical environment is related to the places where people eat, like home, schools, markets and other establishments. These places influence the availability of food, and might be barriers or opportunities for healthy food choices. Macro-level environmental factors are related to food marketing, producers, policies, economy, etc. These factors are more distal, but have a considerable and powerful effect on eating patterns [9].

Overall, these environments are interconnected, and interact, directly and indirectly, influencing eating behaviours. In the case of children, parents play a unique role by shaping their children's eating environment, acting as providers, role models and enforcers [9]. Parental influences in child's eating will be detailed later on in another section.

To sum up, food intake depends on many factors, thus the expression of appetite reflects the complex interactive phenomenon between biological, psychological and environmental processes [4].

### *Assessment of eating behaviours*

As described, appetite regulation is a complex process, thus assessing appetite and eating behaviour is a challenging task, being its assessment possible through both biological and behavioural methods [3].

Biological methods are objective tools in the investigation of food intake regulation and energy balance, and are based on biomarkers of satiation and satiety. In a short-term measurement of satiation, one possibility is to measure the stomach distension and/or the concentrations of cholecystokinin and glucagon-like peptide-1 (GLP-1), whereas for a long-term analysis, it is useful to measure the blood concentrations of leptin, ghrelin and insulin [3]. Besides these, neuronal techniques are used as well, such as functional magnetic resonance imaging and positron emission tomography. These techniques are based on the detection of changes in blood flow and neuronal activation, allowing understanding how appetite modulates brain activity [10, 11].

Despite being more objective and complete, biological methods are more expensive, thus when the goal is to evaluate large sample sizes or at population-level, indirect measures are more feasible, such as through questionnaires [12]. These questionnaires allow the evaluation of different eating constructs, which can be investigated in the laboratory setting or

in individual's daily environment [3, 10, 11]. The main limitation of this type of method, compared to biological methods, is the social desirability bias that may occur [12].

Several questionnaires have been developed to evaluate appetitive behaviours; the Dutch Eating Behaviours Questionnaire [13, 14], the Baby Eating Behaviours Questionnaire [15] and Children's Eating Behaviour Questionnaire (CEBQ) [16] are the most frequently used. The CEBQ is a widely used questionnaire to assess eating behaviours on children and its development was in the United Kingdom (UK) in 2001 by Jane Wardle and colleagues [16]. This questionnaire, designed to be answered by parents or main caregivers, aims to assess eating styles related to obesity risk, using a behavioural perspective rather than be focused on genetics and physiology. It facilitates the investigation of individual differences in eating behaviour that contributes to the development of obesity [16].

The original CEBQ includes 35 items related to eating behaviours among children, and is answered on a 5-point Likert scale, ranging from 1 = "Never" to 5 = "Always". These items are divided into eight subdomains related to appetite, namely Satiety Responsiveness (CEBQ-SR), Slowness in Eating (CEBQ-SE), Emotional Undereating (CEBQ-EUE), Food Fussiness (CEBQ-FF), Food Responsiveness (CEBQ-FR), Desire for Drinks (CEBQ-DD), Emotional Overeating (CEBQ-EOE) and Enjoyment of Food (CEBQ-EF) [16].

Some researchers have organized these eight subdomains into two broader ones: food approach and food avoidance behaviours [11]. Food approach behaviours refer to disinhibited eating, which measures individual's sensitivity to external cues in eating, e.g. a higher food intake motivated by the smell or sight of food. On the other hand, food avoidance refers to restraint behaviours, when there is lower food intake due to internal satiety cues (greater sensitivity to fullness or satiety response capacity) [11]. The dimensions included in each of the above-mentioned food approach and food avoidance behaviours, along with examples of items that compose each subdomain, are described in **Table 1**.

Despite being an indirect method and susceptible of desirability bias, several studies have shown that the CEBQ has good psychometric properties, such as good internal consistency, reproducibility and construct validity [16-18]. Thus, it has been validated and applied in different countries [16, 18-21].

In Portugal, the CEBQ was translated and tested in children from a population-based birth cohort at 7 years of age [22], resulting in a Portuguese version of the questionnaire (P-CEBQ). It revealed good internal consistency, defined as a Cronbach's alpha of 0.7 or higher for each subdomain and a Pearson correlation between subdomains of 0.4 or higher. Cronbach's alpha for the different subdomains in the validated Portuguese CEBQ, ranged from 0.74 to 0.85. Additionally, authors grouped subdomains into two composite factors, obtained by principal component analysis, namely Appetite Restraint and Appetite Disinhibition [22]. The Appetite Restraint factor was related to subdomains measuring internal cues of satiety and

food fussiness, on which loaded mostly CEBQ-FF, CEBQ-EF, CEBQ-SE, and CEBQ-SR, and the Appetite Disinhibition factor was related to subdomains measuring external food cues and emotional responses toward foods, on which loaded mostly CEBQ-FR, CEBQ-EOE, CEBQ-EUE, and CEBQ-DD. The Appetite Restraint factor explained 35% and Appetite Disinhibition factor explained 26% of the total variance [22]. The Pearson correlations between both domains was weak (rho = −0.013), demonstrating their independency [22].

**Table 1**. Description of the subdomains of the Children's Eating Behaviour Questionnaire (CEBQ).

| | Subdomain | Examples of Items/Questions | Meaning |
|---|---|---|---|
| **Food avoidance behaviours** | Satiety Responsiveness | *"My child gets full up easily"* | Refers to the degree of self-regulation of the amount of food intake, based on satiety [18, 23] |
| | Slowness in Eating | *"My child takes more than 30 minutes to finish a meal."* | Measures the speed of eating; reflects a reduced interest in a meal [23] |
| | Emotional Undereating | *"My child eats less when s/he is angry"* | Refers to lower intake during negative emotional states [23] |
| | Food Fussiness | *"My child refuses new foods at first"* | Reflects a lack of interest in food and unwillingness to try new foods; highly selective about the range of foods that are accepted [23] |
| | Food Responsiveness | *"Given the choice, my child would eat most of the time"* | Refers to the extent to which a child indicates an interest in and desires to spend time eating food [24]; assesses the tendency to eat when prompted by external cues [18] |
| **Food approach behaviours** | Desire for Drinks | *"My child is always asking for a drink"* | Measures the desire to drink [16] |
| | Emotional Overeating | *"My child eats more when annoyed"* | Refers to higher intake during negative emotional states [23] |
| | Enjoyment of Food | *"My child loves food"* | Represents a general appetite for food or desire to eat; captures the extent to which a child finds eating pleasurable and desires to eat [18, 24] |

To test reliability (varies between 0 and 1, with higher values representing higher reliability), Albuquerque and colleagues analysed the agreement between the subdomains in 2-time points (CEBQ was answered again 35 days apart) using 2-way random intraclass

correlation coefficients (ICC). The mean ICC of the P-CEBQ ranged from 0.51 (Desire for Drinks subdomain) to 0.85 (Food Fussiness subdomain). Most subdomains presented good reliability (ICC ≥ 0.7), except Desire for Drinks (ICC = 0.51) and Emotional Undereating (ICC = 0.58). Overall, the P-CEBQ demonstrated a good reliability in this Portuguese sample of 7-year-olds (mean ICC = 0.73). Regarding to the construct validity, it was assessed taking into account theoretical hypotheses based on previously described literature, i.e. as higher the children's Body Mass Index (BMI), higher the emotional eating and responsiveness to external cues and lower the food fussiness and responsiveness to internal satiety cues. The mentioned associations were consistent with the theoretical hypothesis, supporting the construct validity of the P-CEBQ scale [22]. More details of the psychometric properties of P-CEBQ can be found elsewhere [22].

### *Problematic eating behaviours*

Problematic eating behaviours are commonly reported by parents or caregivers, with prevalence varying from 2 to 66% according to European population-based birth cohorts [25]. Problematic eating behaviours, either food avoidance behaviours (pickiness, food refusal, neophobia, sensitivity to internal cues of satiety) or food approach behaviours (overeating, disinhibited eating, great sensitivity to external eating cues), arise during childhood [25-27].They may also compromise child's future health [28], since these may lead to a poor diet quality, as some population-based studies have shown [26, 29-31].

Many factors contribute to a poor diet quality, such as having an unbalanced total energy intake, either excessive or insufficient, as well as having a lack of food variety, rejecting some food groups. Unhealthy diets are commonly based on a high consumption of energy-dense foods (for instance fatty and/or sugary foods and beverages) and low consumption of nutrient-dense foods (mainly fruits and vegetables) [25, 26, 30, 32]. These factors may contribute to an unbalanced nutritional status [25, 26, 29, 32-35].

In a previous work of a Portuguese birth-cohort (Generation XXI), authors concluded that children following less healthy dietary patterns during pre-school years may have a greater risk of problematic eating behaviours later in childhood [30]. It is of particular relevance investigating these eating behaviours among children, since evidence suggests a stability of appetitive traits [36] and dietary patterns over time [37].

Eating behaviours seem to have an impact on child's weight status as well. Evidence from different populations has shown a strong positive association between food approaching-related traits and BMI, and a negative association of BMI and food avoidance behaviours [19, 38, 39].

### *Development and determinants of eating behaviours*

Eating behaviours develop early in life. It is believed that their development begins *in utero* and results from a combination of genetic and biological factors, shaped by the surrounding environment [33, 40].

#### *Genetics*

It is known that genetics influence appetite traits in children [41]. Studies with twins concluded that there is a strong genetic effect in eating behaviours, such as Slowness in Eating and Satiety Responsiveness, and a moderate effect in Food Responsiveness and Enjoyment of Food [41, 42]. Furthermore, feeding characteristics may be a precursor of later eating speed [42].

The FTO gene has been investigated in order to explain these genetic predispositions. It is predominantly expressed in the appetite-control areas and it has been described as the gene with the strongest influence on obesity, being associated with an increasing energy intake and reduced satiety [43]. It has been hypothesized that it can be related to pathways that regulate addiction and reward behaviours. There is also an association with the genetic variation in FTO and lower scores of Satiety Responsiveness and higher on Enjoyment of Food and Food Responsiveness in children [44, 45].

#### *Innate Preferences*

It is well accepted that there is a tendency to prefer high-fat and sweet foods early in life, and in the opposite, vegetables are normally unwelcomed [46-48]. Children tend to prefer sweet tastes to bitter or sour. This suggests the existence of innate preferences for sweets and results from an adaptive process in which sweetness indicates that the food is nutritious and safe, whereas bitterness and sourness may indicate the presence of toxins [49, 50].

#### *Familial/Parental influences*

Maternal food consumption during pregnancy and breastfeeding may influence food and tastes preferences of children [51-54].

During pregnancy, the *foetus* swallows amniotic fluids, experiencing tastes and smells from the maternal diet [55]. Afterward, the first experienced taste is the breast milk, being the palatability clearly different between breastfed and formula-fed infant. This can be explained by the fact that the artificial milk has always the same flavour, whereas flavours in maternal milk vary, depending on what the mother eats and on the maturation of the milk, that changes in composition and flavours over the time [56, 57]. It is described that the different flavours experienced during pregnancy and in the first days of life may influence the acceptance of foods during infancy [56, 58, 59].

It is also important to mention that the consumption of breast milk is linked to a better self-regulation of the energy intake. Drinking through the bottle implies less effort and less control of the amount ingested, what may lead to overconsumption [51]. Research has shown that children who were breastfed have a better self-regulation of food intake, resulting into an enhanced response to internal satiety cues [60, 61]. In contrast, shorter breastfeeding duration has been associated with poorer satiety response and higher consumption of foods in absence of hunger [62]. In a recent study, the authors concluded that breastfeeding may be protective against external eating among boys later, during adolescence, and this is explained by the influence of breastfeeding on the enhanced ability to identify emotions [63].

Thus, parents influence children's eating behaviours through genetics and the environment provided by themselves, including their behavioural examples. It begins at the prenatal period and continues during childhood [64].

Parenting styles reflect parental attitudes and behaviours of interplay with the child. To assess these attitudes, a variety of questionnaires have been developed in the last decades. Researchers agree on four dimensions of parenting styles, namely, authoritative, authoritarian, permissive and uninvolved/neglectful [65, 66]. Authoritative parenting style seems to be the most positive style, being associated with better health outcomes in terms of weight status and eating behaviours [67-69].

To measure parenting attitudes, beliefs and practices about child feeding and obesity proneness, Birch and colleagues validated the Child Feeding Questionnaire [70]. This tool was reviewed by other researchers, and translated and tested in different populations[71, 72]. In 2014, a combination of this tool and parenting styles' measure scale was proposed to assess parental feeding practices in Portuguese pre-school children [73].

Parents use a variety of practices that are an attempt to maintain or modify children's eating habits and weight status, such as pressuring the child to eat, restricting certain foods, using food for reward and monitoring food intake [74, 75]. These practices have been reported to have a negative influence on the development of healthy eating habits and child's weight, as well [76-78].

Parents usually restrict the access to foods when they consider that the child should not eat, normally foods with high content in sugar and fat [74, 79]. Contrary to parental intentions, this restriction actually can promote the overconsumption of those foods. In addition, these feeding practices may decrease the regulation of child's internal hunger and satiety signals [33].

Another important factor that contributes to the use of feeding practices is parental perception of child's weight status. Child's weight status usually is a topic of concern to mothers and may lead them to seek to modify or maintain it through feeding practices. Mothers that are concerned about their child's weight (especially daughters) tend to use more restrictive feeding

practices [80]. On the other hand, mothers use pressure to eat when they consider their children as too thin [81, 82].

Besides parent's feeding practices, their own food preferences have also a role on the development of child's food preferences and consumption, as parents use to buy and eat what they like the most [83]. In line with this idea, children eat what is available and is familiar, and what they appreciate [84]. In addition, they tend to imitate what they observe from their parents, once they are the greatest influencing models [64, 85]. Several studies have shown that repeated exposures contribute to the familiarization of a food, which has positive effects on child's acceptance of novel foods [86-88] .

Parents' educational level, the socio-economic context, and weight status also influence children's eating behaviours. Specifically, having a higher education level and belonging to a higher socio-economic context, as well as having a healthy weight status have been associated with healthier children's eating behaviours [89].

Having family meals is also a good habit in child's life. During meals, parents are often managing child behaviours, imposing rules and expectations, and interacting with their children, contributing to the development of their eating patterns [64]. Sharing family meals has been associated with less overweight, eating less unhealthy food and less disordered eating, along with higher consumption of healthy foods [90]. It is important to highlight that watching television during mealtime seems to decrease these benefits [91], because the family tends to interact less, in addition to the great influence of food marketing and advertisements [92, 93]. Evidence supports the association of screen media exposure with unhealthy eating habits among children [94], mainly influenced by marketing of energy-dense and low-nutrient foods and beverages [92, 93].

*Environmental influences*

Besides parents' influence, siblings, friends and peers have also a role on the development of child's eating behaviours. Children tend to reproduce the attitudes of their models [95-97]. Early in life, parents are great influences, but also siblings, especially older ones, and also other children [95, 98-100] may influence child's eating behaviors.

Findings suggest that children's food choices, preferences and consumption are associated with peers in diverse ways. Studies found that peers' food choices for a child's non-preferred food are capable of increasing the preference for that food [95, 101]. The effect of peer modelling may be moderated by gender, age and the perceived status of the role models. It is described that the influence of an older peer is stronger, as well as if it is a girl. In addition, Cruwys *et al.* suggested that modelling is stronger when individuals perceive themselves to be similar to the model. This effect of social modelling is in part mediated through imitation [102]. Thus, children's eating behaviours may be influenced by their peers' food choices,

preferences and modelling and being in large groups of children of different ages may encourage pre-schoolers to eat greater amounts of healthy foods [102, 103].

The accessibility and availability to food is a determinant of children's food intake and parents play a pivotal role in this issue, especially at home. However, they are not the unique responsible; school and child care facilities also provide food, hence these environments should also be spots of intervention [104], besides the influence of other children, as mentioned above.

In 2008, Story and colleagues referred that there was a lack of research and interventions on child care facilities, being this a missed opportunity, once children spent many hours of the week there. Pre-school facilities are favourable environments for conditioning the availability of healthy foods and for nutrition education [9]. Later, in 2011, authors from another review came to the same conclusion, highlighting the importance of additional well-designed studies to inform the implementation of effective interventions, regulations, and policies to improve eating behaviours among children [105].

In the same way, schools are crucial environments, being essential to provide healthy food options. Another important point to consider is the offered options nearby the school, which usually provide mainly unhealthy options (including sugar-sweetened beverages, etc.), that are preferred by children, making the foods available at school less desirable and consumed [106-108]. Some regulations have been implemented by governments aiming to limit the access to these competitive foods [109-112] and make healthier options available for children. In Portugal, schools need to follow standards for school lunch meals since 2013, and also guidelines for food supply in school buffets/cafeterias and vending machines [112, 113]. These and other strategies to promote healthy behaviours at school are considered pivotal interventions during childhood [110, 111].

### *Strategies aiming to improve eating behaviours*

The review from Scaglioni and colleagues published in 2018 provides relevant and helpful information to improve children's eating behaviours. It is well known that children's food preferences and eating behaviours are influenced by several factors, as described above. These factors have been investigated in order to highlight the matter and provide to health professionals practical tools to understand the background of eating behaviour development and to achieve children's nutrition preventive purposes. This work from Scaglioni *et al.* gathers the factors implied on children's eating behaviours, as well as effective strategies to improve them, as described in **table 2** [64].

This table includes different factors that are engaged on children's eating behaviours development, such as parenting styles and practices, children's environments, mainly the

family environment, and selects the most effective practices to promote a healthy eating behaviour according to the current evidence. For instance, providing a home environment with healthy options, avoiding unhealthy ones and attending places where they are available; promoting self-regulation of appetite, serving adequate amounts of food, as well as stimulating children to try new foods, by repeated exposure, may expand the chances of children to appreciate and consume them.

**Table 2.** Strategies to improve eating behaviours

| Strategy | Practices |
|---|---|
| Convert control | • Purchasing healthy foods to have at home<br>• Avoidance of unhealthy stores and fast food |
| Avoid the use of food rewards | • Food maintains the behaviour on which its delivery and acquisition is dependent |
| Promotion-self-regulation | • Recognition of fullness sense<br>• Serving moderate portions<br>• Help in organizing the feeding environment |
| Authoritative parenting style | • Encourage children to try new foods<br>• Parents are the example<br>• Parent models healthy eating and enjoyment of foods<br>• Do not model disliking of foods in front of child<br>• In obesogenic environment, some parental control is likely needed to moderate children's intake of palatable snack foods<br>• Early responsive parenting intervention |
| Family meals | • Expose to a variety of foods<br>• Repeatedly expose child to a food<br>• Allow child to have input into food choices<br>• High frequency of shared family meals<br>• Daily shared breakfast<br>• Socialization during mealtime<br>• Turn off TV at meals |
| Parents focused intervention | • Educationally-based interventions adapted to parents and caregivers<br>• Feeding-related advice<br>• Empowering parents<br>• Social support |
| Family environment | • Early-life experiences with healthy tastes and flavours may promote healthy eating<br>• Give the parental role in food shopping and preparation<br>• Healthy food availability<br>• Reduce screen time and get adequate sleep |

Table adapted from Scaglioni *et al*. 2018 [64]

# Sugar-sweetened beverages

Sugar-sweetened beverages (SSB), by definition, are hot or cold beverages containing added sugar. They include carbonated and non-carbonated soft drinks, ice tea, fruit punches, fruit nectars, energy drinks, sports drinks and others with added sugar, usually not including diet or light versions [114].

SSB have been suggested as one of the main contributors to childhood obesity [115, 116], the biggest concern of public health for this age range. This work will be focused on their relationship with eating behaviours.

*Consumption data*

The consumption of SSB has risen around the decade of 70 across the globe [117-119]. In the recent years, a tendency of decline or replacement of these beverages by options with less sugar, with artificial substitutes and/or smaller portions, has been observed [120, 121]. Even though, the consumption still remains high worldwide, and represents a topic of concern in public health [121-125].

Given the example of United States of America (USA), in 2014, 61% of children reported the consumption of, at least, one SSB per day [122]. In 2006, 37.3% of the pre-schoolers in Brazil consumed soft drinks and artificial juices at least four days in a week [126]. In Portugal, according to the National Food, Nutrition, and Physical Activity Survey (IAN-AF, 2015-2016), the average consumption of soft drinks was 88 grams per day, by the general population, and was the second beverage type most consumed, after the water [127]. The daily average consumption of soft drinks and fruit nectars (≥220g/day) was 18% among the inquired, being the highest in adolescents 42% (199g/day) [127]. Children consumed, in average, 20g/day of fruit nectars and 56g/day of soft drinks, and 22% of them consumed ≥220g of these beverages per day [127]. Considering this, childhood may be a key period to prevent this excessive consumption.

Moreover, an increasing consumption of SSB has been observed in Portugal. Between 1990 and 2012, the consumption of soft drinks doubled, from approximately 100mL to 203.6mL per inhabitant per day [128]. However, a recent report from this year, revealed a decreasing in consumption since 2016, and this is likely a potential consequence of the adopted policies in 2017. In this year, in Portugal, the SSB tax was introduced, as well as the reformulation of the products, with less content in sugar, resulting in decrease of SSB consumption [129, 130]. This and other public health interventions will be discussed in more detail further in this section.

*Relation with appetite*

There is an agreement that sugary beverages contribute to weight gain due to a positive energy balance [131]. However, the mechanisms through which it occurs are less understood.

It has been suggested that SBB imply a higher food intake [132, 133], because liquid forms (like SSB) do not provide subsequent compensation of energy intake, implying a greater intake of the total amount of energy [131, 134]. In other words, liquid forms exert a less satiation effect compared to solid forms. Also, beverages require less oral processing, have a faster gastric-emptying and orocecal transit times [135, 136]. Another hypothesis is related to the smaller increase of GLP-1 and insulin and likewise a smaller reduction in ghrelin after ingestion of liquids, compared with solid forms [137]. Corroborating with this statement, in 2016, Shearrer *et al*. concluded in their work with adolescents that the habitual SSB intake appears to play a key role in moderating fullness responses possibly via ghrelin [138].

In addition, sweet tastes have a special effect on satiation and satiety, and their high palatability could trigger the regulating systems, leading to overconsumption [139]. In more detail, palatable foods (high in sugar and fat) taste more intensively than other foods and this may result in a stronger effect on rewarding systems, leading to a release and/or up-regulation or reward mediators such as serotonin, dopamine and opiates [6].

Thirst sensations are stronger and more consistent than hunger sensations, and drinking to meet hydration needs is more likely than eating just to meet energy needs [140].

Even so, beverages are highly consumed in the population due to their great availability, satiation effect and the social context [141]. It is usual eating while drinking as well. In addition, SSB are more consumed by low-income people, what might indicate their low price and subsequent easy access [142]. Thus, beverages may stimulate energy intake by several ways, and their consumption is associated with greater food intake in children, adolescents and adults [143].

*Heath consequences*

Scientific evidence supports the link of SSB consumption and several health consequences among adults, namely weight gain [117, 144], cardiovascular risk factors [145], insulin resistance and type 2 diabetes [146, 147], non-alcoholic fatty liver disease [148], and metabolic syndrome [149]. Moreover, evidence suggests that SSB may provide greater health risks compared to sugar-containing foods [149].

During childhood, the consumption of SSB has been considered a dietary factor with great impact on obesity [116]. Along with the greater obesity risk in children, SSB consumption has been strongly associated with dental caries, and less consistently with insulin resistance and, probably, type 2 diabetes [122].

Beyond this, more recently, SSB with caffeine have also been associated with negative outcomes, such as poor quality or reduced sleep, headaches, risk-seeking behaviour and depressive symptoms [122]. This is particularly worrisome in energy drinks [122].

Due to these negatives consequences to health some global food companies have begun responding to public health concerns and consumer demands to change their products and portfolios [120].

*Taxation and related policies*

Due to the effects of SSB, particularly on childhood obesity, the WHO highly recommends measures to control this exaggerated consumption [125, 150, 151]. The application of measures that model the accessibility to high sugar content foods, as well as measures of products reformulation along with awareness and population education are suggested [125]. In order to achieve this and because of the great healthcare expenditures due to population's excessive weight [152], governments of different countries (41 countries plus 8 states on United States till May 2019) have implemented taxation legislation [153].

The economic process of policies related to the regulation of SSB is based on making consumers internalize the costs they enforce on themselves and on others result from the high consumption of SSB. Taxation of SSB can lead to improvements that can be divided into three groups: (i) increase of prices, leading to the reduction of purchasing these products; (ii) taxation that may result in a stimulus for manufacturers to reformulate their products formulas adding less sugar or creating new versions; and (iii) the effect on education of consumers, with greater information about the topic in media and public debates, contributing for more awareness of the negative effects of the consumption [154].

Since the implementation of these measures, SSB sales decrease has been reported in many countries [155], including Portugal [156]. It is important to mention that each country has different ways on taxation and this obviously has an influence on the impact of this measure. According to a WHO's report from 2015, price policies applied to food in Hungry, Denmark, Finland and France, influenced what consumers bought and contributed for the improvement of health by changing consumption and supporting healthier diets [157].

Regarding the efficacy of the taxes, reviews [158-160] and reports [161] suggested that an increase between 10 to 20% of the prices is necessary in order to observe significant effects on population's health and, according to a review of experimental studies, the countries with lower effects eating habits at the population level was explained by the insufficient taxation [162]. In another recent review, it was proposed that taxes should increase the price by, at least, 20% to be effective [163]. Evaluations of the impact of taxes in countries where the increase in price of the product was higher than 10% have shown a positive effect on health indicators.

For example, in Mexico, there was a decrease of 6% in average consumption of SSB after one year of the tax implementation [164].

Actual evidence of the SSB taxation impact on consumption is scarce, being the results based on predictive models that take into count the elasticity of food prices [165]. Studies about the effects on health are even scarcer, being also essentially based on predictive models. Despite this fact, the observed effects are positive, and evidence suggests that these measures have significant impacts on populations' health [166].

# State-of-the art: SSB and Eating behaviours

The long-term effects of SSB consumption are poorly studied [138], especially those effects on eating behaviours. According to previous literature, positive associations between the consumption of SSB and food approaching-related traits have been found, and in opposite, the consumption of SSB has been negatively associated with food avoidance traits. However, the majority of studies used a cross-sectional design [134, 167-170].

*Population-based studies*

A cross-sectional study with 3265 Swedish parents and their children found that the intake of SSB was related to lower restrained traits in boys aged between 10 and 13 years. In this study, artificially sweetened soft drinks were also included, and higher external eating (a food approach behaviour) score had a greater association with sugar-sweetened soft drinks than light versions, but this only among girls. In addition, being more sensitive to external stimulus seems to promote the consumption of sugar-sweetened soft drinks instead of the artificially sweetened ones [167]. The consumption of sweets and soft drinks may also be linked to emotional eating in girls and soft drink consumption, specifically, with external eating [168].

Another study observed that higher sensitivity to reward (related to consumption of palatable foods) was significantly and positively associated with higher intakes of unhealthy snacks and SSB [169]. The food approach behaviour Desire for Drinks (CEBQ-DD) seems also to be related to beverage consumption and preferences, as reported in a cross-sectional study with pre-schoolers [134]. Higher CEBQ-DD scores were related to higher preferences for SSB and also greater consumption. The findings suggest that higher CEBQ-DD scores may be related to the desire for sweet taste in the mouth and not simply a matter of thirst or hunger. This can imply an increased total energy intake, if there is no compensation for energy provided in liquid forms [134]. However, in 2017, another study did not find any relationship between CEBQ-DD and the consumption of SSB, but with a higher consumption of fat-containing milk, among 406 children aged 6-8 years [170].

To sum up, positive associations have been found between the consumption of SSB and food approaching traits, however, the majority of the studies are cross-sectional. There is a lack of research that investigates the effects of SSB on appetitive-traits using prospective approaches. To our knowledge, only one study [38] analysed prospectively the associations of some food groups, including SSB, with child's eating behaviours. In this study, conducted in 2009 with a sample of 1275 children at the age of 9, the CEBQ and a Food Frequency Questionnaire (FFQ) were applied, and children were weighted. After one year, these measurements were repeated and associations between SSB and eating behaviours were

found to be inconsistent, and appetitive traits did not predict any changes in child's weight nor food intake [38]. However, it is important to highlight that the follow-up period was just one year, which might have been too short in order to observe significant effects.

# II. Objective

This research aims to investigate the influence of the early consumption of SSB on appetitive behaviours later in childhood using data from the prospective population-based Generation XXI birth cohort. To answer this question, a specific objective was defined:

- ✓ To study the association between the consumption of SSB at 4 years-old and appetitive behaviours, measured by the CEBQ, at 7 years of the age.

**Expected outcomes**

It is expected to find associations between the consumption of SSB and higher scores of food-approaching eating behaviours, and negative associations with food-avoidance eating behaviours.

This data might contribute to a better understanding of the development of eating behaviours. Further, it can provide relevant evidence to formulate health advice related to appetite and food education in childhood.

# III. Paper

**The effect of sugar-sweetened beverages at 4 years of age on appetitive behaviours of 7-years-old children from the Generation XXI birth cohort**

# The effect of sugar-sweetened beverages at 4 years of age on appetitive behaviours of 7-years-old children from the Generation XXI birth cohort

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# Abstract

**Background:** Problematic eating behaviours may arise during childhood. These include a deviation towards food avoidance or food approaching traits, having negative and positive associations with Body Mass Index (BMI), respectively. Behavioural factors are important determinants of eating behaviours development. A high consumption of sugar-sweetened beverages (SSB) has previously showed a potential effect on these eating behaviours, although research relies in cross-sectional designs.

**Aim:** To investigate the prospective association of SSB consumption at 4 years of age on appetitive behaviours of 7-years-old.

**Methods:** Participants are children from a population-based birth cohort study from Northern Portugal (Generation XXI). Data at birth, 4 and 7 years-old were collected by trained researchers using structured questionnaires. SSB consumption was evaluated by a validated Food Frequency Questionnaire (FFQ). Appetitive behaviours were assessed by parental-report using the Children's Eating Behaviour Questionnaire (CEBQ), previously translated and validated. The 8 subdomains were grouped into two-composite factors: Appetite Restraint (related with subdomains measuring internal cues of satiety and food fussiness) and Appetite Disinhibition (related to subdomains measuring external food cues and emotional responses toward foods). Generalized linear models and multinomial logistic regressions were run [β regression coefficients and odds ratio (OR) with 95% confidence intervals (95%CI) with Bonferroni correction were computed] for 3917 participants. Models were adjusted for child's sex, maternal BMI before pregnancy, family structure and media screening time at 4 years-old, maternal age, maternal education and maternal consumption of SSB at age 4 of the child (inclusion of maternal consumption only in model 2).

**Results**: In univariate analysis, children with a higher consumption of SSB scored significantly more in Enjoyment of Food (β=0.038, 95%CI 0.001; 0.075), Food Responsiveness (β=0.047, 95%CI 0.010; 0.084), Emotional Overeating (β=0.039, 95%CI 0.010; 0.069), Desire for Drinks (β=0.137, 95%CI 0.100; 0.174), and in Appetite Disinhibition (β=0.107, 95%CI 0.061; 0.152). In multivariate analysis (model 1), most associations were lost; only the Desire for Drinks subdomain (β=0.097, 95%CI 0.059, 0.136) and the Appetite Disinhibition factor (β=0.076, 95%CI 0.029, 0.124) remained significantly associated with SSB. The inclusion of the consumption of SSB by mother at 4 years-old of the child, weakened these associations, but these remained significant (Desire for Drinks: β=0.075, 95%CI 0.031; 0.119; Appetite Disinhibition factor: β=0.061, 95%CI 0.007; 0.116).

The same associations (with model 2) were tested but according to increasing categories of SSB consumption and increasing scores in the CEBQ subdomains. Consuming ≥1 serving per day of SSB (compared with a lower intake) was prospectively associated with a higher odds

of being at the upper tertiles of the CEBQ-Desire for Drinks scores (2nd tertile: OR=1.262, 95%CI 0.917, 1.736; 3rd tertile: OR=1.345, 95%CI 1.048; 1.727), and of the Appetite Disinhibition factor (3rd tertile: OR=1.218, 95%CI 0.934; 1.588). No significant associations were found with the remaining appetitive behaviours subdomains.

**Conclusions:** A higher consumption of SSB at 4 years of age was associated with disinhibited eating behaviours later in childhood. Familial factors had a great impact on these associations.

**Keywords:** children; pre-schoolers; soft drinks; eating behaviours; feeding behaviours; CEBQ; cohort studies

# Background

Eating behaviours develop early in life. It is believed that their development begins *in utero* and there are several factors leading to a decreased self-regulation ability of food intake, namely genetic predisposition, the first taste experiences and the family food environment [1, 2].

Abnormal/problematic eating behaviours, either food avoidance behaviours (pickiness, food refusal, neophobia, high sensitivity to internal cues of satiety) or food approach behaviours (overeating, disinhibited eating, great sensitivity to external eating cues), may arise during childhood [3-5]. The prevalence of eating problems in pre-school children, according to European population-based cohorts, may vary from 3 to 66% [4].

These eating behaviours may compromise child's future health [6], since these may lead to a poor diet quality, as some population-based studies have shown [3, 7-9]. A poor diet quality involves a lack or excess of food intake in general (total energy) or of specific food groups (e.g. high consumption of energy-dense foods (fatty and/or sugary foods and beverages) and low consumption of nutrient-dense foods (fruits and vegetables), leading to an unbalanced nutritional status [2-4, 7, 10-12]. In a previous work, authors concluded that children following less healthy dietary patterns during pre-school years may have a greater risk of problematic eating behaviours later in childhood [8]. It is of particular relevance investigating these eating behaviours among children, since evidence suggests a stability of appetitive traits [13] and dietary patterns over time [14]. Eating behaviours seem to have an impact on child's weight status as well. Evidence from different populations has shown a strong positive association between food approaching-related traits and Body Mass Index (BMI), and a negative association of BMI with food avoidance behaviours [15-17].

According to the literature, Sugar-sweetened beverages (SSB) have been considered one of the dietary factors with greatest impact in childhood obesity [18]. Along with the greater obesity risk, SSB consumption has been associated with dental caries and insulin resistance [19]. They contribute to a positive energy balance [20], and increased food intake [21, 22] and habitual SSB intake appears to play a key role in moderating fullness, responses possibly via ghrelin [23]. If there is no compensation for energy provided in liquid forms, this can imply in an increased total energy intake [20, 24]. However, the effects of long-term consumption of SSB are poorly studied [23], especially those effects on eating behaviours.

From previous literature, positive associations between the consumption of SSB and food approaching-related traits have been found and, in opposite, the consumption of SSB has been negatively associated with food avoidance-traits. However, the majority of studies were cross-sectional [24-28].

For instance, in a cross-sectional study with 346 pre-schoolers, higher preferences and greater frequency of SSB consumption were associated with higher scores of the Desire for Drinks (CEBQ-DD) subdomain, suggesting that this can be linked to the desire for sweet taste in the mouth and not simply a matter of thirst or hunger [24]. In contrast, in 2017, another study did not find any relationship between CEBQ-DD and the consumption of SSB [28].

Research on the effect of SSB on appetitive-traits is still scarce, especially using a prospective approach. To our knowledge, only one study analysed prospectively the associations of some food groups, including SSB, and children's eating behaviours [16]. This study conducted in 2009 with a sample of 1275 children used the Children's Eating Behaviour Questionnaire (CEBQ) for assessment of eating behaviours, and a Food Frequency Questionnaire (FFQ) for assessment of child's food intake, applied in two time points. Associations between SSB and eating behaviours were inconsistent, and the appetitive traits did not predict changes in child's weight, nor intake, in the one-year follow-up [16]. However, it is important to highlight that the follow-up period might have been too short to observe effects, and it is necessary to study the opposite direction of these associations.

Knowledge from prospective studies may help to better understand how the consumption of SSB shapes eating behaviours, and influences weight and other health indicators in the future. Thus, the aim of this research was to study if the consumption of SSB early in childhood (4 years-old) is prospectively related with appetitive behaviours three years later (at 7 years-old).

# Methods

*Study design and participants*

This study included participants from Generation XXI, a prospective population-based birth cohort, described in detail elsewhere [29]. A total of 8495 women and their 8647 children were recruited from all public maternity units in Porto Metropolitan Area (northern Portugal), between 2005 and 2006. These maternity units were responsible, at enrolment, for 91.6% of the deliveries in the whole catchment population. Of the invited mothers, 91.4% agreed to participate. The second evaluation was between April 2009 and August 2011, when children were aged 4 years-old, and 7459 children were evaluated (86% participation rate). Between April 2012 and March 2014, there was a third evaluation (7 years-old) with the participation of 6882 children (80% participation rate). Other evaluations occurred at 10 years-old (74%) and 13 years-old (on-going since 2018).

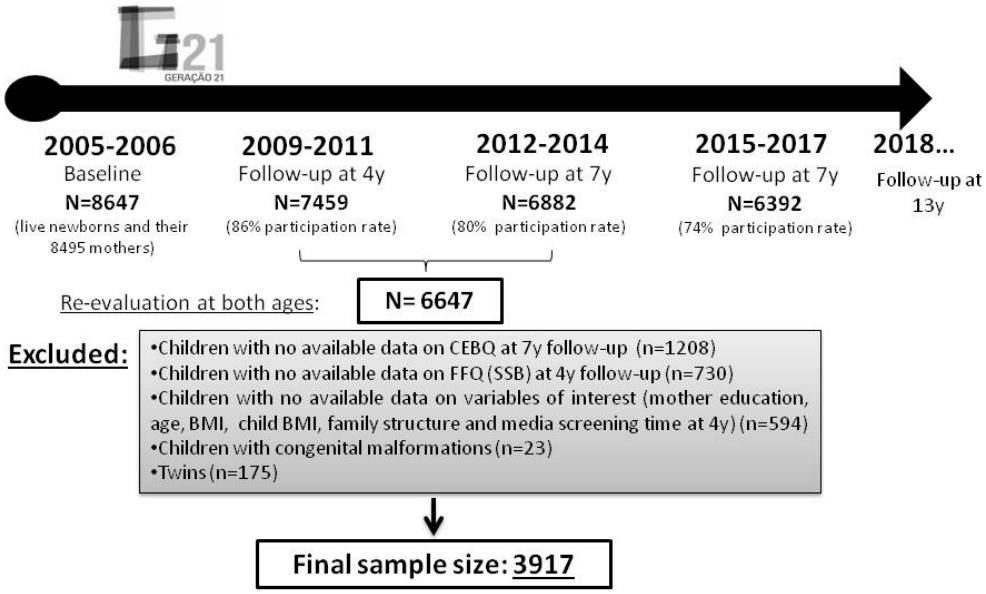


**Figure 1**. Flowchart of participants.

The final sample included participants who had responded to the FFQ at 4 years-old and the CEBQ at 7 years-old. We also included participants with data available on child's sex, maternal BMI before pregnancy, maternal age, maternal education, family structure, the time per day spent in front of the television, maternal consumption of SSB, and child's BMI. Participants with congenital malformations and twins were excluded. Overall this corresponded to 3917 participants (**Figure 1**).

Student's t-test was performed in order to compare the current sample (n=3917 with the non-participant subjects) characteristics at baseline (n=4730). In the current sample, mothers were slightly older [(29,8 years-old ±5,19 compared to 28,3±5,8] and were slightly more educated [(11,3 schooling years ±4,23 compared to 9,7±4,15]. According to Cohen's d effect size values (0.27 for maternal age and 0.38 maternal education), the magnitude of the presented differences is considered low [30], thus it is likely to be due to the large sample size, and less likely because of differences between participants' characteristics.

*Ethics*

Generation XXI was approved by the University of Porto Medical School/ S. João Hospital Centre Ethics Committee and by the Portuguese Data Protection Authority.

All the phases of the study complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki. Accordingly, a written

informed consent from the parents (or legal substitute) and an oral consent from the children were obtained in each evaluation.

*Data Collection and Analysis*

Data was collected in face-to-face interviews by trained researchers using structured questionnaires or retrieved from medical records. Self-reported questionnaires by the main caregiver were also used.

Information on maternal sociodemographic characteristics and birth data were available at baseline.

At 4 years-old, child characteristics were assessed, including family structure (which asked with whom the child was living – parents, siblings, grandparents, other family members or others) and diet. Children's dietary intake was obtained through a FFQ, covering the previous 6 months, filled in by the main caregiver, usually mothers. Response frequency options varied between "more than 4 times per day" and "never", including 9 different frequencies of consumption. From the 35 food groups, five assessed the consumption of sugary beverages. These were: colas and other carbonated-drinks, non-carbonated soft-drinks, ice tea and packed nectar juices. Frequencies of consumption were converted into daily frequencies (e.g., once a week was converted into 1/7 days = 0.14 times/day). This questionnaire was previously validated by comparison with 3-day food records, also completed by the main caregiver(s) of a sub-sample of children from Generation XXI [31]. Maternal diet was also assessed by a FFQ following the same structure described above.

At the 7-years-old evaluation, eating behaviours were assessed using the CEBQ, completed by the main caregiver (94% were mothers). The original CEBQ includes 35 items related to eating behaviours among children, and is answered on a 5-point Likert scale, ranging from 1 = "never" to 5 = "always" [32]. In accordance with the original scale, five of the items were reverse-scored due to opposite phrasing. The scale is composed by eight subdomains related to distinct eating behaviours: Satiety Responsiveness (CEBQ-SR), Slowness in Eating (CEBQ-SE), Enjoyment of Food (CEBQ-EF), Food Responsiveness (CEBQ-FR), Food Fussiness (CEBQ-FF), Desire for Drinks (CEBQ-DD), Emotional Overeating (CEBQ-EOE), and Emotional Undereating (CEBQ-EUE). This questionnaire is a well-established instrument in the study of appetitive behaviours among children, demonstrating stability over time and good psychometric properties [32-34].

In this research, we used the Portuguese version of the CEBQ (P-CEBQ), previously translated and validated in children from Generation XXI [35]. Cronbach's alpha for the different subdomains of the P-CEBQ ranged from 0.74 to 0.85, attesting its good internal consistency, and the reliability assessed by the mean intra-class correlation coefficient was 0.73, attesting its good reliability at 7 years-old [35]. Data from the CEBQ were recovered in questionnaires

with <50% of missing data items, by replacement for the average of the remaining questions within each subdomain of the participant. In a previous work from Albuquerque *et al.* (2017) with a sample from the same cohort, authors grouped subdomains into two composite factors, obtained by principal component analysis: Appetite Restraint and Appetite Disinhibition [35]. The Appetite Restraint factor was related to subdomains measuring internal cues of satiety and food fussiness, on which loaded mostly CEBQ-FF, CEBQ-EF, CEBQ-SE, and CEBQ-SR, and the Appetite Disinhibition factor was related to subdomains measuring external food cues and emotional responses toward foods, on which loaded mostly CEBQ-FR, CEBQ-EOE, CEBQ-EUE, and CEBQ-DD. Factor 1 – Appetite Restraint explained 35% and factor 2 – Appetite Disinhibition explained 26% of the total variance [35]. In this study we also used these factors to investigate the relationship with SSB consumption.

Anthropometrics were also collected at 4 and 7-years-old by trained staff according to standard procedures [36]. Weight was measured in light clothing and without shoes, by using a scale (TANITA®, Arlington Heights, IL, USA) (to the nearest 0.1kg). Height was measured using a stadiometer (SECA®, Hamburg, Germany) (to the nearest 0.1cm). Children were classified according to the age- and sex-specific BMI reference z-scores developed by the World Health Organization [37]. At baseline, mothers were heighted and were asked about their weight before pregnancy.

*Statistical analysis*

Descriptive statistics and univariate and multivariate generalized linear models, computing β regression coefficients and the respective 95% confidence intervals with Bonferroni's correction, were performed to estimate the associations between SSB and the eight subdomains of the P-CEBQ and the two composite factors of Appetite Restraint and Appetite Disinhibition.

Additionally, we performed a multinomial logistic regression to assess the association between the consumption of SSB, dichotomized into <1 and ≥1 servings per day and increasing scores on the P-CEBQ subdomains (divided into tertiles).

The selection of confounders in each model was based on a previous literature review, and tested in the current sample. Thus, we performed adjustments for child's sex, family structure at 4 years (living with both parents or living with one of them or having other type of family structure - without any of their parents), media screening time at 4 years-old and maternal BMI before pregnancy, maternal age, education, and maternal SSB's consumption at 4 years of the child. The effect of child's BMI at 4 years-old was also tested in the model, but the magnitude of the associations did not change (results not shown). For this reason, since BMI may be influenced by eating behaviors and vice-versa, we decided not to include

child's weight status in the tested models. Additionally, an adjustment for other dietary variables, namely fruit and vegetables daily consumption (as an indicator of overall diet quality) and for other sweets, such as cookies, cakes, candies, chocolates, was performed. As the results remained similar the final model did not include these variables.

As the interaction of child's sex in these associations did not show differences, the analysis was not stratified and sex was included in the model as a potential confounder.

Statistical analyses were run using IBM SPSS® (Statistical Package for Social Sciences), version 24.0™.

# Results

**Table 1** shows the mother's and child's characteristics in the whole sample and stratified by categories of SSB consumption. In our sample, the majority of the mothers (62.4%) were between 25 and 34 years-old at baseline, 52.6% were highly educated (≥12 years) and a third had excessive weight before pregnancy. Of the mothers with overweight, almost 35% consumed more than 1 serving of SSB per day.

A third of the children were overweight or obese at 4 years of age and, of these, 33.4% consumed ≥1 servings of SSB per day. Nearly 40.3% of the children consumed SSB between 3 and 4 times a week and 59.1% once or twice a week. The most frequent consumed beverages were ice tea and packed fruit nectars (data not shown).

**Figure 2** presents the mean scores of each P-CEBQ subdomain at 7 years-old by intake of SSB at 4 years-old, stratified by less than 1 and ≥1 serving per day. Mean scores of Food Responsiveness, Desire for Drinks, Emotional Overeating, Enjoyment of Food, Slowness in Eating were statistically different between categories of SSB consumption, with greater scores for those ones who consumed one serving or more, except for slowness in eating, that was lower.

Associations between the consumption of SSB at 4 years-old (as a continuous variable) and eating behaviours assessed at 7 years-old are described in **Table 2**. In univariate analysis, children with a higher consumption of SSB scored significantly more in Enjoyment of Food (β=0.038, 95%CI 0.001; 0.075), Food Responsiveness (β=0.047, 95%CI 0.010; 0.084), Emotional Overeating (β=0.039, 95%CI 0.010; 0.069), and Desire for Drinks (β=0.137, 95%CI 0.100; 0.174). A similar association was found with the Appetite Disinhibition factor (β=0.107, 95%CI 0.061; 0.152). When adjusting for child's sex, BMI before pregnancy, family structure and media screening time at 4 years-old of the child, and maternal age and education (Model 1) most associations were lost; only the Desire for Drinks subdomain (β=0.097, 95%CI 0.059, 0.136) and the Appetite Disinhibition factor (β=0.076, 95%CI 0.029, 0.124) remained significantly associated with SSB. In Model 2**,** the same potential confounders were included

plus consumption of SSB by mothers at 4 years-old of the child. The previous associations were weakened, but remained significant (Desire for Drinks: β=0.075, 95%CI 0.031; 0.119; Appetite Disinhibition factor: β=0.061, 95%CI 0.007; 0.116).

**Table 1 –** Mother's and child's characteristics at baseline and follow-ups at 4y and 7y of age (n= 3917).

| | | Child's daily SSB consumption | | |
|---|---|---|---|---|
| | N (%) | <1 serving | ≥1 servings | p-value* |
| **MOTHER´S CHARACTERISTICS** | | | | |
| Maternal age (y) | | | | |
| <25 | 753 (19.2%) | 422 (15.7%) | 331 (27.0%) | <0.001 |
| 25-34 | 2445 (62.4%) | 1740 (64.7%) | 705 (57.5%) | |
| ≥35 | 719 (18.4%) | 529 (19.7%) | 190 (15.5%) | |
| Maternal education (y) | | | | |
| <9 | 1580 (40.3%) | 909 (33.8%) | 671 (54.7%) | <0.001 |
| 9-11 | 276 (7.0%) | 171 (6.4%) | 105 (8.6%) | |
| ≥12 | 2061 (52.6%) | 1611 (59.9%) | 450 (36.7%) | |
| Maternal BMI** before pregnancy | | | | |
| Under-/normal weight (BMI <25kg/m$^2$) | 2702 (69.0%) | 1900 (70.6%) | 802 (65.4%) | 0.001 |
| Overweight/obese (BMI ≥25kg/m$^2$) | 1215 (31.0%) | 791 (29.4%) | 424 (34.6%) | |
| Amount of SSB | | | | |
| <1 serving/day | 2754 (70.3%) | 2257 (83.9%) | 497 (40.5%) | <0.001 |
| ≥1 servings/day | 1163 (29.7%) | 434 (16.1%) | 729 (59.5%) | |
| **CHILD´S CHARACTERISTICS** | | | | |
| Sex | | | | |
| female | 1934 (49.4%) | 1355 (50.4%) | 579 (47.2%) | 0.073 |
| male | 1983 (50.6%) | 1336 (49.6%) | 647 (52.8%) | |
| Weight status at 4y** | | | | |
| Under-/normal weight (<1 SD) | 2676 (68.3%) | 1859 (69.1%) | 817 (66.6%) | 0.129 |
| Overweight/ obesity (≥1 SD) | 1241 (31.7%) | 832 (30.9%) | 409 (33.4%) | |
| Family structure at 4y | | | | |
| living with both parents | 3492 (89.1%) | 2430 (90.3%) | 1062 (86.6%) | 0.002 |
| living with at least one parent | 398 (10.2%) | 247 (9.2%) | 151 (12.3%) | |
| other family structure | 27 (0.7%) | 14 (0.5%) | 13 (1.1%) | |
| Daily media screening at 4y | | | | |
| ≤120min | 2843 (72.6%) | 2031 (75.5%) | 812 (66.2%) | <0.001 |
| >120min | 1074 (27.4%) | 660 (24.5%) | 414 (33.8%) | |
| TOTAL | **3917 (100%)** | **2691 (68.7%)** | **1226 (31.3%)** | |

*****P-values according to the Chi-squared test. **Z-scores and BMI are defined according to the WHO's classification. BMI: Body Mass Index; SD: Standard Deviation; SSB: sugar-sweetened beverages; y: years.

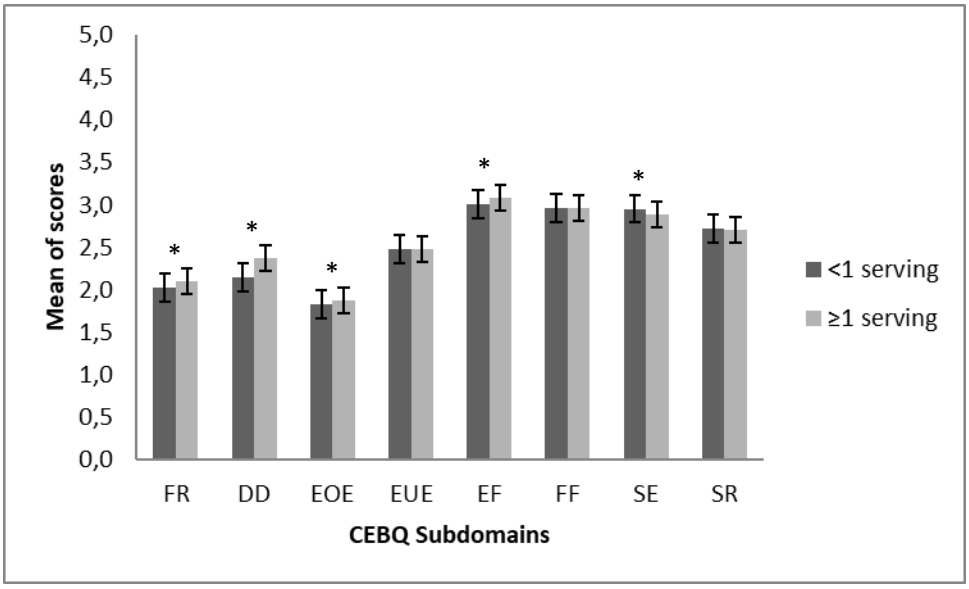


**Figure 2**. Mean (±SE) intake of SSB at 4 years, according to child's appetitive behaviours at 7y (n=3917).

*p<0.05 according to the Student's t-test. CEBQ-FR: Food Responsiveness, CEBQ-DD: Desire for Drinks, CEBQ-EOE: Emotional Overeating; CEBQ-EUE: Emotional Undereating; CEBQ-EF: Enjoyment of Food, CEBQ-FF: Food Fussiness, CEBQ-SR: Satiety Responsiveness; CEBQ-SE: Slowness in Eating; SSB: sugar-sweetened beverages

In **Table 3,** the same associations were tested but according to increasing categories of SSB consumption and increasing scores in the CEBQ subdomains. The multinomial logistic regression analysis shows that consuming ≥1 servings per day of SSB (*vs.* <1 serving per day) at 4 years-old was associated with a higher odds of scoring higher in Desire for Drinks in a dose-response relationship (2nd *vs.* 1st tertile adjusted OR=1.262, 95%CI 0.917, 1.736; 3rd *vs.* 1st tertile adjusted OR=1.345, 95%CI 1.048; 1.727). The association between SSB at age 4 and the Appetite Disinhibition factor at 7 years-old was only significant considering the upper tertile of consumption (3rd *vs.* 1st tertile adjusted OR=1.218, 95%CI 0.934; 1.588). No significant associations were found with the remaining appetitive behaviours subdomains.

**Table 2.** Linear regression models between child's sugar-sweetened beverages consumption at 4 years-old and appetitive behaviours at 7 years-old.

| | CEBQ - EF | CEBQ - FF | CEBQ - SR | CEBQ - SE | Appetite Restraint factor | CEBQ - FR | CEBQ - DD | CEBQ - EOE | CEBQ - EUE | Appetite Disinhibition factor |
|---|---|---|---|---|---|---|---|---|---|---|
| | **β (95% CI)** | | | | | | | | | |
| **Crude** | **0.038 (0.001; 0.075)** | -0.007 (-0.042, 0.029) | 0.004 (-.028, 0.036) | -0.038 (-0.078, 0.002) | -0.024 (-0.071, 0.022) | **0.047 (0.010, 0.084)** | **0.137 (0.100, 0.174)** | **0.039 (0.010, 0.069)** | 0.015 (-0.020, 0.051) | **0.107 (0.061, 0.152)** |
| **Model 1** | 0.006 (-0.032, 0.045) | -0.004 (-0.041, 0.033) | 0.020 (-0.013, 0.054) | -0.029 (-0.071, 0.013) | 0.005 (-0.044, 0.053) | 0.020 (-0.18, 0.058) | **0.097 (0.059, 0.136)** | 0.026 (-0.005, 0.057) | 0.032 (-0.005, 0.068) | **0.076 (0.029, 0.124)** |
| **Model 2** | 0.011 (-0.033, 0.055) | -0.013 (-0.056, 0.029) | 0.006 (-0.032, 0.044) | -0.039 (-0.088, 0.009) | -0.009 (-0.065, 0.046) | 0.013 (-0.031, 0.056) | **0.075 (0.031, 0.119)** | 0.024 (-0.012, 0.059) | 0.030 (-0.012, 0.072) | **0.061 (0.007, 0,116)** |

95% CI with Bonferroni correction (The threshold of statistical significance with Bonferroni correction is 0.006). **Bold** values at <0.05 level.
CEBQ-EF: Enjoyment of Food, CEBQ-FF: Food Fussiness, CEBQ-SR: Satiety Responsiveness, CEBQ-SE: Slowness in Eating, CEBQ-FR: Food Responsiveness, CEBQ-DD: Desire for Drinks, CEBQ-EOE: Emotional Overeating; CEBQ-EUE: Emotional Undereating.
Model 1 - adjusted for child's sex, maternal BMI before pregnancy, family structure at 4y, media screening time at 4y, maternal age and education
Model 2 - adjusted for the same potential confounders as model 1 plus maternal consumption of SSB at 4y of the child

**Table 3.** Multinomial regression analysis between child's sugar-sweetened beverages consumption (≥1 vs. < 1 serving/day – reference category) at 4 years of age and appetitive behaviours at 7 years-old (divided into tertiles – 1st tertile as reference).

| | | CEBQ - EF | CEBQ - FF | CEBQ - SR | CEBQ - SE | Appetite Restraint **factor** | CEBQ - FR | CEBQ - DD | CEBQ - EOE | CEBQ - EUE | Appetite Disinhibition **factor** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Odds Ratio (95% CI)** | | | | | | | | | |
| Crude | 1st tertile | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 2nd tertile | 0.997 (0.773, 1.287) | 1.152 (0.910, 1.458) | 0.853 (0.671, 1.084) | 0.988 (0.776, 1.258) | 0.967 (0.768, 1.219) | 0.914 (0.714, 1.171) | **1.276 (0.962, 1.693)** | 1.154 (0.880, 1.512) | 0.931 (0.727, 1.191) | 1.106 (0.872, 1.404) |
| | 3rd tertile | 1.198 (0.958, 1.498) | 0.994 (0.795, 1.244) | 0.956 (0.757, 1.207) | 0.808 (0.641, 1.019) | 0.857 (0.679, 1.080) | 1.138 (0.899, 1.439) | **1.710 (1.374, 2.127)** | 1.097 (0.875, 1.374) | 0.976 (0.780, 1.220) | **1.430 (1.134, 1.803)** |
| Adjusted model* | 1st tertile | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 2nd tertile | 1.055 (0.789, 1.410) | 1.084 (0.828, 1.417) | 0.851 (0.647, 1.118) | 0.976 (0.741, 1.285) | 1.053 (0.810, 1.370) | 0.936 (0.706, 1.241) | **1.262 (0.917, 1.736)** | 1.100 (0.809, 1.497) | 0.950 (0.717, 1.258) | 1.120 (0.854, 1.469) |
| | 3rd tertile | 1.114 (0.862, 1.439) | 0.993 (0.769, 1.283) | 0.975 (0.746, 1.273) | 0.832 (0.638, 1.085) | 0.936 (0.717, 1.222) | 1.010 (0.771, 1.323) | **1.345 (1.048, 1.727)** | 0.974 (0.753, 1.260) | 1.060 (0.821, 1.368) | **1.218 (0.934, 1.588)** |

95% CI with Bonferroni correction (The threshold of statistical significance with Bonferroni correction is 0.006). **Bold** values at <0.05 level.
CEBQ-EF: Enjoyment of Food, CEBQ-FF: Food Fussiness, CEBQ-SR: Satiety Responsiveness, CEBQ-SE: Slowness in Eating, CEBQ-FR: Food Responsiveness, CEBQ-DD: Desire for Drinks, CEBQ-EOE: Emotional Overeating; CEBQ-EUE: Emotional Undereating.
*****Model adjusted for child's sex, maternal BMI before pregnancy, family structure at 4y, media screening time at 4y, maternal age and education and maternal consumption of SSB at 4y of the child **(model 2**)

# Discussion

This study aimed to examine prospective associations between the consumption of SSB at 4 years-old and appetitive behaviours three years later. First, we hypothesized that a positive association would be found between the consumption of SSB and disinhibited eating behaviours later in childhood and our findings corroborate with this hypothesis. A positive association between SSB at 4 years-old and food-approach behaviours at age 7 was found, particularly between SSB consumption and Desire for Drinks and a composite factor of Appetite Disinhibition behaviours.

Although using a cross-sectional design, previous studies [24-28] have shown a tendency of associations between the consumption of SSB and food-approach behaviours among children. In the study of Sweetman *et al*. (2008), the consumption of SSB was associated with higher scores of Desire for Drinks [24], as also seen in the current results. In this study with a smaller sample size of 346 pre-schoolers, children with greater Desire for Drinks consumed more frequently SSB and had greater preference for them [24]. In contrast, in 2017, Jalkanen *et al.* did not find any relationship between this subdomain and the consumption of SSB; it was only found an association with a higher consumption of fat-containing milk, among children aged 6-8 years [28]. It is important to highlight that in these cross-sectional studies, questions about the preference for different types of beverages were included, and in the current study, participants were asked about the frequency of consumption of four different beverage groups, and not the actual preference for them. In the current study, the subdomain Desire for Drinks was the most consistently associated with SSB consumption. It specifically inquiries about the appetite for drinks, using the following statements: “My child is always asking for a drink”, “If given the chance, my child would drink continuously throughout the day” and “If given the chance, my child would always be having a drink”. However, a higher score in CEBQ-DD does not provide sufficient information about the actual consumption of this food group and the type of beverage consumed.

The consumption of artificially sweetened beverages or light versions are also worthy to highlight, however, in the current study, the FFQ used at 4 years-old did not specify this type of beverages. For instance, in the cross-sectional study of Elfhag *et al.* (2007), higher consumption of artificial sweetened beverages was associated with restrained eating and the sugar sweetened beverages with less restrained behaviours. In addition, being more sensitive to external stimulus seemed to promote the consumption of sugar-sweetened soft drinks instead of the others, but only among girls [25]. In recent years, due to the negative impact of SSB on health, companies

reformulated some products, diminished the portions sizes and introduced formulas with less sugar or artificial sweeteners [38, 39]. In Portugal, this has been observed and become popular since the introduction of sugar taxation regulation in 2017 [40], so their consumption when FFQ was applied was probably irrelevant, because they were probably less common and available in the market.

Authors from a Swedish cross-sectional study found that the consumption of sweets and soft drinks was associated with Emotional Eating in girls [26]. In the present study, no significant association was found between SSB and Emotional Eating, after adjusting for potential confounders. Yet, in the above-mentioned study, children were older (12 years-old) compared to our study (children were 4 years of age at the moment of consumption and when CEBQ was applied they were 7 years-old). Given the substantial age difference, eating behaviours in response to emotions may be different as well. Moreover, we did not investigate if children with higher scores of Emotional Eating consumed more SSB at 7 years-old or later. Instead, we analysed the association in the opposite direction, and no relationship was found between the consumption of SSB at age 4 and Emotional Eating later in childhood.

Considering our initial hypotheses, we were expecting to find positive associations between SSB consumption and subdomains related to appetite disinhibition, i.e. Food Responsiveness and Enjoyment of Food, and negative associations with Satiety Responsiveness. However, no associations were found with the subdomains in isolation, only for the Appetite Disinhibition factor, which summarizes the following subdomains: CEBQ-FR, CEBQ-EOE, CEBQ-EUE, and CEBQ-DD.

The association of SSB consumption with appetitive traits three years later was weakened after adjustment for potential confounders. In model 1, β regression coefficients were adjusted for child's sex, maternal age, education and BMI before pregnancy, family structure and media screening time at 4 years-old. When analysing the covariates individually, we found greater impact when controlling for maternal education, age and media screening time at 4 years (data not shown). In model 2, after further adjustment for mother's consumption of SSB at 4 years of the child, the impact was even greater, giving the example of Desire for Drinks subdomain the β coefficient went from β=0.137 to β=0.096 (only adding this variable, with the complete model: β=0.075). This can be a reflection of parent's influence as role models [41], with children behaving according to their families and peers, especially in early ages, when parents and siblings have a high influence in the development of eating behaviours [42]. Furthermore, this can also be an indicator of the food accessibility and availability in child's environment [41]. If the mother consumes SSB often, it is likely that this type of

food is available in their home, too. Thus, these factors seem to play an important role on child's SSB consumption, as well as on disinhibition behaviours later in childhood.

Research on SSB consumption among children has demonstrated a variety of harmful effects when excessively consumed, such as dental caries, insulin resistance, caffeine-related effects (such as sleeping problems) and weight gain [19]. The mechanisms behind the association of SSB and weight gain are still unclear [19]. Low satiety effect of liquid forms and, consequently, a poor energy compensation, has been suggested as a potential pathway [20].

Due to those effects, particularly on childhood obesity, some countries (41 countries plus 8 states on USA till may 2019) have implemented taxation policies as a public health measure in order to decrease the consumption at population level [43], as recommend by the World Health Organization [44-46]. Other public health measures have been implemented as well. For instance, in Portugal, the National Programme for the Promotion of Healthy Eating includes strategies such as creating healthier food environments, improving quality and consumer accessibility to healthy choices, promoting and developing literacy for consumers' healthy food choices, promoting innovation and entrepreneurship focused on the area of healthy eating promotion (e.g. SSB reformulation) [47]. Since the implementation of these measures, a SSB sales decrease has been reported in many countries [47, 48]; in Portugal, producers have lowered the sugar content of SSB [47].

Considering that eating behaviours may have an important role in the development of childhood obesity [49], tackling children's unhealthy food habits can be an essential target to prevent this health problem. Nonetheless, a bidirectional association between obesity and eating behaviours has been suggested [50].

Findings from a recent study [51] suggest that a higher BMI early in childhood can promote disinhibited eating behaviours later in life. This is concordant with previous work from Steinsbekk and colleagues in 2017, which suggested that higher fat mass predicted greater food responsiveness over time [52]. Thereby it is necessary to be aware of the risks of obesity and eating behaviours simultaneously early in childhood, trying to cease the process of gaining weight as soon as possible.

This study has some limitations that need to be addressed. First of all, the data was collected by face-to-face interviews, which can imply on recall and social desirability bias. However, in relation to the FFQ, the social desirability bias is unlikely to have a great impact, since the instrument was composed by 35 items, instead of being focused only in the SSB consumption, and was previously validated against 3-day food records [53]. Furthermore, we grouped the colas and other carbonated-drinks, non-carbonated soft drinks, ice tea and packed nectar juices, assuming similar contents of sugar, what

could not be entirely factual. Likewise, other sugary beverages were not included, for example chocolate milk. However, once our sample had relatively low consumption of SSB, we decided to group the different beverages for statistical reasons. In addition, the CEBQ was also previously tested in the Generation XXI sample, and showed good psychometric properties [35].

In our study, the isolated effect of a group of drinks was analysed. Nevertheless, diet is complex and the consumption of SSB has been considered a marker of a dietary pattern with poor quality that usually includes other unhealthy foods and beverages [4, 54-56]. In order to test if the effects were specifically from SSB further adjustments for other dietary variables (daily fruit, vegetables and sweets consumption) were done and did not change the observed results.

It is relevant to mention that studies with European populations have shown that Portuguese children have, in general, healthier eating habits [4, 8, 57]. Data from this cohort revealed a relative lower intake of SSB and other energy-dense foods, and a higher intake of fruit and vegetables when compared with other populations [4, 8, 19, 57]. A lower consumption may have implied in weaker associations between SSB and CEBQ scores, not just due to the lower intake of unhealthy foods, but also because of the intake of foods with protective effects (fruits and vegetables), due to their content of micronutrients and phytochemicals [2, 58, 59]. In light of this, the associations between the consumption of SSB and appetitive behaviours could be stronger in populations with higher daily consumption of SSB, and additional associations with other subdomains could also be found. Despite the consumption of SSB in our sample being minor compared to other populations [4, 8, 19, 57], this consumption is still worrisome and an important public health concern.

The main strength of the present study is, to our knowledge, to be the first investigating the association between the consumption of SSB and eating behaviours using a prospective approach. So far, only one study used a prospective approach, but in the opposite direction of associations [16], as cited above. In this study, conducted in 2009 with 1275 children at the age of 9, the CEBQ and the FFQ were applied and, after a one-year follow-up, the procedures were repeated. Associations between SSB and eating behaviours were inconsistent, and the appetitive behaviours did not predict changes in child's weight, nor intake [16]. In our study, the associations were exploited in the opposite direction with three years between evaluations, which permitted a better understanding of cause and effects relationship, in contrast to the previous study. Moreover, this study had a larger sample size and confidence intervals were set with Bonferroni's correction, which focus only in results using a more rigorous cut-off.

# Conclusions

Higher consumption of SSB during pre-school years was associated with disinhibited eating behaviours later in childhood. Familial covariates had the greatest impact in these associations. In such wise, it seems relevant, from early ages, to promote the intake of alternative drinks, such as water and to restrict the availability of other sweetened options, making parents and caregivers aware of the importance of these exposures, once they have a pivotal role in shaping children's eating behaviours.

Longitudinal studies are needed in order to understand better the long-terms effects of SSB consumption, and the effects of different types of SSB on the development of disinhibited eating behaviours later in childhood**.**

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# IV. General Discussion

Research on SSB consumption among children has demonstrated a variety of harmful effects when excessively consumed, such as dental caries, insulin resistance, caffeine-related effects and weight gain [122]. However, few have related SSB consumption with eating behaviours during childhood, and the ones available used a cross-sectional design. As better as we know, so far, this is the first work about this topic using a prospective approach in this direction of association.

In the current work, the consumption of SSB at 4 years-old was positively associated with the Desire for Drinks at 7 years of age, and with the Appetite Disinhibition factor, although with a weaker magnitude. Given this, it makes sense to question if SSB are capable of influencing thirst mechanisms/regulation and/or drinking habits and by which processes. Literature in this field is scarce, at least in humans and for the long-term.

McKiernan and colleagues have studied if thirst and hunger are linked with drinking and eating. In 2008, they concluded that neither absolute values nor changes of hunger or thirst are strong predictors of energy intake. Based on their observations, it was proposed that the high and increasing exposure to palatable, energy yielding beverages facilitates drinking considerable quantities of energy. The marked inter-individual variation in self-reported thirst and hunger sensations indicates that certain individuals may be more at risk from consumption of these beverages than others [171]. In 2009, in another work, they questioned the reliability of homeostatic regulations of hunger and thirst with eating and drinking, due to absence of strong associations between them. These authors suggested an influence of other factors in eating behaviour, such as palatability of foods and beverages or, in other words, hedonic influences [6].

Corroborating with the current results, the cross-sectional study of Sweetman *et al*. (2008), also associated the consumption of SSB with higher scores of Desire for Drinks. In this study with 346 pre-schoolers, children with greater Desire for Drinks consumed SSB more frequently and had greater preference for them. Authors also suggested that this Desire for Drinks may not simple denote thirst or hunger but a preference for the sweet taste, what can implicate preferences and consumption in long term [134]. The results of the present study are in line with this idea, suggesting a long-term effect. Hence, it is essential to understand the biological plausibility of these processes.

Studies with European populations have shown that Portuguese children have, in general, healthier eating habits [25, 30, 172]. In the current sample, 31.3% of the children consumed ≥1 serving of SSB per day, nearly 40.3% of the children consumed SSB between 3 and 4 times a week and 59.1% once or twice a week. Thus, pre-schoolers from Generation

XXI cohort had a relative lower intake of SSB and other energy-dense foods, and a higher intake of fruit and vegetables when compared with other populations, such as from UK, France and USA [25, 30, 122, 172].

Despite this fact, the consumption of SSB is still worrisome and an important public health concern in developed countries, as observed in the National Survey in Portugal that showed that children aged between 3 and 9 years-old consumed in average 76g/day of soft drinks and fruit nectars in 2016 [127].

In light of the great consumption of sugar among children around the world, the WHO has changed the guidelines in the last years, recommending a reduced intake of free sugars. This intake should be less than 10% of total energy intake, for both adults and children [173]. These recommendations for children are based on the current evidence that suggests an association between the consumption of SSB with overweight and obesity. So, the reduction of its consumption is seen as a potential intervention to control childhood overweight and obesity [125].

In order to follow the recommendations of WHO, countries, such as Portugal, have developed policies and interventions.

### *SSB taxation in Portugal*

As stated above, taxation policies seem to have a great impact in consumer's food choices. Given the evidences from other countries [153, 155, 158-160], Portugal SSB taxes were approved by the government in December of 2016 (Decree-law no. 42/2016), simultaneously with the budget for 2017 (Law no.42/2016), and implemented in February 2017 [130, 174]. The taxes apply to non-alcoholic beverages with added sugar or sweeteners, excluding products like milk-, soy-, or rice-based beverages; fruit-, algae-, or veggie-based juices and nectars; cereal- and nut-based drinks; and drinks considered essential for special dietary needs [154]. In Portugal, the funds from these taxes are used to finance health interventions [165]. The initial amount of the tax was 0.08€ per litre of beverages with less than 80 grams of sugar per litre, and 0.16€ for the ones with 80 or more grams per litre. These different breaks are an incentive to manufacturers to reduce the sugar content in the beverages [154]. Some products that used to have 80 grams or more of sugar now have 78-79 g/L, paying the minor tax. The same also happened in other countries, namely in United Kingdom, after the introduction of soda tax a reformulation of products was also noted [175].

After the implementation of the tax, sales decreased 7% [165] and, in 2017, the sales of the beverages with more than 80 grams of sugar per litre had decrease of almost 50% compared to the previous years and this was mainly due to the reformulation of the beverages [174]. Reformulation processes led to a reduction of 11% of total energy intake through

sweetened beverages' consumption by the Portuguese population [176]. The impact of marketing and consumer's awareness should also not be ignored as probable contributors to this effect. In the first report about these measures, authors referred that the reduction in sugar intake through beverages in 2017 was higher than the reduction through education and autoregulation mechanisms, between 2013 and 2016, reinforcing the importance of this kind of intervention [165].

Overall, habitual consumers of these beverages consumed less 5630 tons of sugar in 2017 [174]. In addition, this tax collected about €70 million and all revenue was invested towards the Portuguese National Health Service funding [176].

There was a substantial pass-through of the tax to consumer prices in beverages with lower content of sugar (<80g/L). This is explained by the costs related to reformulations on the products and this is considered the main advantage of the taxes implementation in Portugal [154].

In 2019, the taxes were reformulated, having four levels of taxation: those ones with less than 25g of sugar per litre pay a tax of €0.01 per litre; ≥25g/L and <50g/L pay a tax of €0.06 per litre; ≥50g/L and <80g/L pay a tax of €0.08 per litre; while the ones ≥80g/L pay a tax of €0.20 per litre [177]. This new tax design was created aiming to promote products reformulations towards sugar content [154], and according to the industry's feedback, a decrease of energy intake by, at least, 15% is expected in the Portuguese population until 2020 [176].

It is important to note that this quick adaption is in line with the recommendations for effective health policies. So, policy makers, politicians, and academics must collaborate in order to create an adjustable environment in which health policies can shape according to increasing health challenges in a more effective and efficient way [176]. In such manner, the Portuguese Government created an interministerial taskforce [178], whose function is to evaluate the effectiveness of this tax, to study changes in consumption patterns, industry offering, reformulation of existing products, launch of new products, and competitiveness of national companies versus those overseas. This taskforce counts with the collaboration of the WHO Regional Office (Europe) to analyse the impact of this measure, as well.

In the future, it is expected a decrease of 1600 obese people in a yearly basis, due to the sales reduction and reformulation [165]. WHO used modelling tools (PRIME) to estimate the impact of SSB tax, through population-level changes in non-communicable diseases risk factors on annual deaths. It was estimated that, by introduction of the SSB tax, at least 27 deaths directly related to excessive sugar consumption in Portugal will be avoided or delayed every year, in the next 15 to 20 years. With the introduction of the new taxation scheme, more progressive, promoting further reformulation, it is possible to avoid or delay a total of 48 deaths

every year. Children and adolescents will probably be the most benefited with these measures, once they are the biggest consumers of SSB [179].

As highlighted, taxes have been considered more effective than education and promotion programs on SSB consumption reduction [180, 181]. However, they should make part of other policies/interventions [174, 182].

*Study limitations and future research and interventions*

For a first longitudinal approach, we are confident with the methodology followed, however we believe that some different perspectives could enrich the knowledge in this topic of research, and can be integrated into future work. Although some of these aspects had already been mentioned in the paper, these will be more exploited in this session.

Taking into account the influence that family and peers have in child's food consumption and eating behaviours, aspects such as parenting styles could have been a good contribution for our research. Maternal intake of SSB seems to be a good indicator of children's consumption, and for the considered age-group, the mother should be one of the main influences. Taking into account the mother's consumption of this kind of beverage, it is very likely that SSB are also available in child's household [64, 183].

Beyond the availability and the access to drinks, to explore the preferred ones by children would help to clarify which sugar-sweetened drinks are consumed indeed, once preferences are strong indicators of consumption [183].

Another topic that could be interesting to explore in a different way is the categorization of beverages. In the current study, SSB were grouped into colas and other carbonated drinks, non-carbonated soft drinks, ice tea, and packed nectar juices, assuming similar contents of sugar, which could not be entirely factual. Split by levels of sugar content, as well as include non-sugary versions would be notable. Despite this, the available data and the sample size did not allow to run a stratified analysis by type of beverage consumed.

Additionally, an issue that might deserve attention is the content of artificial sweeteners and caffeine in some of the beverages (such as cola and ice tea). A cross-sectional study with children aged 10-12 years, reported that the consumption of cola was associated with low appetite and sleeping problems [184], which can also indirectly influence appetite [185, 186]. Nevertheless, this is a prospective study and no studies which reported the effect of caffeine on appetite or eating behaviours in a long-term way were found. Moreover, in a review from 2017, associations between caffeine and appetite control were inconsistent [187].

The effect of artificial sweeteners on appetite regulating hormones has been also studied. Although no consistent effects have been found in prospective observational studies

[188], it could be interesting to test, in populations with high degree of exposure, if there are different effects between beverages with and without non-nutritive sweeteners.

As the aim of this research was to study the isolate effect of SSB on eating behaviours, the adjustment of the associations for global dietary patterns could also have been useful. Diet is complex and the consumption of SSB has been considered a marker of a dietary pattern with poor quality that usually includes other unhealthy foods and beverages [25, 189-191]. A previous study has shown that children belonging to an "energy-dense foods" dietary pattern, including SSB, at 4 years-old had more Appetite Disinhibition at 7 years-old [30]. In the current work further adjustments for other dietary variables were tested. Independently of daily fruit, vegetables and sweets consumption at 4 years-old, the effect of SSB on appetitive behaviours at 7 years-old remained.

In this study, child's BMI was not included in the models because when tested as a potential confounder, it did not change any association. Additionally, it seems to have a bi-directional association with eating behaviours [12]. However, including in the models different fat depots measures, such as fat and lean mass, could have been helpful. For instance, Steinsbekk and colleagues found that higher fat mass predicted greater Food Responsiveness over time [192].

In addition, to include further follow-up data from the Generation XXI cohort at 10 and 13 years-old, may also have enriched the research on the longitudinal association of consumption of SSB and later eating behaviours. For instance, it could be interesting to understand if the Desire for Drinks remains high when the consumption is reduced.

In the current work, SSB were measured by a FFQ. Although it showed good psychometric properties when compared with food records [193], the analyses of daily food records would help to understand the context in which the beverages are consumed, if during meals or between them, for example. In the same way, studying if there are differences in total energy intake according to levels of consumption, could contribute to clarify the question related to compensation associated to energy in liquid forms.

Considering these factors, future studies will help to better understand the role of SSB on eating behaviours and weight gain, thus supporting better planning intervention strategies.

Besides the taxation implemented in 2017, other strategies were implemented in the country. In 2012, Portugal implemented the first national food and nutrition programme - The National Programme for the Promotion of Healthy Eating (PNPAS). The purpose of this programme is to improve the nutritional status of the population, stimulating physical and availability of healthy foods, limiting the accessibility to unhealthy options, and creating conditions so that the population can value, appreciate and integrate them into their daily routines [194]

Later on, in 2017, The Integrated Strategy for the Promotion of Healthy Eating (EIPAS) was created, an interministerial promotion strategy, i.e. which involves several sectors beyond the Ministry of Health, being possible a “health in all policies” approach [195].

Briefly, the EIPAS includes four strategic areas [156]:

- Strategic area 1 - Create healthier food environments;
- Strategic area 2 - Improve quality and consumer accessibility to healthy choices;
- Strategic area 3 - Promote and develop literacy for consumers’ healthy food choices:
- Strategic area 4 - Promote innovation and entrepreneurship focused on the area of healthy eating promotion.

Each strategy area is composed of areas of intervention and has several actions to achieve these goals. Here, the main strategies with influence on SSB consumption and/or on children eating habits and behaviours will be briefly mentioned [156].

To start, the SSB tax measure can be integrated into the Strategic area 1, because the main objective of this measure was a reformulation of the products, one of the four priority areas of intervention of this strategic area. In addition, another priority area of intervention is to change food availability in different public settings, including schools, namely to extend the existing guidelines for the provision of food in schools to all levels of education [156].

The strategic area 2 has five priority intervention areas, being one of them restricting the marketing of unhealthy foods to children. Recently, a law (Law nº. 30/2019) that introduces restrictions to food marketing to children under 16 years was approved [196]. These restrictions concern foods that are high in energy, salt, sugar, saturated and *trans* fatty acids, and limits were establish following WHO recommendations and adapted by the Directorate-General of Health (DGS) [197]. The implementation of this measure is expected to initiate in October of this year [198]. In short, this measure prohibits advertising of the mentioned foods within 30 minutes previous and subsequent and during TV programs directed to children as well as online contents intended for children. Also forbids advertisements in a radius of 100 meters from pre-school facilities, schools and children’s playgrounds [196]. This intervention is based on evidence that suggests an influence of marketing on the consumption of unhealthy foods [93] and is highly recommended [199-201].

One of the four priority areas of Strategic area 3 is the improvement of food and nutrition literacy from the paediatric age. In detail, promoting food literacy of pregnant women and parents about the importance of healthy eating in the first 1000 days of life [156].

Thus, since the implementation of a food and nutrition programme, Portugal has adopted important measures to improve the nutritional status of the population, some of them being specific to children and many others influencing their eating behaviours indirectly.

Additionally, as already mentioned in the Introduction of this thesis (*Strategies aiming to improve eating behaviours* chapter) there are several strategies that can be applied to improve children's eating behaviours, once there is sufficient evidence showing their effectiveness. These strategies can be taken into consideration in the elaboration of intervention programmes, as well as been taught to parents and professionals who work with children and that are capable of influencing their behaviours directly or indirectly.

# V. Conclusions

To conclude, higher consumption of SSB during pre-school age was associated with disinhibited eating behaviours later in childhood. Familial influences had a great impact in the association of child's food consumption and eating behaviours,

Longitudinal studies are needed in order to better understand the long-terms effects of the consumption of SSB, and the potential differences of the diverse types of these beverages.

Meanwhile, from a public health point of view, it seems worthy, from early ages, to promote the intake of alternative drinks such as water and to restrict the availability of other sweetened options. It is necessary to aware parents and caregivers of the importance of these exposures, once they have a pivotal role in shaping children's eating behaviours for which effective strategies have been described. Providing healthier foods at home, avoiding unhealthy stores, learning how to recognize child's fullness, serving moderate portions, encouraging children to try new and a variety of foods from early-life and promote repeated exposure, are some of these examples.

Public health interventions that create healthier food environments and improve the quality and accessibility to healthy food choices for all the population are essential. Simultaneously, the improvement of food literacy of the parents and caregivers is an urgent need, considering their role on children's food intake and on the establishment of eating behaviours.

Moreover, all the interventions should be evaluated constantly in order to track their impact and effectiveness, and to be possible to adapt the interventions to population according to their expectations and needs.

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