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Eating behaviours related to appetite during childhood: Determinants from a prospective approach

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

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Esta dissertação tem por base dois manuscritos, nos quais colaborei ativamente na operacionalização das hipóteses, análise e interpretação dos dados e fui responsável pela redação das suas primeiras versões:

I. **Early influences on appetite-related eating behaviours in 7 years old children.**

II. **Children's dietary patterns at 4 years: are they associated with appetite-related eating behaviours at 7 years old?**

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LIST OF ABBREVIATIONS

AGA	Adequate for Gestational Age
BEBQ	Baby Eating Behaviour Questionnaire
BIC	Bayesian Information Criteria
BMI	Body Mass Index
CEBQ	Children's Eating Behaviour Questionnaire
CI	Confidence Interval
DEBQ	Dutch Eating Behaviour Questionnaire
EDF	Energy Dense Foods
FFQ	Food Frequency Questionnaire
FV	Fruit and Vegetables
GLM	Generalized Linear Model
ICC	Intra-class Correlation
kg	Kilograms
m^2	Square meters
PCA	Principal Component Analysis
P - CEBQ	Portuguese version of the Children's Eating Behaviour Questionnaire
R	Reversed item
SD	Standard Deviation
SGA	Small for Gestational Age
SPSS	Statistical Package for Social Sciences
UK	United Kingdom
WHO	World Health Organization

RESUMO

Contextualização e Objetivos

O apetite representa um processo biológico e psicológico dinâmico, que pode ser avaliado indiretamente através de instrumentos psicométricos. O *Children's Eating Behaviour Questionnaire* (CEBQ) é um dos instrumentos psicométricos mais abrangente e consistentemente utilizado para avaliar os comportamentos alimentares de crianças. Este tem mostrado ser um instrumento válido em vários países do mundo, tendo sido relacionado com o desenvolvimento de obesidade em crianças e seus progenitores e com práticas de alimentação infantil. No entanto, existem ainda poucos estudos que avaliem os fatores que precocemente influenciam os comportamentos alimentares relacionados com o apetite, utilizando uma abordagem abrangente e prospetiva. A maioria dos estudos apresenta um desenho transversal, centra-se em dimensões isoladas dos comportamentos alimentares e não leva em linha de conta a complexidade da alimentação, avaliando preferencialmente o efeito de alimentos isolados nestes comportamentos.

Este estudo tem como objetivo estudar os fatores que precocemente influenciam o desenvolvimento de comportamentos alimentares relacionados com o apetite em crianças de 7 anos de idade de uma coorte de nascimento prospetiva de base populacional. Para responder a este objectivo geral, três objectivos específicos foram delineados: a) avaliar as propriedades psicométricas da versão Portuguesa do CEBQ aplicada a crianças de 7 anos de idade da coorte Geração XXI (artigo I); b) estudar as exposições precoces dos comportamentos alimentares relacionados com o apetite identificados aos 7 anos de idade (artigo I); c) quantificar a associação entre os padrões alimentares de crianças estabelecidos aos 4 anos de idade e os seus comportamentos alimentares relacionados com o apetite identificados aos 7 anos de idade (artigo II).

Métodos

Este estudo integra os objetivos científicos da coorte Geração XXI, uma coorte de nascimento prospetiva de base populacional. Os participantes foram recrutados em todas as maternidades públicas do Porto, Portugal, entre 2005 e 2006 (n=8495 mães e 8647 crianças). Todas as famílias foram convidadas a participar na segunda (2009-2011) e terceira (2012-2014) avaliações da coorte, quando as crianças apresentavam 4 e 7 anos de idade, respetivamente. Nas referidas ocasiões, avaliou-se 86% e aproximadamente 80% da coorte inicial. Em cada avaliação, os representantes legais de cada um dos participantes foram informados sobre os benefícios e potencial desconforto, tendo sido obtido um consentimento informado para a recolha de informação.

Entrevistadores treinados, através de questionários estruturados, recolheram informações sobre características sociodemográficas, de saúde e estilos de vida das crianças e das suas famílias e foram ainda efectuadas medições antropométricas das crianças e das suas mães. Aos 4 anos de idade, o consumo alimentar das crianças foi avaliado através de um questionário qualitativo de frequência alimentar (QFA) composto por 35 itens, relativo aos 6 meses anteriores, e por diários alimentares de 3 dias. Na avaliação efetuada aos 7 anos de idade, as mães preencheram a versão portuguesa do CEBQ (composta por 35 itens numa escala de respostas de Likert de 5 pontos).

No artigo I, para testar as propriedades psicométricas da versão Portuguesa do CEBQ, foram efetuadas duas Análises de Componentes Principais e calculou-se o Alfa de Cronbach para avaliar a consistência interna da escala. A reprodutibilidade foi testada através do coeficiente de correlação intra-classe (ICC) numa subamostra de 150 crianças (com um segundo CEBQ preenchido aproximadamente 35 dias após o primeiro preenchimento) e recorreu-se ainda à fórmula de previsão de Spearman-Brown para estimar uma versão com menos itens. Modelos de regressão linear generalizados foram utilizados para estimar a associação entre as características das crianças e maternas e os comportamentos alimentares, após ajuste para idade e escolaridade maternas, Índice de Massa Corporal (IMC) antes da gravidez, sexo da criança, estrutura familiar e número de irmãos. A amostra final incluiu apenas um gémeo por família, selecionado aleatoriamente e participantes com informação completa para todas as variáveis de interesse (n=3562 crianças).

No artigo II, com base nas frequências de consumo de 17 grupos de alimentos avaliados pelo QFA, identificaram-se três padrões alimentares, através de Análise de Classes Latentes, genericamente designados de: *Saudável*, *Baixo em alimentos saudáveis* e *Alimentos densamente energéticos - Produtos Lácteos, representando grupos mutuamente exclusivos de crianças que partilham o mesmo padrão alimentar.* Para estimar as associações entre os padrões alimentares das crianças e os comportamentos alimentares relacionados com o apetite, utilizaram-se modelos de regressão linear generalizados, após ajuste para idade, escolaridade e índice de massa corporal (IMC) maternos antes da gravidez. A amostra final incluiu apenas um gémeo por família, selecionado aleatoriamente, crianças sem anomalias congénitas ou doenças que pudessem influenciar a ingestão alimentar e com informação completa para todas as variáveis de interesse (n=4148 crianças). Testou-se ainda um possível efeito de interação, tendo-se efectuado análises estratificadas subsequentes.

Resultados

No artigo I, os comportamentos alimentares avaliados pelo CEBQ, agregaram-se em dois domínios, explicando 62% da variância total, com boa consistência interna (o Alpha de Cronbach foi de 0,77 para o fator 1 e de 0,64 para o fator 2). O factor 1 foi designado de *Restrição do Apetite* [média = 2,88, Desvio Padrão (DP) =0,61], incluindo os sub-domínios (do inglês) *Satiety Responsiveness, Slowness in Eating, Food Fussiness and Enjoyment of Food* e o fator 2 foi designado de Desinibição do Apetite (média=2,15; 0,52), incluindo os sub-domínios *Food Responsiveness, Emotional Overeating, Emotional Undereating and Desire for Drinks*. Estes domínios mostraram boa validade de construto e boa reprodutibilidade (ICC 0,51-0,85). Foi ainda proposta uma versão do CEBQ com 20 itens.

Em análise multivariada, as crianças com maior *Restrição do Apetite* aos 7 anos de idade parecem ser prevenientes de famílias com maior nível de escolaridade, com ambos os pais a viver com a criança e sem irmãos, mas apresentando também estilos de vida menos saudáveis aos 4 anos de idade (mais horas de visualização de multimédia por dia e sem prática regular de actividade física). Adicionalmente, crianças e mães com parâmetros antropométricos mais elevados na avaliação dos 4 anos apresentaram menor Restrição do Apetite aos 7 anos de idade. Por outro lado, crianças de um contexto educacional mais baixo, que vivem em famílias menos estruturadas, com estilos de vida mais sedentários (mais horas diárias de visualização de multimédia) e com parâmetros antropométricos menos favoráveis aos 4 anos de idade apresentaram uma pontuação mais elevada de *Desinibição do Apetite* aos 7 anos.

No artigo II, em análise multivariada, as crianças que aos 4 anos seguiam os padrões alimentares designados de *Alimentos densamente energéticos - Produtos Lácteos* e *Baixo em alimentos saudáveis* obtiveram maior pontuação na dimensão *Restrição do Apetite aos 7 anos, comparadas com aquelas que seguiam o padrão alimentar Saudável.* As pontuações na dimensão *Desinibição do Apetite* foram gradualmente superiores em crianças caracterizadas pelos padrões *Baixo em alimentos saudáveis* e *Alimentos densamente energéticos - Produtos Lácteos*. Encontrou-se um efeito de interação entre o perímetro da cintura das crianças aos 4 anos de idade e os padrões alimentares (p=0,020).

Conclusões

Com base numa versão Portuguesa do CEBQ, foram definidos dois domínios do Apetite neste estudo: *Restrição do Apetite* (mais relacionado com os sub-domínios que avaliam sinais internos de saciedade e *food fussiness*) e Desinibição do Apetite (mais relacionado com os sub-domínios que avaliam sinais externos e respostas emotivas face à

alimentação). Esta solução de 2 fatores mostrou apresentar boas propriedades psicométricas em crianças Portuguesas de 7 anos de idade e permitiu identificar fatores que podem influenciar precocemente os comportamentos alimentares das crianças. O ambiente familiar mostrou influenciar os dois domínios do apetite em direções diferentes; por um lado, um nível superior de escolaridade materna e uma melhor estrutura familiar associaram-se positivamente com a pontuação no domínio *Restrição do Apetite* e negativamente com a pontuação no domínio *Desinibição do Apetite* aos 7 anos de idade. Pelo contrário, as crianças com parâmetros antropométricos menos favoráveis aos 4 anos de idade apresentaram menor pontuação no domínio Restrição do Apetite e maior no domínio *Desinibição do Apetite* aos 7 anos. Além disso, as crianças com comportamentos mais sedentários (mais horas de visualização de multimédia) e hábitos alimentares pouco saudáveis (consumo aumentado de alimentos densamente e diminuído em alimentos saudáveis) no início da sua vida desenvolveram comportamentos alimentares mais problemáticos no final da infância.

Os resultados deste estudo poderão ser úteis em investigação futura e no desenvolvimento de recomendações e estratégias educativas que visem comportamentos saudáveis e a prevenção da obesidade infantil. Para isso é essencial promover no ambiente familiar atitudes e comportamentos saudáveis desde idades precoces que suportem o desenvolvimento de hábitos e comportamentos alimentares saudáveis durante a infância.

Palavras-chave: CEBQ, crianças, estudos de coorte, comportamentos alimentares, hábitos alimentares, comportamentos em saúde

ABSTRACT

Background and Aims

Appetite is a biological and psychological dynamic process, which can be indirectly measured by psychometric instruments. One of the most reliable and comprehensive psychometric tools to assess eating behaviours is the Children's Eating Behaviour Questionnaire (CEBQ), which has been successfully validated in countries worldwide and linked to childhood and parental obesity and feeding strategies. Nevertheless, there are few studies evaluating the early influences of appetite-related eating behaviours, from a comprehensive and prospective approach. Most studies have a cross-sectional design, are focused on few dimensions of eating behaviours, and rely on the effect of single foods, rather than assessing diet as a more complex exposure.

This research aims to study the early influences of appetite-related eating behaviours in 7 years old children from a prospective population-based birth cohort. To answer this question, three specific objectives were defined: a) to evaluate the psychometric properties of a Portuguese version of the CEBQ in 7 years old children from the Generation XXI cohort (paper I); b) to study the early influences of the appetite-related eating behaviours identified at 7 years of age (paper I); c) to quantify the association between dietary patterns established at 4 years old and eating behaviours related to appetite identified at 7 years of age (paper II).

Methods

This study is embedded in Generation XXI, a prospective population-based birth cohort. Participants were recruited in all public maternity units of Porto, Portugal between 2005 and 2006 (n=.8495 mothers and their 8647 children). All families were invited to attend the second (2009-2011) and third (2012-2014) cohort evaluations, when children were aged 4 and 7 years old, respectively. On both occasions, 86% and approximately 80% of all children were re-evaluated. A written informed consent was obtained from legal representatives of each participant for the collection of information at each follow-up assessment.

Trained interviewers administered a structured questionnaire on socio-demographic characteristics, children's health and lifestyles. Anthropometrics of both children and mothers were measured. Children's food intake at 4 years old was also assessed, through a qualitative 35-item food frequency questionnaire (FFQ), assessing usual consumption in the previous six months, and 3-day food records. A Portuguese version of the original CEBQ (35-items in a 5-point Likert scale) was self-completed by mothers at the 7 years old follow-up assessment.

In paper I, to test the psychometric properties of a Portuguese version of the CEBQ, two Principal Component Analysis were conducted, and the Cronbach's Alpha was computed to evaluate the internal consistency of the CEBQ scale. Reliability was tested through Intra-class Correlation coefficients (ICC) in a subsample of 150 children (with a second CEBQ completed within 35 days post-evaluation) and Spearman-Brown prediction formula was used to estimate a version with fewer items. Generalized linear models were used to estimate the associations between children and maternal characteristics and eating behaviours, after adjustment for maternal age, education and body mass index (BMI) before pregnancy, child's sex, family structure and number of siblings. The final sample included only one twin randomly selected and participants with complete data on key variables (n=3562 children).

In paper II, based on frequencies of consumption of 17 food groups of the FFQ, 3 dietary patterns were fitted by Latent Class Analysis: Healthier, Lower in Healthy Foods and Energy Dense Foods (EDF) – Dairy, representing mutually exclusive groups of children sharing the same dietary pattern. Generalized linear models were used to estimate the associations between children's dietary patterns and appetite-related eating behaviours, after adjustment for maternal age, education and body mass index (BMI) before pregnancy. The final sample included only one twin randomly selected, children with no diseases that might influence dietary intake and with complete data for variables of interest (n=4148 children). Interaction was tested and stratified analyses were further conducted.

Results

In paper I, the eating behaviours measured by the CEBQ were aggregated into two broad domains, explaining 62% of the total variance, with good internal consistency (Cronbach's Alpha was 0.77 for factor 1 and 0.64 for factor 2). The factor 1 was named Appetite Restraint (mean=2.88; standard deviation (SD)=0.61), including the sub-domains Satiety Responsiveness, Slowness in Eating, Food Fussiness and Enjoyment of Food and the factor 2 was named Appetite Disinhibition (mean=2.15; 0.52), including the sub-domains Food Responsiveness, Emotional Overeating, Emotional Undereating and Desire for Drinks. These domains showed good construct validity and good reliability (ICC from 0.51 to 0.85). A 20-item CEBQ version was proposed.

In multivariate analysis, children with higher Appetite Restraint scores at 7 years of age seem to come from families with a higher educational level, with both parents living in the child's home and with no siblings, but presenting also more unhealthy lifestyles at 4 years of age (more hours of media screening, no regular physical activity practice). In addition, children and mothers with higher anthropometrics at 4 years presented lower Appetite

Restraint scores at 7 years of age. On the other hand, children with a lower educational background, living in less structured families, with more sedentary behaviours (more hours of media screening) and with less favourable anthropometrics at 4 years of age presented higher Appetite Disinhibition scores later in childhood.

In paper II, in multivariate analysis, children belonging to the EDF-Dairy and to the Lower in Healthy Foods pattern at 4 years scored higher on Appetite Restraint at 7 years old, compared with children in the Healthier dietary pattern. Scores in the Appetite Disinhibition dimension were increasingly higher in children following the Lower in Healthy Foods and also the EDF-Dairy dietary patterns. An interaction effect between dietary patterns and children's waist circumference at 4 years old was found (p=0.020).

Conclusions

Two domains of Appetite, based on a Portuguese version of the CEBQ, were defined in the current study: Appetite Restraint (more related with sub-domains measuring internal cues of satiety and food fussiness) and Appetite Disinhibition (more related with sub-domains measuring external food cues and emotional responses towards foods). This 2-factor solution showed good psychometric properties in 7 years old Portuguese children, and allowed to identify early influences of children's appetite-related eating behaviours. The family environment showed to influence the two appetite domains in different directions; while a higher maternal educational level and more structured families increased the Appetite Restraint scores, they were negatively associated with the Appetite Disinhibition scores at 7 years of age. Oppositely, children with less favourable anthropometrics at 4 years of age presented lower Appetite Restraint scores, but higher Appetite Disinhibition scores at 7 years. In addition, children following more sedentary behaviours (more hours of media screening) and unhealthy dietary patterns (high in EDF and lower in healthy foods) early in life developed more problematic eating behaviours in later childhood.

The current results may be useful in future research and in the development of prevention guidelines and educational strategies improving healthy behaviours and childhood obesity. It is important to promote, at the home environment, early family feeding practices and behaviours supportive of healthy dietary habits and eating behaviours during childhood.

Keywords: CEBQ, children, cohort studies, feeding behaviours, food habits, health behaviour.

INTRODUCTION

Appetite is commonly used to refer to the sensation of hunger (or the subjective 'urge to eat') or even to a trait-like characteristic, as when individuals are described as 'having good or poor appetites'[1]. Nevertheless, this is a more complex concept and these manifested sensations and behaviours are based upon a dynamic biological process. A more objective approach defines appetite as 'the internal driving force for the search, choice, and ingestion of food'[2]. Interpreting the duality of this concept has been a challenging and fruitful research task over time and several appetite features have been highlighted, namely during childhood.

The present work will focus primarily on behavioural aspects of appetite which cannot be, however, dissociated from the fundamentals of its biological mechanisms, briefly described below.

1. Appetite Regulation and Expression

Despite a strong environmental influence, the biological mechanisms interfering in the regulation of appetite and food ingestion play an important role in human eating behaviour. The majority of models explaining this process suggest a relationship between homeostatic and hedonic pathways. The homeostatic pathway controls energy balance by increasing the motivation to eat in consequence of depletion of energy stores. The hedonic pathway, in turn, acts predominantly in periods of relative energy abundance and overcomes the homeostatic pathway in the sense that increases the desire to consume highly palatable foods[3]. The interaction between these regulatory pathways acts towards the balance between eating to satisfy a need and eating for pleasure and reward[4].

1.1. Homeostatic mechanisms

The psychobiological model, proposed by Blundell in 1991[5], conceptualizes appetite regulation in terms of a network based on three independent but inter-related domains: the domain of psychological experiences (e.g. sensations of hunger and satiety, cravings and hedonics), their conceptualization (through meal intake, snacking and food choices) and consequences (energy intake and macronutrient composition of food consumed); the domain of peripheral physiology and metabolic events (which arises as a consequence of nutrient absorption, utilisation and storage) and the domain of neurotransmitter and metabolic signal interactions in the central nervous system (figure 1).

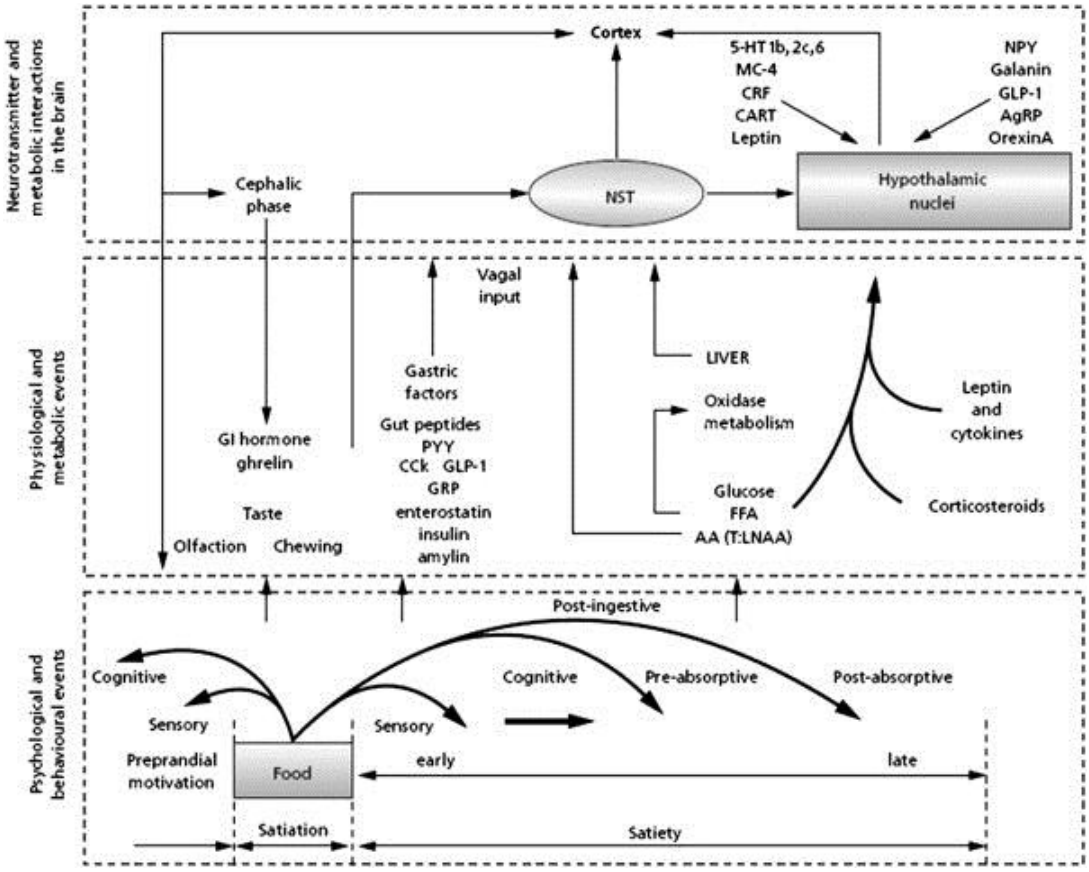

Figure 1. The psychobiological appetite regulating network conceptualized in three domains: psychobiological and behavioural events, psychological and metabolic events and neurochemical brain activity (Source: Harrold, 2012[4]).

5-HT - serotonin; AA - aminoacids; AgRP - agouti-related peptide; CART – cocaine and amphetamine-regulated transcript; CCK – cholecystokinin; CRF – corticotrophin releasing factor; FFA – free fatty acids; GI – Gastrointestinal; GLP-1 – glucagon-like peptide-1; GRP – gastric releasing peptide; MC - melanocortin; NPY – neuropeptide Y; NST – nucleus tractus solitarius; PPY – petide YY; T:LNAA – Tryptophan large neutral amino acid ratio.

In the domain of the psychological experiences, the "satiety cascade" represents the processes comprised between the stimulation (pre-prandial) and the termination (post-prandial) of eating. *Hunger* and *Satiety* are the psychological experiences determining the eating behaviour, meal by meal. Blundell defines *hunger* as the motivation to seek and consume foods, initiating a feeding episode[5]. In turn, *satiation* (the set of processes that bring episodes of eating behaviour to an end) leads to a state of s*atiety*, in which the hunger drive and eating behaviour are inhibited[5]. In the pre-prandial phase, the sight and smell of foods act as pre-prandial hunger signals and stimulate food intake, preparing the body for ingestion[6]. Later, in the prandial phase, the contact with food in the mouth generates sensory information which is carried to the brainstem, in the central nervous system, signalling hunger and promoting food intake. In this phase the central nervous system also receives post-ingestive sensory signals from the gut, such as mechano-receptors signal gastric

distension caused by the presence of food (providing, thus, an indication of the amount of food consumed), and chemo-receptors that detect the presence of nutrients providing information on the nutritional composition of foods consumed. Finally, the detection of nutrients absorbed from the gastrointestinal tract into the peripheral circulation generates prandial and post-prandial signals[4].

The central nervous system is a relevant structure in the appetite regulation, which receives information generated by the sensory experience of eating and by the periphery as a result of ingestion, absorption, metabolism and energy storage. This information reaches the central nervous system via: a) signals from peripheral receptors in the gut and metabolic changes in the liver (energy conversion and energy status); b) signals from receptors in the brain stem, detecting circulating levels of nutrients, their metabolites and other factors within the periphery and c) substances, such as glucose and neurotransmitter precursors, crossing the blood brain barrier and entering the brain where they directly alter central nervous system neurochemical activity in key regulatory sites[4,5]. These signals are recognized by specific neurones within the central nervous system, which help to determine energy intake and expenditure. Some of the brain regions involved in the food intake regulation are the brainstem, the hypothalamus, the amygdala and the nucleus accumbens of the cortico-limbic system[4].

The peripheral signals generated after an episode of food ingestion are coordinately integrated in the central nervous system and can be classified as episodic or tonic[7], differing in the duration of their effect. While episodic factors are short-term inputs generated by recent consumption, tonic factors provide long term signalling and are generated by the constant metabolic need for energy[5]. Episodic signals of hunger are generated during the cephalic phase of appetite control, which occurs in the mouth (saliva production), stomach and small intestine. The chemical (i.e. taste) and physical (i.e. mouth feel - oiliness and viscosity) contacts of food with receptors in the mouth and nose mucosa set up orosensory effects of food stimuli. Important satiety episodic signals are the stomach distension and the concomitant release of chemicals (mostly neurotransmitters) sensible to the nutritional content along with the gastrointestinal tract, such as the cholecystokinin that is released from duodenal mucosal cells in response to protein and fat content and the Glucagon-like pepetide-1, released from the gut into the bloodstream in response to intestinal carbohydrate[4]. Other peripheral satiety signals include peptides such as enterostatin, neurotensin, peptide YY and amylin[4]. Episodic satiety signals are also associated with an increase in the glucose metabolization and fatty acid oxidation in the hepatoportal area, triggered by changes in hepatic Adenosine Triphosphate (ATP) concentration[8,9]. Changes in blood glucose levels may also provide a signal for meal initiation[10]. Ghrelin, a hormone found in high concentration in the gut, is considered a potent episodic meal initiator. This substance

is responsive to the nutritional status, and its concentration peaks just before the meal initiation[11] and falls following intake, dependent on the energy value of the food consumed[12].

Tonic signals are generated in tissue stores, indicating the state of depletion or repletion, to drive intake if energy reserves are low. In comparison to episodic signals, they are far more responsive to deficits or excesses of energy stored in the adipose tissue, establishing a more accurate representation of the body's energy needs[4]. Organs implicated in energy storage include the liver, the pancreas and the adipose tissue, which produce various circulatory factors known to act as determinants on food intake such as leptin, insulin and glucagon[13]. According to the lipostatic hypothesis of appetite control[14], tonic appetite control is achieved via lipostatic signals (mostly leptin, but also insulin), that inform hypothalamic neuropeptide pathways controlling the stimulation and inhibition of food intake[15] about the size of the adipose tissue stores.

Circulating levels of leptin reflect the status of body fat deposition and increase with the level of adiposity, demonstrating the responsiveness of endogenous leptin to weight gain and energy status. Its action is generally associated with reduced food intake and increased metabolic expenditure, resulting in weight loss[4]. Obese individuals present increased leptin production, which can reach four times more the normal values of non-obese individuals, but they also show a state of leptin-resistance[16]. This apparent state is proposed to be a result of defective leptin entry into the central nervous system, whether by saturation of its blood-brain transport or alterations on leptin receptors in the brain (in the plexus choroid)[16]. As a consequence, occurs a decreased inhibitory action on food intake[17]. It is due to the leptin-resistance that most obese have an exaggerated appetite (hyperphagia), but other implications of these high-circulating levels of leptin are still not very clear[18].

1.2. Hedonic mechanisms

Compared to the homeostatic pathway that regulates eating behaviour, hedonic (or food reward) mechanisms are considerably less understood. It is known, however, that they are triggered by palatability cues, particularly taste and smell[19], which increase motivation to eat.

The ingestion of palatable foods has shown resemblances with the use of drugs of abuse and addiction, as they converge on the mesolimbic dopaminergic pathway to mediate motivated behaviours[20,21]. In response to the ingestion of these foods, dopamine is released, acting on the motivation to the achievement of food reward (in aspects as expectation, pleasure and memory/conditioned learning - remembering food-associated stimuli)[3]. The level of released dopamine is correlated with the level of obtained pleasure through intake[22].

Another neurotransmitter with action in the mesolimbic pathway towards the release of dopamine is serotonin, providing a sense of well-being and improvement of mood and promoting motivated-food intake[19]. Curiously, medicines that act on serotonin, of which Sibutramine® is a good example, began to be used in the depression treatment, and are now used to treat obesity as well.

High palatable foods also modulate the expression of metabolic signals of hunger and satiety, extending the duration of eating episodes, due to a high content in sugar and fat.[23] The sweet taste of sugar is capable of triggering the food reward system itself, through taste receptors in the tongue, but its combination with fat is particularly efficient in inducing motivated-food intake. Despite there are no taste receptors for fat, the palatability of this nutrient seems to be due to its associated texture and flavour[24]. Studies have shown a higher preference for sweet and fatty foods in individuals with higher body mass index (BMI)[25], contributing to an expected association between obesity and a dietary pattern characterized by the high consumption of these foods.

In resume, "Food reward" is believed to be composed by three dimensions: *liking*, *wanting* and *learning*[26]. The *liking* component is a hedonic reaction expressed by behaviour and neural signals and appears before the pleasure obtained by eating high palatable foods. *Wanting* is the motivational component usually triggered by stimuli of reward (e.g. visual or olfactory) and induces a demand for foods, resulting in increased appetite, craving, and other behaviours associated with an increased motivation for food. *Learning* is associated with memory and with remembering food-associated stimuli. The mesolimbic dopamine pathway is particularly important for the *wanting*.

Although the *liking* and *wanting* are closely associated, reward-motivated behaviours can occur in the absence of pleasure, as seen in individuals with addiction to chemical drugs[23]. It was found that overweight individuals present increased *wanting* (dopaminergic activation) and energy intake in the absence of hunger and in response to food pictures[27] and decreased post-prandial 'liking'[28], comparatively to normal weight individuals in situations of stress. Also, the frequent ingestion of high palatable foods was shown to lead to the attenuation of dopamine signalling[23]. Altogether, these findings suggest a difficulty in overweight individuals in obtaining reward (pleasure), what probably leads to an excess demand for it, resulting eventually in weight gain[29].

2. Assessment of Appetite and related Eating Behaviours

As a complex and subjective construct, appetite's assessment has been challenging over time. A variety of methods to investigate appetitive traits have been developed generally

based on the appetite regulation mechanisms, already described, and related whether with satiation or satiety. These methods include both biological and behavioural (laboratory and questionnaires) instruments.

2.1. Biological methods

Biological markers of appetite involve most of the neural structures and substances participating in the process of appetite regulation and expression. They can measure satiation, satiety or both and depend whether their action is peripheral or in the central nervous system[2]. With respect to peripheral biomarkers, physical and chemical measures of stomach distension and blood plasma concentrations of cholecystokinin and Glucagon-like pepetide-1 are useful in the evaluation of short-term satiation (meal termination)[2]. Concentrations of leptin, ghrelin and insulin are useful in the long-term mechanisms regulating eating behaviours[2].

Neural measures, techniques of functional Magnetic Resonance Imaging (fMRI) and Positron Emission Tomography (PET), are also available to study brain responses related to appetite[2], although they are less used. These neuroimaging procedures measure brain activity by detecting associated changes in blood flow, relying on the fact that cerebral blood flow and neuronal activation are coupled and, when a brain area is in use, blood flow to that region increases[30]. Several studies of brain activation triggered by food cues have highlighted a number of brain structures within reward and motivation circuits important in the phenomenon of Food Responsiveness in children and adolescents[31]. These studies could be considered neural measures of food cue responsiveness[31]. Growing neuronal research in humans, especially in children, may provide more insights into the individual appetitive traits in the coming years.

2.2. Behavioural methods

Behavioural methods are indirect measures of appetite (as they evaluate behaviours) which were developed to assess different eating style constructs, whether in the laboratory setting or psychometrically, through questionnaires and scales.

Laboratory methods have been broadly organized into two categories: food approach or food cue responsiveness and food avoidance measures. Food cue responsiveness can be defined as the degree to which external food cues, such as the sight or smell of foods, encourage an individual to eat, generally in excess[31]. In the food avoidance construct, the *satiety sensitivity* or the *satiety responsiveness* (the degree to which individuals are capable

of ceasing consumption in response to internal signals[31]) is the main aspect being measured (Table 1).

The dimension (i.e. domain or construct) of *food cue responsiveness* is linked with behaviours such as *Eating in the Absence of Hunger, Intake after exposure to food cues, Reinforcing value of food, Delay of gratification* and *Implicit assessments of food cue responsivity*[31]*. The food avoidance* dimension has been mostly studied through *Caloric Compensation* and *Eating Rate*[31].

Table 1. Description of behavioural laboratory methods to assess Appetite-related Eating Behaviours.

Food approach or Food cue responsiveness	
Eating in the Absence of Hunger	Individuals eat until satiety and then are offered simultaneously a number of palatable snack foods to consume *ad libitum* and desirable play activities (e.g. access to a selection of toys or games in the case of children), for a certain amount of time. The calories consumed within this period provide an estimation of how much the individual ignores internal satiety signals in favour of responding to the external cue of the presentation of the palatable food[32].
Intake after exposure to food cues	Individuals are exposed to different types of food cues (e.g. food odour[33] or food branding[34]) and are presented with palatable snacks or a meal. The difference between the exposure vs. non-exposure to these cues provides an estimation of their influence on individual's food intake.
Reinforcing value of food	It is the extent to which the child continues to work for food in relation to that for an alternative reward (which can also be a food) that provides an index of the reinforcing value of food[31].
Delay of gratification	Assesses children's self-control by asking to choose between a small immediate reward or to wait to earn a more desirable reward (e.g. two cookies in 15 minutes vs. one cookie now)[35].
Implicit assessments of food cue responsivity	Children deal with tasks concerning games of food words[36,37] or pictures[38], for example, and later their memory is evaluated when asked to recall what was previously shown. It was designed to test underlying attentional biases towards food[31].
Food avoidance	
Caloric Compensation	It is evaluated how well children are able to adjust food intake and calories in an *ad libitum* meal, subsequently to the consumption of a preload (meal, snack or beverage). It was originally developed in laboratory setting[39], but more recent variants were developed (e.g.: assessment in a free-living environment or intake assessment through dietary recall[40]).
Eating Rate	It is based on the principle that if someone is sensitive to internal satiety signals occurring in the intake episode, it would be expected that their rate of eating would slow down[31]. It is usually defined as total energy (kcal) or mouthfuls of food consumed within a given time interval during a laboratory test meal and measured as kcal/min or bites/min[41].

Psychometric instruments have been also developed for the purpose of detecting individual differences in eating behaviours, among adults and children. Throughout time, some authors created different scales and questionnaires, but more recent studies are generally based into three questionnaires: the Dutch Eating Behaviour Questionnaire

(DEBQ)[42,43], the Children's Eating Behaviour Questionnaire (CEBQ)[44] and the Baby's Eating Behaviour Questionnaire (BEBQ)[45].

The DEBQ is available for both child-reporting (validated for children 7-12 years old)[42] and parent-reporting[43] and includes scales measuring External Eating (eating in response to food *stimuli* without regard to internal hunger or satiety[43]), Emotional Eating (excessive eating in response to negative emotional states such as anger, fear or anxiety[43]) and Cognitive Restraint (the tendency to consciously restrict food intake to control body weight[43]). The BEBQ was designed for completion by mothers of 0–3 months old infants, during the breast/bottle-feeding stage, or retrospectively, after complementary feeding. The items were derived from the CEBQ, with subscales (or sub-domains) covering four aspects of appetite: Enjoyment of Food, Food Responsiveness, Slowness in Eating and Satiety Responsiveness[45]. The CEBQ is probably the most widely used questionnaire to assess eating behaviours of children and will be described in detail just below.

2.3. The Children's Eating Behaviour Questionnaire (CEBQ)

The CEBQ is generally accepted as one of the most comprehensive psychometric tools in the assessment of eating behaviours. It was developed and first validated by Wardle and collaborators[44] for school-aged children in the UK, with the objective of capturing individual differences in aspects (dimensions or domains) of eating behaviours that have been hypothesized to contribute to both underweight and overweight[1]. The included dimensions were obtained by literature research on children's and adults' eating behaviours, followed by qualitative interviews with parents about their child's eating behaviours.

The CEBQ is a parent-report questionnaire of 35 items, rated in a 5-point Likert frequency scale (1 = never to 5 = always). It is organized onto eight dimensions (subscales or sub-domains): Enjoyment of Food, Food Responsiveness, Emotional Overeating, Desire for drinks, Satiety Responsiveness, Slowness in Eating, Emotional Undereating, and Food Fussiness. The first four sub-domains are known to measure the Food Approach dimension and the latter four the Food avoidance dimension[46]. The "Food Approach" sub-domains have been positively associated with children's weight, whereas the "Food Avoidance" sub-domains have been negatively associated with children's relative weight[46,47]. Table 2 describes the different sub-domains of this questionnaire, as well as the items to which they relate.

Table 2. Description and items of each CEBQ sub-domains.

Dimension	Sub-dimension	Definition	#	Items
Food Approach Eating Behaviours	EF	Represents a General interest in food[1]. Captures the extent to which a child finds eating pleasurable and desires to eat[48].	1.	My child loves food.
			5.	My child is interested in food.
			20.	My child looks forward to mealtimes.
			22.	My child enjoys eating.
	FR	Measures eating in response to environmental food cues[1], referring to the extent to which a child indicates an interest in and desire to spend time eating food[48].	12.	My child is always asking for food.
			14.	If allowed to, my child would eat too much.
			19.	Given the choice, my child would eat most of the time.
			28.	Even if my child is full up, s/he finds room to eat his/her favourite food.
			34.	If given the chance, my child would always have food in his/her mouth.
	EOE	Represents increased eating in response to negative emotions, such as anger and anxiety[49,50].	2.	My child eats more when worried.
			13.	My child eats more when annoyed.
			15.	My child eats more when anxious.
			27.	My child eats more when s/he has nothing else to do.
	DD	Aims to identify desire to have drinks, particularly sugar-sweetened drinks[44].	6.	My child is always asking for a drink.
			29.	If given the chance, my child would drink continuously throughout the day.
			31.	If given the chance, my child would always be having a drink.
Food Avoidance Eating Behaviours	SE	Measures the speed of eating during the course of a meal, with higher scores on this sub-domain reflecting a gradually reduced interest in a meal[49,50].	4.	My child finishes his/her meal very quickly.
			8.	My child eats slowly.
			18.	My child takes more than 30 minutes to finish a meal.
			35.	My child eats more and more slowly during the course of a meal.
	SR	Represents the degree to which a child regulates the amount of food eaten, based on their perceived fullness[1,49].	3.	My child has a big appetite.
			17.	My child leaves food on his/her plate at the end of a meal.
			21.	My child gets full before his/her meal is finished.
			26.	My child gets full up easily.
			30.	My child cannot eat a meal if s/he has had a snack just before.
	EUE	Represents decreased eating in response to negative emotions, such as anger and anxiety[49,50].	9.	My child eats less when s/he is angry.
			11.	My child eats less when s/he is tired.
			23.	My child eats more when s/he is happy.
			25.	My child eats less when s/he is upset.
	FF	Reflects a lack of interest in food and unwillingness to try new foods (food neophobia), leading to an inadequate variety of foods[49].	7.	My child refuses new foods at first.
			10.	My child enjoys tasting new foods.
			16.	My child enjoys a wide variety of foods.
			24.	My child is difficult to please with meals.
			32.	My child is interested in tasting food s/he hasn't tasted before.
			33.	My child decides that s/he doesn't like food, even without tasting it.

- Item's position in the original CEBQ; DD – Desire for Drinks; EF – Enjoyment of Food; EOE – Emotional Overeating; EUE – Emotional Undereating; FF – Food Fussiness; FR – Food Responsiveness; SE – Slowness in Eating; SR – Satiety Responsiveness

Several studies have been conducted to validate the CEBQ in different settings, demonstrating good psychometric properties, such as good internal consistency, concurrent validity with actual eating behaviour and reproducibility or test-retest reliability (table 3). This questionnaire was tested in Dutch children with 6–7 years of age[50]; in Canadian children aged 4-5 years[51], in Swedish children aged 1–6 years[49], in Chinese children aged 12–18 months[52] and in Australian children aged 24 months – 5 years[53]. In a study conducted in the USA[54], the original CEBQ model failed to be replicated, raising the possibility of a low sensitivity to cultural differences, also highlighted in the Chinese and Australian versions. The CEBQ was even validated (with adjustments due to age and cultural differences) as a self-report method in Malaysian adolescents[55], showing good psychometric properties. As referred previously, the same authors who originally developed this questionnaire have later demonstrated moderate convergent validity of three of the sub-domains of the CEBQ (Satiety Responsiveness/Slowness in Eating, Food Responsiveness and Enjoyment of Food) with four aspects of eating behaviours: “Eating without hunger”, “Caloric Compensation”, “Eating Rate” and “Energy intake at a meal”, in a UK sample of 4–5 years old children (n = 111)[1]. Eating without hunger, mean eating rate and mean total energy intake accounted for 56%, 33% and 40% of the variance in sub-domains, respectively.

This questionnaire was also tested in Portuguese children aged 3–13 years[56], resulting in a 6-factor model explaining 60.5% of the variance. Cross-sectional associations with BMI and age showed a tendency of the Food Approach sub-domains to show positive associations with BMI and of the Food Avoidance sub-domains to show negative associations with BMI; older children tended to score lower on Food Fussiness and Slowness in Eating[56].

CEBQ has been successfully used to measure diverse associations of eating behaviours, namely with BMI and/or waist circumference[46,50,56-58], with parental feeding strategies[59-67], with child temperament[68], to compare appetite preferences in children of lean and obese parents[69] and to test stability in children’s eating behaviours across time[57,70]. These associations will be described in more detail in the next chapter.

Table 3. Resume of the results obtained in previous CEBQ validation studies.

Study			Sample		Validation					
Author	Year	Country	Age	n	Method	No. Factors	No. Items	Internal consistency		Test - retest reliability
								Explained Variance (%)	Cronbach's Alpha	
Wardle et al	2001	UK	2 - 9 years	308	PCA	7	35	69.0	0.72 - 0.91	0.52 - 0.87*
Carnell and Wardle[1]	2007	UK	4 - 5 years	111	--	--	--	--	--	--
Viana et al	2008	Portugal	3 - 13 years	240	PCA	6	35	60.5	0.70 - 0.89	--
Sleddens et al	2008	Netherlands	6 -7 years	135	PCA	7	35	62.8	0.67 - 0.90	--
Spence et al	2010	Canada	4 - 5 years	1730	--	--	--	--	0.76 - 0.92	--
Svenson et al	2011	Sweden	1-6 years	174	PCA	7	35	61.2	0.71 - 0.90	--
Santos et al	2011	Chile	6 – 12 years	294	PCA	7	35	61.8	0.70 – 0.89	
Cao et al	2012	China	12 - 18 months	219	PCA	7	19	52.1	0.52 - 0.80	--
Sparks	2012	USA	2 - 5 years	229	PCA	3	15	--	--	--
					CFA (Poor fit)	7	34	--	--	--
Mallan et al	2013	Australia (a)	24 months	244	CFA (Good fit)	8	35	--	0.73 - 0.91	--
		Australia (b)	1 - 5 years	203		8	35	--	0.61 - 0.88	--
		Australia (c)	1-4 years	254		8	33	--	0.61 - 0.87	--
Loh et al	2013	Malaysia	13 years	362	PAF	8	32	54.2	0.48 - 0.72	0.72 - 0.90**
				646	CFA	8	30	--	--	--

* Pearson Correlation; ** Intra-Class-Correlation;
[1] - Study of convergent validity between the CEBQ sub-dimensions Satiety Responsiveness/Slowness in Eating, Food Responsiveness and Enjoyment of Food and 4 aspects of eating behaviours: "Eating without hunger", "Caloric Compensation", "Eating Rate" and "Energy intake at a meal".
(a) – Australian Sample; (b) – Indian sample; (c) – Chinese sample
CFA – Confirmatory Factor Analysis; PAF – Principal Axis Factoring; PCA – Principal Components Analysis; USA – United States of America; UK – United Kingdom;

2.4. Considerations to the assessment methods

When evaluating appetite in children, each method has its strengths and weaknesses and thus there is no ideal tool. Whilst biological markers have the capacity to capture the various biological components of the appetite response more completely[31], behavioural methods are more vulnerable to social desirability bias and may only give a partial picture of an individual's food response. When comparing behavioural methods, laboratory experiments ascertain more objectively eating behaviour measures than psychometric instruments[31]. However, the majority of behavioural experiments uses only a single measure, at a single occasion, which limits generalisation from that instance of the behaviour to the underlying trait[71]. Also, implementing behavioural tasks with an adequate number of participants to provide sufficient statistical power is associated with logistic difficulty and expense[31].

Psychometric measures of behavioural traits, in turn, are cheaper, convenient to administer on a large scale and may evaluate several eating behaviours with one single application. Respondents understand that the questions relate to 'usual' behaviour, however, self-reports of behaviour are subjective, which raises questions about their reliability as indicators of the latent trait[31]. Thus, the psychometric proprieties of each scale must be evaluated in different samples. Parent-reports may even promote socially desirable responses, and have the disadvantage of constituting a second-hand account of child's behaviours based only on eating occasions that the parent is able to observe. Nevertheless, parents have privileged observational access to their children and are in a strong position to contribute to the assessment of behavioural traits[1]. Child-report methods require comprehension skills and self-awareness, being only available for older children and not indicated for children who may lack these competences (e.g. children with cognitive developmental delays)[1], but are considered a plausible alternative to parent-report methods. Considering all these reasons, the CEBQ is assumed to be a comprehensive psychometric tool widely used in the assessment of eating behaviours among young children.

3. Development of Eating Behaviours and their determinants

Childhood, especially the first five years of life, is a period with particular developmental characteristics, such as rapid growth and transitions in digestive behaviour[72,73]. During this period (until the two years of age) occurs a transition between breast or formula milk to complementary foods, which takes an important part in shaping infants' eating behaviours[74]. Eating behaviours emerge as an interaction between biological (e.g. genetic predispositions

and natural food responses) and behavioural factors, to meet with health and growth requirements[72]. Behavioural aspects as food preferences, food intake and energy regulation are strongly influenced by the environment provided by the family and other caregivers, particularly by the adoption of certain feeding practices, as they decide what, when, and how children eat[72,74,75].

Eating behaviours, as studied by the CEBQ, have shown some promising (and in some cases controversial) results, but are still few, underlying the need of more insight into the development of children's eating behaviours. One study evaluating stability of these traits showed that during the first five years, there is no continuity in eating behaviours[57]; yet two other studies contradict this hypothesis, having demonstrated that eating behaviour in early childhood shows stability from 2 to 5 years old[76] and throughout childhood[70]. Older children tend to have lower scores on Food Fussiness and Slowness in Eating sub-domains[56].

3.1. Genetic predisposition

Food preferences are important in understanding future eating behaviours and can either be innate or modelled by learning[75,77]. Children's preferences to basic tastes (sweet, salt, bitter, sour and umami) are a good example of these genetic predispositions. In neonates, the observational study of facial expressions has demonstrated a universal preference for sweet tastes, along with the aversion to sour or bitter tastes[77]. Preferences for salt appear at approximately four months of life[78]. Umami taste is also associated with unlearned positive responses[72]. These predispositions result from an adaptive process in which sweetness encourages the consumption of energy-dense foods, whereas bitterness or sourness discourages the ingestion of toxins[79]. Generally, sweet foods such as fruits, flavoured yogurts and juices are readily accepted by infants, while foods such as vegetables, containing bitter components, are initially rejected, which is indicative of a predisposed preference for flavours present in energy-dense foods (more palatable)[80,81]. In addition, twin studies have shown coefficients of heritability for food types, translated in strong heritability for protein foods and moderate for fruit, vegetables and desserts[82].

Twin studies have greatly contributed to the progress in the study of the heritability of appetitive traits in children. In a population-based sample of twin infants from the *Gemini Cohort,* who were exclusively milk fed, a large genetic effect was found for Slowness in Eating (in 84% of the subjects) and Satiety Responsiveness (in 72% of the subjects) and moderate for Food Responsiveness and Enjoyment of Food (in 59% and 53% of the subjects, respectively) during the first three months of life[83]. In another study, conducted within the *Twins Early development Study*, heritability was studied in 8-11 years old children[84] in relation to Satiety Responsiveness and Food Responsiveness, demonstrating,

respectively, 63 and 75% of heritability. In the same cohort, Eating Rate was shown to be heritable in 62% 10-12 years old twins[85]. These predispositions are becoming to be explained by the field of Molecular Genetics. One of the most plausible hypothesis specifies the involvement of the *FTO* gene, predominantly expressed in the appetite control areas of the hypothalamus[86], which has been shown to be associated with Satiety Responsiveness and Food Responsiveness in children[87,88]. The *FTO* gene has also shown to strongly influence obesity risk[89]. Finally, two twin studies have revealed strong heritability of food avoidance traits. A study within a Canadian twin cohort (Quebec Newborn Twin Study)[90] of 9 years old found strong heritability for traits as "refusing to eat" (84%) and "being fussy about food" (85%), converging with previous findings[91] from a study within the *Twins Early development Study* which revealed an heritability of 78% for food neophobia in children aged 8-11 years.

3.2. Early taste experiences

Children's acceptance of solid foods is early influenced, namely *in utero.* Several studies revealed that the foetus is exposed to flavours from the maternal diet presented in the amniotic fluid (e.g. cumin, curry[92] and garlic[93]), which provides a "flavour bridge"[72] and allows the infant to become familiar with these "transmittable" flavours. The concept of "familiarization", plays a key role in the acquisition of food and flavour preferences throughout childhood[94].

Research has shown that flavours from the maternal diet are also present in breast milk (garlic[95], alcohol[96], and vanilla[97]) and influence infant's food consumption and preferences. For instance, breast milk flavoured with garlic[95] and vanilla[97] increased infant sucking time when compared to breast milk without garlic or vanilla flavour. These same researchers demonstrated that repeated exposure to carrot flavours in breast milk increased infant's acceptance and enjoyment of carrot flavoured cereal[98]. At the complementary feeding period there is, yet, limited evidence. However, a short-term longitudinal study[99] examined the influence of milk feeding regimen and repeated exposure on acceptance of infant's first pureed vegetable. Participants were randomly assigned to be repeatedly fed one vegetable (pureed peas or green beans) and results revealed that the initial intake of vegetables did not differ between breastfed and formula-fed infants, but the breastfed infants increased their intake more rapidly than formula fed infants, continuing to consume significantly more vegetables after ten exposures. Altogether, these findings suggest that early experiences with flavour in breast milk modify the infants' acceptance and enjoyment of foods of the modified adult diet, especially those foods consumed by the mother during

lactation[98]. Breastfeeding can also promote acceptance of solid foods more easily, comparatively to formula feeding, due to its sensory properties[99].

3.3. Early feeding practices

Breastfeeding has been associated with several health benefits, contributing to a better growth and future health, reason why it is recommended as the optimal feeding method in the first six months and, at least, in the first year of life[100]. In some studies, it has been associated with lower risk of obesity among school-aged children and adolescents, but systematic reviews report that the available findings about whether duration of breastfeeding is protective of overweight and obesity later in life are still inconsistent[101,102]. The mechanisms by which breastfeeding may exert a protective effect on weight status are not well known yet, but apparently, it influences the development of the anatomy and physiology of the gastrointestinal tract differently from formula feeding[72]. Two other hypotheses are currently accepted in the literature: the effects on food acceptance and on the self-regulation of food intake[72]. A growing body of evidence suggests that infants have some ability to self-regulate caloric intake by adjusting the volume of milk consumed. It has been shown that formula-fed infants consume more milk and present more rapid growth and weight gain than breastfed infants[103]. One of the first researchers focusing on this subject, Fomon, speculated that this differential growth would be due to differences in milk intake, with higher intakes by formula-fed infants resulting in overfeeding as a consequence of maternal control over the infant's intake[104]. In formula-feeding, the mother can monitor the remaining quantity in the bottle and may control how much the infant eats and encourage the infant to finish the bottle. The formula-fed infant is, therefore, passive in the feeding process, having fewer opportunities to control the amount consumed, in opposition to the breastfed infant, who may have a more active role with more control over the size of the feed, as this feedback is not available to the mother[72,105].

As the child grows, there is a need of starting complementary feeding, in which exposure appears to have a great effect on the development of flavours, but also food preferences[106]. In this period, other foods besides milk are new to the infant and it is essential to promote the acceptance of a variety of solid foods. As referred previously, predetermined preferences and taste experiences *in utero* and during breastfeeding can affect food preferences in early infancy and throughout childhood. However, even minimal exposure can promote liking during this early period[107], shaping predetermined preferences. It has been shown that experiences with a variety of flavours in pureed foods promote acceptance of other unfamiliar flavours[106]. Adding to the fact that "neophobia" increases with age, peaking around the age of four, and gradually decreasing[108] thereafter, complementary

feeding appears to represent an “opportunity window” for developing flavour preferences[107] and for determining young children’s food preferences and eating behaviours.

3.4. Family environment

Parents and main caregivers are believed to have a central role in shaping child’s eating behaviours. They act as role models, providing children with their first food learning experiences, controlling the availability and exposure to foods and using content-specific parenting strategies, that play a critical role in the development of children’s taste preferences, eating habits, nutrition and eventually weight status[109,110].

One way children learn about food is by observing the eating behaviour of others (adults and peers). Children show a tendency to taste unfamiliar foods more readily when they observe adults eating them than when the food is merely offered to the child[111]. Also, children who observe peer models eating a food they dislike presented willingness to choose and eat that food subsequently[112]. Though, considering home environment, parents and siblings[113] (especially if they are older[114]) strongly influence the development of children’s eating behaviours.

Parenting child feeding practices have evolved over time as parental responses to environmental threats to children’s health and well-being. Through most of Human History these major threats have been food scarcity and infectious disease and feeding practices developed to address situations of food insecurity have been passed from one generation to the next and implemented as traditional practices routinely used by parents, without questioning[72]. Additionally, the Nutrition Transition[115] introduced changes in the food supply, by increasing the availability of palatable, energy-dense and inexpensive foods. Presently, traditional feeding practices include offering food as a first response to infant’s crying and distress, feeding frequently when food is available, providing large portions, offering foods designed especially for infants, preferred foods if possible and encouraging children to eat as much as possible, often pressuring them[116]. Differences between and within cultures are mostly influenced by children’s individual characteristics (age, sex, weight status and eating behaviour) and the goals parents set for their children[72]. Despite the fact that these attitudes reveal good intentions of parents, growing evidence has been shown that certain traditional feeding practices may compromise the development of children’s food preferences, intake, diet quality, growth and weight status[72,77]. Also, these practices can foster both “picky eating” and “overeating”, hindering the development of self-regulation of intake[32,72,74,117]. Low demanding or permissive parents are associated with more “chaotic” attitudes, as inconsistency in food education, erratic eating schedules or harder to predict eating patterns in terms of healthiness[118,119]. Modelling, exposure and involvement, in contrast, have been

considered as positive feeding practices and behaviours, which may help to promote healthy eating attitudes and behaviours in children[118].

Other family aspects may be important in defining behavioural determinants of appetite-related eating behaviours. A prospective study concluded that family income was associated with both picky eating and overeating[113]. In this study, overeater children were more likely to be male, to live in a single parent home, have experienced food insufficiency, having immigrant mothers and both parents overweight or obese. Parental BMI, actually, is correlated in diverse studies with children's eating behaviours. In a cross-sectional study, mothers with higher BMI had children with lower scores in the 'food avoidance' sub-domains (Emotional Undereating and Food Fussiness) and fathers with higher BMI had children showing higher 'overeating' scores[49]. In another study, conducted with pre-schoolers, children with obese parents showed higher Emotional Overeating and Food Responsiveness[69].

3.5. Obesogenic environment

A permissive obesogenic environment is a recognized contributor to the development of unhealthy eating behaviours in children. Evidence on the effects of several behavioural/lifestyle characteristics of children on appetite and eating behaviours have been highlighted in recent years.

A cross-sectional study investigating associations between sleep and eating behaviours has found that short sleep duration was associated with higher levels of external eating and poor sleep continuity with emotional eating, suggesting that sleep loss may be associated with decreased self-regulation of appetite in children and increased risk of overeating and obesity[120].

Another cross-sectional study has found an association between emotional and external eating and spending much time with media screening[42]. This was explained by the fact that this activity is often paired with mindless eating (in which less attention is being paid to increasing feelings of fullness in the stomach because the child is focused on the computer or television screen)[121]. When becomes a habit, it may promote loss of awareness of hunger feelings and an increase of emotional and external eating. Despite physical activity has been understudied in research involving eating behaviours among children, this same study[42] also found a positive association between practicing sports and restrained eating among boys and further between practicing sports for slimming and restrained eating, among boys and girls.

Food intake characteristics as type, amount and/or composition of foods consumed may be also relevant in the context of obesogenic environment and have brought some clarity into the development of eating behaviours. Picky eating was associated, in girls, with lower intake of fruits and vegetables but also fat and sweets[122]. In this sample, picky eaters did not compensate the lower fruit and vegetable intake with consuming more palatable foods. In turn, emotional eating in girls[123] has been associated with a higher sugar intake and external eating in adolescents with more positive attitudes towards different types of foods[124]. In another cross-sectional study in children, both Emotional and External Eating have been associated with a higher consumption of sweets (emotional eating particularly evident in girls) and External Eating was additionally associated with soft drink consumption[125]. In this study, children's intake was clearly explained by their parents' intake.

Some of the research has used the CEBQ to assess the association between eating behaviours and food intake in children. One of the first studies[126] revealed lower food neophobia and greater food enjoyment of food to be associated with higher intake of fruits and vegetables. Accordingly, a study focusing on cross-sectional and prospective associations found that Food Approach eating behaviours (Enjoyment of Food, Food Responsiveness, Emotional Overeating and Desire for Drinks) were positively associated with fruit intake, whereas Food Avoidance eating behaviours (Food Fussiness, Emotional Undereating, Satiety Responsiveness and Slowness in Eating) were negatively associated with these foods[127]. The associations between appetitive traits and the consumption of snacks or sweetened beverages are less evident; positive associations were found between Satiety Responsiveness and Desire for Drinks with snacks and between Slowness in Eating and sugar-sweetened beverages[127]. Enjoyment of Food was negatively associated in this study with snack intake. More recently, it was designed a "fussy eater" profile in 4 years old children, characterized by less consumption, at 14 months, of vegetables, wholegrain products, fish and meat than children not exhibiting this eating behaviour, but also by higher consumption of confectionary, snacks and fast-food, in comparison with non-fussy eaters[128]. In other study, higher Desire for Drinks was associated, among pre-schoolers[129], with a greater preference for sugar-sweetened soft drinks, fruit squash and milk and with a more frequent consumption of sugar-sweetened soft drinks and low calorie soft drinks, suggesting that this CEBQ sub-domain may be measuring a desire for sweet things in the mouth rather than thirst or hunger. Lastly, energy intake has been inversely associated with Satiety Responsiveness, and positively associated with Enjoyment of Food and with Food Responsiveness[1].

3.5.1. Dietary patterns analysis

From a methodological point of view, food intake assessment has been heterogeneously approached, revealing a limitation that has been emphasized frequently: the complexity of diet, which is not captured in studies based on single nutrients or foods[130]. Efforts have been made, however, to overcome these limitations and dietary patterns have become increasingly accepted as a tool to characterize overall diet[131], by expressing cumulative and interactions effects of foods and nutrients, rather than their individual and non-independent effect[132].

Two general approaches have been used to define dietary patterns in observational studies: a hypothesis-oriented approach and an exploratory approach. Hypothesis-oriented dietary patterns (often named as *a priori* patterns, indexes or scores) are defined as a composite score from various food items or nutrients[133-135]. The patterns' definition is based on available scientific evidence for specific diseases, which may be drawn from studies on single dietary components or from studies on overall dietary habits. It is also common to use the patterns' scores as summary measures of the degree to which an individual's diet is in agreement with specific dietary recommendations. Such summary variables involve some subjective decisions, namely the identification of foods to be included, as well as the scoring method to be applied. Despite these constraints, *a priori* dietary patterns seem to represent simple methods of summarizing total diet, with easily reproducibility, comparability, interpretation and translation to the general public[136-138], which could be highly useful for public health messages.

In contrast, exploratory methods rely totally on the data at hand and derive patterns purely empirically, and for that reason are often named as *a posteriori* dietary patterns. Principal component analysis/exploratory factor analysis and cluster analysis are the main exploratory methods applied in nutritional epidemiology[139]. Principal component analysis or factor analysis identifies foods that are frequently consumed together. They aggregate food items or food groups on the basis of the degree to which they are correlated with one another. The goal is to identify linear composites of food items or food groups which account for the largest amount of variation in diet between individuals. While in principal component analysis the component score represents a mathematical transformation (a linear combination) of the observed variables, factor scores, computed in factor analysis, are considered only estimates of where individuals stand on the actual underlying and unobservable factor[139]. Cluster analysis aggregates individuals into relatively homogeneous subgroups (clusters) with similar diets[139]. Therefore, it defines mutually exclusive clusters of individuals, based on distance measures between observations, which can be frequency of food consumption, average grams of food intake or percentage of energy provided by each food or food group.

Dietary patterns are influenced by sociocultural factors and food availability and are population-specific[140]. Furthermore, dietary patterns established during childhood have shown to track into adulthood[141], and are closely connected with eating behaviours, namely through the social interactions surrounding food and eating moments.

Research on this topic is very scarce; one cross-sectional study[127] assessed the association between dietary patterns (sweet foods, fatty foods, snacks, and FV) and emotional eating in school-aged children, but no association was found. The influence of early dietary patterns on eating behaviours should be explored in future studies. These will enable to clarify the global effect of diet in the establishment of appetite-related eating behaviours.

OBJECTIVES

This research aims to study the early influences of appetite-related eating behaviours in 7 years old children from a prospective population-based birth cohort. To answer this question, specific objectives were defined:

- To evaluate the psychometric properties of a Portuguese version of the CEBQ in 7 years old children from the Generation XXI cohort.
- To study the early influences of the appetite-related eating behaviours identified at 7 years of age.
- To quantify the association between dietary patterns established at 4 years old and eating behaviours related to appetite identified at 7 years of age.

PAPER I

Early influences on appetite-related eating behaviours in 7 years old children.

Early influences on appetite-related eating behaviours in 7 years old children

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Abstract

Objectives: To evaluate the psychometric properties of the Children's Eating Behaviour Questionnaire (CEBQ) in 7 years old Portuguese children and to assess the early influences of the appetite dimensions identified

Methods: Participants are part of the Generation XXI population-based birth cohort, which recruited 8495 mothers and their 8647 children. Approximately 80% of all children participated in the follow-up assessment at 7 years of age. Trained interviewers administered a structured questionnaire on socio-demographic characteristics, children's health and lifestyles, and anthropometrics of both children and mothers were measured. A Portuguese version of the original CEBQ (35-items in a 5-point Likert scale) was self-completed by mothers (a sample of 3562 children were included in the final analysis). Principal Component Analysis was conducted; Cronbach's Alpha was computed to evaluate the internal consistency of the CEBQ scale. Reliability was tested through Intra-class Correlation coefficients (ICC) and Spearman-Brown prediction formula was used to estimate a version with fewer items. Generalized linear models were used to estimate the associations between children and maternal characteristics and eating behaviours, after adjustment for maternal age, education and body mass index (BMI) before pregnancy, child's sex, family structure and number of siblings.

Results: A 2-factor final solution was identified, explaining 62% of the total variance, with good internal consistency (Cronbach's Alpha was 0.77 for factor 1 and 0.64 for factor 2). The factors were named Appetite Restraint (factor 1, mean=2.88; SD=0.61), including the sub-domains Satiety Responsiveness, Slowness in Eating, Food Fussiness and Enjoyment of Food and Appetite Disinhibition (factor 2, mean=2.15; 0.52), including the sub-domains Food Responsiveness, Emotional Overeating, Emotional Undereating and Desire for Drinks. These domains showed good construct validity and good reliability (ICC from 0.51 to 0.85). A 20-items CEBQ version was proposed.

In multivariate analysis, children with higher Appetite Restraint scores at 7 years of age seem to come from families with a higher educational level, with both parents living in the child's home and with no siblings, but presenting also more unhealthy lifestyles at 4 years of age (more hours of media screening, no regular physical activity practice). In addition, children and mothers with higher anthropometrics at 4 years presented lower Appetite Restraint scores at 7 years of age. On the other hand, children with a lower educational background, living in less structured families, with more sedentary behaviours (more hours of media

screening) and with less favourable anthropometrics at 4 years of age presented higher Appetite Disinhibition scores later in childhood.

Conclusions: The CEBQ showed good psychometric properties in 7 years old Portuguese children and allowed to identify early influences of children's appetite-related eating behaviours, which can be useful in future research and in the development of prevention guidelines and educational strategies involving families, aimed at improving healthy eating behaviours and childhood obesity.

Keywords: CEBQ, children, cohort studies, feeding behaviours, health behaviour.

Introduction

Eating behaviours develop early in life and result from a complex interplay between genetic predispositions, natural food responses and food preferences, the latter greatly influenced by the exposure to foods within the family environment[1,2]. In previous literature, individual differences in eating behaviours and appetite-related traits have been associated with differences in children's weight[3-5]. Obese children demonstrate more positive responses towards food (enjoyment, rapid consumption and responsiveness to palatability and other food cues), hypothesized to promote food intake, while leaner children express more sensitivity to internal cues of satiety and fussiness about food, that are likely to reduce food intake[4-7].

Previous literature has performed an attempt to understand which factors are underlying the development of appetite-related eating behaviours. Some birth-related and early infancy characteristics have been found to predict future eating behaviours. Low birth weight[8,9], preterm birth and a restrictive intrauterine growth[10] have been associated with pickiness (the consumption of an insufficient amount or an inadequate variety of foods through rejection of food items[11,12]) and a higher risk of low appetite and food enjoyment in children. Early feeding practices have also been associated with appetite-related traits later in life. Breastfeeding, hypothesized to promote an early learning self-regulation of energy intake, was associated with higher satiety responsiveness at 2 years old[13] and in later adolescence[14]. On the other hand, slow sucking frequency during breastfeeding and formula feeding were associated with picky eating[15] and neophobia (reluctance to eat or the avoidance of novel foods)[16].

Family plays a strong influence on the development of children's eating behaviours with parents serving as models[17] in this learning process. In young girls, higher maternal disinhibition was associated with greater eating in the absence of hunger[18] and a restrictive feeding style has been associated with overeating[18], disinhibition[19] and picky eating and diminished self-regulation of intake[20-22] in children. Additionally, the socioeconomic familial environment could exert their impact. A prospective study[9] concluded that overeater children were more likely to be male, to live in a single parent home, have experienced food insufficiency, having immigrant mothers and both parents overweight or obese and that family income was associated with both overeating and picky eating. In other studies, low maternal age and parental financial problems have been identified as risk factors for picky eating[9,15], as well as having siblings has been shown to be both protective[23] and a risk factor[15] on the development of picky eating. Other family and social aspects may be

important in defining early determinants of appetite-related eating behaviours and should be highlighted in future research.

This previous research has focussed on few dimensions of eating behaviours, independently evaluated. But even that few, there are standardised psychometric instruments available to assess children's eating behaviours from a global approach. The Children's Eating Behaviour Questionnaire[24] (CEBQ) assesses eight aspects of children's appetite – Enjoyment of Food, Food Responsiveness, Emotional Overeating, Satiety Responsiveness, Slowness in Eating, Emotional Undereating, Food Fussiness and Desire for Drinks. The CEBQ has been successfully validated in countries worldwide[25-32], used to study the stability of children's eating behaviours[33-35] and to measure their association, namely with childhood obesity[4,5,25,26,34,36], parental obesity[25,30,37] and feeding practices[18,38,39].

As the first years of life are characterized by critical periods in children's growth[40], children become a target group for intervention on the acquisition of healthy eating behaviours. It is crucial to understand which factors are underlying appetite development, namely through prospective approaches (and covering a wide range of eating behaviours), which are still lacking[41] and become necessary to hamper reverse causality. The aim of this prospective study is to evaluate the psychometric properties of the CEBQ scale in the Generation XXI sample at 7 years of age and to assess early exposures influencing eating behaviours related to appetite in this school-aged group of children.

Methods

This study is embedded in Generation XXI, a prospective population-based birth cohort, previously described elsewhere[42]. Participants were recruited in all public maternity units of Porto, Portugal between 2005 and 2006. Of the invited mothers, 91.4% agreed to participate totalling a sample of 8495 mothers and their 8647 children. All families were invited to attend the second (2009-2011) and third (2012-2014) cohort evaluations, when children were aged 4 and 7 years old. On both occasions, 86% and approximately 80% of all children were re-evaluated, respectively.

The study protocol was approved by the local ethical committee (Ethical Committee of São João Hospital/University of Porto Medical School) and by the Portuguese Authority of Data Protection. Legal representatives of each participant were informed about the benefits and potential discomfort, and written informed consent was obtained for the collection of information at each follow-up assessment.

Children and maternal characteristics

Data were collected in face-to-face interviews by trained researchers. At baseline, the following variables were collected: maternal age and education, self-reported body mass index (BMI) before pregnancy (from self-reported height and weight; recoded into under/normal weight and overweight/obese according to the World Health Organization (WHO) criteria[43]), smoking habits during pregnancy (ever and never smoking), type of delivery (vaginal or caesarean), children's sex, birth weight and gestational age. Weight for gestational age was defined according to the sex-specific population-based Kramer growth standards[44]. Small for gestational age (SGA) and large for gestational age (LGA) were defined as below the 10th and above the 90th percentile, respectively; appropriate for gestational age (AGA) was deemed to be within these thresholds. These standards refer only for single births.

Variables collected at the 4-years evaluation include: child's number of siblings (none, one, two or more), family structure (two-parent family, one-parent family or other family structure), any breastfeeding duration (recoded into never or less than 4 months, between 4 and 5 months, 6 months or more), physical activity (defined as the regular practice of physical exercise) and the number of media screening hours (subdivided in less than 120 minutes and 120 minutes or more per day). Timing of introduction to complementary feeding and the first food eaten by the infant in this period were asked at 7 years old.

Anthropometric information on height, weight, body fat and waist circumference were measured during the physical examinations at 4 and 7 years old. Weight was measured in light clothing and without shoes using a digital scale (TANITA®) and was recorded to the nearest 0.1 kg. Height was measured as the distance from the top of the head to the bottom of the feet without shoes using a fixed stadiometer (SECA®) to the nearest 0.1 cm. The children's BMI was defined as weight in kg divided by height in m^2. Each child was then classified according to the age- and sex-specific BMI standard z-scores developed by the WHO[45] and recoded into under/normal weight (BMI <2 SD) and overweight/obese (BMI $\geq$2 SD). Waist circumference (WC) was measured at the umbilicus level, with abdomen relaxed, to the nearest 0.1 cm, with the child in a standing position, arms at the sides and feet positioned together. This variable was recoded into thirds (<50.5 cm; 50.5–53.5 cm; ≥53.6 cm). Bioelectric impedance analysis was performed using a tetra-polar device (BIA 101 Anniversary, Akern, Florence, Italy). The unit was calibrated before testing using the 500-ohm resistor provided by the manufacturer. After cleaning all skin contact areas with alcohol, four surface electrodes were placed on the right wrist (bisecting the head of the ulna), on the right ankle (bisecting the medial and lateral malleoli) and on the right hand and foot at the

base of the metacarpal-phalangeal joint with the child lying horizontally. Measurements were performed, whenever possible, at least 30 minutes after the last meal. Fat free mass was determined using the equation published by Schaefer et al.[46]; fat mass was derived accordingly and recoded into thirds (<12.8%; 12.8–19.5%; ≥19.5%).

Maternal height and weight were also collected at children's 4 years old follow-up (measured by trained researchers). Maternal BMI was calculated with the previously described formula and classified according to the WHO criteria into under/normal weight (BMI <24.9 kg/m^2) and overweight/obese (BMI ≥25.0 kg/m^2)[43]. It was further created another variable, BMI change, representing the change in maternal BMI between the pre-pregnancy period and 4 years after birth (deemed into "maintaining under/normal weight or decreasing of BMI category" and "maintaining overweight/obesity or increasing of BMI category").

Children's Eating Behaviours

Eating behaviours were assessed by self-reported questionnaires (CEBQ), filled during the evaluation at 7 years old (94% of the questionnaires were answered by mothers). The original CEBQ[24] was designed to assess variation in eating styles among children and includes 35 items. This questionnaire consists of eight sub-domains related to distinct eating behaviours, in which items are answered on a five-point Likert scale, ranging from 1=never to 5=always. Satiety Responsiveness reflects the ability to regulate the amount of food eaten, based on perceived fullness[27,28] ; Slowness in Eating measures the speed of eating during the course of a meal and reflects a gradually reduced interest in a meal[27]; Enjoyment of Food represents a general interest in food and Food Responsiveness measures eating in response to external food cues[28]. Food Fussiness measures a lack of interest in food and unwillingness to try new foods and the sub-domain Desire for Drinks, increased desire to have drinks, particularly sugar-sweetened drinks[47]. The sub-domains Emotional Overeating and Emotional Undereating are characterized by either increased or decreased eating in response to negative emotions, such as anger and anxiety[26,27]. The CEBQ has demonstrated stability over time and good psychometric properties (internal consistency, concurrent validity with actual eating behaviour and reliability[24-28,33-35]). A version of this questionnaire has been previously validated and adapted to Portuguese children[25]. However, in that version researchers included children aged 3-13 years and some items were slightly different from those in the scale developed by Wardle and collaborators. We opted for re-translating the original CEBQ into Portuguese (Appendix), resulting in a new Portuguese version (P-CEBQ). One of our tasks in the present study was to test the psychometric properties of the CEBQ in the Generation XXI sample (restricted to 7 years old children), and to propose a short-version to be further applied in future research.

Responses to this questionnaire were available for 5786 children. Of these, 4485 questionnaires had all items fulfilled and after retrieving missing data 5449 were considered valid. In individuals with missing data in less than 50% of the items, data was recovered by replacement for the average of the remaining questions within each sub-domain. The sample used in the analysis of the psychometric properties comprises children with complete data on the CEBQ (n=4485). For a sub-sample of 150 children we asked to re-answer this questionnaire at home, within an average period of 35 days post-evaluation to assess the reliability of this tool.

In the analysis of the determinants of children's eating behaviours, only one twin was randomly included; thus, 103 children were excluded from analysis. The sample was further restricted to participants with complete information on the variables of interest; thus, 1887 were excluded, remaining 3562 participants. For timing of complementary feeding, first food eaten at complementary feeding and children's fat percentage estimated by bioelectric impedance the sample sizes are lower (n=2698; n=2668; n=3012, respectively).

We compared characteristics of the present study sample (n=3562) with the remaining cohort (n=5085) at baseline, and no significant differences were found for child's sex (50.7% vs. 51.2% of boys, p=0.661), and maternal BMI before pregnancy (mean 23.86 vs. x 23.89 kg/m^2, p=0.769). However, differences were found for maternal age and education: mothers in this study were slightly older [mean = 29.85; standard deviation (SD) = 5.22 vs. mean = 28.47; SD = 6.26] and more educated (mean = 11.27; SD = 4.25 vs. mean = 9.88; SD = 4.17) than mothers in the remaining cohort. The Cohen's effect size values were lower than 0.35[48], suggesting that the magnitude of the differences was not high (i.e. differences were at most part due to the large sample size than to large differences between participant's characteristics)[48].

Statistical Analysis

A Principal Component Analysis (PCA) with Oblimin Rotation was firstly performed on all items of the CEBQ to understand if the original factor structure (with 8 factors, representing 8 sub-domains) would be replicated in the present sample. A second PCA was later conducted on the emerged sub-domains and the scree plot supported an alternative 2-factor solution (representing 2 domains). The items with absolute factor loading of 0.3 or higher were interpreted as having meaningful part on each factor. To assess the internal consistency of the CEBQ scale, Cronbach's Alpha coefficients for each sub-domain and Pearson correlation coefficients between sub-domains were calculated. Good internal consistency was defined by a Cronbach's Alpha of 0.7 or higher and a Pearson Correlation of 0.4 or higher. For each sub-domain defined through the PCA, descriptive statistics (mean,

SD) were calculated. A higher score in a sub-domain indicated a higher presence of the eating behaviour. In the first PCA, in accordance with the original CEBQ, the scores of five items were reversed due to opposite phrasing (Appendix). In the second PCA, only the score of the Enjoyment of Food sub-domain was reversed (factor 1).

Construct validity was performed on the two factors emerged in the second PCA, by comparing the mean values of each one by children's BMI and maternal pre-pregnancy BMI, using one-way ANOVA.

Reliability was tested by analysing the agreement between the sub-domains in the two moments using two-way random Intra Class Correlation (ICC)[49]. ICC varies between 0 and 1, with higher values representing higher reliability.

It was also conducted a Spearman-Brown prediction formula to estimate the hypothetical Cronbach's Alpha in a new version of the CEBQ scale with less items. It was set to retain in each sub-domain only the necessary number of items to achieve a Cronbach's Alpha of at least 0.7.

Descriptive statistics and generalized linear models (GLM) (β regression coefficients and the respective 95% confidence intervals (95%CI)) were performed to estimate the association between the predictor variables and the scores in the 2 factors (domains) identified. Two models were drawn: one represented crude associations between each predictor variable and both factors; the second represented the same associations adjusted for socio-demographic characteristics (maternal age and education and child's sex), family structure (family structure and number of siblings) and maternal BMI before pregnancy. Further adjustments for smoking during pregnancy, child's BMI z-score and media screening were also tested, but did not change the magnitude of the associations.

An interaction of the child's sex in these associations was studied by conducting stratified analysis, but no significant interaction was found; thus, results are reported for both sexes together.

Significance level was set at 5%. SPSS (Statistical Package for Social Sciences) Statistics 21.0 (SPSS Inc., Chicago, IL, USA) was used to perform all the statistical analyses, with the exception of the PCA, conducted with the software R 3.0.1.

Results

Factor analysis and internal consistency of the P-CEBQ

The first PCA revealed an 8-factor structure that explained 67% of the total variance (table 1). Most of the items loaded on the expected sub-domains, as previously reported[24-

[27,36]. Internal consistency coefficients (Cronbach's Alpha) for the different sub-domains ranged from 0.74 to 0.85, which means a good internal consistency.

The second PCA revealed a 2-factor solution that explained 62% of the total variance, with factor 1 and factor 2 explaining 35% and 26% of the total variance, respectively. The sub-domains Food Fussiness, Enjoyment of Food, Slowness in Eating and Satiety Responsiveness loaded mostly on factor 1 (loadings: 0.64; -0.78; 0.74 and 0.88) and the sub-domains Food Responsiveness, Emotional Overeating, Emotional Undereating and Desire for Drinks loaded mostly on factor 2 (loadings: 0.65; 0.82; 0.66 and 0.65) (table 1). Cronbach's Alpha was 0.77 for factor 1 and 0.64 for factor 2, indicating that this structure has also a good internal consistency. The average score in factor 1 was 2.88, SD=0.61 and in factor 2 was 2.15, SD=0.52. Factor 1 was subjectively named "Appetite Restraint" and factor 2 "Appetite Disinhibition".

The correlation between factor 1 and 2 was weak (rho=-0.013), indicating that these domains are independent from each other, representing very different aspects of eating behaviours.

Construct validity of the P-CEBQ

Construct validity was assessed considering theoretical hypotheses, based on previously described literature: as higher the children's BMI, higher the emotional eating and responsiveness to external cues[18,39,50] and lower the food fussiness[24,25] and responsiveness to internal satiety cues. A similar relation was hypothesized with higher maternal BMI[9].

As expected, children's BMI was negatively and significantly associated with Appetite Restraint (factor 1) and positively associated with Appetite Disinhibition (factor 2); overweight/obese children scored significantly lower on Appetite Restraint [mean=2.60 (SD=0.55) vs 3.05 (0.58); p<0.001] and higher on Appetite Disinhibition [2.07 (0.48) vs 2.28 (0.57), p<0.001]. Regarding maternal BMI, children with overweight/obese mothers also scored significantly lower on factor 1 [2.82 (0.61) vs 2.91 (0.60); p<0.001] and significantly higher on factor 2 [2.17 (0.55) vs 2.14 (0.51); p=0.026]. The referred associations were consistent with the theoretical hypothesis, supporting the construct validity of the P-CEBQ scale.

Reliability and Short version of the P-CEBQ

The mean ICC of the P-CEBQ ranged from 0.51 (Desire for Drinks sub-domain) to 0.85 (Food Fussiness sub-domain). Most sub-domains presented good reliability (ICC $\geq$0.7), except Desire for Drinks (ICC=0.51) and Emotional Undereating (ICC=0.58). Overall, the P-CEBQ presented a good reliability in this 7 years old Portuguese sample (mean ICC= 0.73).

A total of 20 items (in replacement of the original 35 items) were retained in the short-version of the P-CEBQ. Our analysis shows that only two items would be enough to represent the sub-domains Enjoyment of Food, Food Responsiveness, Desire for Drinks and Slowness in Eating and three items would be enough to represent the the sub-domains Emotional Undereating, Emotional Overeating, Satiety Responsiveness and Food Responsiveness. The Cronbach's Alpha of this version ranged from 0.68 to 0.72, demonstrating a good internal consistency, as the original CEBQ scale. As there is no consensus on a method to define which items to retain, i.e. to include in the short-version, we therefore suggest that this decision should be taken by each research group, concerning two main criteria: loadings and pertinence of the items in each sub-domain.

Associations with Eating Behaviours related to Appetite

Table 2 presents participant's characteristics included in this study (n=3562). Approximately 51% were boys, most of them lived in a two-parent home (89.1%) and had none (46.7%) or one sibling (43.4%) at 4 years of age. Most had a vaginal delivery (62.6%), and were born with an adequate weight for gestational age (82.0%). Almost 50% were breastfed for at least six months and complementary feeding started mostly between 4 and 5 months (48.8%). Seventy per cent of children, at 4 years, watched less than 120 minutes of media per day (71.3%) and practiced regular physical activity (68.5%); approximately one third (31.4%) was overweight or obese at 4 years old. At baseline, most mothers were aged between 25 and 35 years (66.3%), had between 9 and 12 years of education (42.8%), were under or normal weight before pregnancy (69.3%) and 51.6% of them maintained under/normal weight or decreased BMI from before pregnancy to 4 years after birth; a fifth smoked during pregnancy (20.7%). Appetite Restraint and Appetite Disinhibition scores ranged from 1-5; descriptives (mean, SD) of these scores according to each characteristic are detailed in table 2.

Associations of children and maternal characteristics with eating behaviours assessed in 7 years old children are presented in tables 3 (univariate model) and 4 (multivariate model). In univariate analysis, a positive association was found between maternal age, maternal education, a caesarean birth, and starting complementary feeding with foods other than soup and the Appetite Restraint domain (factor 1). Children from mothers who maintained high or increased BMI (from pregnancy to 4 years after birth) also presented a higher probability of scoring high in the Appetite Restraint domain. On the other hand, a negative association was found between living in a one-parent family (compared with two parents), having siblings, being a boy, being overweight or obese and having higher waist circumferences and fat mass % at 4 years old and the Appetite Restraint dimension. Children

from mothers with increased maternal BMI before pregnancy and who smoked during pregnancy also showed lower scores in this domain.

Children with higher scores in the Appetite Disinhibition domain were more often boys, from a one-parent family, with more sedentary behaviours (media screening at least 120 minutes per day), overweight or obese, with higher waist circumferences and fat mass %, at 4 years old. Maternal characteristics, such as a high BMI before pregnancy and smoking during pregnancy also showed a positive association with this domain. Conversely, a negative association was also found with maternal age, education and longer breastfeeding duration.

In multivariate analysis (table 4), higher maternal age (≥35 vs. <25 years: β=0.127, 95%CI: 0.054; 0.201) and education (≥12 vs. <9 years: β=0.109, 95%CI: 0.055; 0.164) were associated with higher Appetite Restraint scores. Children from mothers maintaining high or increasing maternal BMI (from pregnancy to 4 years after birth) also presented with higher Appetite Restraint scores (β=0.043, 95%CI: 0.003; 0.083). Similarly, children starting the complementary feeding with foods other than soup (β= 0.077, 95%CI: 0.030; 0.124) and watching at least 120 minutes of media per day at 4 years (β=0.048, 95%CI: 0.004; 0.092) showed higher Appetite Restraint scores at 7 years of age. In contrast, children living in a one-parent family (one-parent vs. two-parent: β=-0.074, 95%CI: -0.140; -0.007), with higher number of siblings (≥2 vs. 0: β=-0.152, 95%CI: -0.224; -0.081), boys (β=-0.053, 95%CI: -0.092; -0.014), practicing regular physical activity at 4 years old (yes vs. no: β=-0.048, 95%CI: -0.091; -0.005), as well as being overweight or obese (β=-0.368, 95%CI: -0.410; -0.327), with higher waist circumference (3^{rd} vs. 1^{st} tertile: β=-0.418, 95%CI: -0.46; -0.371) and fat mass % at 4 years old (3^{rd} vs. 1^{st} tertile: β=-0.276, 95%CI: -0.329; -0.224) presented significantly lower Appetite Restraint scores at 7 years.

The characteristics significantly associated with higher Appetite Disinhibition scores at 7 years of age were living with one parent (one-parent vs. two-parent: β=0.090, 95%CI: 0.034; 0.147) and being a boy (β=0.051, 95%CI: 0.018; 0.085), watching at least 120 minutes of media per day (β=0.055, 95%CI: 0.018; 0.093), being overweight or obese (β=0.183, 95%CI: 0.146; 0.219) and having higher waist circumference (3^{rd} vs. 1^{st} tertile: β=0.188, 95% CI 0.147; 0.229) and fat mass % at 4 years old (3^{rd} vs. 1^{st} tertile: β=0.160, 95%CI: 0.114; 0.206). In addition, children from overweight/obese mothers before pregnancy (β=0.046, 95%CI: 0.009; 0.083) and smokers during pregnancy (β=0.082, 95%CI: 0.039; 0.124) also presented higher Appetite Disinhibition scores. In turn, maternal age (≥35 vs. <25 years: β=-0.187, 95%CI: -0.250; -0.125) and education (≥12 vs. <9 years: β=-0.056, 95%CI: -0.102; -0.009) were significantly negatively associated with Appetite Disinhibition scores.

Discussion

The current study showed that the Portuguese version of the CEBQ used in 7 years old children from the Generation XXI birth cohort has good psychometric properties. The eating behaviours measured by the CEBQ were aggregated into two broad domains, Appetite Restraint (more related with sub-domains measuring internal cues of satiety and food fussiness) and Appetite Disinhibition (more related with sub-domains measuring external food cues and emotional responses towards foods).

The study of the early influences of these appetite-related eating behaviours highlighted diverse children and maternal characteristics associated with both domains. A higher maternal education, a more structured family, but also more unhealthy lifestyles at 4 years of age (more hours of media screening, no regular physical activity practice) seem to influence higher Appetite Restraint scores at 7 years of age. In addition, children and mothers with higher anthropometrics at 4 years presented lower Appetite Restraint scores at 7 years of age. On the other hand, children with a lower educational background, living in less structured families, with more sedentary behaviours (more hours of media screening) and with less favourable anthropometrics at 4 years of age presented higher Appetite Disinhibition scores later in childhood.

Concerning socio-demographics, we found that a high level of maternal education and also higher maternal age were associated with high Appetite Restraint and low Appetite Disinhibition scores at 7 years of age. These results concur with a previous study which has prospectively demonstrated an association between high maternal education and lower drive to eat[10] in children. Moreover, a more recent study found low maternal education and maternal age to be associated with overeating[9] in children, which corroborates our associations found with the Appetite Disinhibition domain.

In addition, children living in a one-parent family presented more Appetite Disinhibition and less Appetite Restraint at 7 years old than did children living in a two-parent family. Children with siblings also showed a lower likelihood of developing Appetite Restraint at 7 years old, and the association was stronger with a higher number of siblings. To our knowledge, only one study[9] has addressed the influence of the family structure in children's eating behaviours and found that living in a one-parent home was predictive of overeating (more related with the Appetite Disinhibition domain of our study). According to the literature, single parenthood is associated with lower household income, parenting quality and maternal time spent with children[51], factors which may significantly impact on children's well-being and behavioural development. One possible explanation for the obtained results is the association between lower income (not specified in the current work) and children's overeating[9]. Additionally, lower parenting quality and time spent with children may lead to

emotional general parenting and feeding practices, which have been correlated with emotional overeating in children[52]. The findings on the siblings' influence are in accordance with a previous prospective study[23]. Having a higher number of siblings may decrease parental attention towards each child during mealtimes, which can benefit children's eating behaviours; being less pressurized to eat decreases the risk of developing pickiness[11,23] and also enables the child to be more autonomous during meals and responsive to internal satiety cues (characteristics of our Appetite Restraint domain). Decreased parental attention during mealtimes may also be a consequence of single parenthood. Altogether, the present findings indicate that a higher educational background and more cohesive family structures may be positively associated with Appetite Restraint and negatively associated with Appetite Disinhibition, showing that a more favourable familial background could have different effects on children's eating behaviours, increasing the likelihood of a possible deregulation of internal cues of satiety (represented by higher scores in the Appetite Restraint domain), but decreasing the likelihood of Appetite Disinhibition.

Sex differences were also found in the current study: boys presented less Appetite Restraint and more Appetite Disinhibition scores at 7 years old, than girls. Previous studies have reported sex differences, with girls presenting less appetite than boys, in both prospective[9,10] and cross-sectional analyses[30,36,53]. In cross-sectional analyses, boys scored higher on Emotional Overeating[53] and Food Responsiveness[30], sub-domains included in our Appetite Disinhibition domain and lower on Food Fussiness, Satiety Responsiveness and Slowness in Eating[36] (sub-domains included in our Appetite Restraint domain).

Our results revealed a no consistent association of eating behaviours with feeding habits of early infancy. The association with breastfeeding duration was only significant in univariate analysis. It showed that infants breastfed for 6 months or longer presented lower Appetite Disinhibition at 7 years old. Previous literature revealed that breastfed children show increased Satiety Responsiveness at 2-years[13,54] and 16-years[14]. These findings suggest that breastfeeding may allow infants to learn how to regulate their appetite and respond to internal cues of satiety[13], protecting from the susceptibility towards external cues of intake (as in this case, maternal judgement of timing, volumes and energy density in each feeding). However, after further adjustment for child's sex, socio-demographics and family structure, the association was no longer significant. Future prospective studies should clarify this association.

In the present study, timing at introduction to complementary feeding did not significantly predict eating behaviours at 7 years old. However, when studying the type of food firstly introduced, children who firstly consumed cereal porridge and fruit presented more Appetite Restraint at 7 years old than did children whose first food was the vegetable soup. Traditionally, the first food consumed by Portuguese infants is cereals porridge, but

recommendations[55] are increasing towards the introduction to complementary feeding by a vegetable broth or puree, due to the higher energy density and sweetness of cereals porridge. Infants have an innate preference for sweet tastes[56], and thus the introduction of solid foods through cereals porridge and fruits may be more easily accepted, but the early absence of repeated exposure to vegetables and unsweetened fruits may also induce a low capacity of tasting different flavours later in life[20], and consequently promoting picky eating. Also, the preference for more sweet and energy-dense foods may compromise the development of self-regulation intake mechanisms, in which eating is initiated in response to hunger and terminated in response to satiation signals[20], supporting the positive association with the Appetite Restraint domain found in our study.

More sedentary behaviours, such as no regular practice of physical activity, but particularly a high media screening time (≥120 minutes/day) were associated with higher Appetite Restraint and Appetite Disinhibition. A previous cross-sectional study[57] found that emotional and external eating were associated with spending much time with screen media, as in our study. This association was explained by the fact that this activity is often paired with mindless eating (in which one pays less attention to increasing feelings of fullness, because is focused on the computer or television screen[58], eating more in response to external food cues and also losing their good awareness of feelings of hunger and satiety)[59].

As to children's anthropometrics, the current study showed that overweight and obese children and those with higher adiposity at 4 years old presented low Appetite Restraint and High Appetite Disinhibition at 7 years old. In the literature, obese children are characterized by having lower responsiveness to internal satiety cues[50,60], being less fussiness about food[5], eating faster during the course of a meal[3], presenting more food enjoyment[4,5], being more sensitive to external food cues[5,25,61] and emotional states[5] and having higher desire for drinks[5] than healthy weight children. Consistently, in the previous Portuguese study conducted with children and adolescents[25], overweight/obese children revealed higher responsiveness to food cues and more emotional eating and lower satiety responsiveness and food fussiness than healthy-weight children. However, most of these studies have a cross-sectional design, and do not clarify the actual direction of these associations. Our findings reinforce the previous literature and add that weight status/adiposity in pre-school children may be a determinant of appetite later in childhood.

The same reported associations for children were found concerning maternal BMI, and in addition, children from mothers who smoked during pregnancy presented high Appetite Disinhibition at 7 years old. These findings may indicate that unhealthy maternal lifestyles are predictors of problematic eating behaviours in children. Research has shown a tendency of overweight mothers with eating concerns of their own, to develop more restrictive feeding styles when they perceived their children as overweight or at risk for developing weight

problems[22,62]. Parental restriction has been associated with children's preference for energy-dense foods and disruption of energy intake regulation on one hand, but also with narrowing children's acceptance of a variety of foods and restrained eating behaviour[20-22,56]. These latter findings appear to fit those reported in our study: mothers maintaining high BMI or increasing BMI from pregnancy to 4 years after birth have children with higher Appetite Restraint scores. However, opposite results were found when we studied the maternal BMI before pregnancy. Despite a little contradiction, they may represent a wider link between parental weight, self-image, concerns about their child's weight and feeding practices and children's eating behaviours. Further investigation over the referred characteristics should be considered, as it might bring some insight into the present findings.

Regarding smoking during pregnancy, it has been demonstrated an association with higher overweight risk in children[63]. Proposed mechanisms for this association highlight the intrauterine exposure to inhaled smoke products (namely nicotine), which act on neurotransmitters associated with reward decreasing appetitive learning of responsiveness to internal cues[64]. Behavioural associations linking parental smoking habits with other unhealthy lifestyles, particularly dietary habits, are also important to refer[63]. Concurring with these theories, children with smoker mothers during pregnancy may have, thus, an appetite more susceptible to the action of external food cues (characteristics of the Appetite Disinhibition domain), as found in the current study.

None of the studied birth characteristics showed a significant association with Appetite Restraint or Appetite Disinhibition, after adjustment for potential confounders, suggesting that other factors, probably acquired after birth, have a more important effect in determining appetite-related traits during childhood.

Some issues related with the specific use of the CEBQ scale must be further discussed. This CEBQ version showed various resemblances with previous studies. The 8-factor structure was replicable, similarly to the Canadian[29] and Australian[32] versions. The P-CEBQ showed also a good internal consistency (Cronbach's Alpha of the 8-factor structure ranged from 0.74 to 0.85 and of the 2-factor solution was 0.77 and 0.64), explaining 67% and 62% of the total variance. This was a very close result to the original CEBQ[24] and superior to the previous Portuguese[25] and other versions[26,27,29,30].

The original CEBQ sub-domains have been previously divided into 2 factors: 'Food Approach' (Enjoyment of Food, Food Responsiveness, Emotional Overeating, Desire for Drinks) and 'Food Avoidance' (Slowness in Eating, Satiety Responsiveness, Emotional Undereating, Food Fussiness) eating behaviours, due to their association with children's weight demonstrated in previous studies[4-6,24-26]. In our study, however, the aggregation into 2 domains was based on the structure suggested by the PCA, distinguishing between the responsiveness to internal hunger and satiety cues and to emotions and external food cues.

To our knowledge, the CEBQ's reliability has been tested only in a few studies[24,65] and our results suggest that our questionnaire is measuring the same in different time periods. A novelty in this study was the definition of a short-version of the CEBQ, with 20 items. The CEBQ-20 will be of great utility in future studies of eating behaviours within the Generation XXI cohort, possibly allowing a higher response rate, due to its less extension and thus faster self-completion.

The current study has some limitations worth to be acknowledged. As in other population-based studies, questionnaires to be self-completed often present low response rates, thus in the present study a relatively high number of children had incomplete or no information on the CEBQ and, when compared with the whole cohort, children in the current sample had mothers slightly older and more educated, which may reveal a selective response bias. However, according to the Cohen coefficient[48] these differences were not relevant, and we were able to recover missing data in cases with more than 50% of the items fulfilled, by replacement for the average of the remaining questions within each sub-domain. Nevertheless, we hope to overcome this barrier in future cohort evaluations by introducing the shorter version defined in this study. In addition, the assessment of eating behaviours was based on parental report, which may introduce measurement error due to inherent subjectivity. However, parents have privileged observational access to their children over diverse situations and may give valid judgements of their child's appetite[28]. Social desirability, despite commonly highlighted in parental reports, should not be of concern in this study as CEBQ has shown good correspondence with objective measures[28]. Another limitation is that some variables, namely related with breastfeeding and complementary feeding were asked at the 4 and 7 years old follow-up assessments, being measured retrospectively and dependent on parent's memory, introducing a possible recall bias. Nonetheless, this is one of the few studies prospectively investigating early influences of appetite-related eating behaviours in children. The fact that this study relies on a representative sample of the population allows for the generalization of findings (external validity).

Conclusions

In conclusion, two domains of Appetite were defined in the present study: Appetite Restraint and Appetite Disinhibition, which showed good psychometric properties in 7 years old children. The study of their early influences suggests that children with higher Appetite Restraint scores at 7 years of age seem to come from families with a higher educational background, with both parents living in the child's home and with no siblings, but presenting also more unhealthy lifestyles at 4 years of age (more hours of media screening, no regular physical activity practice). Starting the complementary feeding period with foods other than

soup seems also to influence higher Appetite Restraint scores at 7 years of age. In addition, children and mothers with higher anthropometrics at 4 years presented lower Appetite Restraint scores at 7 years of age. On the other hand, children with a lower educational background, living in less structured families, with more sedentary behaviours (more hours of media screening) and with less favourable anthropometrics at 4 years of age presented higher Appetite Disinhibition scores later in childhood.

These results provide relevant insights into some early influences of appetite-related eating behaviours in children, which can be useful in future research and in the support for the development of prevention guidelines and educational strategies involving families, aimed at improving healthy eating behaviours and childhood obesity.

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Table 1. Factor Loadings, from the Principal Components Analysis, for the 35 items of the Child Eating Behaviour Questionnaire (CEBQ) resulting in the original eight-factor structure and the two-factor final solution of the Portuguese-CEBQ used in 7 years old children (n = 4485)

#	Item	Original eight-factor structure					Two-factor solution	
		Sub-domain	Loading	α	Mean	SD	Loading Factor 1	Loading Factor 2
7	My child refuses new foods at first.		-0.80					
10	My child enjoys tasting new foods.		0.87					
16	My child enjoys a wide variety of foods.		0.63					
24	My child is difficult to please with meals.	**FF**	-0.32	0.84	2.95	0.76	**0.64**	0.19
32	My child is interested in tasting food s/he hasn't tasted before.		0.87					
33	My child decides that s/he doesn't like food, even without tasting it.		-0.73					
	Explained variance:		**10%**					
12	My child's always asking for food.		0.41					
14	If allowed to, my child would eat too much.		0.58					
19	Given the choice, my child would eat most of the time.		0.75					
28	Even if my child is full up, s/he finds room to eat his/her favourite food.	**FR**	0.64	0.85	2.06	0.78	-0.48	**0.66**
34	If given the chance, my child would always have food in his/her mouth.		0.77					
	Explained variance:		**10%**					
1	My child loves food.		0.67					
5	My child is interested in food.		0.74					
20	My child looks forward to mealtimes.	**EF**	0.30	0.83	3.03	0.79	**-0.78**	0.32
22	My child enjoys eating.		0.67					
	Explained variance:		**9%**					
4	My child finishes his/her meal very quickly.		-0.82					
8	My child eats slowly.	**SE**	0.86					
18	My child takes more than 30 minutes to finish a meal.		0.81	0.81	2.92	0.86	**0.74**	-0.05
35	My child eats more and more slowly during the course of a meal.		0.63					
	Explained variance:		**8%**					
2	My child eats more when worried.		0.81					
13	My child eats more when annoyed.	**EOE**	0.84	0.79	1.83	0.63	-0.09	**0.81**
15	My child eats more when anxious.		0.83					
27	My child eats more when s/he has nothing else to do.		0.33					
	Explained variance:		**8%**					
3	My child has a big appetite.		-0.12					
17	My child leaves food on his/her plate at the end of a meal.		0.69					
21	My child gets full before his/her meal is finished.	**SR**	0.81	0.74	2.70	0.68	**0.88**	0.13
26	My child gets full up easily.		0.69					
30	My child cannot eat a meal if s/he has had a snack just before.		0.57					
	Explained variance:		**8%**					
9	My child eats less when s/he is angry.		0.78					
11	My child eats less when s/he is tired.	**EUE**	0.79	0.76	2.46	0.76	0.47	**0.66**
23	My child eats more when s/he is happy.		0.63					
25	My child eats less when s/he is upset.		0.73					
	Explained variance:		**7%**					
6	My child is always asking for a drink.		0.77					
29	If given the chance, my child would drink continuously throughout the day.	**DD**	0.89	0.82	2.21	0.81	0.12	**0.64**
31	If given the chance, my child would always be having a drink.		0.89					
	Explained variance:		**7%**				**35%**	**26%**
	Total explained variance:		**67%**				**62%**	

- Item's position in the original CEBQ; α – Cronbach's Alpha; SD – Standard Deviation
DD – Desire for drinks; EF – Enjoyment of Food; EOE – Emotional Overeating; EUE – Emotional Undereating; FF – Food Fussiness; FR – Food Responsiveness; SE –Slowness in Eating; SR – Satiety Responsiveness

Table 2. Descriptive Statistics (mean, SD) of the 2-factors identified in the Portuguese Child Eating Behaviour Questionnaire according to children and maternal characteristics (n=3562)

		n	%	Factor 1 Appetite Restraint Mean	Factor 1 Appetite Restraint SD	Factor 2 Appetite Disinhibition Mean	Factor 2 Appetite Disinhibition SD
Sociodemographics and family structure	**Maternal age**						
	< 25 years	**541**	**15.2**	2.803	0.633	2.297	0.545
	25 – 34 years	**2361**	**66.3**	2.905	0.597	2.116	0.510
	≥ 35 years	**660**	**18.5**	2.914	0.603	2.094	0.495
	Maternal education						
	< 9 years	**944**	**26.5**	2.824	0.592	2.174	0.550
	9-11 years	**1524**	**42.8**	2.870	0.617	2.161	0.522
	≥12 years	**1094**	**30.7**	2.979	0.588	2.079	0.476
	Family structure at 4 years						
	Two-parents	**3174**	**89.1**	2.899	0.602	2.127	0.508
	One-parent	**365**	**10.2**	2.820	0.627	2.248	0.589
	Other family structure	**23**	**0.70**	2.919	0.542	2.160	0.412
	Number of siblings at 4 years						
	0	**1664**	**46.7**	2.930	0.618	2.143	0.524
	1	**1545**	**43.4**	2.872	0.588	2.142	0.512
	≥ 2	**353**	**9.90**	2.789	0.600	2.112	0.512
	Child's sex						
	Girls	**1755**	**49.3**	2.916	0.608	2.115	0.518
	Boys	**1807**	**50.7**	2.867	0.601	2.164	0.516
Birth Characteristics	**Type of delivery**						
	Vaginal	**2231**	**62.6**	2.874	0.601	2.144	0.523
	Caesarean	**1331**	**37.4**	2.920	0.610	2.131	0.507
	Weight for gestational age						
	Small	**515**	**14.5**	2.926	0.654	2.120	0.516
	Adequate	**2921**	**82.0**	2.886	0.596	2.142	0.515
	Large	**126**	**3.50**	2.871	0.601	2.152	0.570
Child's lifestyle	**Any Breastfeeding duration**						
	Never or < 4 months	**1437**	**40.3**	2.870	0.616	2.159	0.539
	4 – 5 months	**452**	**12.7**	2.913	0.605	2.164	0.513
	≥ 6 months	**1673**	**47.0**	2.903	0.595	2.116	0.498
	Introduction to complementary feeding*						
	< 4 months	**240**	**6.7**	2.896	0.583	2.161	0.573
	4 – 5 months	**1737**	**48.8**	2.926	0.614	2.148	0.500
	≥ 6 months	**721**	**20.2**	2.890	0.574	2.150	0.490
	Complementary feeding - 1st food*						
	soup	**981**	**27.5**	2.847	0.602	2.171	0.530
	cereals porridge/fruit/other	**1687**	**47.4**	2.947	0.595	2.132	0.487
	Media screening at 4 years						
	< 120 min per day	**2540**	**71.3**	2.884	0.603	2.118	0.512
	≥ 120 min per day	**1022**	**28.7**	2.909	0.610	2.192	0.526
	Regular physical activity at 4 years						
	No	**1121**	**31.5**	2.902	0.603	2.150	0.526
	Yes	**2441**	**68.5**	2.886	0.605	2.135	0.513
Child's Anthropometrics	**Child's BMI z-score (WHO) at 4 years**						
	Under/Normal weight	**2445**	**68.6**	3.011	0.589	2.079	0.479
	Overweight/Obese	**1117**	**31.4**	2.629	0.553	2.273	0.570
	Child's waist circumference at 4 years						
	<50.5 cm	**1192**	**33.5**	3.092	0.590	2.055	0.490
	50.5 – 53.5 cm	**1201**	**33.7**	2.915	0.563	2.112	0.503
	≥ 53.6 cm	**1169**	**32,8**	2.661	0.583	2.255	0.551
	Child's fat % at 4 years*						
	<12.8%	**973**	**27.3**	2.994	0.566	2.088	0.486
	12.8 – 19.4%	**1018**	**28.6**	2.917	0.587	2.122	0.495
	≥ 19.5%	**1021**	**28.7**	2.740	0.617	2.232	0.584
Maternal characteristics	**Maternal BMI before pregnancy**						
	Under/Normal weight	**2470**	**69.3**	2.920	0.602	2.126	0.503
	Overweight/Obese	**1092**	**30.7**	2.825	0.606	2.171	0.546
	Maternal BMI change from before pregnancy to 4 years after birth						
	Maintaining under/normalweight or decreasing	**1838**	**51.6**	2.861	0.607	2.151	0.534
	Maintaining high BMI or increasing	**1724**	**48.4**	2.923	0.601	2.127	0.499
	Smoking during pregnancy						
	Never smoker	**2825**	**79.3**	2.904	0.601	2.117	0.510
	Ever smoker	**737**	**20.7**	2.839	0.616	2.226	0.536

*These variables do not add to 3562 due to missing data;

BMI – Body Mass Index; CI – Confidence Interval; WHO – World Health Organization; SD: standard deviation

Table 3. Univariate associations of children and maternal characteristics with Eating Behaviours related to Appetite of 7 years old children (n = 3562)

		Appetite Restraint			Appetite Disinhibition		
		β	95% CI		β	95% CI	
Sociodemographics and family structure	**Maternal age**						
	< 25 years	ref			ref		
	25 – 34 years	**0.101**	**0.045**	**0.158**	**-0.181**	**-0.229;**	**-0.133**
	≥ 35 years	**0.111**	**0.042**	**0.179**	**-0.203**	**-0.261;**	**-0.145**
	Maternal education						
	< 9 years	ref			ref		
	9-11 years	0.046	-0.003	0.095	-0.013	-0.055;	0.029
	≥12 years	**0.155**	**0.102**	**0.207**	**-0.095**	**-0.140;**	**-0.050**
	Family structure at 4 years						
	Two-parents	ref			ref		
	One-parent	**-0.079**	**-0.144**	**-0.013**	**0.121**	**0.065;**	**0.177**
	Other family structure	0.020	-0.228	0.268	0.033	-0.179;	0.245
	Number of siblings at 4 years						
	0	ref			ref		
	1	**-0.058**	**-0.100**	**-0.017**	-0.001	-0.037;	0.035
	≥ 2	**-0.141**	**-0.210**	**-0.072**	-0.031	-0.091;	0.028
	Child's sex						
	Girls	ref			ref		
	Boys	**-0.050**	**-0.089**	**-0.010**	**0.049**	**0.015;**	**0.083**
Birth Characteristics	**Type of delivery**						
	Vaginal	ref			ref		
	Caesarean	**0.046**	**0.005**	**0.087**	-0.013	-0.048;	0.022
	Weight for gestational age						
	Small	ref			ref		
	Adequate	-0.041	-0.097	0.016	0.022	-0.026;	0.071
	Large	-0.055	-0.173	0.062	0.032	-0.069;	0.133
Child's lifestyle	**Any Breastfeeding duration**						
	Never or < 4 months	ref			ref		
	4 – 5 months	0.043	-0.021	0.107	0.005	-0.049;	0.060
	≥ 6 months	0.034	-0.009	0.076	**-0.043**	**-0.080;**	**-0.007**
	Introduction to complementary feeding*						
	< 4 months	ref			ref		
	4 – 5 months	0.030	-0.051	0.111	-0.012	-0.081;	0.056
	≥ 6 months	-0.007	-0.095	0.081	-0.010	-0.084;	0.063
	Complementary feeding - 1st food*						
	soup	ref			ref		
	cereals porridge/fruit/other	**0.101**	**0.054**	**0.148**	-0.038	-0.078;	0.001
	Media screening at 4 years						
	< 120 min per day	ref			ref		
	≥ 120 min per day	0.025	-0.018	0.069	**0.073**	**0.036;**	**0.111**
	Regular physical activity at 4 years						
	No	ref			ref		
	Yes	-0.016	-0.059	0.027	-0.015	-0.052;	0.022
Child's Anthropometrics	**Child's BMI z-score (WHO) at 4 years**						
	Under/Normal weight	ref			ref		
	Overweight/Obese	**-0.381**	**-0.422**	**-0.340**	**0.194**	**0.158;**	**0.230**
	Child's waist circumference at 4 years						
	<50.5 cm	ref			ref		
	50.5 – 53.5 cm	**-0.177**	**-0.224**	**-0.131**	**0.057**	**0.016;**	**0.098**
	≥ 53.6 cm	-0.431	-0.478	-0.385	0.200	0.159;	0.241
	Child's fat % at 4 years*						
	<12.8%	ref			ref		
	12.8 – 19.4%	**-0.076**	**-0.128**	**-0.025**	0.034	-0.011;	0.079
	≥ 19.5%	**-0.254**	**-0.306**	**-0.202**	0.144	0.098;	0.189
Maternal Characteristics	**Maternal BMI before pregnancy**						
	Under/Normal weight	ref			ref		
	Overweight/Obese	**-0.095**	**-0.138**	**-0.052**	**0.045**	**0.008;**	**0.082**
	Maternal BMI change from before pregnancy to 4 years after birth						
	Maintaining under/normal weight or decreasing	ref			ref		
	Maintaining high BMI or increasing	**0.062**	**0.023**	**0.102**	-0.023	-0.057;	0.011
	Smoking during pregnancy						
	Never smoker	ref			ref		
	Ever smoker	**-0.065**	**-0.114**	**-0.016**	**0.110**	**0.068;**	**0.151**

*For these variables, sample sizes are lower (as described in table 2)
BMI – Body Mass Index; CI – Confidence Interval; WHO – World Health Organization;
Significant associations are in bold type.

Table 4. Multivariate associations of children and maternal characteristics with Eating Behaviours related to Appetite 7 years old children (n = 3562)

		Appetite Restraint			Appetite Disinhibition		
		β*	95% CI		β*	95% CI	
Sociodemographics and family structure	**Maternal age**						
	< 25 years	ref			ref		
	25 – 34 years	**0.083**	**0.024**	**0.142**	**-0.161**	**-0.212;**	**-0.110**
	≥ 35 years	**0.127**	**0.054**	**0.201**	**-0.187**	**-0.250;**	**-0.125**
	Maternal education						
	< 9 years	ref			ref		
	9-11 years	0.028	-0.021	0.078	-0.017	-0.059;	0.025
	≥12 years	**0.109**	**0.055**	**0.164**	**-0.056**	**-0.102;**	**-0.009**
	Family structure at 4 years						
	Two-parents	ref			ref		
	One-parent	**-0.074**	**-0.140**	**-0.007**	**0.090**	**0.034;**	**0.147**
	Other family structure	0.007	-0.239	0.253	-0.001	-0.211;	0.209
	Number of siblings at 4 years						
	0	ref			ref		
	1	**-0.077**	**-0.120**	**-0.040**	0.028	-0.009;	0.064
	≥ 2	**-0.152**	**-0.224**	**-0.081**	-0.006	-0.067;	0.055
	Child's sex						
	Girls	ref			ref		
	Boys	**-0.053**	**-0.092**	**-0.014**	**0.051**	**0.018;**	**0.085**
Birth Characteristics	**Type of delivery**						
	Vaginal	ref			ref		
	Caesarean	0.028	-0.013	0.069	0.002	-0.033;	0.037
	Weight for gestational age						
	Small	ref			ref		
	Adequate	-0.039	-0.096	0.017	0.029	-0.019;	0.077
	Large	-0.043	-0.160	0.074	0.042	-0.058;	0.142
Child's lifestyle	**Any Breastfeeding duration**						
	Never or < 4 months	ref			ref		
	4 – 5 months	0.029	-0.035	0.092	0.021	-0.033;	0.075
	≥ 6 months	0.021	-0.021	0.064	-0.025	-0.061;	0.012
	Introduction to complementary feeding*						
	< 4 months	ref			ref		
	4 – 5 months	-0.004	-0.084	0.077	0.008	-0.059;	0.076
	≥ 6 months	-0.026	-0.112	0.061	0.012	-0.067;	0.078
	Complementary feeding - 1st food*						
	soup	ref			ref		
	cereals porridge/fruit/other	**0.077**	**0.030**	**0.124**	-0.018	-0.057;	0.022
	Media screening at 4 years						
	< 120 min per day	ref			ref		
	≥ 120 min per day	**0.048**	**0.004**	**0.092**	**0.055**	**0.018;**	**0.093**
	Regular physical activity at 4 years						
	No	ref			ref		
	Yes	**-0.048**	**-0.091**	**-0.005**	0.005	-0.032;	0.042
Child's Anthropometrics	**Child's BMI z-score (WHO) at 4 years**						
	Under/Normal weight	ref			ref		
	Overweight/Obese	**-0.368**	**-0.410**	**-0.327**	**0.183**	**0.146;**	**0.219**
	Child's waist circumference at 4 years						
	<50.5 cm	ref			ref		
	50.5 – 53.5 cm	**-0.171**	**-0.217**	**-0.125**	**0.052**	**0.011;**	**0.092**
	≥ 53.6 cm	**-0.418**	**-0.465**	**-0.371**	**0.188**	**0.147;**	**0.229**
	Child's fat % at 4 years*						
	<12.8%	ref			ref		
	12.8 – 19.4%	**-0.092**	**-0.144**	**-0.041**	**0.047**	**0.002;**	**0.092**
	≥ 19.5%	**-0.276**	**-0.329**	**-0.224**	**0.160**	**0.114;**	**0.206**
Maternal Characteristics	**Maternal BMI before pregnancy**						
	Under/Normal weight	ref			ref		
	Overweight/Obese	**-0.077**	**-0.12**	**-0.033**	**0.046**	**0.009;**	**0.083**
	Maternal BMI change from before pregnancy to 4 years after birth						
	Maintaining under/normal weight or decreasing	ref			ref		
	Maintaining high BMI or increasing	**0.043**	**0.003**	**0.083**	-0.020	-0.055;	0.014
	Smoking during pregnancy						
	Never smoker	ref			ref		
	Ever smoker	-0.045	-0.095	0.004	**0.082**	**0.039;**	**0.124**

* β adjusted for maternal age, education and BMI before birth, family structure and number of siblings at 4 years old and child's sex).

** For these variables, sample sizes are lower (as described in table 2); BMI – Body Mass Index; CI – Confidence Interval; WHO – World Health Organization; Significant associations are in bold type.

Appendix

Correspondence between the original items of the Child Eating Behaviour Questionnaire (CEBQ) and the current Portuguese version (P-CEBQ) used in 7 years old children

Original Items of the CEBQ	Items of the P-CEBQ	CEBQ sub-domains
1. My child loves food.	1. O meu filho(a) adora comida.	EF
2. My child eats more when worried.	2. O meu filho(a) come mais quando anda preocupado(a).	EOE
3. My child has a big appetite. **R**	3. O meu filho(a) tem muito apetite. **R**	SR
4. My child finishes his/her meal very quickly. **R**	4. O meu filho(a) termina as refeições muito rapidamente. **R**	SE
5. My child is interested in food.	5. O meu filho(a) interessa-se por comida.	EF
6. My child is always asking for a drink.	6. O meu filho(a) anda sempre a pedir para beber.	DD
7. My child refuses new foods at first.	7. Perante novos alimentos o meu filho(a) começa por recusá-los.	FF
8. My child eats slowly.	8. O meu filho(a) come lentamente.	SE
9. My child eats less when s/he is angry.	9. O meu filho(a) come menos quando está zangado(a).	EUE
10. My child enjoys tasting new foods. **R**	10. O meu filho(a) gosta de experimentar novos alimentos. **R**	FF
11. My child eats less when s/he is tired.	11. O meu filho(a) come menos quando está cansado(a).	EUE
12. My child's always asking for food.	12. O meu filho(a) está sempre a pedir comida.	FR
13. My child eats more when annoyed.	13. O meu filho(a) come mais quando está aborrecido(a).	EOE
14. If allowed to, my child would eat too much.	14. Se o deixassem o meu filho(a) comeria demais.	FR
15. My child eats more when anxious.	15. O meu filho(a) come mais quando está ansioso(a).	EOE
16. My child enjoys a wide variety of foods. **R**	16. O meu filho(a) gosta de uma grande variedade de alimentos. **R**	FF
17. My child leaves food on his/her plate at the end of a meal.	17. O meu filho(a) deixa comida no prato no fim das refeições.	SR
18. My child takes more than 30 minutes to finish a meal.	18. O meu filho(a) demora mais que 30 minutos para terminar uma refeição.	SE
19. Given the choice, my child would eat most of the time.	19. Se tivesse oportunidade o meu filho(a) passaria a maior parte do tempo a comer.	FR
20. My child looks forward to mealtimes.	20. O meu filho(a) está sempre à espera da hora das refeições	EF
21. My child gets full before his/her meal is finished.	21. O meu filho(a) fica cheio/saciado(a) antes de terminar a refeição.	SR
22. My child enjoys eating.	22. O meu filho(a) tem prazer em comer.	EF
23. My child eats more when s/he is happy.	23. O meu filho(a) come mais quando está feliz.	EUE
24. My child is difficult to please with meals.	24. O meu filho(a) é difícil de agradar com as refeições.	FF
25. My child eats less when s/he is upset.	25. O meu filho(a) come menos quando anda chateado(a).	EUE
26. My child gets full up easily.	26. O meu filho(a) fica cheio/saciado(a) muito facilmente.	SR
27. My child eats more when s/he has nothing else to do.	27. O meu filho(a) come mais quando não tem nada para fazer.	EOE
28. Even if my child is full up, s/he finds room to eat his/her favourite food.	28. Mesmo se já está cheio o meu filho(a) arranja espaço para comer um alimento preferido.	FR
29. If given the chance, my child would drink continuously throughout the day.	29. Se tivesse oportunidade o meu filho(a) passaria o dia a beber continuamente.	DD
30. My child cannot eat a meal if s/he has had a snack just before.	30. O meu filho(a) é incapaz de comer a refeição se antes tiver comido alguma coisa.	SR
31. If given the chance, my child would always be having a drink.	31. Se tivesse oportunidade o meu filho(a) passaria a maior parte do tempo a beber.	DD
32. My child is interested in tasting food s/he hasn't tasted before. **R**	32. O meu filho(a) interessa-se por experimentar alimentos que nunca provou antes. **R**	FF
33. My child decides that s/he doesn't like food, even without tasting it.	33. O meu filho(a) decide que não gosta de um alimento mesmo que nunca o tenha provado.	FF
34. If given the chance, my child would always have food in his/her mouth.	34. Se tivesse oportunidade o meu filho(a) estaria sempre com comida na boca.	FR
35. My child eats more and more slowly during the course of a meal.	35. O meu filho(a) come cada vez mais devagar ao longo da refeição.	SE

R – Reversed item; DD – Desire for drinks; EF – Enjoyment of Food; EOE – Emotional Overeating; EUE – Emotional Undereating; FF – Food Fussiness; FR – Food Responsiveness; SE – Slowness in Eating; SR – Satiety Responsiveness

PAPER II

Children's dietary patterns at 4 years: Are they associated with appetite-related eating behaviours at 7 years old?

Children's dietary patterns at 4 years: are they associated with appetite-related eating behaviours at 7 years old?

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Abstract

Introduction: Previous observational evidence, supported by plausible biological mechanisms, have highlighted a possible relation between children's dietary intake and certain aspects of eating behaviours, but most studies rely on the effect of single foods and have a cross-sectional design.

Objective: To quantify the association between dietary patterns established at 4 years old and eating behaviours related to appetite identified at 7 years of age.

Methods: Participants are part of the Generation XXI population-based birth cohort (n=8647 children). Approximately 86% and 80% of all children participated in the follow-up assessments at 4 and at 7 years of age, where trained interviewers administered a structured questionnaire on socio-demographic characteristics, children's health and lifestyles. Anthropometrics of both children and mothers were measured. Children's food intake at 4 years old was assessed by a Food Frequency Questionnaire and 3 dietary patterns were fitted by Latent Class Analysis: Healthier, Lower in Healthy Foods and Energy Dense Foods (EDF)- Dairy. A Portuguese version of the original Children's Eating Behaviour Questionnaire (CEBQ) was self-completed by mothers at 7 years old. This version has previously showed good psychometric properties and allowed to aggregate the 8 CEBQ sub-domains in 2 wider dimensions: Appetite Restraint (including the sub-domains Satiety Responsiveness, Slowness in Eating, Food Fussiness and Enjoyment of Food) and Appetite Disinhibition (including the sub-domains Food Responsiveness, Emotional Overeating, Emotional Undereating and Desire for Drinks). Generalized linear models were used to estimate the associations between children's dietary patterns and appetite-related eating behaviours, after adjustment for maternal age, education and body mass index (BMI) before pregnancy (complete data available for 4148 children). Interaction was tested and stratified analyses were further conducted.

Results: In multivariate analysis, children belonging to the EDF-Dairy (β=0.072, 95%CI: 0.021; 0.124) and to the Lower in Healthy Foods pattern (β=0.139, 95%CI: 0.097; 0.182) scored higher on Appetite Restraint, compared with children in the Healthier dietary pattern Scores in the Appetite Disinhibition dimension were increasingly higher in children following the Lower in Healthy Foods (β=0.072, 95%CI: 0.036; 0.107) and also the EDF-Dairy (β=0.137, 95%CI: 0.093; 0.180) dietary patterns. An interaction effect between dietary patterns and children's waist circumference at 4 years old was found (p=0.020). Stratified

analysis revealed that the positive association between the EDF-Dairy pattern and Appetite Restraint was only significant among children within the highest waist circumference tertile.

Conclusions: Children following more unhealthy dietary patterns early in life may develop more problematic eating behaviours in later childhood. It seems relevant to promote, at the home environment, early family feeding practices supportive of healthy dietary habits and eating behaviours during childhood.

Keywords: CEBQ, children, cohort studies, feeding behaviours, food habits.

Introduction

Eating behaviours develop early in childhood and show stability through this stage of life[1-3]. Dietary habits and patterns are also established early and persist into adulthood[4]. Children's diet and eating behaviours are closely connected, namely through the social interactions surrounding food and eating moments. The transmission of cultural and familial beliefs, attitudes and feeding practices allows children to learn what, when and how much to eat[2].

A heathy eating behaviour is characterized by eating when feeling hungry and at regular moments, to allow physiological growth and energy expenditure[5]. It is increasing, however, the prevalence of problematic eating behaviours, which may vary from picky eating (the consumption of an insufficient amount or an inadequate variety of foods through rejection of food items[6]) to overeating and disinhibited eating[7,8]. The relation between several aspects of diet and these eating behaviours has been little explored in previous research and some inconsistencies between their findings are yet to be clarified. A cross-sectional study has found that picky eater girls at 9 years old consumed significantly fewer servings of fruits and vegetables (FV), but also fatty and sweet foods[6]. A more recent prospective study[9], however, found that 4 years old picky eater children consumed, at 14 months, less vegetables, wholegrain products, fish and meat than children not exhibiting this eating behaviour, but also more confectionary, snacks and fast-food. Emotional eating (excessive eating in response to negative emotional states such as anger, fear or anxiety[10]) has been cross-sectionally associated with a higher consumption of sweet foods among 9 - 12[13] and 9 - 10[14] years old children, especially in girls[11] and also with snacks[16], fatty foods[10] and sugar-sweetened beverages[12] in adolescents, while others did not find this association[13]. Cross-sectional studies also revealed associations between external eating (eating in reaction to external food stimuli)[13] and sweet foods[14,15] and even with soft drink consumption[15] in adolescents.

These associations with more palatable and pleasurable foods (comfort foods) have been under studied and it has been hypothesized that emotions may regulate eating, but the opposite may be also true[16]. Diet composition and dietary components have showed to influence certain mechanisms of appetite, namely those related with satiety[17]. Proteins, for example, are known to induce satiety, increase secretion of gastrointestinal hormones and increase diet-induced thermogenesis[18]. Also, a deregulation of caloric intake towards a positive energy balance has been showed to depend on the energy density and palatability of the diet[19]. Sugar-sweetened beverages could be also on the basis of body fat deregulation, as they may have a relatively high contribution to energy intake in children, particularly if consumed between meals[20].

Most associations found between diet and eating behaviours have been focused on the effect that single foods have in eating behaviours, not taking into account the complexity of dietary intake. Dietary patterns have become increasingly accepted as a reliable tool to characterize overall diet[21], by expressing cumulative and interactions effects of foods and nutrients, rather than their individual and non-independent effect[22]. Research on this topic is very scarce; one cross-sectional study[23] assessed the association between dietary patterns (sweet foods, fatty foods, snacks, and FV) and eating behaviours in school-aged children, but no association was found.

Our hypothesis is that dietary patterns established at 4 years old children are associated with certain appetite-related eating behaviours in later childhood (at 7 years of age).

Methods

Study Design and Participants

This study is embedded in Generation XXI, a prospective population-based birth cohort, previously described elsewhere[24]. Participants were recruited in all public maternity units of Porto, Portugal between 2005 and 2006. Of the invited mothers, 91.4% agreed to participate totalling a sample of 8495 mothers and their 8647 children. All families were invited to attend the second (2009-2011) and third (2012-2014) cohort evaluations, when children were aged 4 and 7 years old, respectively. On both occasions, 86% and approximately 80% of all children were re-evaluated, respectively.

The study protocol was approved by the local ethical committee (Ethical Committee of São João Hospital/University of Porto Medical School) and by the Portuguese Authority of Data Protection. Legal representatives of each participant were informed about the benefits and potential discomfort, and written informed consent was obtained for the collection of information at each follow-up assessment.

Children and maternal characteristics

Trained researchers administered in face-to-face interviews, a structured questionnaire on socio-demographic characteristics, children's prenatal care, health and lifestyles. At baseline, the following variables, with interest for the current study, were collected: maternal age (categorized into <25 years, 25-29 years and >29 years) and education (number of completed schooling years at delivery, categorized into ≤9 years, 10-12 years and >12 years). Self-reported body mass index (BMI) before pregnancy (through self-reported height

and weight; recoded into under/normal weight and overweight/obese according to the World Health Organization (WHO) criteria[25]) and child's sex were also recorded.

Child's anthropometrics on height, weight and waist circumference were measured during the physical examinations at 4 and 7 years old. Weight was measured in light clothing and without shoes using a digital scale (TANITA®) and was recorded to the nearest 0.1 kg. Height was measured as the distance from the top of the head to the bottom of the feet without shoes using a fixed stadiometer (SECA®) to the nearest 0.1 cm. The children's BMI was defined as weight in kg divided by squared height in m^2. Each child was then classified according to the age- and sex-specific BMI standard z-scores developed by the WHO[26] and recoded into under/normal weight (BMI <2 SD) and overweight/obese (BMI ≥2 SD). Waist circumference was measured at the umbilicus level, with abdomen relaxed, to the nearest 0.1 cm, with the child in a standing position, arms at the sides and feet positioned together. This variable was recoded into thirds (<50.5 cm; 50.5–53.5 cm; ≥53.6 cm).

Children's Eating Behaviours

Eating behaviours were assessed by self-completion of the Children's Eating Behaviour Questionnaire (CEBQ), during the evaluation at 7 years old (94% of the questionnaires were answered by mothers). The original CEBQ[27] was designed to assess variation in eating styles among children and includes 35 items, answered on a five-point Likert scale ranging from 1=never to 5=always. This questionnaire consists of eight sub-domains related to distinct eating behaviours. Satiety Responsiveness reflects the ability to regulate the amount of food eaten, based on perceived fullness[28,29]; Slowness in Eating measures the speed of eating during the course of a meal and reflects a gradually reduced interest in a meal[29]; Enjoyment of Food represents a general interest in food and Food Responsiveness measures eating in response to external food cues[28]. Food Fussiness measures a lack of interest in food and unwillingness to try new foods and the sub-domain Desire for Drinks, increased desire to have drinks, particularly sugar-sweetened drinks[30]. The sub-domains Emotional Overeating and Emotional Undereating are characterized by either increased or decreased eating in response to negative emotions, such as anger and anxiety[29,31]. The CEBQ has demonstrated stability over time and good psychometric properties (internal consistency, concurrent validity with actual eating behaviour and reliability[1,3,27-29,31-33]).

The psychometric characteristics of the CEBQ were assessed in a previous study (results not published yet) and it has showed to have good psychometric properties in 7 years old Portuguese children. In the Portuguese version of the CEBQ (P-CEBQ), a 2-factor final solution was identified, explaining 62% of the total variance, with good internal consistency (Cronbach's Alpha was 0.77 for factor 1 and 0.64 for factor 2). The factors were

named Appetite Restraint (factor 1, mean=2.88; SD=0.61), including the sub-domains Satiety Responsiveness, Slowness in Eating, Food Fussiness and Enjoyment of Food and Appetite Disinhibition (factor 2, mean=2.15; 0.52), including the sub-domains Food Responsiveness, Emotional Overeating, Emotional Undereating and Desire for Drinks. These domains showed good construct validity and good reliability (intra-class correlation coefficient (ICC) varied from 0.51 to 0.85).

In the Generation XXI sample, responses to the P-CEBQ were available for 5786 children. Of these, 4485 questionnaires had all items fulfilled, and after retrieving missing data 5449 were considered valid. In individuals with missing data in less than 50% of the items, data was recovered by replacement for the average of the remaining questions within each sub-domain.

Children's Dietary Patterns

Children's dietary patterns within the Generation XXI cohort have been defined at 4 years old children in a previous study (results not published yet). Children's dietary intake was evaluated through a 35-item qualitative food frequency questionnaire (FFQ), assessing usual consumption in the previous six months. Nine response options were considered in face-to-face interviews, varying between never to 4 or more times per day. To the definition of children's dietary patterns, 12 food items and 5 food groups were further established: fruit, vegetable soup, vegetables on a plate, cheese, fish, red meats, processed meats, rice-pasta-potatoes, bread, butter-margarine, crisps, pizza-burger, milk (whole, semi-skimmed, skimmed), yoghurt (sugared, non-sugared), white meat and eggs (poultry, rabbit, eggs), sweets (cakes, chocolate, sugar, candies) and soft drinks (sweetened beverages, carbonated or not). In a sub-sample of approximately 300 children, 3-day food records were also completed, and correlations between key food groups measured by the FFQ and food diaries were calculated to assess the validity of the FFQ. Pearson's coefficients showed a weak-to-moderate correlation for most food groups (results not showed).

Dietary patterns were identified by latent class analysis and the number of patterns was defined according to the Bayesian information criteria (BIC), representing mutually exclusive groups of children sharing the same dietary pattern. The analysis used the 17 food items/groups, categorized into lower (1st quintile), intermediate (2nd-4th quintiles aggregated) and higher (5th quintile) consumption frequencies. Profiles of probabilities in 17 food items/groups each item response (lower, intermediate or higher consumption frequencies), conditionally on pattern membership, were used to interpret children's dietary patterns.

Three dietary patterns were identified, based on the item profiles within each class and on the respective mean daily consumption frequencies. Pattern 1, Energy Dense Foods

(EDF)-Dairy, was characterized as a dietary pattern higher in EDF (as crisps, pizza-burgers, sweets, and soft drinks), dairy (milk, yoghurt, cheese), red and processed meats, bread and butter or margarine intake; pattern 2, Lower in Healthy Foods, was characterized as a dietary pattern lower in healthy foods, as FV, fish, white meat and eggs, bread, butter or margarine intake and intermediate in EDF; and pattern 3, Healthier was characterized by higher intake of FV (vegetables category including vegetable soup and vegetables on the plate), fish, white meat and eggs, and lower intake of red and processed meats and EDF.

The final sample included in the current study includes 4148 children, for whom the CEBQ at the evaluation at 7 years old was filled, and for which complete information on dietary patterns at 4 years old and on key variables (maternal age, education and maternal BMI before pregnancy) was available. Exclusion criteria dropped out 154 twins and 26 subjects with congenital anomalies or diseases that might influence dietary intake (cerebral palsy, celiac disease, food allergy, food intolerance and phenylketonuria).

We compared characteristics of the present study sample (n=4148) with the remaining cohort (n=4499) at baseline, and no significant differences were found for child's sex (50.5% vs. 51.4% of boys, p=0.439) and maternal BMI before pregnancy (mean = 23.92 vs. mean = 23.83 kg/m^2, p=0.534). However, differences were found for maternal age and education: mothers in this study were slightly older (mean = 28.80; standard deviation (SD) = 5.21 vs. mean = 28.34; SD = 6.38) and more educated (mean = 11.27; SD = 4.25 vs. mean = 9.69; SD = 4.12) than mothers in the remaining cohort. The Cohen's effect size values were lower than 0.4[34], suggesting that the magnitude of the differences was not high (i.e. differences were at most part due to the large sample size than to large differences between participant's characteristics)[34].

Statistical Analysis

Descriptive statistics (mean, SD and proportions, whenever applicable) of children and maternal characteristics at baseline, 4 and 7 years old were computed.

Generalized linear models (GLM) (β regression coefficients and the respective 95% confidence intervals (95%CI)) were performed to estimate the association between children's dietary patterns at 4 years old and the scores in the two Appetite domains previously identified (Appetite Restraint and Appetite Disinhibition) at 7 years old. Three models were drawn: one represents crude associations between the dietary patterns and both dimensions (table 3, model 0), the second represents the same associations adjusted for socio-demographic characteristics (maternal age and education) and maternal BMI before pregnancy (table 3, model 1), and the third evaluates a further adjustment for child's BMI z-score at 4 years (table 3, model 2). Furthermore, adjustments for child´s sex, family structure

and number of siblings were also tested, but the associations remained unchanged (results not showed).

In addition, it was examined whether some exposures modified the association between dietary patterns and appetite-related eating behaviours. This effect was tested by adding interaction terms into the regression analysis (model 1); only an interaction between child's waist circumference and dietary patterns at 4 years old in the association with eating behaviors was found. Stratified analysis concerning this variable was further conducted. Significance level was set at 5%. SPSS Statistics 21.0 (SPSS Inc., Chicago, IL, USA) was used to perform all the statistical analyses.

Results

Participant's characteristics included in this study (n=4148) are presented in Table 1. At baseline, the majority of mothers were aged between 25-34 years old (66.4%) and had between 9 and 11 years of education (42.8%). Approximately one third were overweight or obese before pregnancy (30.8%). Approximately 51% of children were boys and more than one third (37.2%) were overweight or obese at 7 years old. Within the present sample, 41.4% of children were characterized by having the Healthier dietary pattern, 38.0% by having the Lower in Healthy Foods dietary pattern and 20.6% the EDF-Dairy dietary pattern at 4 years old. At 7 years old, children's mean score in the Appetite Restraint dimension was 2.89 (SD = 0.61) and in the Appetite Disinhibition was 2.15 (SD = 0.52); the score could range from 1 to 5.

The mean score of each eating behaviour dimension, according to dietary patterns, is described in Table 2. Significant differences in Appetite Restraint and Appetite Disinhibition mean scores (assessed at 7 years) were found. Children belonging to the Lower in Healthy Foods pattern scored significantly higher ($p<0.001$) in Appetite Restraint (mean = 2.96; SD = 0.60), followed by the EDF-Dairy (2.87; SD = 0.60) and the Healthier dietary patterns (2.85; SD = 0.62). In contrast, scores in the Appetite Disinhibition dimension were significantly higher ($p<0.001$) in children following the EDF-Dairy dietary pattern (2.25; SD = 0.54), compared with children in the Lower in Healthy Foods (2.17; SD = 0.51) and Healthier (2.08; SD = 0.50) dietary patterns.

Univariate regression analysis (table 3, model 0) shows a significant positive association between the dietary pattern Lower in Healthy Foods and the Appetite Restraint dimension ($\beta=0.104$, 95%CI: 0.063; 0.146), using as reference children following the Healthier dietary pattern. Significant positive associations were also found between this

dietary pattern (β=0.089, 95%CI: 0.054; 0.124) and Appetite Disinhibition and between the EDF-Dairy dietary pattern and Appetite Disinhibition (β=0.168, 95%CI: 0.126; 0.210).

In multivariate analysis (table 3, model 1) (associations were controlled for maternal age, education and maternal BMI before pregnancy), comparatively to the Healthier dietary pattern, children belonging to the EDF-Dairy (β=0.072, 95%CI: 0.021; 0.124) and to the Lower in Healthy Foods pattern (β=0.139, 95%CI: 0.097; 0.182) scored higher on Appetite Restraint. Scores in the Appetite Disinhibition dimension were increasingly higher in children following the Lower in Healthy Foods (β=0.072, 95%CI: 0.036; 0.107) and also the EDF-Dairy (β=0.137, 95%CI: 0.093; 0.180) dietary patterns. Further adjustment for child's BMI z-score at 4 years old (table 3, model 2), child's sex, family structure and number of siblings did not change the magnitude of the associations (results not showed).

An interaction effect between child's waist circumference and EDF-Dairy dietary pattern at 4 years old on the Appetite Restraint dimension was found (p=0.020). For the Appetite Disinhibition dimension, no interaction effect was found (p>0.05 for the EDF-Dairy and for the Lower in Healthy Foods pattern). Subsequent stratified analysis revealed that the positive association between the EDF-Dairy pattern (Healthier dietary pattern as reference category) and the Appetite Restraint was only significant among children within the highest waist circumference tertile (table 4).

Discussion

This study examined the association between children's dietary patterns at 4 years old and eating behaviours related to appetite at 7 years old in a large sample of children belonging to a population-based cohort. Our results suggest that children presenting the Lower in Healthy Foods and the EDF-Dairy dietary patterns at 4 years of age, when compared with those in the Healthier dietary pattern, showed higher Appetite Restraint and Appetite Disinhibition scores at 7 years of age. These findings, suggest that children following more unhealthy dietary patterns early in life may develop more problematic eating behaviours in later childhood. It was also highlighted an interaction between dietary patterns and child's waist circumference at 4 years old on the Appetite Restraint dimension.

The dietary pattern Lower in Healthy Foods is characterized by a low food intake in general, fact that may explain the positive association with the Appetite Restraint dimension, defined by fussiness about food, slowness in eating, responsiveness to internal satiety cues and low enjoyment of food. Among the less eaten food products in this dietary pattern are fish, bread, butter and FV. Concurring with the present findings, FV intake have been negatively associated with picky eating[35], food neophobia[36] and Slowness in Eating, Satiety Responsiveness and Food Fussiness[23]. This dietary pattern is also characterized by an

intermediate consumption of EDF, such as fast-food, crisps, sweets and soft drinks. Interestingly, a recent study[9] has drawn a profile of "fussy eater" (with appetitive traits very similar to the ones met in the Appetite Restraint dimension) in which it was evidenced, along with a low consumption of wholegrain products, fish, meat and vegetables, a high intake of confectionary and chips and fast-food, corroborating our results.

In addition, the intermediate and high consumption of EDF, characteristic of both dietary patterns identified (Lower in Healthy Foods and EDF-Dairy, respectively) may help to justify the positive associations with the Appetite Disinhibition dimension, that is strongly based on the influence of emotional and external cues as the food smell and palatability in triggering appetite. Previous studies in children and adolescents have associated emotional eating with the consumption of sweet foods[10,14,15,37] and drinks[12] (and even fatty foods[10,38]) and external eating with the consumption of sweet foods[14,15] and soft drinks[15].

In particular, the EDF-Dairy dietary pattern is characterized by a high intake of dairy, bread, butter and EDF and an intermediate FV intake and was the most positively associated with Appetite Disinhibition. Similar findings have been reported previously. Emotional Overeating and Desire for Drinks, two sub-domains of this appetite dimension, have been positively associated with snacks intake in children of 7 - 10 years old[23]. The sub-dimension Desire for Drinks has been also associated with a higher consumption of sweetened[23,30] and low-calorie[30] soft drinks and failure to compensate energy intake provided by these drinks[30], suggesting the co-existence in these children of a low responsiveness to internal food cues and a higher one to external food cues (namely food sweetness). Desire for Drinks was also associated in the latter study with the consumption of milk, highlighting the relation between a high consumption of dairy and higher appetite disinhibition found in the present study.

An interaction effect between the EDF-Dairy pattern and waist circumference on the Appetite Restraint dimension was found. The findings suggest that, among children presenting the EDF-Dairy pattern at 4 years old, those with the highest waist circumference and thus higher adiposity, develop significantly more Appetite Restraint at 7 years old. Previous studies described that parent's concerns with their child's weight and high adiposity may lead to restrictive feeding practices over child's diets (namely restricting more EDF intake) and also using more pressure to eat more healthy foods[39]. Despite in the short-term such practices may promote an healthier consumption (e.g. of vegetables), in the long-run they have been associated with reduced preference for these foods[40]. Also, parental control may have the counterproductive effect of enhancing preferences for high-fat foods and EDF and disrupt children's regulation of energy intake, diminishing responsiveness to internal cues of hunger and satiety[2,39,41].

The association between dietary patterns and eating behaviours was approached prospectively in this study, enabling to overcome some limitations of previous studies. The

present study has as strengths the definition of dietary patterns to characterize food intake (instead of studying single foods) and its large sample size, arising from the fact that this study is based on a population-based birth cohort. Some limitations, however, should be mentioned. First, both dietary intake and eating behaviours were assessed subjectively, based on parental reports (FFQ and CEBQ, respectively). This may lead to social desirability bias, associated, in the case of dietary patterns, with over-reporting of healthy foods and/or under-reporting of unhealthy foods, possibly leading to an underestimation of the studied associations. However, for most food groups, weak-to-moderate correlations between the FFQ and 3-day food diaries were found, supporting the validity of the FFQ. As to the assessment of eating behaviours, this should not be of concern in this study as CEBQ has showed good correspondence with objective measures[28]. In addition, self-completed questionnaires often present low response rates, as occurred in the present study regarding CEBQ, and despite it was possible to recover missing data in cases with more than 50% of the items fulfilled, this fact limited the size of the study sample. Also, when compared with the whole cohort, children in the current sample had mothers slightly older and more educated, which may reveal a selective response bias. According to the Cohen coefficient[34] however, these differences were not relevant.

Conclusions

In conclusion, the Lower in Healthy Foods and the EDF-Dairy dietary patterns at 4 years of age were associated with increased Appetite Restraint and Appetite Disinhibition scores at 7 years of age, comparatively to the Healthier dietary pattern.

These findings highlight that children following more unhealthy dietary patterns early in life may develop more problematic eating behaviours in later childhood. The interaction between dietary patterns and waist circumference at 4 years old may suggest a possible influence of parental feeding practices on these associations. Further studies focusing on the associations between children's dietary patterns and eating behaviours and parental feeding practices should clarify some findings from the current study. Above all, it seems relevant to promote, at the home environment, early family feeding practices and behaviours supportive of healthy dietary habits and eating behaviours during childhood.

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Table 1. Children and maternal characteristics of the study Generation XXI sample (n=4148).

Maternal age at baseline (years), n (%)		
< 25 years	634	(15.3)
25 – 34 years	2755	(66.4)
≥ 35 years	759	(18.3)
Maternal education at baseline (years), n (%)		
< 9 years	1101	(26.5)
9-11 years	1777	(42.8)
≥12 years	1270	(30.6)
Maternal BMI before pregnancy (kg/m^2), n (%)	23.92	(4.23)
Under/Normal weight	2871	(69.2)
Overweight/Obese	1277	(30.8)
Child's sex, n (%)		
Girls	2050	(49.4)
Boys	2098	(50.6)
Child's BMI z-score (WHO) at 7 years old, n (%)*		
Underweight/Normal weight	2599	(62.7)
Overweight/Obesity	1541	(37.2)
Child's Waist circumference at 4 years old, n (%)**		
Lowest tertile (<50.5 cm)	1344	(32.8)
2nd tertile (50.5–53.5 cm)	1393	(34.0)
Highest tertile (≥53.6 cm)	1362	(32.2)
Dietary patterns at 4 years old, n (%)		
Healthier	1717	(41.4)
Lower in Healthy Foods	1575	(38.0)
EDF- Dairy	856	(20.6)
Eating Behaviours appetite-related at 7 years old, mean (SD)		
Appetite Restraint	2.89	(0.61)
Appetite Disinhibition	2.15	(0.52)

* - For this variable, sample size is lower (n = 4140); ** - For this variable, sample size is lower (n = 4099)
BMI – Body Mass Index; WHO – World Health Organization; EDF – Energy Dense Foods; SD – Standard Deviation

Table 2. Descriptives (mean, SD) of the 2-factors identified in the Portuguese Child Eating Behaviour Questionnaire at 7 years according to their dietary patterns at 4 years old (n=4148).

Dietary patterns at 4 years old			Appetite Restraint at 7 years old		Appetite Disinhibition at 7 years old	
	n	%	mean	SD	mean	SD
Healthier	1717	41.4	2.85	0.62	2.08	0.50
Lower in Healthy Foods	1575	38.0	2.96	0.60	2.17	0.51
EDF-Dairy	856	20.6	2.87	0.60	2.25	0.54
p-value:			**<0.001**		**<0.001**	

EDF – Energy Dense Foods; SD – Standard Deviation

Table 3. Univariate and multivariate associations between children's dietary patterns at 4 years old and eating behaviours related to appetite at 7 years old (n= 4148).

Dietary patterns (4 years)	Appetite Restraint (7 years)		Appetite Disinhibition (7 years)	
Model 0 (unadjusted)	**β**	**95% CI**	**β**	**95% CI**
Healthier	ref		ref	
Lower in Healthy Foods	**0.104**	**0.063; 0.146**	**0.089**	**0.054; 0.124**
EDF-Dairy	0.018	-0.031; 0.068	**0.168**	**0.126; 0.210**
Model 1*				
Healthier	ref		ref	
Lower in Healthy Foods	**0.139**	**0.097; 0.182**	**0.072**	**0.036; 0.107**
EDF-Dairy	**0.072**	**0.021; 0.124**	**0.137**	**0.093; 0.180**
Model 2**				
Healthier	ref		ref	
Lower in Healthy Foods	**0.141**	**0.100; 0.181**	**0.072**	**0.036; 0.107**
EDF-Dairy	**0.073**	**0.024; 0.123**	**0.137**	**0.093; 0.180**

Significant associations are in bold type.

***** β coefficients adjusted for maternal age, education and maternal BMI before pregnancy.

** β coefficients adjusted for maternal age, education, maternal BMI before pregnancy and child's BMI z-score at 4 years old; This model includes 4106 children for w hom complete information is available.

EDF – Energy Dense Foods; CI – Confidence Interval; ref - reference class

Table 4. Association between children's dietary patterns at 4 years old and eating behaviours related to Appetite at 7 years old according to child's waist circumference at 4 years old (n= 4099).

Waist circumference*	Dietary Patterns at 4 years old			Appetite Restraint at 7 years old		Appetite Disinhibition at 7 years old	
		n	%	β	95% CI	β	95% CI
Lowest tertile (<50.5 cm)	**Healthier**	553	13.5	ref		ref	
	Lower in Healthy Foods	528	12.9	**0.084**	**0.011; 0.157**	**0.103**	**0.036; 0.170**
	EDF-Dairy	263	6.4	-0.028	-0.119; 0. 063	**0.118**	**0.037; 0.1989**
2nd tertile (50.5–53.5 cm)	**Healthier**	582	14.2	ref		ref	
	Lower in Healthy Foods	518	12.6	**0.121**	**0.054; 0.189**	**0.062**	**0.002; 0.122**
	EDF-Dairy	293	7.1	0.073	-0.009; 0.154	**0.133**	**0.061; 0.205**
Highest tertile (≥ 53.6 cm)	**Healthier**	557	13.6	ref		ref	
	Lower in Healthy Foods	516	12.6	**0.178**	**0.108; 0.249**	0.056	-0.001; 0.114
	EDF-Dairy	289	7.1	**0.142**	**0.057; 0.227**	**0.161**	**0.090; 0.232**

***** β coefficients adjusted for maternal age, education and maternal BMI before pregnancy.

EDF – Energy Dense Foods; CI – Confidence Interval; ref - reference class; Significant associations are in bold type.

CONCLUSIONS

In this thesis, early influences of eating behaviours related to appetite were assessed among children, using a prospective approach. This research allowed the establishment of some conclusions.

The psychometric evaluation of the Portuguese version of the CEBQ applied in the Generation XXI sample at 7 years of age demonstrated good properties, namely factor structure, internal consistency, construct validity and reliability. Also, the study allowed the proposal of a short-version of this questionnaire, with 20 items, useful for future research. The definition of two global domains of appetite, *Appetite Restraint* (more related with sub-domains measuring internal cues of satiety and food fussiness) and *Appetite Disinhibition* (more related with sub-domains measuring external food cues and emotional responses towards foods) through the re-aggregation of the CEBQ sub-domains, supported by statistical reasoning, simplified the study of their early determinants.

The family environment in early childhood was revealed, in this study, as a key influence on the emergence of these appetite dimensions at 7 years old. On one hand, children from more structured families and higher educational background developed higher Appetite Restraint and lower Appetite Disinhibition. On the other hand, children with less favourable anthropometrics developed lower Appetite Restraint and higher Appetite Disinhibition. Moreover, those children following more sedentary behaviours (more hours of media screening) and unhealthy dietary patterns (high in EDF and lower in healthy foods) at four years old developed more problematic eating behaviours (more Appetite Restraint and Appetite Disinhibition) in later childhood.

This thesis presents evidence to support the contribution of children, maternal and familial factors to the development of eating behaviours related to appetite during childhood. To my knowledge, this is one of the few studies prospectively evaluating the influence of a broad range of factors on childhood eating behaviours. Moreover, the influence of early children's dietary patterns, supported by plausible biological mechanisms, has been also demonstrated in the current study, whereas most studied have relied in cross-sectional associations with single foods. However, future analysis of other possible factors interfering in the development of eating behaviours related to appetite is needed to better understand the ways in which diverse determinants interact and influence appetite in children.

Emphasis towards the expansion of knowledge on this research area is of particular relevance, for supporting the development of strategies promoting children's health (and the inherent behaviours). The consolidation and proper divulgation of findings on childhood appetite should be useful for researchers and clinicians, but especially for parents. Most factors analysed in the current study, with significant interference in the development of appetite, namely dietary patterns, depend on family characteristics. Thus, education strategies involving parents and families since early should be addressed, in order to prevent the development of unhealthy eating behaviours, associated with later poor health outcomes, as obesity.

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